Exploring the Behavioural and Brain Basis of the Link Between Traumatic Brain Injury and Parkinson's disease

ING CHEE WEE

Discipline of Anatomy and Pathology
School of Biomedicine
The University of Adelaide

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Contents

Abstract

Accurately identifying individuals at risk for Parkinson's disease (PD) is crucial for developing effective treatment strategies. The recognition of non-motor symptoms preceding classical motor PD symptoms highlights the existence of the prodromal stage—an early phase marked by symptoms and signs before an official PD diagnosis, and emphasises the need for research to unravel the risk factors predicting PD onset. While the etiology of PD remains largely unknown and likely multifaceted—encompassing aging, genetics, and environmental influences —this thesis aims to explore the mechanisms via which two of the most widely reported risk factors, traumatic brain injury (TBI) and pesticide exposure, as well as the synergistic combination of the two, may drive the progression of PD pathogenesis. The first part investigates the impact of different severities of traumatic brain injury (TBI) on long-term motor performance, alterations in catecholaminergic signalling and chronic neuroinflammation, as such underlying abnormalities may set the stage for the later emergence of PD. The results from this thesis revealed that TBI severity is associated with persistent motor deficits, presented in a dose-dependent pattern in balance, gait and speed over 10 years post-injury. The exploration of neuropathology aligns with this pattern, with moderate-severe TBI demonstrating the most notable alterations in neuroinflammation and noradrenaline signalling in the nigrostriatal pathway, accompanied by subtle deficits in cognitive flexibility at 12 months post-injury. A different effect was observed, with single mild TBI animals displaying altered dopamine signalling in the prefrontal cortex that was found to be associated with reduced anxiety. To explore further, the second part of the thesis aims to develop a novel animal model for studying the contribution of low-level exposure to the pesticide rotenone and moderate-severe diffuse TBI, alone and in combination, to PD pathogenesis by conducting a comprehensive behavioural assessment at 1-month following injury in this model. It reveals that dual environmental risk factors lead to more pronounced deficits in balance, motor

coordination, and learning ability than individual risks alone. While other functional tests assessing motor, cognition, and prodromal symptoms are preserved (with the possible exception of constipation), this may hint at an increased risk for future PD development. Taken together, this thesis demonstrates a possible association between TBI and the risk of developing PD, with this risk heightened with more severe TBI and the addition of environmental risk factors. This thesis also provides preliminary insights into predicting PD after such exposures, underscoring the significance of future investigations into the complex interplay of multiple risk factors, which is critical for advancing our knowledge in PD progression and enhancing the accuracy of PD diagnosis.

Declaration

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ING CHEE WEE
Date: 18th December 2023

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Academic outcomes

Publications

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3. **Wee, I.C**., Arulsamy, A., Corrigan, F., Collins-Praino, L., 2024. Long-Term Impact of Diffuse Traumatic Brain Injury on Neuroinflammation and Catecholaminergic Signaling: Potential Relevance for Parkinson's Disease Risk. Molecules 29, 1470.

Conference Presentations

Wee I.C., Corrigan F., and Collins-Praino L.E, **3 Minute Thesis Competition (3MT)-Faculty Heat,** Oral Presentation, The University of Adelaide, Aug 2019- 'Is Tremor a little too late?'

Wee I.C., Corrigan F., and Collins-Praino L.E, **14th Florey International Postgraduate Research Conference**, Poster Presentation, September 2020- 'Long-term alterations in Motor Performance following varying severities of Traumatic Brain Injury-A Systematic Review'

Wee I.C., Corrigan F., and Collins-Praino L.E, **Australian Society for Medical Research Annual Scientific Meeting**, Oral Presentation, June 2021- 'Chronic motor performance following different traumatic brain injury-, A Systematic Review'

Wee I.C., ArulsamyA., Corrigan F., and Collins-Praino L.E, **15th Florey International Postgraduate Research Conference**, Poster Presentation, September 2021- 'The relationship between prefrontal cortex morphology and executive function at 12-months following different severities of traumatic brain injury.'

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Thesis explanation

This thesis is structured with chapters presented in both publication-style and narrative formats. It consists of a general introduction, four experimental chapters and an overall discussion, with bridging narratives provided prior to each experimental chapter, in order to provide rationale for the reader about the study to follow. Chapter 1 provides a general introduction, outlining environmental risk factors associated with Parkinson's disease (PD), with a specific focus on rotenone exposure and its relationship to traumatic brain injury (TBI) in PD development. Followed by a narrative, underlining the crucial need for a systematic review to evaluate long-term motor impairments in TBI survivors across varying severity. Chapter 2 presents a comprehensive systematic review encompassing both clinical and preclinical studies, that delves into chronic motor impairment following different severities of TBI. While it remains a challenge of determining the relationship between the changes in chronic motor function following injury and the risk of PD development, another narrative, highlighting that this relationship could probed further by investigating the shared mechanisms of TBI and PD, particularly the catecholamine and neuroinflammatory pathways at 12 months post-injury. This sets the stage for experimental exploration of this topic in Chapter 3. The next bridging narrative explores the heterogeneity of PD and the potential synergistic effects seen by combining major environmental risk factors in an animal model. Chapter 4 focuses on developing a novel animal model for studying the contribution of low-level exposure to the pesticide rotenone and moderate-severe diffuse TBI, while Chapter 5 delves into the effect of these environmental risk factors alone and in the combination, to PD pathogenesis by conducting a comprehensive behavioural assessment at 1-month following injury in this model. Finally, Chapter 6 presents a comprehensive discussion, summarising the work presented

throughout this thesis. This is followed by a references section and appendices. For chapters prepared in a publication format, they are prefaced by a signed statement of authorship.

Abbreviations

α-syn- α-synuclein
ADRA1A- Alpha 1A Adrenoceptor
ADRA2A-Alpha 2A Adrenoceptor
ADRB1-Beta-1 adrenergic receptor
ANOVA- Analysis of variance
AOR- Adjusted Odd Ratio
AT- Adhesive Removal Test
BFT- Buried Food Test
BM- Barnes Maze
BW- Beam Walking
CCI - Controlled Cortical Impact
CCI-CS- Controlled Cortical Impact-Closed Skull
CAT- Catalase
CHIMERA- Closed Head Impact Model of Engineered Rotational Acceleration
CNS- Central Nervous System
COMT- Catechol-O-methyltransferase
CSF- Cerebrospinal Fluid
CTE- Chronic Traumatic Encephalophathy
DA- Dopamine
DAB- 3,3'-Diaminobenzidine tetrahydrochloride
DAMPs- Damage-Associated Molecular Patterns
DAT- Dopamine transporter
DβH-Dopamine Beta Hydroxylase
DMSO- Dimethyl Sulfoxide
DMV -Dorsal Motor neuron of the Vagus
DPI- Day Post Injury
DRD1- Dopamine D1 receptor
ELISA- Enzyme-Linked Immunosorbent Assay
EPM- Elevated Plus Maze
LFP- Lateral Fluid Percussion
GABA- γ-aminobutyric Acid
GCS- Glasgow Coma Scale
GFAP- Glial fibrillary acidic protein
GST- Grip Strength Test
GT- Gait test
GSH- Glutathione
HPLC- High Performance Liquid Chromatography
HR- Hazard Ratio
HY- Hoehn and Yahr scale
IBA1- Ionized calcium binding adaptor molecule 1
iNOS- Nitride Oxide Synthase

IHC- Immunohistochemistry
JT- Jaw Tremor Test
LB- Lewy Bodies
LC- Locus Coeruleus
LN -Lewy Neurites
LOC- Loss Of Consciousness
LRRK2- Leucine Rich Repeat Kinase 2
MAPK- Mitogen-Activated Protein Kinase
MMNS- Mild Motor- Non-motor Subtype
MND- Motor Neuron Disease
MPTP- 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine
MRI- Magnetic Resonance Imaging
MSA- Multiple System Atrophy
msTBI- Moderate – Severe Traumatic Brain Injury
mUPDRS- Modified UPDRS
NA- Noradrenergic
NFkB- Nuclear-Factor-kB
NFL- National Football League
OP- Open Field
OCT- Optimum Cutting Temperature Compound
PMT- Pasta Manipulation Test
ROT-Rotenone
PBBI- Penetrating ballistic like Impactor
PBS- Phosphate- buffered Saline
PD- Parkinson's Disease
PET- Positron Emission Tomography
PFC- Prefrontal Cortex
PIGD- Postural Instability-Gait Disturbance
PPMI- Parkinson’s Progressive Markers Initiative
PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PRRs- Pattern Recognition Receptors
PTA- Post-Traumatic Amnesia scale
PTSD-Post Traumatic stress disorder
RAGE- Receptor for Advanced Glycation End products
RevMan- Review Manager
rmTBI- Repetitive Mild Traumatic Brain Injury
RNS- Reactive Nitrogen Species
ROS- Reactive Oxygen Species
SMNS- Severe Motor-Non-motor Subtype
smTBI- Single Mild Traumatic Brain Injury
SN- Substantia Nigra
ST- Forelimb Adjusting Step Test
SOD- Superoxide Dismutase
SPC- Streptavidin Peroxidase Conjugate

SPECT- Single-Photon Emission Computerised Tomography
STR- Striatum
SYRCLE- Systematic Review Centre for Laboratory Animal Experimentation
TBI- Traumatic brain injury
TBST – Tris buffered saline with tween
TD- Tremor Dominant
TH- Tyrosine Hydroxylase
TNF- Tumour Necrosis Factor
VEH-Vehicle
WD- Weight Drop
UPDRS- Unified Parkinson's Disease Rating Scale

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01

General Introduction: Parkinson's Disease, Environmental Factors, and Pathogenesis

Chapter prepared by **WEE, IC.**, CORRIGAN, F., COLLINS-PRAINO, L.E

ABSTRACT

With formal characterisation of the prodromal stage in PD, marked by non-motor symptoms that precede presentation of the cardinal motor symptoms, research has been redirected toward uncovering the risk factors that predict the onset of clinical PD. Emerging evidence suggests that environmental factors such as pesticide exposure and traumatic brain injury (TBI) are key players in increasing the risk of PD development. However, as the focus of PD research has largely been on motor presentation for decades, the intricate connections between these factors and disease pathogenesis remain largely unexplored. This review aims to investigate the environmental risks associated with PD, specifically focusing on of the mechanisms via which they may drive PD pathogenesis. Despite extensive research that has showcased the impact of TBI on PD risk, even decades following the initiating injury, a consensus regarding the mechanisms via which TBI may be associated with this heightened risk is yet to be reached. A similar scenario is observed in studies of pesticide exposure, where the majority utilise this neurotoxin to induce a moderately advanced PD model, displaying severe parkinsonian symptoms, rather than a more clinically relevant progression-based model. To bridge this gap and elucidate the connection of these factors to PD onset, future studies should prioritise understanding the time course of symptom progression, as well as how risk factors may act synergistically to drive the risk of PD development.

1. INTRODUCTION

Parkinson's Disease (PD) is the fastest-growing neurodegenerative disease, surpassing Alzheimer's Disease in its escalating prevalence. Between 1990 and 2015, the incidence of PD surged by 118%, resulting in approximately 6.3 million cases worldwide (Feigin et al., 2021). In Australia alone, a conservative estimate reveals that by 2015, there were 70 thousand individuals living with Parkinson's, and on average, 32 new diagnoses were made each day (Deloitte, 2015). Notably, 80% of these cases occurred in individuals aged 65 and above, underscoring the mounting challenge as the population ages and life expectancy rises (Deloitte, 2015; Wanneveich et al., 2018). In fact, Dorsey and Bloem projected in 2018 that PD incidence is expected to exceed 12 million cases globally by 2040 (Dorsey and Bloem, 2018). When considering additional factors like increasing longevity, industrialisation, and a decrease in smoking rates, the burden of PD could potentially escalate to a staggering 17.5 million cases by that time (Dorsey and Bloem, 2018).

This escalating prevalence carries significant implications, particularly its economic impact on society (Dorsey and Bloem, 2018; Wanneveich et al., 2018). On average, an Australian living with Parkinson's for 12 years incurs a lifetime financial cost of approximately $161k AUD (Deloitte, 2015). A comprehensive 2017 review by Yang *et al.* consisting of factors like society, patients and families emphasised that the combined economic burden posed by PD in the United States amounted to a direct medical cost of $25.4 billion USD, coupled with an additional 26.5 billion USD in other non-medical expenses for the approximately 1 million Americans diagnosed with PD (Yang et al., 2020). With the increasing prevalence of PD, this burden is expected to rise significantly, surpassing a substantial $79 billion USD by 2037 within the United States alone (Yang et al., 2020).

Developing interventions that can slow or halt the progression of PD or prevent its development is paramount to mitigate the future economic impact of this burgeoning epidemic.

However, a major obstacle in discovering effective therapies lies in the fact that upon PD diagnosis, patients already exhibit crucial motor symptoms, with more than half of substantia nigra (SN) cells already lost, accounting for an approximately 80% dopaminergic denervation in the striatum (STR) (Fearnley and Lees, 1991; Greffard et al., 2006). Hence, early detection of PD, or at least before the emergence of motor dysfunction, is important (Soman et al., 2023). In this context, comprehending the pathogenesis of PD is key, especially understanding how recognised environmental risk factors contribute to its development (Chen and Ritz, 2018).

In this introductory chapter, we will first delve into the current understanding of PD, exploring its pathophysiological mechanisms. We will then discuss how major environmental factors, including traumatic brain injury (TBI) and pesticide exposure, could serve as pathological triggers and give rise to PD development.

2. BRIEF OVERVIEW OF PARKINSON'S DISEASE

PD was first described as a 'Shaking Palsy' or 'Paralysis Agitans' by James Parkinson in 1817 (Parkinson, 2002), portraying it primarily as a disorder impacting movement, consisting of the well-recognised motor-related symptoms (tremor, bradykinesia, rigidity, postural instability). However, as our comprehension of PD has evolved, it has transcended this initial motor-centric perception to encompass a wide spectrum of non-motor symptoms, with some of these features preceding the onset of motor manifestations in PD (Hughes et al., 1992).

In the early stages, autonomic dysfunction can emerge, leading to issues such as orthostatic hypotension and constipation. Concurrently, sleep disturbances, including insomnia and fragmented sleep, may appear and evolve into more intricate sleep disorders like restless legs syndrome and REM sleep behavior disorder as the disease advances. Sensory symptoms like hyposmia and gastrointestinal dysfunction are often present during the prodromal phase. Additionally, mood alterations and cognitive changes, such as depression, anxiety, and mild

cognitive impairment, may manifest. These psychiatric symptoms may progress to more pronounced forms, like hallucinations and delusions, in later PD stages (for review, see (Aarsland and Kurz, 2010; Postuma and Berg, 2019)). Similarly, the cognitive symptoms can also progress significantly, with up to 80% of individuals with PD going on to develop dementia (for review, see (Russell et al., 2014)) (Figure 1).

The progression of both motor and non-motor symptoms can vary among individuals, with three distinct subtypes proposed through multimodal analysis of clinical, cognitive, motor and neuroimaging data; namely, cognitive-motor, cognitive dominant and motor dominant (Albrecht et al., 2022) Other subtypes have been put forward in other studies, including the classic tremor dominant (TD) vs. postural instability-gait disturbance (PIGD) subtypes, based on motor symptoms alone (Jankovic et al., 1990), and the more recent proposal of brain-first vs. body-first PD, based on the initial presentation of alpha-synuclein pathology (Horsager et al., 2020). Currently, there is no agreed upon method to subtype those with PD, although it has been argued that more standardised clustering analysis methods are needed in order to increase the ability to reproduce subtypes and improve comparability across studies (Shakya et al., 2022). Using such a method (i.e. a standardised k-means cluster analysis approach) in the analysis of 408 *de novo* PD patients with complete clinical data from the Parkinson's Progressive Markers Initiative (PPMI), Shakya and colleagues (2022) identified two distinct PD subtypes: a severe motor-non-motor subtype (SMNS) and a mild motor- non-motor subtype (MMNS). Importantly, these individuals differed in terms of time of symptoms onset, severity of symptoms, striatal dopamine binding values on SPECT imaging and progression of symptoms (Shakya et al., 2022). Thus, accurate subtyping can have important implications for both predicting the course of disease for an individual, as well as the development of personalised treatment modalities.

2.1 Mechanisms and Pathological Hallmarks of PD

PD is a progressive neurodegenerative disease, with its pathological events unfolding in a step-wise manner. Emerging evidence suggests that the intricate interplay of various pathological processes, such as mitochondrial dysfunction, lysosomal dysfunction, and excitotoxicity, initiated by various risk factors (aging, genetics and environmental factors), may act as potential triggers, nudging the neurological system towards the development and manifestation of PD (Pang et al., 2019). Among these factors, the two most interconnected pathophysiological mechanisms, neuroinflammation and oxidative stress, are believed to underlie and play a central role in disease development—specifically influencing dopaminergic neuron degeneration and the aggregation of α-synuclein.

2.1.1 Loss of Dopaminergic Neurons in Substantia Nigra

Underlying the development of these motor and non-motor symptoms is the hallmark of PD, loss of dopaminergic (DA) neurons in the substantia nigra pars compacta. Compared to healthy controls, post-mortem examination of brains from individuals with PD consistently reveals a notable reduction in the pigment neuromelanin in DA neurons in the SNpc (Dauer and Przedborski, 2003; Fabbri et al., 2017; Marsden, 1983). Studies have indicated that by the time motor symptoms are present, there is an approximate 40-60% loss of DA neurons within the SNpc, and as the disease progresses, this neuronal loss can escalate to 70% or even higher (Kordower et al., 2013; Ma et al., 1997).

Notably, the loss of DA SN pars compacta neurons results in substantial reductions in dopamine levels within the STR, the primary target region of the nigrostriatal pathway. This pathway, crucial for precise movement control, governs functions such as movement initiation and regulation (Bernheimer et al., 1973; de la Fuente-Fernández, 2013; K et al., 2011). Supporting this correlation, a study utilising neuromelanin magnetic resonance imaging (MRI)

and dopamine transporter single-photon emission computerised tomography (SPECT) to quantify the severity of PD motor symptoms, as determined by Hoehn and Yahr scale (HY) in 40 PD patients, revealed that both the signal-to-noise ratio in the SN pars compacta on neuromelanin MRI and the striatal specific binding ratio on dopamine transporter SPECT were significantly greater in the individuals in early-stage PD group than those in the advanced group (Hoehn and Yahr, 2001; Takahashi et al., 2019). These results indicate that the dopamine levels in the nigrostriatal pathway are closely associated with the severity of motor symptoms and are involved in the development of PD (Takahashi et al., 2019).

In addition to the depletion of DA cells in SN pars compacta and decrease of DA levels in STR, emerging literature suggests a widespread neuronal loss in other brain regions, including the hypothalamus, locus coeruleus (LC), the dorsal motor nucleus of the vagus, and the olfactory bulb (Giguère et al., 2018). These regions play a regulatory role involving various neurotransmitters, including γ-aminobutyric acid (GABA), acetylcholine, serotonin, adrenaline, glutamate, and adenosine (Barone, 2010; Giguère et al., 2018). Disruption in these systems potentially contributes to the development of non-motor symptoms of PD that do not respond well to dopamine replacement therapy (Yadav and Kumar, 2022). Of these, strong evidence suggests LC noradrenergic neurodegeneration occurs earlier than that of neurons in the SN pars compacta and is critical to the pathogenesis of several non-motor symptoms and can also exacerbate dopaminergic neurodegeneration in PD rodent models (Szot et al., 2012; Zarow et al., 2003).

2.1.2 Spread and Accumulation of a-Synuclein

The spread and accumulation of misfolded α-synuclein (α-syn) represents another fundamental factor in the pathogenesis of PD and stands as one of the most significant hypotheses explaining the degeneration of nigrostriatal neurons (Maries et al., 2003). Under normal conditions, α-syn

is a small, acidic, natively unfolded and soluble protein found both in the cytosol and presynaptic terminal, where it plays a crucial role in regulating synaptic function (Atias et al., 2019). However, in PD, α-syn aggregates into structures known as Lewy bodies (LB) and Lewy neurites (LN), along with other proteins like neurofilament protein, tau, ubiquitin, and α-B crystallin (Spillantini et al., 1997), disrupting normal cellular functions and leading to the malfunction and death of neurons.

A study conducted by Braak and colleagues revealed that PD-related α-syn-immunopositive LB and LN initially spread from the olfactory bulb or dorsal motor neuron of the vagus (DMV) to the raphe nucleus and then to the LC, the basal nucleus of Meynert, and eventually extend throughout the brain to the temporal cortex and neocortex (Braak et al., 2003b). However, the fact that a-syn could be detected in peripheral tissue before PD diagnosis (Hilton et al., 2014; Stokholm et al., 2016), coupled with evidence from Holmqvist *et al.* That demonstrated that different forms of α-syn (monomeric, oligomeric, and fibrillar) from PD lysates could travel from the intestine along the vagus nerve to reach the brain (Holmqvist et al., 2014), indicating that the initiation site of α-syn could begin in the gut. This led to the recent proposal of brain-first versus body-first PD, with initial multimodal neuroimaging data supporting this hypothesis (Horsager et al., 2020). In brief, in brain-first PD, the initial site of α-synuclein pathology is within the central nervous system (likely rostral to the SNc) and spreads via interconnected structures, eventually reaching the autonomic nervous system. In contrast, in body-first PD, the initial site of α-synuclein pathology is within the enteric nervous system and spreads in a caudo-rostral direction to reach the autonomic and eventually the central nervous system. A recent review of the literature by Horsager and colleagues (2022) supports this subtyping and highlights differences in clinical and neuroimaging between the two groups, but further characterisation is needed (Horsager et al., 2022), particularly in terms of differences in disease progression and treatment responsiveness, between the two groups.

Although it remains a source of much debate, the hypothesis that α-syn could self-propagate and spread progressively between brain regions has received a great deal of support (Masuda-Suzukake et al., 2014; Recasens et al., 2014). For example, this concept finds support in emerging studies that demonstrate the ability of neurons to release α-syn aggregates into the extracellular environment, which can then be internalised by healthy neurons, resulting in neuron dysfunction and death (Danzer et al., 2009; Froula et al., 2019; Jan et al., 2021). However, the precise relation to cell loss in each region and the mechanism of cell-to-cell transmission remains largely unknown. Nonetheless, it is widely believed that the spreading pattern of the disease and cell-to-cell transmission of α-syn is closely associated with the onset and progression of PD symptoms (Stefanis, 2012).

Recently, α-syn conformational strain has also been proposed to play a key role in driving the heterogeneity of symptom progress in PD (for review, see (So and Watts, 2023)). Structural differences among α-synuclein aggregates can lead to unique biochemical and pathological properties in both human patients and experimental models.(So and Watts, 2023) In line with this, a study examining the α-syn aggregates obtained from the CSF of patients with PD, multiple system atrophy (MSA) and control subjects demonstrated clear structural variations in α-syn aggregates across these disease conditions, which can be distinguished by the size and number of protease-resistant bands (Shahnawaz et al., 2020). When subjected to the MTT assay, the amplified α-syn aggregates from the same patient cohorts demonstrate differential cytotoxicity levels, with the α-syn aggregates derived from MSA being more toxic to RK13cells and neuronal precursor cells derived from human induced pluripotent stem cells than PD-derived α-syn aggregates, revealing the nuanced structural and biochemical properties within α-syn (Shahnawaz et al., 2020).

2.1.3 Neuroinflammation

Neuroinflammation, a complex process involving the activation of immune cells within the central nervous system (CNS), such as microglia and astrocytes, is considered a crucial contributor to the pathogenesis of several neurodegenerative diseases, including PD. This process involves glial cells releasing various inflammatory molecules and cytokines that signal other immune cells, initiating an inflammatory response to eliminate threats and remove damaged cells (Glass et al., 2010). While this mechanism is an essential as part of the body's defence mechanism to protect the nervous system from injury or toxic environments, chronic or excessive neuroinflammation can be detrimental.

Increasing evidence from post-mortem examinations of PD brains has revealed the activation of microglia (Imamura et al., 2003; McGeer et al., 1988) and an increased density of astrocytes within the SN (Damier et al., 1993). In the analysis of biological fluids, elevated levels of pro-inflammatory cytokines, including tumour necrosis factor (TNF) (El-Kattan et al., 2022; Scalzo et al., 2009), interleukin (IL)-1β (Williams-Gray et al., 2016)and IL-6 (Blum-Degena et al., 1995; Diaz et al., 2022), were also observed in the cerebrospinal fluid and blood serum of PD patients. Positron emission tomography (PET) imaging study utilising [11C](R)-PK11195 as a microglial marker further supports these findings, demonstrating ongoing and static microglial activation in multiple brain regions in clinically diagnosed idiopathic PD patients (Gerhard et al., 2006).

While it is well acknowledged that neuroinflammation contributes to neuronal cell loss (Ouchi et al., 2005; Tansey and Goldberg, 2010), it resembles an intercellular feedback loop: activation of neuroinflammation results in cell degeneration, and the subsequent neuronal death releases proinflammatory cytokines, thereby triggering further inflammatory responses; however, whether neuroinflammation initiates neurodegeneration or is a consequence of it still remains an area of debate within the field (Tansey and Goldberg, 2010). Similarly, concerning the connection between α-syn aggregation and inflammation, *in vitro* studies provide evidence

that α-syn aggregations can induce microglial activation in rat primary mesencephalic neuron-glia cultures (Zhang et al., 2005) and microglia obtained from mice neonates (Reynolds et al., 2008). Conversely, findings indicate that anti-inflammatory agents suppress α-syn aggregation and spreading in mice (Kim et al., 2022) and the absence of microglial affects α-syn cell-cell transfer in a mouse model injected with human α-syn into the SN (George et al., 2019). The precise relationship, including whether α-syn aggregation acts as a driving force for initiating inflammation or if inflammation triggers the development of α-syn pathology, remains unclear (Lema Tomé et al., 2013).

2.1.4 Oxidative stress

Oxidative stress is another pathological mechanism that plays an important role in dopaminergic toxicity. It arises when there is an increased production and accumulation of free radicals, such as superoxide (O_2-), hydroxyl (-OH), and hydrogen peroxide (H_2O_2), collectively defined as reactive oxygen species (ROS), or peroxynitrite (ONOO-) and nitrogen dioxide (NO_2), defined as reactive nitrogen species (RNS) (Sies and Jones, 2020). ROS and RNS are highly reactive molecules that can induce oxidative damage to cellular components, such as lipids, proteins, and DNA, eventually leading to cell death (Li et al., 2003). Factors like neuroinflammation (Taylor et al., 2013), environmental exposures (Samet and Wages, 2018) and aging (Liguori et al., 2018) can further contribute to elevated ROS and RNS levels. To counteract these harmful effects, the brain employs antioxidants to maintain equilibrium.

However, when there is an excess of free radicals and insufficient levels of antioxidants to counteract the effects, this imbalance could also induce oxidative stress and culminate in the occurrence of PD pathogenesis. In support of this, a mutation in the gene encoding the antioxidant protein DJ-1, also known as PARK7, has been linked to elevated ROS levels and abnormalities in oxidative stress biomarkers (Di Nottia et al., 2017). DJ-1 knock-out in mice

increased protein oxidation, leading to nigrostriatal dopaminergic neuron loss (Goldberg et al., 2005; Guzman et al., 2010). This gene mutation is also identified as a causative factor in α-synucleinopathy (Taipa et al., 2016) and contributes to the progression of early-onset recessive PD (Bonifati et al., 2003). There are also mutations in genes, such as Parkin and PINK1, which are found to be associated with oxidative stress and are also linked to familial forms of PD (Hauser and Hastings, 2013; Lee Mosley et al., 2006; Norris et al., 2015).

Furthermore, accumulating evidence from post-mortem studies consistently demonstrates a significant reduction in an important antioxidant –glutathione (GSH)- in the brains of individuals with PD (Zeevalk et al., 2008). Specifically, this decrease in GSH levels is pronounced in the SN (Riederer et al., 1989; Sian et al., 1994; Sofic et al., 1992) and is observed in nigral dopamine neurons (Pearce et al., 1997). In addition to altered GSH levels, superoxide dismutase (SOD) I and II—an enzyme responsible for the conversion of superoxide radical (O2-) into oxygen and hydrogen peroxide- was similarly higher in the SN of individuals with PD compared to controls, reflecting a heightened superoxide-radical environment (Marttila et al., 1988; Saggu et al., 1989). For further discussion of the role of oxidative stress in PD, several comprehensive reviews are available (Algarni and Stoessl, 2016; Dias et al., 2013; Ikawa et al., 2021)).

2.2 PD staging

PD can be classified into three main stages: the pre-clinical stage, the prodromal stage, and the clinical stage (Figure 1).

I) **Pre-clinical stage:** The pre-clinical stage represents the earliest phase of PD, during which individuals may exhibit subtle DA degeneration and synucleinopathy in the brain without displaying overt clinical signs of the disease (Berg et al., 2015).

II) **Prodromal stage:** The prodromal stage, conversely, is characterised by the presence of non-motor symptoms, such as hyposmia, sleep abnormalities, constipation, anxiety, depression, as well as subtle motor changes, such as facial akinesia, upper limb rigidity and loss of balance, that do not meet the criteria for a clinical PD diagnosis (Kalia and Lang, 2015; Postuma et al., 2012). This phase is primarily associated with Braak stages 1-2 (Braak et al., 2003b), marked by the presence of synucleinopathy in lower brainstem regions and the olfactory bulb. Additionally, it is during this stage that DA neuronal loss and subtle alterations in the DA system become apparent and can be detected by neuroimaging (Heng et al., 2023; Jennings et al., 2014). Understanding these subtle changes in the prodromal stage is crucial for predicting the future development of PD and offers valuable insights for assessment and screening.

III) **Clinical stage:** The clinical stage begins with the current accepted diagnostic criteria that define PD, which requires the presence of bradykinesia alongside at least one of either resting tremor or rigidity. Pathological progression aligns with Braak stages 3-4 (Braak et al., 2003b), involving the emergence of synucleinopathy affecting the SN and

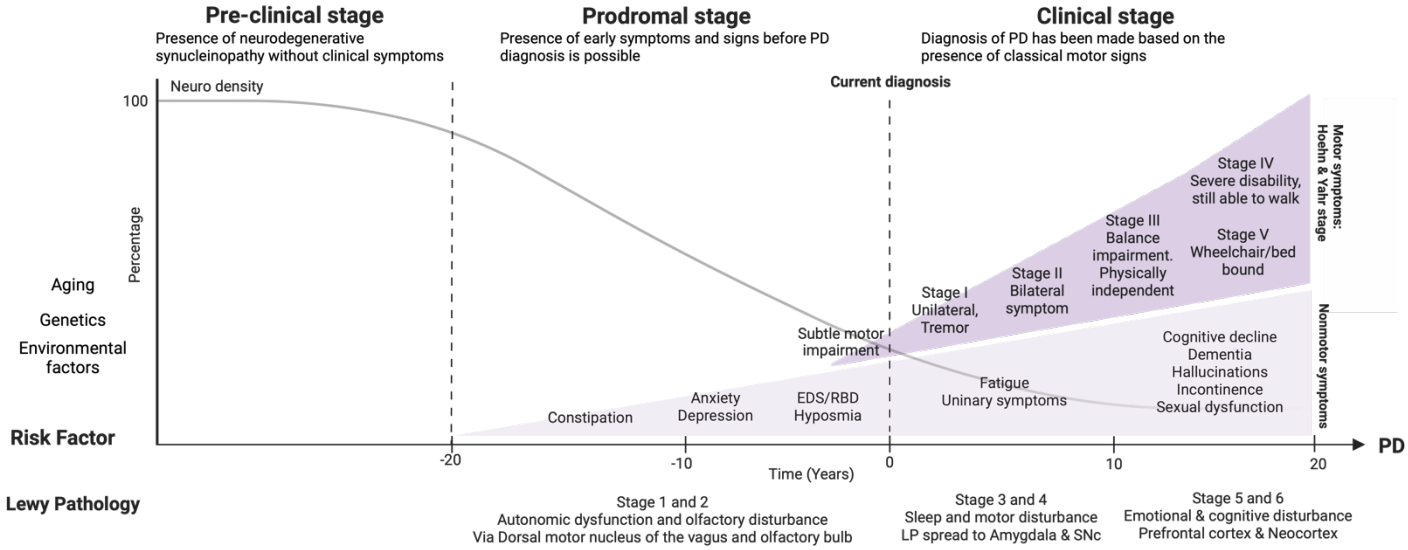

Figure 1. A comprehensive schematic timeline delineating key aspects of Parkinson's Disease progression, including the evolution of clinical symptoms, time course, Lewy body pathologies, dopaminergic neuron loss, and the Hoehn and Yahr Scale. The figure has been adapted and modified from Kalia and Lang's work in 2015.

other limbic areas. The symptoms in the early clinical stage are mild, progressing from unilateral to bilateral (HY stages 1-2) (Hoehn and Yahr, 2001). However, the degeneration of DA neurons at this stage is relatively rapid, with almost total loss of dopamine axon terminals in the dorsal putamen detected after 4 years and extensive loss of dopamine cell bodies in the SN by 5 years (Kordower et al., 2013).

As PD progresses into the mid-clinical stage (HY stage 3) (Hoehn and Yahr, 2001), motor symptoms intensify, significantly impacting daily activities and mobility. This temporal progression aligns with Braak stages 4-5 (Braak et al., 2003b), characterised by further advancement of synucleinopathy affecting the SN and additional brain regions. This exacerbates the loss of DA neurons and leads to a worsening of motor symptoms.

In the advanced clinical stage (HY stages 4-5)(Hoehn and Yahr, 2001), conspicuous clinical features include pronounced postural instability, gait disturbances, and cognitive impairments. This stage aligns with Braak stage 6 (Braak et al., 2003b), characterised by extensive α-syn pathology spread throughout the brain, particularly impacting the neocortex. This widespread α-syn pathology gives rise to the emergence of deficits in higher-order cognitive functions, sensory perception, and motor coordination. The substantial loss of DA neurons in the SN and the degeneration of more than 90% of the DA terminals significantly contribute to both motor and non-motor symptoms, delineating an advanced and challenging phase of PD (Lewis et al., 2023).

3. ENVIRONMENTAL FACTORS AS PATHOLOGICAL TRIGGERS IN PD

PD is generally considered an idiopathic disorder, with its likelihood of development hinging on a complex interplay of aging, genetic predisposition, environmental exposures, and lifestyle

factors (Kouli et al., 2018). Although approximately 10-15% of PD cases are attributed to genetic factors (Klein and Westenberger, 2012; Thomas and Beal, 2007), extensive research has revealed a number of specific environmental risks associated with PD development (Ball et al., 2019; Deng et al., 2018). In a comprehensive meta-analysis examining 30 potential risk factors for PD, 6 environmental risk factors, including pesticide exposure, head injury, rural living, beta-blocker usage, agriculture, and well water drinking, were identified as significantly linked to an increased risk of PD (Noyce et al., 2012). Notably, among these factors, exposure to pesticides and a history of prior head injury stand as two of the most compelling environmental risk factors for PD (Noyce et al., 2012). Beyond their role as environmental factors, they also manifest as pathological triggers in the development and progression of PD, as thoroughly reviewed by Mckee and Daneshvar (Mckee and Daneshvar, 2015) and demonstrated by Van Laar *et al.* (Van Laar et al., 2023).

Exposure to pesticides, such as rotenone and paraquat, has been frequently implicated in PD development due to their structural resemblance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound associated with parkinsonism in illicit drug users (Heikkila et al., 1985). Indeed, in a case-control study investigating pesticide use, both rotenone (odds ratio=2.5; 95% CI, 1.3-4.7) and paraquat (odds ratio=2.5; 95% CI, 1.4-4.7) showed a positive association with an increased risk of PD (Tanner et al., 2011). However, despite structural similarities to MPTP and comparable risks of inducing PD, rotenone has been more frequently utilised as a neurotoxin to create PD animal models (Betarbet et al., 2000; Johnson and Bobrovskaya, 2015). This preference is attributed to its demonstrated ability to induce PD pathology and replicate functional deficits through the inhibition of mitochondrial function (Richardson et al., 2005). For this reason, the focus of the discussion will now center on examining the relationship between rotenone and the pathogenesis of PD in the subsequent sections. Building on the evidence of rotenone's association with an elevated risk of PD, recent

epidemiological studies by Furlong *et al.* and Pouchieu *et al.*, have underscored this substantial link (Furlong 2015: hazard ratio=3.7, 95% CI: 1.7-8.1; Pouchieu 2018: hazard ratio=1.57, 95% CI: 1.08-2.29), particularly in individuals with prolonged exposure (Furlong et al., 2015; Pouchieu et al., 2018).

Furthermore, an increasing body of evidence suggests that individuals with a history of head injuries face an elevated risk of developing PD (Bower et al., 2003; Goldman et al., 2006). A meta-analysis encompassing 34 articles investigating the association between head trauma and PD demonstrated a notable 57% increase in the risk of developing PD in individuals with a history of head injury (hazard ratio=1.57, 95% CI: 1.35–1.83) (Jafari et al., 2013). A more recent meta-analysis of 23 studies using a random-effect model supported this association, showing a 1.50 odd ratio (95% CI: 1.23–1.83) of TBI among individuals with PD compared to controls (Balabandian et al., 2023). The risk appears particularly pronounced among athletes, including boxers, American football and rugby players, as well as military veterans exposed to frequent head impacts (Daneshvar et al., 2011). Indeed, both United States military veterans (Gardner et al., 2018) and former rugby players (Russell et al., 2022) exhibited a higher incident rate of PD diagnosis (TBI in veteran: hazard ratio=1.71, 95% CI: 1.53-1.92; TBI in athlete: hazard ratio=3.04, 95% CI: 1.51-6.10) over a 12 to 32-year follow-up period.

Compelling evidence demonstrates the association between these environmental factors (rotenone exposure and prior head injury) and an elevated risk of PD. Importantly, these environmental factors not only amplify the susceptibility to PD, but also mirror the disease's hallmark pathological features and clinical manifestations.

4. ROTENONE EXPOSURE IN THE PATHOGENESIS OF PD

Rotenone is a naturally occurring substance found in the seeds and stems of several plants, like the jicama vine and the roots of the Fabaceae family. Recognised for its broad-spectrum

insecticide, pesticide and nonselective piscicide (lethal to fish) properties, rotenone is extensively used in agriculture and is prevalent in agricultural lands and aquatic environments (Chandler and Marking, 1982; Marking and Bills, 1976). Rotenone is generally considered relatively safe to use due to its short half-life in both water and soil, typically lasting between 1 to 3 days and losing its toxicity within 5-6 days of exposure to spring sunlight or 2-3 days of exposure to summer sunlight (Marking and Bills, 1976). Despite this, concerns persist regarding the potential environmental and public health impacts of its use.

Rotenone can enter the body through multiple routes, such as inhalation, dermal absorption/eye contact, and ingestion (Wood et al., 2005). Commonly reported symptoms include irritation, nausea, gastric pain, coughing and choking. While neurological symptoms are infrequent, a few cases have reported peripheral neuropathy, numbness, or tremors (Gupta, 2014). According to a 2006 report by the U.S. Environmental Protection Agency, no fatalities or systemic poisonings have been reported in connection to the 'basic use' of rotenone (USEPA, 2006). However, chronic exposure is linked to PD development, with one recent meta-analysis by Yan *et al.* proposing that the duration and level of exposure to pesticides, including rotenone, are associated with a 1.05 (95% CI: 1.02-1.09) and 1.11 (95% CI: 1.05-1.18) augmented risk of developing PD, respectively (Yan et al., 2018).

4.1 Pathology overlap between rotenone exposure and PD

Greenamyre and colleagues demonstrated for the first time that chronic systemic exposure to rotenone in rats could induce highly selective degeneration of nigrostriatal dopaminergic neurons, accompanied by the formation of Lewy body-like cytoplasmic inclusions and behavioural manifestations resembling PD, such as hypokinesia, rigidity and tremor (Betarbet et al., 2000). Subsequent research then actively engaged in exploring various rotenone dosages (ranging from 0.4μg-100mg/kg, depending on the administration route) and routes of

administration, including oral (Inden et al., 2011), intranasal (Sharma et al., 2023), inhalation (Rojo et al., 2007), intracranial (Xiong et al., 2009), subcutaneous (Sherer et al., 2007; Zhang et al., 2017), intravenous (Fleming, 2004) and intraperitoneal (Cannon et al., 2009), to replicate and establish an optimal preclinical PD model.

Undoubtedly, these studies have consistently resulted in the successful induction of DA neuronal loss (Johnson and Bobrovskaya, 2015) with progressive loss of dopaminergic nerve terminals of the striatum and cell bodies in the SN (Betarbet et al., 2000; Norazit et al., 2010; Rocha et al., 2022; Van Laar et al., 2023) that are akin to the progressive degeneration observed in clinical PD (Cheng et al., 2010). As mentioned above, PD pathology is not restricted to the DA neuron loss in SNpc and striatum, with the effect of rotenone also found to induce noradrenergic neuronal loss in the LC (Jing et al., 2021), cholinergic neuronal loss in the dorsal motor nucleus of the vagus (Miyazaki et al., 2020) in mice, as well as serotonergic neuronal loss in midbrain neuronal cultures (Ren and Feng, 2007).

Rotenone exposure also increases α-syn cytoplasmic inclusion and expression in the SN (Betarbet et al., 2006; Cannon et al., 2009; García-García et al., 2005; Inden et al., 2011; Rocha et al., 2022; Xiong et al., 2009), with a dose-response increase in α-syn expression (Zhang et al., 2017). It is worth noting that current research regarding α-syn pathology following rotenone exposure outside the SN is limited. While a study by Chen *et al.* showed elevated intestinal α-syn levels in old mice treated with low-dose rotenone gavage for 2 months (Chen et al., 2022), and a more recent study by Sharma *et al.* revealed increased α-syn pathology in the olfactory bulb with intranasal rotenone administration (Sharma et al., 2023), reaching a conclusive correlation between α-syn pathology and Braak stage or gut-first theory (Braak et al., 2003a) remains challenging.

Behaviourally, chronic exposure to rotenone in animal models effectively replicates several critical motor and non-motor symptoms commonly observed in PD. Many of these studies have

detected motor deficits, including reduced motor activity and rearing(Rocha et al., 2022; Zhang et al., 2017), impaired balance and motor coordination (Miyazaki et al., 2020; Zhang et al., 2017), decreased grip and muscle strength (Fathalla et al., 2016; Zhang et al., 2017), and abnormal gait (Fathalla et al., 2016; Ruan et al., 2022). The onset of these motor deficits is notably rapid in animals exposed to rotenone, especially when administered high dosages through daily injections. For instance, in the study by Cannon *et al.*, 25% of adult rats receiving daily injections of 3mg/kg rotenone exhibited parkinsonian signs by day 4, with all demonstrating these signs by day 9. In contrast, lower dosages (2.75mg/kg) took twice as long for all animals to display Parkinsonian signs (Cannon et al., 2009). While many of these studies assessed motor deficits during or shortly after the rotenone dosing regimen, a study by Van Laar *et al.* demonstrated potential long-term consequences of rotenone exposure (Van Laar et al., 2023). This study revealed that motor deficits peaked during rotenone administration, but completely recovered after a 9-day period of rotenone cessation. Interestingly, however, these motor abnormalities resurfaced at the 3-month mark and continued to worsen over the course of 9 months following the initial rotenone exposure, suggesting that transient rotenone exposure may lead to delayed motor deficits, potentially correlating with the progressive loss of DA neurons in the SNpc observed in the same study (Van Laar et al., 2023).

In the context of non-motor symptoms, studies employing the rotenone rodent model have successfully detected gastrointestinal dysfunction (Drolet et al., 2009; Greene et al., 2009), marked by delayed gastric emptying and reduced stool frequency, closely mirroring the constipation symptoms frequently associated with PD (Abbott et al., 2001; Gao et al., 2011). Additionally, delayed olfactory impairment (Sharma et al., 2022), rapid eye movement, sleep behaviour (Du et al., 2021), symptoms of depression and anxiety (Ujvári et al., 2022), as well as learning and memory deficits (Guo et al., 2022). While these symptoms may manifest

differently depending on the administration routes, collectively, the rotenone model adeptly replicates the diverse spectrum of non-motor symptoms often observed in PD.

4.2 Rotenone Mechanism

The mechanism driving this association is not fully known, with rotenone functioning as a mitochondrial complex I inhibitor in the electron transport chain (Sherer et al., 2003). Complex I, also known as NADH dehydrogenase, plays a crucial role in transferring electrons from NADH to ubiquinone, a key step in the production of ATP. Rotenone disrupts this electron transfer process by binding to complex I, causing electron leakage and the subsequent generation of free radicals within the mitochondria. This disturbance in the electron transport chain, coupled with an increased production of free radicals (Fato et al., 2009; Heinz et al., 2017), contributes to oxidative stress and triggers neuroinflammatory responses (Gao et al., 2002; López-Armada et al., 2013). These effects can have widespread effects on cellular components and functions, potentially leading to cellular damage and, in the context of neurodegenerative research, contributing to neurological conditions like PD, where both of these processes are known to be involved in the pathophysiology of the disease (Betarbet et al., 2000; Gao et al., 2003).

4.2.1 Neuroinflammation following rotenone exposure

Neuroinflammation has been identified as a crucial mechanism that mediates rotenone-induced neurological damage. In *in vitro* studies, rotenone was found to significantly activate the nuclear factor kappa B (NFκB) signalling pathway through p38 mitogen-activated protein kinase (MAPK) in the BV2 microglia cell line, leading to the production of inflammatory cytokines, along with an upregulation of inflammatory mediators like nitride oxide synthase (iNOS) and caspase-1 (Gao et al., 2013; Liang et al., 2017). These inflammatory molecules

promote a toxic environment for neurons. This is also evident when rotenone is introduced into rodent models, as it triggers these inflammatory processes by releasing proinflammatory cytokines (TNF-α, IL-6 and IL-1β), accompanied by an increase in both microglia and astrocyte populations, resulting in a reduction of DA nerve fibers in the striatum (Azimullah et al., 2023).

Importantly, the neuroinflammatory processes initiated by rotenone exposure have the potential to persist and worsen over time. A study by Van Laar and colleagues demonstrated that the population of activated microglia within the SN of rat models treated with 2.8mg/kg intraperitoneal rotenone for five consecutive days exhibited progressive increases for up to 9 months following the final injection (Van Laar et al., 2023). In the same study, this gradual elevation of neuroinflammation was found to be associated with the progressive accumulation of endogenous α-Syn and the loss of DA neurons within the SN (Van Laar et al., 2023). This chronic neuroinflammatory response could thus be a key contributing factor in the subsequent development of PD.

4.2.2 Rotenone-induced mitochondrial dysfunction and oxidative stress

Rotenone functions as a mitochondrial complex I inhibitor, capable of increasing the production of ROS, particularly the superoxide radical and hydrogen peroxide within the mitochondria (Li et al., 2003). In response to varying concentrations of rotenone (10nM to 1μM), a dose-dependent decline in ATP levels, accompanied by an increase in ROS production and oxidative damage (measured by protein carbonyl levels), has been shown in various *in vitro* studies (Han et al., 2014; Radad et al., 2006; Sherer et al., 2003), suggesting that the primary source of free radicals contributing to oxidative stress during rotenone exposure is linked to induced mitochondrial dysfunction. In addition to ROS production, rotenone has also been reported to decrease the activity of antioxidant enzymes, like superoxide dismutase (SOD)

and catalase (CAT) in the midbrain ((Ojha et al., 2016; Radad et al., 2019). Indeed, Sherer *et al.* demonstrated that continuous exposure to rotenone at a dose of 3.0mg/kg/day for 5 weeks, administered via the subcutaneous mini-osmotic pump, resulted in selective oxidative damage, as indicated by elevated carbonyl levels, in various brain regions, including midbrain, striatum, olfactory bulb and cortex of male Lewis rat model (Sherer et al., 2003). Of note, this effect was accompanied by dopaminergic cell loss within the nigrostriatal pathway (Sherer et al., 2003). Similarly, increased levels of oxidised proteins and peroxynitrite in the striatum, along with the loss of nigrostriatal cells and apoptotic cell death in both the striatum and SN, have been reported at 14 and 28 days utilising the same method and animal model used in Sherer 2003 (Lin et al., 2012).

Interestingly, oxidative stress may also promote the misfolding and accumulation of various proteins, including α-syn (Bae et al., 2014; Scudamore and Ciossek, 2018). ROS interact with specific amino acid residues in α-syn, particularly sulphur-containing residues methionine or tyrosine (Glaser et al., 2005), inducing conversion and random cross-linking, leading to misfolding and an increased propensity for aggregation, which, over time, may set in motion the pathogenic accumulation of α-syn and the formation of Lewy bodies and Lewy neurites seen in PD (Betarbet et al., 2000).

5. TRAUMATIC BRAIN INJURY IN THE PATHOGENESIS OF PD

Traumatic brain injury (TBI) is a type of injury that occurs when an external force results in a direct or indirect injury to the brain (Finnie and Blumbergs, 2002). This force can arise from various events like accidents, falls, sports injuries, assaults, or any other incident that causes sudden brain movement. It is estimated that approximately 10 million individuals experience a new TBI event each year (Hyder et al., 2007) and the number of reported TBI cases surged to 27 million individuals during 2016 alone (James et al., 2019). Depending on the magnitude of

damage to the brain post-injury, TBI can also be categorised into three main severity levels: mild, moderate, and severe depending on the extent of brain damage sustained after injury. This classification is determined by assessing factors such as the Glasgow coma scale (GCS), the duration of loss of consciousness, and the duration of post-traumatic amnesia (Esselman and Uomoto, 1995; Sherer et al., 2007; Teasdale and Jennett, 1974). Mild TBI, often referred to as a concussion, is characterized by a GCS score of 13-15. Moderate TBI is identified by a GCS score ranging from 9 to 12, while severe TBI is associated with a GCS score of 3-8. Interestingly, studies have shown a positive correlation between the severity and frequency of TBI and the risk of developing PD (Gardner et al., 2018, 2015). Gardner and colleagues (2018) reported, for example, that all-severity TBI is associated with increased risk of PD (hazard ratio= 1.71, 95% CI: 1.53-1.92), with moderate-severe TBI patients having higher risk (hazard ratio=1.5, 95% CI: 1.35-1.66) of developing PD then those with mild TBI (hazard ratio=1.24, 95% CI: 1.04-1.48) (Gardner et al., 2018). Similarly, the likelihood of developing PD also increases in individuals who have experienced more than one TBI (hazard ratio=1.87, 95% CI: 1.58-2.21), compared to those who experienced a single TBI (hazard ratio= 1.45, 95% CI: 1.30-1.60) (Gardner et al., 2015).

5.1 Role of TBI in promoting the later development of PD

Chronic TBI pathology can vary; while some patients experience a full recovery, others might suffer physical and cognitive decline (Ruet et al., 2019; Stocchetti and Zanier, 2016), with an increased risk of developing neurodegenerative diseases like PD (Gardner et al., 2015; Padmakumar et al., 2022). Clinical research has consistently shown abnormal α-syn aggregates in swollen axons following TBI (Uryu et al., 2007), as well as abundant α-syn deposits in neurons and glial cells within the temporal cortex (Ikonomovic et al., 2004), as well as both the SN and LC (Crane et al., 2016), following injury. Elevated levels of α-syn have also been

observed using ELISA in the cerebrospinal fluid (CSF) taken from individuals who have experienced severe TBI up to 8 days after the injury (Mondello et al., 2013). Preclinical studies using animal models have further validated this link, where a mixed focal and diffuse moderate TBI leads to an increased accumulation and aggregation of α-syn in the SNpc and striatum, mirroring the observations in PD (Acosta et al., 2019, 2015; Hutson et al., 2011; Uryu et al., 2003).

Furthermore, both PD and TBI are characterised by neuronal degeneration within the nigrostriatal pathway (Sidaros et al., 2009). Preclinical investigations utilising a mild-blast TBI rat model revealed a notable loss of the DA neurons in the SNpc one week following the injury (Acosta et al., 2019). More chronically, Hutson *et al.* demonstrated a progressive loss of DA-neurons, increasing from 15% to 30% between day 11 to 26 weeks in the adult rat model after moderate TBI induced by lateral fluid percussion injury (Hutson et al., 2011). This loss disrupts the nigrostriatal pathway and correlates with a subsequent reduction in dopamine levels within the striatum, suggesting that TBI might lead to lasting detrimental effects on this pathway that may prime an individual for the development of PD (Hutson et al., 2011). In clinical contexts, studies have revealed pronounced neurodegeneration and a reduction in total brain volume that persists for up to 35 years post-injury (Ng et al., 2008; Raymont et al., 2010). Alterations in dopaminergic neurotransmission are further supported by the results of SPECT imaging studies. In one such study, Donnemiller and colleagues (2000) reported significantly lower striatal/cerebellar beta-CIT and IBZM binding ratios, indicating impaired striatal dopamine transporter and D2R binding, in 10 individuals with a history of TBI (GCS = 5.8+/-4.2) compared to age-matched controls (Donnemiller et al., 2000). Interestingly, this occurred even in the absence of overt structural change within the striatum using CCT/MRI. Similarly, in a study of 12 individuals with a history of moderate-severe TBI, approximately one-year post-injury, using the PET tracers [(11)C]βCFT and [(11)C]raclopride, age-adjusted reductions in

DAT-binding reduction in the caudate, putamen, and ventral striatum, as well as small increases in D2 binding in the ventral striatum, were reported compared to healthy controls, lending support to the idea that alterations to dopamine neurotransmission may persist for a lengthy period of time following the initiating injury (Wagner et al., 2014).

Behaviourally, individuals who have experienced TBI can present a spectrum of motor and non-motor symptoms that also show overlap with the symptoms of PD (Moon, 2022; Nicholl and LaFrance, 2009). A study investigating 398 severe TBI survivors revealed that approximately 22.6% developed a movement disorder, with tremors the most commonly reported, followed by dystonia (Krauss et al., 1996). Slowness of movement (Incoccia et al., 2004), stiffness and resistance to limb movement (signs of muscle rigidity) and postural instability, particularly difficulty maintaining balance(Geurts et al., 1996), have all also been reported following TBI. Despite this, understanding of the time course of motor changes following TBI remains elusive. To address this, a comprehensive systematic review of the clinical and preclinical literature on chronic motor changes will follow in Chapter 3 (Corrigan et al., 2023). Moreover, beyond motor symptoms, non-motor manifestations, including cognitive changes affecting memory (Paterno et al., 2017), mood disorders (such as depression(Choi et al., 2022) and anxiety(Al-Kader et al., 2022)), sleep disturbance (insomnia, hypersomnia, excessive day time sleepiness), autonomic dysfunction (related to impaired blood pressure regulation, digestion, and sweating), and a reduction or loss of sense of smell, have also all been reported following TBI (Temmel et al., 2002). Despite this, the time course of these symptoms, including how long they persist following injury, remains unclear.

5.2 TBI mechanism

TBI is a physical insult to the brain resulting from direct mechanical forces and can be diffuse or focal in nature. Diffuse injury is the result of rapid acceleration/deceleration forces towards

the head, causing widespread injury. In contrast, focal injuries, like skull fractures and contusions, are region-specific and commonly caused by a direct blow to the head(Andriessen et al., 2010; Masel and DeWitt, 2010). This primary injury sets off a cascade of secondary events that can significantly worsen brain damage from tissues to cells (Mckee and Daneshvar, 2015). This secondary brain injury involves a complex array of systemic complications that evolve over time, persisting and potentially intensifying months or even years after the initial injury. This includes calcium influx, oxidative stress, inflammation, excitotoxicity (due to the unregulated release of glutamate), mitochondrial dysfunction, and ultimately cell death (Acosta et al., 2013; Katayama et al., 1990; Kumar and Loane, 2012; Lizhnyak and Ottens, 2015; Xiong et al., 1997). These processes collectively contribute to the overall severity of the injury. They can lead to the manifestation of different symptoms spanning from physical impairments to sensory disturbances, cognitive decline, emotional changes, and alterations in behaviour (Al-Hassani et al., 2018; Grandhi et al., 2017). This wide array of symptoms can have a profound and lasting impact on an individual's health, well-being, and overall quality of life (Corrigan et al., 2017; Stocchetti and Zanier, 2016).

5.2.1 Neuroinflammation following TBI

Neuroinflammation is a key secondary response that occurs following TBI. When the brain encounters TBI, it initiates an inflammatory response, beginning with the recognition of damage signals or damage-associated molecular patterns (DAMPs) by microglia and astrocytes through pattern recognition receptors (PRRs) (Mckee and Daneshvar, 2015). Active PRRs, particularly members of the Toll-like receptor family, such as TLR4, and the receptor for advanced glycation end products (RAGE), induce the NFkB and MAPK pathway, leading to the release of pro-inflammatory cytokines like TNF-α, IL-6, IL-1β and IL-12 to clear tissue

debris and promote wound healing (Lawrence, 2009; You et al., 2013; Ziebell and Morganti-Kossmann, 2010).

An increasing body of evidence indicates that in some TBI patients, however, this inflammatory response may become maladaptive, persisting long-term and leading to progressive secondary damage (Johnson et al., 2013; Lee et al., 2009). Evidence of chronic inflammation, including microglial activation and the release of pro-inflammatory cytokines, has been described in the serum, cerebrospinal fluid and brain tissue of TBI patients (Juengst et al., 2014; Kumar et al., 2015; Ramlackhansingh et al., 2011; Smith et al., 2013). Notably, PET studies in TBI survivors using the ligand [11C](R)PK11195 have revealed the presence of activated microglia up to 17 years after a TBI in multiple brain regions, including the thalami, putamen, occipital cortices and posterior limb of the internal capsule (Ramlackhansingh et al., 2011). Importantly, prolonged microglia activation following TBI has been linked to reduced brain volume. In post-mortem studies of 25 TBI individuals with survival periods ranging from 1 to 47 years post-injury, activated microglia were observed, along with a reduction in tissue thickness in the corpus callosum (Johnson et al., 2013).

In preclinical work, Acosta *et al.* demonstrated that microglial activation persisted, specifically in the SN of a rat model, for 60 days following a moderate focal TBI (Acosta et al., 2015). This sustained activation of MHCII-positive microglia coincided with a marked increase in α-Syn accumulation and the loss of dopaminergic neurons, replicating PD pathology(Acosta et al., 2015). Furthermore, neuroinflammatory processes were observed to persist in the ipsilateral cortex, corpus callosum and thalamus for up to one year following a single moderate-level focal TBI in adult male mice and were found to accompany progressive cortical lesion expansion, hippocampal neurodegeneration and demyelination (Loane et al., 2014).

5.2.2 TBI-induced oxidative stress

Oxidative stress is another key mediator in the secondary response following TBI (Fesharaki-Zadeh, 2022). Both experimental and clinical investigations have demonstrated that there is an increase in the production of oxidative stress-inducing agents, encompassing both ROS (Cernak et al., 2000; Tyurin et al., 2000) and RNS, following TBI (Cherian et al., 2004; Hall et al., 1999). The primary source of these free radicals is attributed to mitochondrial dysfunction (Hiebert et al., 2015). Following TBI, the initial physical insult leads to the disruption of brain fibres and membranes, resulting in an uncontrolled elevation in glutamate concentration, which facilitates calcium influx, thereby promoting the opening of the mitochondrial permeability transition pore, culminating in the overproduction of free radicals within the mitochondria. Additionally, subsequent inflammatory processes involving microglial activation and the recruitment of macrophages and neutrophils are responsible for additional free radicals, collectively contributing to oxidative damage (Teleanu et al., 2022).

This trauma-induced oxidative stress perpetuates a cycle, triggering further disruption in mitochondrial function and concurrently acting as a signalling mechanism for chronic neuroinflammation, persisting over years to decades (Galluzzi et al., 2012). In a repeated mild fluid percussion rat model, levels of lipid peroxidation in the injured cortex were significantly higher than those in the sham control at 12 weeks following the injury (Webster et al., 2015). However, it's important to note that long-term experimental studies in this area are limited. Despite this limitation, a clinical study investigating 15 individuals with brain injury, ranging from 1 to 35 years, revealed a seven-fold increase in the concentration of lipid peroxidation by-products within the serum compared to control subjects (Mackay et al., 2006). This elevation in lipid peroxidation potentially plays a pivotal role in the development of PD, as there is evidence demonstrating increased levels of lipid peroxidation in the cerebrospinal fluid of experimental PD models (Qiu et al., 2023), its presence in the blood serum of PD patients

(Sanyal et al., 2009), and its localisation in the brain stem Lewy bodies in post-mortem PD tissue (Castellani et al., 2002).

Over time, persistent neuroinflammatory responses and oxidative stress following TBI, along with their associated pathological mechanisms, could potentially contribute to the emergence of PD. However, the ongoing investigation of these mechanisms in relation to the development and progression of PD is insufficient, leaving these questions unanswered.

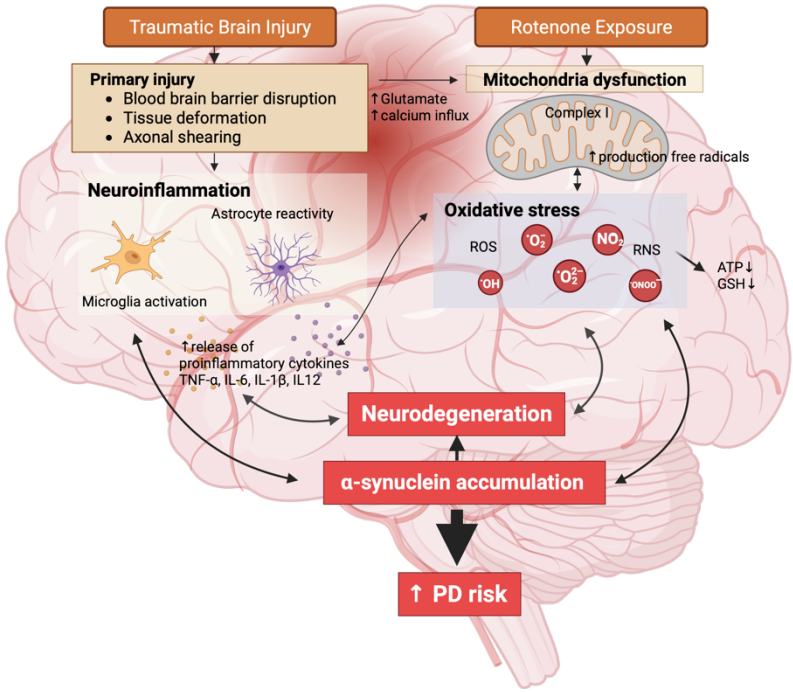

Figure 2. Illustration of the complex interplay between TBI and rotenone-induced mechanisms and potential link to PD pathogenesis. Created by Biorender.

6. CONCLUSION AND FUTURE STUDIES

PD stands as a complex and heterogeneous neurodegenerative disease. Despite the increasing recognition that environmental risk factors are associated with later PD development, the specific mechanisms are not known. Furthermore, why some individuals develop PD, while others do not, is yet to be established. Particularly noteworthy is the limited observation duration in the majority of experimental TBI studies, typically extending only up to 1-month post-injury. Together with the scarcity of clinical TBI studies, and the limited consideration for

factors such as TBI frequency, severity and the type of TBI in existing research, poses a considerable challenge in comprehending the intricate mechanisms linking TBI, the progression of PD pathologies, and the risk of developing PD.

In contrast to the relatively comprehensive approach taken in TBI studies, existing rotenone, or neurotoxin research in general, has primarily focused on mature PD models rather than adopting a step-wise, progressive PD model that could provide insights into the developmental progression from rotenone exposure to PD. This gap extends to clinical investigations, with no studies to date exploring the impact of pesticides on human subjects. Future research on this area should prioritise the study of individuals consistently exposed to pesticides or the analysis of post-mortem tissue, mirroring the approach adopted in TBI studies. Additionally, delving into an immature rotenone-induced PD model is essential to unravel the underlying mechanisms that drive the transition from rotenone exposure to PD.

It is also important to remember that in individuals, exposure to environmental risk factors associated with PD does not occur in a vacuum. Indeed, it is likely that over their lifespan, individuals may be exposed to several of these factors, which could act synergistically to elevate risk. In support of this notion, a study examining the interplay between TBI and modifying factors, in this case, pesticide exposure, revealed a three-fold higher risk of developing PD (adjusted odd ratio=3.01, 95% CI=1.51–6.01) in participants exposed to both TBI and pesticide exposure than those with neither risk factor (Lee et al., 2012). Preclinical work by Hutson *et al.* demonstrated a similar synergistic pattern in the loss of dopaminergic neurons in animals exposed to both the pesticide and experimental TBI (Hutson et al., 2011). However, while intriguing, these studies have not systematically explored the intricate relationship between rotenone exposure and TBI in the context of PD risk and pathogenesis. This comparison is particularly vital, given the shared potential mechanisms driving PD pathologies between rotenone exposure and TBI highlighted in this review (Figure 2). Hence,

future studies should comprehensively investigate both functional deficits and neuropathological changes, along with the underlying mechanisms accounting for these changes, over an extended period of time. This can be achieved by employing preclinical models, to more fully elucidate the individual contribution and potential synergistic effect of known environmental risk factors. Only through such integrated research, can we then take a critical step toward revolutionising early identification and unravelling the nuanced progression of symptoms in PD, and advance our understanding of the intricate mechanisms underlying disease progression, ultimately facilitating the development of interventions aimed at modifying or halting the progression of the disease in its earliest stages.

The Imperative For Investigating Long-Term Motor Function In Traumatic Brain Injury Survivors

TBI has been identified as a known risk factor for the later development of PD(Goldman et al., 2006; Jafari et al., 2013). TBI typically results from external forces or trauma to the head (Prins et al., 2013) and can range in severity, from mild to severe, depending on the nature of the initiating force (Jain and Iverson, 2020; Teasdale and Jennett, 1974). Importantly, neuronal injury persists beyond the initial insult and can lead to a variety of motor symptoms, depending on the location of the injury (Bramlett and Dietrich, 2015).

Although acute motor deficits have been extensively characterised and encompass balance and coordination deficits, paralysis, postural instability, gait abnormalities, and reduced fine motor control (Khan et al., 2003; McMillan et al., 2012; Stålnacke et al., 2019), a substantial gap in understanding persists. A considerable body of research focuses on the early impact and subsequent rehabilitation; however, this acute-centric approach leaves us with limited insight into the evolution, recovery, or exacerbation of motor impairments over an extended period following the injury. Long-term studies are, therefore, crucial, not only to elucidate the nature of motor deficits that persist or develop beyond the acute phase, but also to shed light on the overlap in motor symptoms that manifest and potentially set the stage for progression into PD.

To address this knowledge gap, a comprehensive review of the existing literature concerning TBI and long-term motor function (defined as >1 month following injury in pre-clinical models and >1 year post-injury in the clinical population) is needed. Existing research in the field of TBI and its long-term effects on motor function is scattered, and, to date, there has not been a comprehensive synthesis of findings at these long-term time points. A systematic review offers a structured approach to assess and analyse the relevant literature. By systematically reviewing the data, evaluating methodologies and identifying patterns, a

systematic review bridges the gaps in our understanding and provides a holistic view of the topic. It is a fundamental step toward shedding light on current gaps in knowledge and establishing a strong foundation for future research methods investigating to the long-term motor implications of TBI.

All three authors (Frances Corrigan, Ing Chee Wee and Lyndsey Collins-Praino) contributed to the conceptual design of the study, reviewed articles for inclusion, contributed to the results and participated in drafting the manuscript. Specifically, ICW contributed significantly to literature collection, preliminary analysis and organised the database, laying a fundamental effort in initiating the work. It is important to acknowledge that a large portion of the data synthesis and interpretation was conducted by Frances Corrigan, which represents a substantial portion of the work that qualifies a systematic review as a novel contribution to the literature. This is subsequently reflected in the author list. Consequently, this upcoming systematic review chapter is positioned not as an original experimental chapter for ICW; rather, it is presented as an introductory guide, setting the stage for the subsequent in-depth original investigations that follow.

02

Chronic Motor Performance following Different Traumatic Brain Injury Severity-A Systematic Review

Chapter prepared in publication by CORRIGAN, F., **WEE, IC.**, COLLINS-PRAINO, L.E

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Principal Author

Name of Principal Author (Candidate)	Frances Corrigan
Contribution to the Paper	Conceptualised the study, supervised the work, reviewed articles for inclusion, results interpretation and analsys, wrote the manuscript.
Overall percentage (%)	
Certification:	This paper ~~reports on original research I conducted during the period of my Higher Degree by Research candidature and~~ is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 18/12/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Ing Chee Wee
Contribution to the Paper	literature collection, reviewed articles for inclusion, preliminary results interpretation, organised database and manuscipt drafting
Signature	Date:

Name of Co-Author	Lyndsey Collins-Praino
Contribution to the Paper	Conceptualised the study, supervised the work, provided assistance with data interpretation, contributed to writing and editing the manuscript
Signature	Date: 18/12/23

Please cut and paste additional co-author panels here as required.

Chronic motor performance following different traumatic brain injury severity -A systematic review

Frances Corrigan[1], Ing Chee Wee[2], Lyndsey E. Collins-Praino[2].

[1]Head Injury Lab, School of Biomedicine, The University of Adelaide, Adelaide, SA, AUSTRALIA

[2]Cognition, Ageing and Neurodegenerative Disease Lab, School of Biomedicine, The University of Adelaide, Adelaide, SA AUSTRALIA

Correspondence:

Lyndsey E. Collins-Praino

lyndsey.collins-praino@adelaide.edu.au

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ABSTRACT

Traumatic brain injury (TBI) is now known to be a chronic disease, causing ongoing neurodegeneration and linked to increased risk of neurodegenerative motor diseases, like Parkinson's disease and amyotrophic lateral sclerosis. While the presentation of motor deficits acutely following traumatic brain injury is well documented, however, less is known about how these evolve in the long-term post-injury, or how the initial severity of injury affects these outcomes. The purpose of this review therefore was to examine objective assessment of chronic motor impairment across the spectrum of TBI in both preclinical and clinical models.

PubMed, Embase, Scopus, and PsycINFO databases were searched with a search strategy containing key search terms for TBI and motor function. Original research articles reporting chronic motor outcomes with a clearly defined TBI severity (mild, repeated mild, moderate, moderate-severe, severe) in an adult population were included. 97 studies met the inclusion criteria, incorporating 62 preclinical and 35 clinical studies. Motor domains examined included neuroscore, gait, fine-motor, balance and locomotion for preclinical studies and neuroscore, fine-motor, posture and gait for clinical studies. There was little consensus among the papers presented, with extensive differences both in assessment methodology of the tests and parameters reported. In general, an effect of severity was seen, with more severe injury leading to persistent motor deficits, although subtle fine motor deficits were also seen clinically following repeated injury. Only six clinical papers investigated motor outcomes beyond 10 years post-injury and 2 preclinical studies to 18-24 months post-injury, and, as such, the interaction between a previous TBI and aging on motor performance is yet to be comprehensively examined.

Further research is required to establish standardised motor assessment procedures to fully characterise chronic motor impairment across the spectrum of TBI with comprehensive outcomes and consistent protocols. Longitudinal studies investigating the same cohort over

time are also key for understanding the interaction between TBI and aging. This is particularly critical, given the risk of neurodegenerative motor disease development following TBI.

Keywords: Traumatic brain injury, Neurodegenerative movement disorder, Motor performance, Long-term outcomes, Systematic Review.

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FC, IC, and LC-P contributed to conception and design of the study, reviewed articles for inclusion, contributed to the results, and wrote sections of the manuscript. IC performed the search and organized the database. All authors contributed to manuscript revision, read, and approved the submitted version.

1.0 INTRODUCTION

Although once thought of as an acute event, it is now well recognized that traumatic brain injury (TBI) leads to long-lasting disability in a subset of individuals (Brett et al., 2022; Corrigan and Hammond, 2013; Masel and DeWitt, 2010), including persistent impairments in memory, decision making and motor function. Following even mild TBI, 53% of individuals still report functional limitations at 12 months post-injury (Nelson et al., 2019); such impairments significantly impact an individual's quality of life, affecting social relationships and ability to return to work (Stocchetti and Zanier, 2016). Mobility, in particular, has been shown to be an important mediator of the relationship between TBI and quality of life following injury (Kalpinski et al., 2013) with more functional impairment associated with decreases in life satisfaction (Williamson et al., 2016).

Acutely, TBI leads to a number of neuromotor deficits which are injury severity dependent. Mild TBI most commonly presents with balance disturbance and poor coordination (Gera et al., 2018; Lin et al., 2015), whilst severe TBI can lead to spastic paralysis, impaired motor co-ordination with postural instability and gait abnormalities, alongside reduced fine motor control (Walker and Pickett, 2007). Motor impairment has been particularly well-characterized to occur following moderate-severe TBI, with nearly 78% of individuals reporting some level of impairment on gross neuromotor examination during acute rehabilitation (Walker and Pickett, 2007). Studies focused on the first year post-injury in moderate-severe TBI have shown that most motor recovery is reached within 6 months post-injury (Agrawal and Joshi, 2014; Patil et al., 2017; Swaine and Sullivan, 1996), with patients not showing significant functional improvement over the latter part of the year (Agrawal and Joshi, 2014; Hart et al., 2014; Sandhaug et al., 2015). In line with this, 30% of individuals reported difficulty in walking unaided up to two years following moderate-severe injury (Dikmen et al., 1993), with 25% of individuals still reporting upper- or lower-limb motor

difficulty and 43% reporting balance difficulties, even four years after a severe brain injury (Jourdan et al., 2016). Conversely, following a mild TBI, impairments generally resolve within days to weeks post-injury, although some level of motor dysfunction may persist in at least a subset of individuals, see (Chmielewski et al., 2021) for review. In support of this, slowed motor execution speed and impaired postural control have been reported up to 9-months following concussion in university football players, compared to healthy, non-concussed controls (De Beaumont et al., 2011).

Despite evidence that motor impairment may persist chronically following TBI, however, examination of the evolution of specific motor deficits long-term following TBI has received comparatively little attention in the literature. Indeed, particularly in clinical research, published TBI outcome studies are skewed toward global measures and/or measures within the behavioral and cognitive, rather than physical, domains. Additionally, of studies that do report physical outcomes, most utilize gross functional or disability instruments, rather than dissecting specific types of motor impairment. For example, utilizing the Rivermead Concussion scale, Theadom found 28.5% of participants reporting dizziness at 12 months following mild TBI (Theadom et al., 2016), which is in line with an earlier Ponsford *et al.* study, where, on structured interview 2 years post-TBI, 36% of patients reported dizziness (Ponsford et al., 1995). Studies where specific motor impairments are reported typically examine only one motor domain; for example, Williams *et al.* examined chronic gait dysfunction following severe TBI (Williams et al., 2010a, 2013; Williams and Schache, 2016) and Pearce *et al.* the effects of prior concussion on fine motor performance (Pearce et al., 2014a, 2018a). Even in preclinical studies, the behavioral batteries employed typically only consist of 1-2 motor-specific tasks (Adkins et al., 2015; Bolton Hall et al., 2016; Cline et al., 2017a; Daglas et al., 2019a; Henry et al., 2020a; Mouzon et al., 2014, 2018; Pruitt et al., 2014; Rana

et al., 2020a; Ritzel et al., 2020), and thus cannot provide a comprehensive overview of how TBI influences motor performance as a whole.

Understanding the persistent nature of motor impairment following TBI is critical, as impaired motor control following concussion has been shown to increase risk for subsequent musculoskeletal injury (Chmielewski et al., 2021) and falling (Klima et al., 2019). TBI is also linked to an elevated risk of developing neurodegenerative diseases associated with motor symptoms, including motor neuron disease (Chen et al., 2007) and Parkinson's disease (PD) (Crane et al., 2016). For example, multiple studies have established a link between TBI and the later development of PD, with Gardner and colleagues (Gardner et al., 2017) recently reporting that mild TBI increases risk of PD by 56%, whilst moderate/severe TBI increases PD risk by 83%. More recently, Russell and colleagues reported in a retrospective cohort study that Scottish former rugby players had a higher incident rate of neurodegenerative diseases, including both PD (HR/OR (95% CI) = 3.04 (1.51-6.10)) and motor neuron disease (HR/OR (95% CI) = 15.17 (2.10-178.96) compared to a matched comparison group from the general population over a 32 year median follow up period from study entry (11.4 vs 5.4%) (Russell et al., 2022). This is consistent with growing neuroimaging evidence that TBI leads to ongoing neurodegeneration. In the months to years following injury, progressive lesion expansion occurs concomitant with white and grey matter atrophy and loss of white matter integrity (Adnan et al., 2013; Bendlin et al., 2008; Green et al., n.d.; Greenberg et al., 2008). Importantly, structures affected include those critical for motor function, such as the striatum (Poudel et al., 2020), thalamus (Harris et al., 2019) and cerebellum (Harris et al., 2019).

Considering the high prevalence of TBI, a fuller description of neuromotor deficits, stratified by motor domain, in gross or fine motor will provide insight into the global recovery process and rehabilitation needs of persons with TBI. Additionally, given that motor function may play a crucial role in linking TBI to the later emergence of neurodegenerative movement

disorders, examining specific motor changes that occur long-term following injury could serve as a novel method for identifying risk of these diseases. As such, the aim of this systematic review was to review all original research reports that assessed chronic motor outcomes following TBI, stratified by injury severity in both preclinical models and patient populations.

2.0 METHODS

2.1 Search Strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Liberati et al., 2009) were used. A comprehensive literature search was performed in May 2019, with an updated search undertaken in March 2022, using the electronic databases PubMed, Embase, Scopus, and PsycINFO to identify relevant publications. The search strategy was developed based on an initial scoping search and in consultation with a health and medical sciences librarian. The search terms used were 'traumatic brain injury', 'Parkinson's disease', 'motor neuron disease' and 'motor performance,' or variations thereof, that were combined using 'AND' and 'OR' search operators. The developed search strategy is depicted in Supplementary Table 1. Further searches were performed in the reference lists from the included studies.

2.2 Study selection: Inclusion and exclusion criteria

Following the search, identified articles were imported into EndNote X9.3.3 and duplicates were removed either by the EndNote "delete duplicates" function or deleted manually. Titles and abstracts were then screened, with clinical studies reporting motor outcomes >1 year post-injury and preclinical studies reporting motor outcomes >30 days post-injury retained. For articles that passed this preliminary assessment, the full-text article was retrieved and screened for eligibility against the inclusion and exclusion criteria. Eligibility of articles was assessed by two independent reviewers. Any conflicts were resolved via discussion, and if a consensus could not be reached, a third reviewer was consulted. A flowchart with reasons for the exclusion of studies is displayed in Figure 1.

2.3 Inclusion criteria:

The following inclusion criteria were utilised:

(i) Original research article published in English

(ii) Investigated an adult population (Preclinical: 8 weeks or older; Clinical:18 years or older) with a prior history of TBI;

(iii) Assessed long term motor performance (Preclinical: >30 days post-injury; Clinical: mean time since TBI > 1 year);

(iv) Clear classification of TBI severity (Preclinical: required a comprehensive description of the TBI model and parameters used to induce injury; Clinical: Provided Glasgow Coma Scale (GCS), Westmead Post-Traumatic Amnesia scale (PTA), and/or loss of consciousness (LOC) duration)

(v) Compared motor performance with a control group.

The search had no restrictions on the year of publication; however, only English language publications were included. Databases were searched from inception.

2.4 Exclusion criteria:

Studies were excluded from further consideration if they:

(i) Reported outcome measurements which were not purely motor (e.g., cognition, visuomotor integration/coordination, social preference, or quality of life);

(ii) Did not specifically state month/time post-TBI, injury severity or motor outcomes assessed;

(iii) Pilot studies that had a sample size of a single group less than 5;

(iv) Studies were single case reports/expert options; or

(v) Were review articles or conference abstracts.

(vi) No specific statistical comparison was reported for injured compared to sham/naïve animals in treatment studies, with treatment effects not the focus of the current review

2.5 Data extraction and synthesis

Data were extracted from the included studies by two independent reviewers. Data extracted included study characteristics (author, year of publication, study design, motor function measurement, injury method in preclinical studies, description of TBI severity for clinical studies); participant/TBI preclinical model characteristics (sample size, age, sex, history of

TBI/frequency of injury, time-point assessed post-injury, mechanism of injury and TBI severity); primary methods/functional tests used to measure motor performance, and primary or secondary outcome(s) of motor performance. A copy of the data extraction template can be found in Supplementary table 2.

Due to the large diversity of motor outcome measures used across the study, the measurements were categorized into different motor functions and analysed separately. The categorization of the motor outcome measures is outlined in Supplementary table 3. In order to assess the effect of TBI severity on motor performance, outcomes were further stratified by injury severity. The evaluation of TBI severity was classified as described in Supplementary Tables 4 and 5. Injury severity in preclinical studies were separated into five groups, (i) single mild; (ii) repetitive mild; (iii) moderate; (iv) moderate-severe; (v) severe. A similar classification system was used for clinical TBI, with minor modifications. As some individuals had experienced more than one injury, the following groups were used (i) single mild, (ii) the combination of single and repetitive mild (prior TBI history ranged from 1 to more), (iii) repetitive mild (>1 prior TBI), (iv) moderate-severe, and (v) severe.

2.6 Assessment of methodological quality

Articles included in the study were assessed for methodological quality by one reviewer (IW), with confirmation provided by a second reviewer (LCP or FC), by using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool (preclinical)(Hooijmans et al., 2014) and the Cochrane Risk of Bias Tool (clinical) (Higgins et al., 2011). Studies were judged as having a low, unclear, or high risk of bias in the following domains: selection bias, performance bias, detection bias, attrition bias, reporting bias and other biases. The overall risk of bias for each included study was categorized as “strong quality” if the risk of bias was low in 70% or more of the criteria, “low quality” if the risk of bias was

high in at least 30% of the criteria, and “moderate quality” if the risk of bias fell between these two parameters. Disagreements were resolved by consensus. Summary graphs were created in Review Manager (RevMan) ([Computer program], Version 5.4.1 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

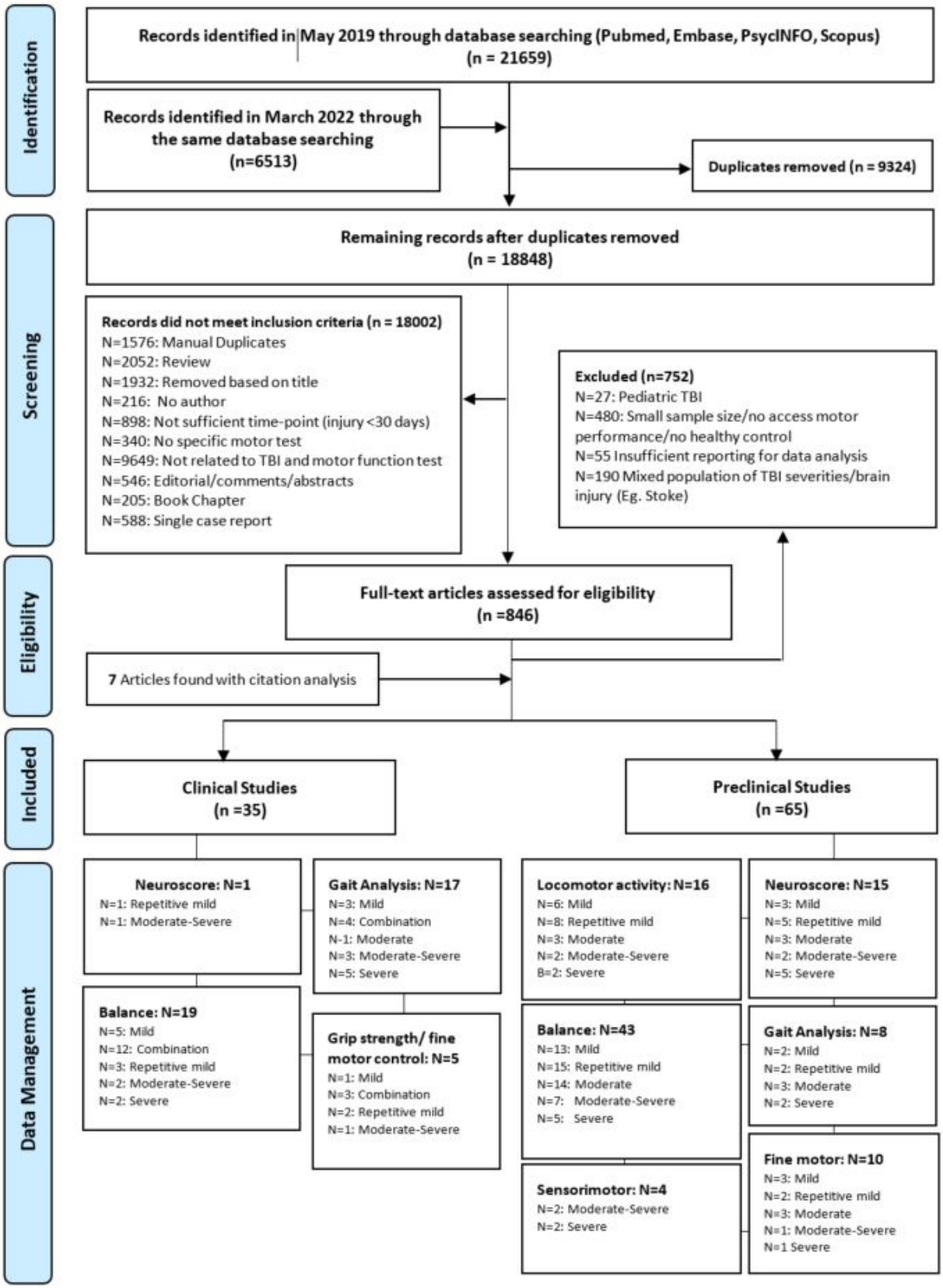

Figure 1. PRIMSA flow diagram outlining the article selection and screening process and subsequent data management. Sum of each domain indicates the total number of publications investigated that motor function.

3.0 RESULTS

3.1 Search outcomes

The initial search yielded 28,172 articles. From these, 9,324 duplicates were removed with the Endnote function, and another 18,002 were excluded after reviewing title and abstracts (Figure 1). Full-text analysis was then performed on the remaining 846 articles, of which only 93 met inclusion criteria, with 7 additional articles identified from a search of the citation lists of included studies. All articles (n = 100) were then separated into preclinical (n=65) and clinical (n=35) subgroups for further analysis. Stratification based on motor outcomes and injury severity was performed as described above. Details of this process are described in the PRISMA diagram (Figure 1).

3.2 Study characteristics

The earliest included study was published in 1997, and the rate of publications/year was low until 2016, when the rate of publications increased markedly, particularly for preclinical studies (Fig 2A). In the preclinical studies, there was an even split between use of mice (50%) and rats (50%). The vast majority of preclinical studies used male animals (82%), with only 11.3% reporting use of female animals, a further 1.7% using both sexes and 4 studies (5%) not mentioning the sex of the animals used. In clinical studies, the majority of studies (23/35) had more than 60% male TBI participants (Arce et al., 2004a; Burton et al., 2002; De Beaumont et al., 2011; Gardner et al., 2017a; Johnston et al., 2020a; Ledwidge et al., 2017; Martini et al., 2011, 2021; Pan et al., 2015a; Pearce et al., 2014a, 2018a; Pitt and Chou, 2020; Sosnoff et al., 2011; Useros Olmo et al., 2020a; Vanderploeg et al., 2007a; Vasudevan et al., 2014a; Walker et al., 2018b, 2018a; Williams et al., 2004, 2009, 2010a, 2013; Williams and Schache, 2016), including 5 studies with only male participants (Arce et al., 2004b; De Beaumont et al., 2011; Pearce et al., 2014a, 2018a; Vanderploeg et al., 2007), 9 studies had a TBI cohort with 20-45%

female participants (Degani et al., 2017; Geurts et al., 1999; Lee et al., 2020; Reilly et al., 2020a; Rosenblum et al., 2020; Stuart et al., 2020a; Ustinova, 2017; Wright et al., 2018a; Zhang et al., 2002) and 3 studies did not state the sex of TBI participants (Buster et al., 2013a; De Beaumont et al., 2009a; Helmich et al., 2016a).

Sample sizes varied across the studies ranging from 6-49 animals in a group in pre-clinical studies. The sample size for the included clinical studies was consistently low, with the majority ranging from 16-66 participants, with only a few moderately sized (111-453 individuals) (Gardner et al., 2017b; Johnston et al., 2020b; Martini et al., 2021; Rosenblum et al., 2020; Sosnoff et al., 2011; Stuart et al., 2020b; Walker et al., 2018b, 2018a) and one large study (4007 subjects) (Vanderploeg et al., 2007). The source populations of clinical studies varied widely including rehabilitation institutes (Williams et al., 2009, 2010a, 2013, 2013; Zhang et al., 2002), professional athletes (Pearce et al., 2014a, 2018a), college students (Helmich et al., 2016a; Lee et al., 2020; Martini et al., 2021), college athletes (De Beaumont et al., 2009a, 2011; Johnston et al., 2020a; Ledwidge et al., 2017; Rosenblum et al., 2020; Sosnoff et al., 2011; Ustinova, 2017), military veterans (Gardner et al., 2017; Pan et al., 2015a; Pitt and Chou, 2020; Vanderploeg et al., 2007; Walker et al., 2018b, 2018a; Wright et al., 2018a), and the community (Arce et al., 2004b; Burton et al., 2002; Buster et al., 2013a; Degani et al., 2017; Martini et al., 2021; Reilly et al., 2020a; Stuart et al., 2020a; Vasudevan et al., 2014a).

In preclinical studies, there was a relatively even split across the injury severities, with 20% mTBI, 24% rmTBI, 29% moderate TBI, 13% mod-severe TBI, and 14% severe TBI (Fig 2B). In comparison, the majority of clinical studies examined a combination of single and repetitive mild TBI (42.5%), with a further 17% reporting single mTBI and 12.5% rmTBI. Mod-severe TBI was included in only 12.5% of studies, and severe TBI in just 15% of studies (Fig 2C). The motor domain most commonly examined in preclinical studies was balance

(43/63 studies), with a much smaller number examining locomotion (16), neuroscore (15) fine motor (10) and gait (8) (Fig 2D). In contrast, in clinical studies, balance (19) and gait (15) were most commonly tested, with fewer studies investigating fine motor control (6) or a neuroscore (1) (Fig 2E).

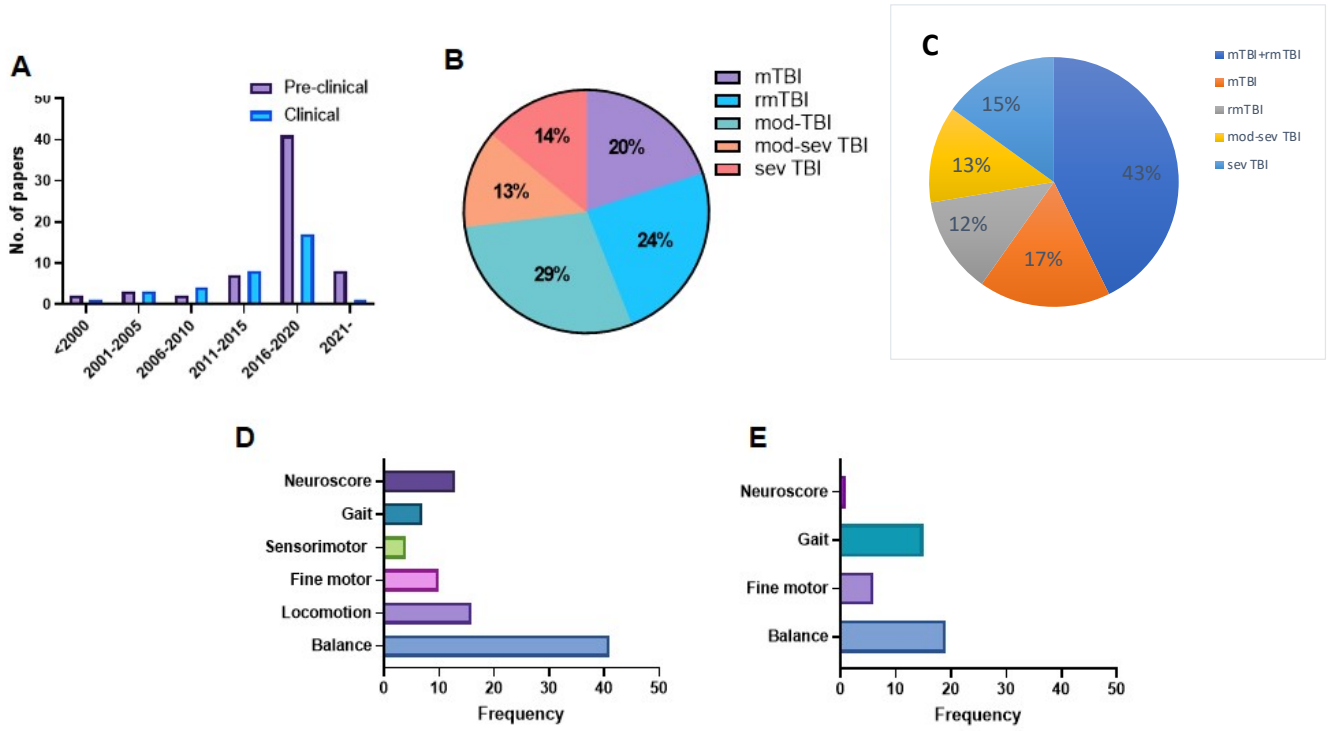

Figure 2. Number of publications per year (A). Pie charts indicating the representation of different injury severities in preclinical (B) and clinical studies (C). Frequency charts indicating the number of studies that reported on each motor domain in preclinical (D) and clinical studies (E).

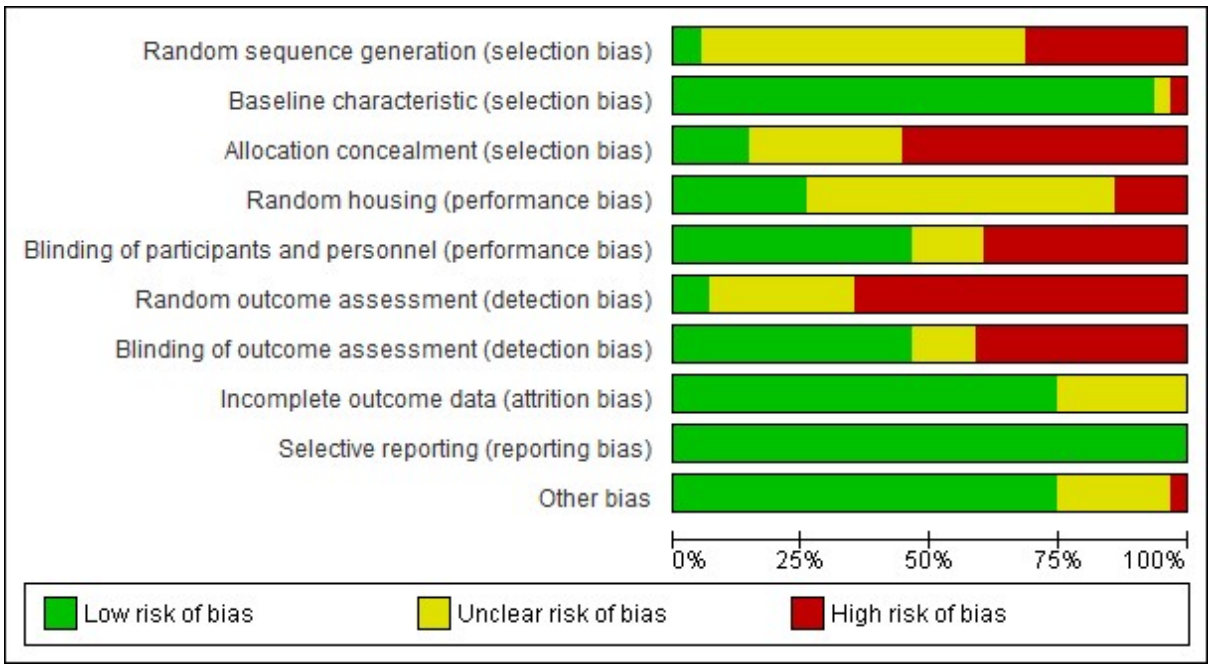

Figure 3. Risk of bias graph-Preclinical. Review authors' judgement about each risk of bias item presented as percentages across all included studies.

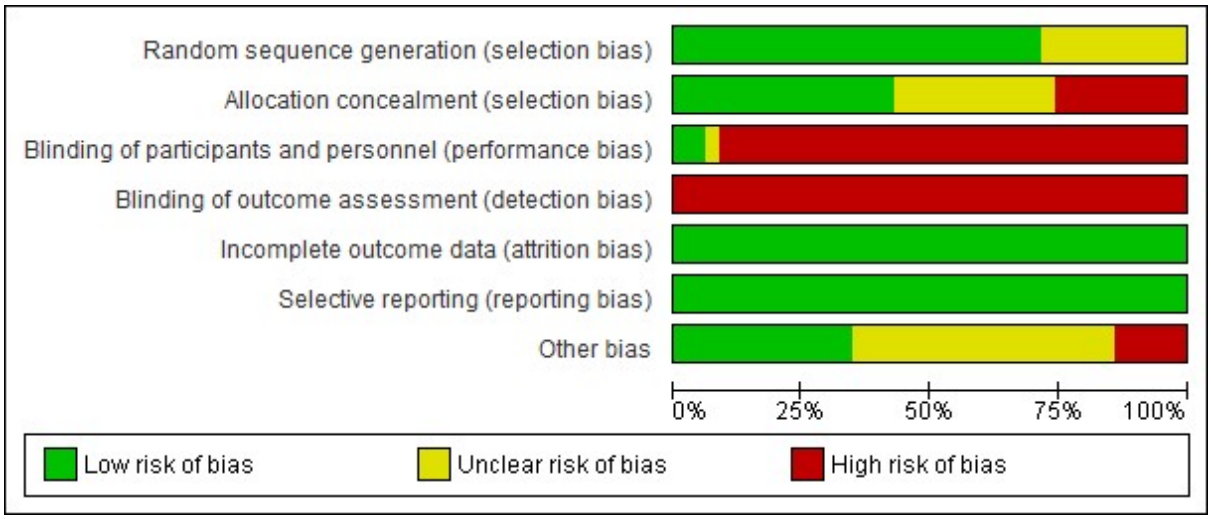

Figure 4. Risk of bias graph-Clinical. Review authors' judgement about each risk of bias item presented as percentages across all included studies.

3.3 Risk of bias

Examination of risk of bias found that of the preclinical studies, only seven were of strong quality, 24 of moderate quality and 31 studies of low quality (Supplementary Figure 1) for complete evaluation, Fig 3 for summary data. Of the clinical studies, only 1 was of strong quality, 1 of moderate quality and the remaining 32 were of low quality (Fig 4). Key sources of bias for preclinical studies included selection bias and detection bias, whereas for clinical studies, they included selection, performance and detection bias.

3.4 Preclinical motor outcomes

3.4.1 Neuroscore

Overall, a focal injury was required to lead to persistent decreases in neuroscore, with minimal changes seen with diffuse injury. It should be noted the neurological severity scoring system varied widely between the 15 included studies, with the standard neurological severity score (Fehily et al., 2019; Laurer et al., 2001; Nissinen et al., 2017; Pierce et al., 1998; Segovia-Oropeza et al., n.d.; Sell et al., 2017; Zhang et al., 2005a), modified neurological severity score (Huynh et al., 2020; Mountney et al., 2017; Zhang et al., 2021), and revised neurological severity score (Feng et al., 2021) all represented. Even papers that used the same names for

their scoring systems incorporated different tasks within their behavioural battery (Supplementary Table 6). The most commonly included items were those from the standard neurological severity score, including forelimb and hindlimb flexion on tail suspension (10 of 15 studies) (Feng et al., 2021; Laurer et al., 2001; Nissinen et al., 2017; Pierce et al., 1998; Segovia-Oropeza et al., n.d.; Sell et al., 2017; Shear et al., 2010; Wang et al., 2020a; Zhang et al., 2005a, 2021), forelimb/hindlimb placement on a flat surface (5/15) (Laurer et al., 2001; Nissinen et al., 2017; Wang et al., 2020a; Zhang et al., 2005a, 2021), and resistance to lateral pulsion (6/15) (Laurer et al., 2001; Nissinen et al., 2017; Pierce et al., 1998; Segovia-Oropeza et al., n.d.; Shear et al., 2010; Wang et al., 2020a). Other tests that were included were simple reflexes (limb, tail, corneal, startle), with different combinations used in 7 studies (Daglas et al., 2019a; Fehily et al., 2019; Feng et al., 2021; Mountney et al., 2017; Sell et al., 2017; Thau-Zuchman et al., 2021; Zhang et al., 2021). Measures of hemiparesis, either via direct assessment of hemiplegia (Daglas et al., 2019a; Fehily et al., 2019; Huynh et al., 2020) or circling behaviour (Fehily et al., 2019; Huynh et al., 2020; Shear et al., 2010; Zhang et al., 2021), were included in 6 studies. Balance was examined as ability to stay on a round beam (Fehily et al., 2019; Huynh et al., 2020; Zhang et al., 2021), a flat surface (Fehily et al., 2019; Huynh et al., 2020), or an inclined plane (Laurer et al., 2001; Pierce et al., 1998; Zhang et al., 2005a), as well as the ability to walk across beams of different widths (1,2 and 3 cm) (Fehily et al., 2019; Feng et al., 2021; Huynh et al., 2020; Mountney et al., 2017; Thau-Zuchman et al., 2021), with a measure of balance incorporated into 9 of the 15 studies. General activity was assessed either directly (Wang et al., 2020a) or via ability to exit a circle (Huynh et al., 2020) or seeking behaviour (Huynh et al., 2020; Thau-Zuchman et al., 2021) in 3 studies. Direct assessment of gait as performance on the treadmill was included in one study (Daglas et al., 2019a). Given the different combinations of tests, reflecting different types of motor behaviour, included in the neurological severity score across the studies, direct comparisons between

studies is difficult. Nonetheless, in studies utilizing focal or mixed moderate (Daglas et al., 2019a; Sell et al., 2017; Zhang et al., 2021), moderate-severe (Shear et al., 2010; Wang et al., 2020b), and severe TBI models (Nissinen et al., 2017; Pierce et al., 1998; Segovia-Oropeza et al., n.d.; Thau-Zuchman et al., 2021; Zhang et al., 2005a), a consistent impairment was found in neuroscore, regardless of the scoring system implemented, out to 8 months post-injury, with no further deficits noted in the one study that included a 12 month time-point (Table 1) (Pierce et al., 1998). In general no chronic deficits in neuroscore were noted in preclinical models of smTBI (Fehily et al., 2019; Laurer et al., 2001) or rmTBI (Fehily et al., 2019; Feng et al., 2021; Huynh et al., 2020; Laurer et al., 2001), with 7 months the latest time-point examined. Only one study utilizing a projectile concussive impact where a steel ball is projected at the rat head which is protected by a steel helmet found chronic neuroscore deficits at 3 months post-injury in both single and repeated (4 x mTBI 1 hr apart) animals (Mountney et al., 2017).

3.4.2 Gait analysis

Gait was analysed post-TBI using automated systems like the CatWalk, requiring animals to actively walk across a platform (Cline et al., 2017a; Henry et al., 2020a; Mountney et al., 2017; Namdar et al., 2020; Ritzel et al., 2020; Schönfeld et al., 2017), or the DigiGait apparatus, which utilizes a treadmill at a steady speed (Bolton Hall et al., 2016; Daglas et al., 2019a). In general, the studies were selective in reporting gait parameters, not providing a comprehensive overview of the different measures in the Methods and often only reporting selective results, with some exceptions (Mountney et al., 2017)(Table 2). No single impact study reported chronic changes in speed or cadence following TBI, with this either directly reported (Cline et al., 2017b; Ritzel et al., 2020; Schönfeld et al., 2017), or not included within the results (Bolton Hall et al., 2016; Daglas et al., 2019a; Henry et al., 2020a; Namdar et al., 2020), with an acute decrease in speed only noted at 24 hrs following severe focal injury (Cline et al., 2017a). Indeed

persistent reductions in cadence were only noted when four impacts were delivered 1 hr apart, with this deficit persisting to 3 months post-injury (Mountney et al., 2017).

However, following even single mTBI, subtle gait alterations were noted with free ambulation of the CatWalk including a decreased front base of support seen acutely and persisting up to three months post-injury (Namdar et al., 2020), with a decrease in hind base of support also developing at the three month time-point (Mountney et al., 2017). Repeated injury led to more prominent gait abnormalities, with the 4 x 1h apart injury model leading to persistent deficits to three months post-injury in single stance time, stride length, stand time and front base of support, with the deficit in hind base support again only developing at three months post-injury (Mountney et al., 2017). In contrast, on a treadmill, repeat injury (5x mTBI 24 or 48 hrs apart) led to no deficits in gait either acutely or at one month, although different gait parameters were reported across studies, including gait symmetry, paw contact area and hindlimb shared stance (Bolton Hall et al., 2016).

Mixed results were also found for gait following more severe injury. Following focal moderate CCI injury, no deficits were detected from 24 hrs to one month post-injury on the CatWalk, with reporting of measures like paw contract area, stance, swing speed, base of support and interlimb co-ordination (Henry et al., 2020a). In contrast, Ritzel *et al* only examined gait at 26 weeks post-moderate CCI injury and reported a number of impairments, including reduced stride length of the contralateral right hindlimb and reduced swing speed in the right hind and forelimb, but no overall change in average speed (Ritzel et al., 2020). Similarly, Daglas *et al* utilizing the DigiGait treadmill apparatus found significant reductions in contralateral swing duration of the right hindlimb with compensatory right forelimb propulsion duration from 1- 32 weeks post moderate CCI injury (Daglas et al., 2019a). Surprisingly, minimal chronic deficits were reported following severe CCI injury, with Cline *et al.* only detecting deficits at 24hrs, but not one month, post-injury, with the exception of

swing duration of the contralateral hindlimb (Cline et al., 2017a). Similarly, Schonfeld *et al.* also found no alterations in stride length, base of support or three limb support at 1 month following injury (Schönfeld et al., 2017).

3.4.3 Sensorimotor control

Sensorimotor function was primarily evaluated with the adhesive removal test (Bouet et al., 2009), with severe injury required to produce chronic deficits. The number of trials evaluated varied between studies ranging from 1-6, as did the presentation of results, which included % sham (Hoffman et al., 2003), latency to remove the adhesive (Alwis et al., 2012; Zhang et al., 2005a) and difference in performance between preferred and non-preferred paw (Schönfeld et al., 2017) (Table 3). Severe injury, either focal (Schönfeld et al., 2017) or mixed (Zhang et al., 2005a), led to persistent deficits in adhesive removal out to 16 weeks post-injury, the latest time-point assessed. In contrast, following moderate-severe injury, neither focal (Hoffman et al., 2003) nor diffuse (Alwis et al., 2012) injury led to chronic deficits in adhesive removal, with deficits noted up to 6 days following diffuse injury and 3 weeks following focal injury, with no further impairment noted to 41 days post-injury, the latest time-point assessed. Interestingly the whisker-evoked forepaw placement task did detect chronic deficits in the diffuse injury moderate-severe model with impaired forepaw placement out to 41 days post-injury (Alwis et al., 2012), indicating potential task dependent effects. To date, no studies have evaluated sensorimotor function chronically following either a single mTBI or repeated mTBI.

3.4.4 Grip strength and fine motor

Grip strength and fine motor ability were assessed via the grip strength meter (Daglas et al., 2019a; Dhillon et al., 2020a; Evans et al., 2014, 2015; Namdar et al., 2020; Tabet et al., 2022) isometric pull task (Pruitt et al., 2014, 2017), pellet reaching tasking (Adkins et al., 2015), and

Montoya staircase test (Schönfeld et al., 2017) (Table 3). The grip strength meter requires minimal pre-training, whereas the other tasks involve more extensive training with food restriction, with the test relying on the animal's desire to obtain sugar pellets as a reward for completing the task successfully. Overall, with single diffuse injury, moderate (Pruitt et al., 2014, 2017; Rana et al., 2020b), but not mild, injury (Evans et al., 2015, 2014; Namdar et al., 2020) led to chronic grip strength deficits. However, the protocol for assessing grip strength varied between studies, with the number of trials examined ranging from 1-10. Following diffuse single mTBI, no deficits were detected on grip strength up to 12 weeks post-injury (Evans et al., 2015, 2014; Namdar et al., 2020), with only one study finding an acute deficit at 1 week post-injury (Evans et al., 2014). In contrast, diffuse moderate TBI did produce deficits in grip strength at 28 days post-injury, the latest time-point assessed (Pruitt et al., 2014, 2017; Rana et al., 2020b). For repeated mild injury, bilateral mTBI weekly for 5 weeks led to impaired grip strength at 40-45 weeks post-injury (Dhillon et al., 2020a), whereas 3 impacts 24 hrs apart led to no deficits at 30 days post-injury (Tabet et al., 2022), with these the only time-points assessed in these studies. Notably, with the negative result, the average of three trials relative to body weight was recorded (Tabet et al., 2022), whereas the positive result was only one trial reported as maximum force achieved, introducing a potential confound (Dhillon et al., 2020a). To date, no study has assessed grip strength alterations chronically following a severe TBI. Another test of strength, the isometric pull task, was used in two studies of focal moderate TBI, in which the lesion was specifically located over the motor cortex (Pruitt et al., 2014, 2017). In this task, rats were trained prior to injury to reach a force threshold of at least 120g within 2 secs on a pull lever in order to receive a food reward. Following injury, rats had a decrease in maximal force produced, a decrease in the % successful trials and decrease in the speed of force generation from weeks 1-6 post-injury (Pruitt et al., 2014, 2017). However, in successful trials, the time taken to reach the 120g threshold was only significantly increased in

Weeks 1-2, returning to sham level from Week 3. This mirrored results for total trials, which were significantly decreased from Weeks 1-2 before returning to sham level, potentially indicating reduced motivation as well (Pruitt et al., 2014). However, given the focal nature of the injury in these studies, it is difficult to know if these findings would transfer to a diffuse injury model.

Fine motor skills were assessed by Atkins *et al.* (Adkins et al., 2015) and Schonfeld *et al.* (Schönfeld et al., 2017) using variations of a pellet reaching task following moderate-severe and severe focal injury respectively, with significant deficits found out to 6 weeks post-injury. In the Adkins *et al* study, the pellets were located on a flat surface in front of the animals (Adkins et al., 2015), whilst Schonfeld *et al.* (Schönfeld et al., 2017) used a staircase with pellets placed on increasing higher steps to enhance difficulty. In the pellet reaching task, injured animals had a decrease in the % successful reaching attempts to 42 days post-injury (Adkins et al., 2015). In the Montoya staircase task, injured rats obtained significantly fewer pellets across all steps, made less reaching attempts and misplaced more pellets on the upper steps (Schönfeld et al., 2017).

3.4.5 Locomotor activity

The open-field test, the most commonly used task to measure general locomotor activity levels by examining the total distance traveled over a test period (5-60 minutes), was used across all included studies (Table 4). Following diffuse mTBI, either single or repeated, locomotor results varied depending on the species (rat versus mice), strain, protocol and apparatus employed (Table 5). Utilization of a smaller apparatus (19 x 11cm) following weight drop TBI in Swiss mice over a 60 minute period found persistent hyperactivity from 48 hours to 12 weeks following injury (Homsi et al., 2010). In contrast, closed skull CCI injury in C57BL/6J mice led to no changes in locomotion over 30 mins in a larger 49 x 36 cm arena either acutely or

chronically up to 90 days post-injury (Bajwa et al., 2016). Indeed, repeated impacts over a short interval (Morriss *et al.*: 5 x 24h; Tucker *et al.*: 3 x 24h) were required to replicate this hyperactivity in mice in a larger arena (40 x 40 cm), with this behaviour developing at 3 months post-injury (Morriss *et al.*, 2021; Tucker *et al.*, 2019) and persisting to 12 months post-injury (Tucker *et al.*, 2019), the latest time-point examined. With 2 CCI-CS impacts over 3 days, changes in locomotion were no longer observed in mice in a similar size arena over 30 mins on day 1, day 7 or 12 weeks post-injury (Bajwa *et al.*, 2016).

Varied results have been reported in rat studies. Mild weight drop TBI in Sprague Dawley rats found no difference in locomotion in a 60 x 60cm arena over 10 mins at either 1 or 4 weeks post-injury (Namdar *et al.*, 2020). Although a decrease in locomotion has been reported at 6 weeks, resolving by 12 weeks post-injury in a larger 1 x 1m enclosure over 5 mins (McAteer et al., 2016a). In contrast, with repeated injury (3 x 5 days), both a decrease in locomotor activity at 6 and 12 weeks (Corrigan et al., 2017) and an increase in locomotor activity at 12 weeks (Corrigan et al., 2017), have been reported utilising the same testing parameters. Notably, the decrease was recorded with manual counting of squares crossed (McAteer et al., 2016a), whereas the increase was detected using automated software of distance travelled (Corrigan et al., 2017). Nonetheless, no differences in locomotion were noted at 12 months post-injury in the same injury model (Arulsamy et al., 2019a), nor in a repeated CHIMERA model (2 x 3 days) in a smaller open field (40cm x 40cm) from day 1 to 12 weeks post-injury (Feng et al., 2021).

Increasing injury severity had little effect on chronic locomotor activity. Moderate focal CCI injury in C57/BL6 mice found a transient decrease in distance travelled at 7 days, but this resolved by 12 weeks (Bajwa et al., 2016). Leconte *et al.* similarly showed no difference compared to naïve animals at 5 months following CCI injury in rats (Leconte et al., 2020). Even with more severe injury, locomotor performance was unchanged at 10 weeks following

injury in young mice, with a significant difference only seen in mice injured at 18 months of age (Islam et al., 2021). Similarly, mixed focal/diffuse injury via LFP had no effect on locomotor activity, as measured via distance travelled over 5 minutes out to 6 months post-injury (Komoltsev et al., 2021; Rowe et al., 2016). The pattern of deficits differed slightly with moderate-severe diffuse injury, with no differences noted at 4 weeks, a decrease in distance travelled at 12 weeks (Arulsamy et al., 2018), with recovery by 12 months, the latest time-point examined (Arulsamy et al., 2019a). A similar pattern was seen following a single blast injury (Arun et al., 2020). No changes were seen from day 1 to 3 months post-injury, but this was followed by a subsequent significant decrease in total distance travelled over 60 mins in a 40 x 40 cm arena at 6 and 9 months, with recovery by 12 months post-injury (Arun et al., 2020). Indeed, two 19 PSI injuries delivered within two minutes were required to lead to a persistent decrease in locomotor activity at 12 months, with deficits seen within the first three days post-injury, resolving at 4 weeks post-injury, prior to re-emerging at 3 months and then persisting to the 12 month time-point (Arun et al., 2020).

3.4.6 Balance and Coordination

Balance and coordination were the most common motor domains evaluated in preclinical studies via tasks encompassing the balance beam (Alwis et al., 2012; Bajwa et al., 2016; Barrett et al., 2020; Dhillon et al., 2020a; Hanscom et al., 2021; Henry et al., 2020a; Lai et al., 2019; Nissinen et al., 2017; Rowe et al., 2016; Sell et al., 2017; S.f et al., 2020; Soblosky et al., 1997a; Wang et al., 2020b; W. G. Wright et al., 2017a; Xie et al., 2019; Xu et al., 2019; Zhang et al., 2005a), ladder (Chen et al., 2016; Fehily et al., 2019; Namdar et al., 2020; Tabet et al., 2022), rotating pole (Arun et al., 2020; Laurer et al., 2001; Zhang et al., 2005a), gridwalk (Bajwa et al., 2016; Cline et al., 2017a), string suspension (Albayram et al., 2017), pole climbing (Leconte et al., 2020; Tabet et al., 2022), and rotarod tasks (Albayram et al., 2017; Alwis et al.,

2012; Arulsamy et al., 2018; Arun et al., 2020; Bajwa et al., 2016; Dhillon et al., 2020a; Evans et al., 2014, 2014, 2015, 2015; Feng et al., 2021; He et al., 2020; Henry et al., 2020a; Hou et al., 2017; Mannix et al., 2014, 2017; McAteer et al., 2016a; Morriss et al., 2021; Mountney et al., 2017; Mouzon et al., 2014, 2018; Namdar et al., 2020; Sabbagh et al., 2016; S.f et al., 2020; Tan et al., 2020; Toshkezi et al., 2018; Tucker et al., 2019; Vogel et al., 2020; Wang et al., 2020b; W. G. Wright et al., 2017a; Zhang et al., 2005a) (Table 5). How the tests were conducted varied between studies including variation in the size of the beam and speed of the rotarod and rotating pole. Furthermore, the parameters examined varied between studies. For example, beam performance was analysed via time to traverse beam (Lai et al., 2019; Rowe et al., 2016; Sell et al., 2017; W. G. Wright et al., 2017b; Xu et al., 2019), number of foot-faults (Bajwa et al., 2016; Barrett et al., 2020; Cline et al., 2017b; Henry et al., 2020a; Laurer et al., 2001; Rowe et al., 2016; Soblosky et al., 1997; W. G. Wright et al., 2017b), or a ranking scale for performance (Albayram et al., 2017; Alwis et al., 2012; Nissinen et al., 2017; Sell et al., 2017; Wang et al., 2020; Zhang et al., 2005b). For the rotarod performance was evaluated on one trial (Alwis et al., 2012; Arulsamy et al., 2019; Evans et al., 2015; McAteer et al., 2016; Namdar et al., 2020) or an average across up to eight trials (Albayram et al., 2017; Feng et al., 2021; Hou et al., 2017; Mannix et al., 2017, 2014; Mouzon et al., 2018, 2014; Namdar et al., 2020; Tucker et al., 2019) which may influence results.

Following diffuse TBI, moderate to severe injury was more likely to lead to acute balance deficits as seen as impaired rotarod performance (Alwis et al., 2012; Arulsamy et al., 2018), time to traverse a beam (Alwis et al., 2012) or a score evaluating performance encompassing foot-faults or falls (Albayram et al., 2017). In contrast, acute deficits were not seen in most models of mTBI (Hou et al., 2017; McAteer et al., 2016a; Namdar et al., 2020), with only one diffuse mTBI study reporting acute deficits (< 72 hrs) on the rotarod (Evans et al., 2015), with these deficits persisting to 1 month following injury and resolving by 3 months

(Evans et al., 2014). Nonetheless following diffuse injury, the overall consensus was that no chronic deficits were seen on the rotarod, regardless of injury severity, from 3 to 24 months post-injury (Arulsamy et al., 2018; Bajwa et al., 2016; Evans et al., 2014; McAteer et al., 2016a; Mountney et al., 2017; Mouzon et al., 2018, 2014). In fact, only a single study found long-term impairment on the rotarod following either mild or moderate diffuse TBI (Hou et al., 2017). In the mild diffuse TBI group, deficits emerged at 8 weeks following injury and persisted to 18 weeks, whereas with a moderate injury deficits emerged at 4 weeks and similarly persisted to 18 weeks, the latest time-point examined (Hou et al., 2017). Use of other balance tests did detect balance deficits up two months post-injury following mTBI on both time to traverse a 0.5cm beam (Lai et al., 2019) and increased missteps on the Erasmus ladder (Namdar et al., 2020). Furthermore, following mod-sev diffuse TBI, worse performance was noted both on an 0.8cm beam, with performance scored from 0-3 depending on how mice were able to traverse the beam and number of falls and foot-faults, and on a string suspension assay (Albayram et al., 2017). However, this has not been consistently reported, with other studies investigating mild (Bajwa et al., 2016; Fehily et al., 2019) and moderate to severe diffuse TBI (Alwis et al., 2012) showing no deficits when traversing larger beams (0.65-2cm) (Alwis et al., 2012; Bajwa et al., 2016) or on forelimb placement in the ladder task (Fehily et al., 2019) up to 3 months post-injury, indicating task dependent effects and that more difficult tasks are required to detect subtle motor deficits.

Compared to diffuse injury, focal injury was more likely to cause chronic balance deficits. Following moderate focal injury, deficits on the grid walk, balance beam and rotarod tasks were noted to 3 months post-injury (Bajwa et al., 2016; Barrett et al., 2020; Henry et al., 2020b, 2020a; Toshkezi et al., 2018; Xie et al., 2019; Xu et al., 2019), the latest time-point assessed. Differing results were seen on the pole test, with an increase in time to turn only emerging at 4.5 months post-injury, with recovery by 6.5 months, which persisted to 9 months

post-injury (Leconte et al., 2020). These results were supported by studies in moderate-severe focal TBI, where Hanscom *et al.* found significantly increased foot faults on the ledged beam from one day to two months post-injury (Hanscom et al., 2021), and a focal punch injury similarly resulted in impaired beam performance up to six weeks post-injury (Wang et al., 2020b). With severe focal injury, balance deficits were consistently noted up to 10 weeks post-injury, the latest time-point assessed on the balance beam, rotarod, cylinder test and gridwalk tasks (Cline et al., 2017a; He et al., 2020; Schönfeld et al., 2017; Xu et al., 2019). In contrast, two studies did not report chronic balance deficits following moderate-severe focal TBI, although these used a larger beam (Soblosky et al., 1997a) and altered rotarod parameters (mice were placed on the rotarod already spinning at 36 RPM, rather than gradually increasing speed from 3 RPM) (Vogel et al., 2020). The larger beam would have reduced the complexity of the task, whereas the increased rotarod starting speed may have made the task too difficult for the shams, masking any injury effect.

With a mixed focal and diffuse injury via LFP, mixed results for balance and coordination were seen with a moderate injury. Wright *et al*. noted increased foot faults and decreased time to cross the beam (W. G. Wright et al., 2017a) and Tan *et al.* found impaired rotarod performance at 3 months post-injury (Tan et al., 2020). Rowe *et al.* found a similar pattern in animals injured at 2 months of age, with increased foot faults and time to cross the beam at both 1- and 3-months post-injury, but interestingly not in animals injured at 4 or 6 months (Rowe et al., 2016). In contrast, Carron *et al.* noted acute deficits in performance on the rotarod and tapered ledged beam, which had recovered by one week following injury, with no further deficits seen to 2 months post-injury (S.f et al., 2020). However, with more severe injury, LFP resulted in impaired performance on both the balance beam and rotating pole tasks out to 4 months post-injury, the latest time-point examined (Nissinen et al., 2017; Zhang et al., 2005).

In models of repeated mTBI, a higher number of injuries or a shorter interval between injuries was generally associated with more persistent balance deficits. Following 5-7 injuries, chronic deficits on the balance beam, string suspension and rotarod were noted up to 12 months post-injury (Albayram et al., 2017; Dhillon et al., 2020; Mannix et al., 2014; Morriss et al., 2021), although no deficits were seen by 24 months post-injury (Mouzon et al., 2018). Only the Mannix *et al*. 2017 and the Mouzon *et al.* 2014 studies failed to find chronic balance deficits following 4-7 impacts (Mannix et al., 2017, 2014). Following three injuries with a 24hr interval between injuries, increased latency to fall on the rotarod was seen up to 6 months post-injury (Tabet et al., 2022; Tucker et al., 2019), with recovery by 12 months (Tucker et al., 2019). Extending the interval between injuries to 5 days meant that 3 injuries no longer led to deficits on the rotarod up to 3 months post-injury (McAteer et al., 2016a). Interestingly. unlike the rotarod, no deficits were noted on forelimb placement in the ladder walk (Fehily et al., 2019) nor in pole climbing time (Tabet et al., 2022) with 3 injuries, 24hrs apart. With two injuries spaced 24hrs apart, the number of foot faults on a rotating pole were increased at 3 days post-injury, before returning to sham levels from 7-28 days, before a deficit re-emerged at 2 months following injury (Laurer et al., 2001). By increasing the interval between the two injuries to 3 days, deficits were no longer noted on the balance beam, rotarod or grid walk tasks either acutely or chronically up to 2 months-post-injury (Bajwa et al., 2016).

Finally, neither single nor repeated blast injury was sufficient to produce persistent motor deficits at six months post-injury, regardless of initial injury severity. With a mild blast injury at 19psi, a single injury led to no balance deficits on the rotating pole or rotarod task to 6 months post-injury (Arun et al., 2020). In contrast, when two 19 PSI injuries were delivered within two minutes, balance deficits emerged at 6 days, persisted to 4 weeks, with recovery and no further impairment noted following this time point up to six months post-injury (Arun et al., 2020). Similarly, following a single moderate blast impact (50 PSI), acute deficits in

latency to fall on the rotarod were noted, which had recovered by 6.5 weeks post-injury (Sabbagh et al., 2016).

3.5 Clinical studies

3.5.1 Motor function test

Overall long-term motor function following TBI was assessed in a single study using the Unified Parkinson's Disease Rating Scale (UPDRS) Motor Examination, which was used to calculate a modified UPDRS (mUPDRS) global motor score, as well as four domain scores: tremor, rigidity, bradykinesia and posture/gait (Gardner et al., 2017). In retired military veterans (M: 76.4±10.0 years of age) who self-reported TBI (median TBIs = 2 (1.2), 53.2±18.1 years since first TBI, 37.0±22.5 years ago since last TBI), those with a history of moderate-severe, but not mTBI, had a significantly worse mUPDRS global motor score, as well as a worsened score for posture/gait, but not for tremor, rigidity, or bradykinesia, compared to those without a history of TBI (M: 79.4±8.2 years of age)(Gardner et al., 2017) (Table 6).

3.5.2 Grip strength and fine motor control

Six studies identified herein (Burton et al., 2002; De Beaumont et al., 2009a, 2011; Pearce et al., 2014a, 2018a; Walker et al., 2018a) evaluated chronic alterations in grip strength or fine motor control (Table 7). Grip strength was assessed only in one study comparing individuals with a history of TBI 1-26 years earlier to healthy controls. No differences were seen following either mild or moderate-severe TBI, in either the dominant or non-dominant hand, although the mTBI group were significantly more variable than healthy controls across 10 trials.(Burton et al., 2002) This study also investigated finger dexterity as time taken to touch each of their fingers to their thumb three times. The moderate-severe TBI group at 12.2 years post-injury (range 1-25), but not the mild TBI group at 7.1 years post-injury (range 1-27), had slower finger

dexterity in both the non-dominant and dominant hands over 10 trials compared to healthy controls (Burton et al., 2002). This was not related to age, given that both groups had a similar mean age (35.4 versus 37.6 years). Similarly, no effects of at least one mTBI (range 1-12, median=2) in military veterans at a median of 8 years post the most recent TBI were noted in the grooved pegboard task, where pins must be manipulated and rotated to fit a hole (Walker et al., 2018a). With a higher number of repeated concussions and a longer time-period post-injury, however, deficits in fine motor control were seen chronically. Retired rugby league players (mean 8.5 concussions) at almost 20 years post-injury took longer in the O'Connor Finger Dexterity Test, where time taken to place pegs in holes is recorded compared to controls (Pearce et al., 2018a), with similar findings in amateur Australian football players (mean 3.2 concussion) at 22.12±6.73 years following their last injury on the same task (Pearce et al., 2014a). Similarly, chronic, but not acute, deficits were seen in a RAM task consisting of rapid wrist supination-pronation movements. Significantly lower movement velocity was found in athletes who sustained their last concussion 30 years earlier (range 27-41 years)(De Beaumont et al., 2009a), but not in those who had sustained their last concussion only 9-34 months earlier (De Beaumont et al., 2011). Importantly, both groups had the same range of 1-5 concussions, suggesting that these effects may be due to time elapsed since injury, rather than number of injuries.

3.5.3 Gait

A number of characteristics can be used to assess gait, including spatiotemporal factors, such as cadence, stride length and single and double support time, kinematics in regards to the motion of joints and kinetics to describe the measurement of the forces required to make a movement (Williams et al., 2010). Clinical studies varied widely in regards to the gait parameters examined, the tests employed and equipment used (Table 8).

No difference in gait speed over a distance of 3-4m was seen either in a group of military veterans (Walker et al., 2018a) or in a cohort recruited from the general population (Burton et al., 2002) on average a decade following their last injury. Conversely, more sophisticated analysis employing an 8m electronic walkway found that students with a history of concussion with a mean time since injury of 6.32 years had greater time in double-leg stance support, and less time in single-leg stance support, throughout the gait cycle (Martini et al., 2011). The more difficult task of heel to toe walking was also found to be affected by previous mTBI, with veterans with a history of mTBI approximately 16 years ago being three times as likely as normal controls to have their performance ranked as abnormal by a neurologist (Vanderploeg et al., 2007). Other studies investigating mTBI specifically recruited patient populations with persistent symptoms. Stuart *et al* aimed to develop a model to describe differences in gait seen in individuals with a history of mTBI sustained approximately 18 months ago (median 551 days) who had self-reported balance instability. Participants walked over a 13 m distance for 2 mins with inertial sensors detecting gait. Differences in the mTBI cohort compared to healthy controls related to increased variability, decreased rhythm and reduced pace in parameters like stride length and time, alongside increased turn duration and velocity (Stuart et al., 2020). These results were only partially supported by another study, which recruited individuals with a history of mTBI with symptoms persisting > 3 months, but did not require these symptoms to be specifically balance related. Participants were on average a year from injury, with statistically significant differences only detected in pace and turning, but not in rhythm and variability, over a ~200m walk with multiple 180° turns. It should be noted that the Stuart *et al* study did not report p values, but rather investigated effect size only, which could also account for the differences between these studies. Conversely, a much smaller study (n=16) of symptomatic veterans with a history of mTBI 3.5 ± 1.7 years previously found no difference in gait speed or stride length over 10m (Wilkerson et al., 2021). Given that the Stuart *et al* study

included 111 participants (Stuart et al., 2020) and the Martini *et al* study 68 (Martini et al., 2011), any differences may relate to the small sample size.

Indeed, in comparison to symptomatic mTBI, moderate-severe TBI at least 18 months earlier (mean: 35.5±20.2 months) found no difference in cadence on a treadmill at 3km/hr for 2 mins (Buster et al., 2013). Similar findings were found more chronically, with a moderate TBI (range 1-26 years prior) not producing deficits in walking or turning speed on a walkway(Burton et al., 2002) or treadmill or elliptical trainer task (Buster et al., 2013). With a more difficult task, participants were placed on split-belt treadmill, such that the speed required for each leg could be varied (Vasudevan et al., 2014). Those with a history of moderate-severe injury an average of 2.9±1.7 years prior took longer to adapt to the belts being at different speeds, seen as an decrease in step symmetry, but were no different in the baseline task or post-adaptation when the two belts were at the same speed (Vasudevan et al., 2014).

Self-selected walking (Williams et al., 2010) and running speed (Williams et al., 2013) were slower in individuals with a previous history of severe TBI an average of 5-6 years earlier, with participants chosen for their ability to walk and run independently respectively, whilst still attending physiotherapy for mobility limitations. When healthy controls matched these speeds, no difference in either cadence or stance time was seen with walking (Williams et al., 2010), whereas, with running, a previous history of TBI led to increased cadence and shorter stride length to produce the same speed (Williams et al., 2013). In the only study that did not report a difference in walking speed, 19/52 participants were unable to walk at the faster speed, negating the measurement (Williams et al., 2010). Nonetheless, numerous kinetic and kinematic alterations were associated with both running and walking following severe TBI, including alterations in ankle power generation (Williams et al., 2010; Williams and Schache, 2016) and knee stability (Williams et al., 2013), which likely drive these alterations in gait. In a follow up study, these authors followed patients for six months following severe TBI an

average of two years earlier with access to a rehabilitation program (Williams and Schache, 2016). At baseline, previous TBI was again associated with significantly slower self-selected walking speed than healthy controls. However, at six month follow up, walking speed had significantly improved, such that there was no longer any difference compared to healthy controls, with an associated improvement in ankle power generation, indicating that these deficits can improve (Williams and Schache, 2016).

In addition to assessing gait alone, a number of studies investigated the effect of increasing difficulty via inclusion of obstacles (Martini et al., 2021) and/or cognitive tasks (Martini et al., 2021, 2011; Pitt and Chou, 2020) on gait following mild TBI (combined single and multiple). In symptomatic individuals < 5 years following the last injury, adding an auditory Stroop task had little effect on gait (Martini et al., 2021; Pitt and Chou, 2020). In a community cohort, changes in rhythm were seen in the dual versus single task in those with a history of mTBI compared to controls (Martini et al., 2021), whereas in a small cohort of military veterans no additive effect was seen with alterations in center of mass displacement seen in both walking alone and the dual task (Pitt and Chou, 2020). Adding obstacles or a spatial memory task actually reduced the performance in healthy controls, such that differences seen in speed and double stance support time with walking alone compared to those with a history of TBI were no longer present (Martini et al., 2011). Indeed, only combining the memory task with obstacles while walking re-introduced the increase in double stance support time in those with a history of mTBI 6 years earlier (Martini et al., 2011). Hence abnormalities in gait following mTBI may be detected by single task alone, without requiring the more difficult combined tasks.

3.5.4 Posture and balance

The effects of a prior TBI on chronic alterations in static balance were assessed under a number of conditions, with the majority of studies only investigating 1-5 years post-injury (Arce et al., 2004a; Geurts et al., 1999; Helmich et al., 2016b; Johnston et al., 2020b; Ledwidge et al., 2019; Rosenblum et al., 2020; Sosnoff et al., 2011; Useros Olmo et al., 2020b; Walker et al., 2018b, 2018b, 2018a, 2018a; Wright et al., 2018b; Zhang et al., 2002) and no studies looking at greater than 10 years post-injury (Table 9). In healthy populations, maintenance of postural stability does not require large amount of conscious effort and is regulated by subconscious reflexive actions of the CNS to interpret and act in accordance to perceived sensory feedback information from the visual, somatosensory and vestibular systems (Rasman et al., 2018). Manipulating the type and/or amount of information being processed by these three sensory feedback systems increases the difficulty of balance tasks and can reveal injury effects (Caccese et al., 2021; Kędziorek and Błażkiewicz, 2020; Surgent et al., 2019). Across the included studies, postural control was examined primarily by evaluating alterations in center of mass whilst standing, with concomitant manipulation of the sensory information available via altering the standing surface, closing eyes or altering the visual surroundings. Studies also incorporated the effects on balance in functional reaching involving either the leg (Johnston et al., 2020) or arm,(W. G. Wright et al., 2017a; Zhang et al., 2002) as well as the effects of bimanual lifting of weights.(Arce et al., 2004)

A history of asymptomatic mTBI <5 years earlier had no effects on postural control whilst standing, regardless of alterations in the support surface or visual feedback in a variety of cohorts including military veterans (Pan et al., 2015b; W. G. Wright et al., 2017a), college athletes (Rosenblum et al., 2020) and university students (Helmich et al., 2016). This included measures of center of pressure sway area (Wright et al., 2018b), sway path length (Pan et al., 2015), trunk pitch and roll angle (Pan et al., 2015) and the degree of anterior-posterior sway (Rosenblum et al., 2020). In contrast, in an arm functional reach task, mTBI an average of 5.8

years earlier was associated with reduced postural angular velocity, although no changes in angular displacement were noted (Ustinova, 2017). In contrast, symptomatic mTBI was associated with greater alterations in balance, with increased postural sway both during quiet standing (Geurts et al., 1999; Pan et al., 2015) and when suddenly perturbed (Pan et al., 2015) Repeat mild injury, either investigated as a separate cohort (Rosenblum et al., 2020; Wright et al., 2018b) or via combining those with a history of single and repeated injuries (De Beaumont et al., 2009a; Degani et al., 2017; Johnston et al., 2020b; Ledwidge et al., 2019; Lee et al., 2020; Reilly et al., 2020b; Rosenblum et al., 2020; Sosnoff et al., 2011; Wright et al., 2018b), had more mixed effects on balance. In general, more difficult tasks were required to detect differences between those with a history of mTBI (1 or more) and healthy controls (Helmich et al., 2016b; Lee et al., 2020; Reilly et al., 2020b; Sosnoff et al., 2011; Walker et al., 2018b; Wright et al., 2018b). For example, in college students, no changes in amplitude, velocity, frequency or regularity were seen in quiet standing in those with a mean time since last injury of 7.1 years (mean 2.5 injuries) (Lee et al., 2020). However, two studies were able to detect postural changes with quiet standing alone, with collegiate athletes 19 months since last injury (range 1-5 injuries) exhibiting an increase in center of mass oscillation irregularity (De Beaumont et al., 2009) and a cohort recruited from the community with one or more mTBIs also at 19 months post-injury having a larger body sway area, a larger displacement amplitude in the medio-lateral direction, a slower body oscillation in both directions and a more irregular pattern of body oscillation (Degani et al., 2017). In contrast, Wright *et al* only noted a difference compared to healthy controls in individuals with more than one mTBI with the last injury at least a year ago in the most difficult condition, where participants were required to stand on a foam surface with a dynamic visual surrounding, leading to increased center of pressure sway area (Wright et al., 2018b). Similarly, Helmich *et al* only saw an increase in effort of balance in symptomatic individuals within a mean of 2 years post-injury during

balance on an unstable surface, with eyes closed or a combination of both, but not on a stable surface or with eyes open (Helmich et al., 2016). At a year post-injury, Reilly *et al.* also found no effect of previous mTBI (combined single and multiple) on bipedal or unipedal stance alone, but did see an increase in sway and decreased regularity when combined with a cognitive task (Reilly et al., 2020). Conversely, Rosenblum *et al.* found no differences on the Sensory Organization test at 2-3 years following the last injury in a population of collegiate athletes (Rosenblum et al., 2020). The Sensory Organization test evaluates quiet standing under six different conditions (either fixed/sway surface, eyes open/closed, or surrounding normal/sway-referenced), thus involving increasing task difficulty. This was regardless of whether analysis looked at single versus multiple injuries compared to healthy controls, or when those with a history of single or rmTBI were combined (Rosenblum et al., 2020). In contrast, the same test in military veterans at an average of 10 years post-injury did find task specific effects, with a decrease in equilibrium score in the eyes closed/fixed surface and eyes open, fixed surface/sway surroundings conditions only when looking at combined single and rmTBI and in these conditions, alongside the sway surface with eyes open or closed conditions, when analysis investigated the rmTBI cohort separate from single injuries (Walker et al., 2018b, 2018a). Only one study utilised a different task, the Y Balance test, where participants stand on one leg and reach the other in an anterior, posteromedial or posterolateral direction (Johnston et al., 2020b). Acute deficits, with an increased amplitude of center of mass in the posteromedial and posterolateral direction in those with a history of TBI a median of 294 days ago, had resolved in those with their last injury a median of 3.5 years ago, with no deficits noted compared to healthy controls (Johnston et al., 2020b).

With more severe injury, the same level of analysis examining multiple parameters like regularity, amplitude, frequency and velocity changes in center of pressure has yet to occur, but consistent alterations in balance have been reported out to 10 years post-injury. A prior

moderate-severe TBI a mean of 3 years earlier was associated with increased center of pressure displacement across three tasks: standing only, standing with a numerical task and standing with a spatial memory task, with no significant alterations in performance between the different tasks (Useros Olmo et al., 2020b). Similarly, a prior severe TBI 10 years earlier led to a decrease in dynamic posturography scores, where postural changes in response to a tilt platform were examined, alongside a higher Berg balance score (Buster et al., 2013b). A functional reach task where participants were able to sit in a wheelchair while moving their arm to touch a target that appeared in either a predictable or unpredictable fashion found a medium-large effect size of prior moderate-severe TBI on stability ratio during the task (Zhang et al., 2002). In a bimanual lifting task, although a history of previous severe TBI 2-10 years earlier was associated with greater instability in the quiet stance phase, the postural adjustments that occurred to lift 4 or 8kg weights were not different from that of healthy subjects (Arce et al., 2004a). Thus, balance changes appear to be similarly evident, particularly with quiet standing following more severe injury.

4.0 DISCUSSION

This systematic review investigated chronic motor outcomes following TBI and the effect of injury severity. The results of this work provide a comprehensive overview of the current state of understanding of motor changes following TBI, highlighting limitations and gaps of existing research that are critical to fill in order to suggest guidelines for rehabilitation programs following TBI. There was little consensus across the papers presented, with a wide variety of motor domains examined, as well as significant differences in the methodology of the tests utilised and parameters reported. Indeed, the lack of consensus in the approaches used in assessing and reporting chronic motor outcomes in both preclinical and clinical models of TBI limits the generalisability of the findings. In the future, more standardised testing parameters and protocols for motor tasks would assist in comparing findings. For example, the development of common data elements for both preclinical and clinical studies would be of benefit, given that standardisation and harmonisation of data collection are of paramount importance (Meeuws et al., 2020).

Overall, there was a paucity of clinical studies investigating motor outcomes beyond 10 years post-injury, with only six identified within this review. The majority of these papers investigated fine motor control (Burton et al., 2002; De Beaumont et al., 2009b; Pearce et al., 2014a, 2018a), meaning that long-term effects of TBI on gross motor functions, like gait and postural control, have not been extensively studied. Furthermore, there was a lack of longitudinal clinical studies investigating how motor performance changes over time in the same cohort in the chronic phase post-injury. Similarly, in preclinical work, only 9 studies were investigated to 12 months post-injury (Arulsamy et al., 2019a; Arun et al., 2020; Dhillon et al., 2020a; Mouzon et al., 2014, 2018; Pierce et al., 1998; Sell et al., 2017; Tucker et al., 2019) and, of these, only 2 studies to 18-24 month post-injury (Mouzon et al., 2018, 2014). More chronic studies are therefore needed to understand how a history of TBI interacts with normal aging to

affect motor performance. Imaging studies have suggested that a history of TBI accelerates the rate of brain atrophy (De Beaumont et al., 2009a; Gardner et al., 2017; Pearce et al., 2014a, 2018a; Vanderploeg et al., 2007; Walker et al., 2018b) and studies investigating cognition have suggested TBI is associated with an earlier age of cognitive decline, not necessarily associated with a specific neurodegenerative disorder (Iacono et al., 2021). Whether TBI similarly leads to earlier physical decline needs further investigation. This is particularly relevant given the growing literature linking a history of TBI to the later risk of neurodegenerative motor disorder development, particularly motor neuron disease (MND) and PD. For example, Wright and colleagues reported ALS-like pathological changes, accompanied by persistent motor deficits, at 12 weeks, but not 1 week, following a moderate experimental TBI in rats (D. K. Wright et al., 2017). This suggests that TBI may begin an insidious neurodegenerative process that predisposes an individual to the later development of motor neuron disease. This is in line with several previous studies conducted with professional athletes, including National Football League (NFL) (Daneshvar et al., 2021; Lehman et al., 2012), soccer (Belli and Vanacore, 2005; Chiò et al., 2005; Mackay et al., 2019; Pupillo et al., 2020), and rugby union players (Russell et al., 2022). Overall, meta-analyses have suggested a 1.3 to 1.7 fold increase in motor neuron disease risk due to a prior history of TBI (Chen et al., 2007; Gu et al., 2021; Liu et al., 2021); however, not all literature has been consistent (Watanabe and Watanabe, 2017). Similar findings have also been reported for PD, with a doubling of deaths due to PD in former professional soccer players compared to a matched control group drawn from the general population (Mackay et al., 2019). Even a mild TBI has been shown to increase risk of PD by 56% in US military veterans, after adjusting for demographics and comorbidities (Gardner et al., 2017). Several potential biological mechanisms have been proposed to explain this link, including chronic neuroinflammation, metabolic dysregulation and pathological up-regulation of several key PD linked proteins, including alpha-synuclein, hyperphosphorylated tau,

amyloid precursor protein, TDP-43 and, more recently, Leucine Rich Repeat Kinase 2 (LRRK2) and its Rab protein substrates (see Delic et al. 2020 for review(Delic et al., 2020a)). Motor dysfunction may also play a role in other neurodegenerative diseases linked to TBI, including chronic traumatic encephalophathy(CTE), which is characterised by the accumulation of hyperphosphorylated tau aggregates.170 Clinical data from 298 donors diagnosed with CTE identified motor symptoms in a large portion of cases, with gait and balance disturbance noted in 51% and signs of parkinsonism in up to 28% of cases.171 Thus, tracking alterations in motor function longitudinally in those with a prior history of TBI may allow for earlier identification, and subsequently treatment, of those at risk for the development of MND, PD or CTE, currently a major area of clinical need.

Despite this significant gap, key findings from clinical studies conducted to date of chronic motor alterations following TBI suggest that measures of balance, including postural control and gait, could differentiate between levels of injury severity injury in those who had suffered there injury in the last 10 years and, importantly, could discriminate between symptomatic and asymptomatic mTBI sufferers. Balance requires multiple input and integration centers spanning the entire brain, with damage to any of these structures or their associated white matter networks resulting in balance impairment (Surgent et al., 2019). A key feature of post-concussion syndrome may be disruption of these networks, subtlety impairing balance control. Given that stressing the sensorimotor integration centers of the brain elicited the greatest degree of impairment, it suggests that, following injury, there may be limited access to neural resources capable of compensating for reductions in sensory feedback information (either visual, vestibular or somatosensory), as opposed to gross decreases in musculoskeletal or aerobic functional capacities (Caccese et al., 2021). Entropy measures of postural sway parameters were particularly shown to be affected by symptomatic mTBI, with these measuring the regularity of center of pressure oscillations (Kędziorek and Błażkiewicz,

2020). From a motor control perspective, more regular values are interpreted as indicating a less stable system, as damage to neural tissue results in a reduced capacity for the complex oscillatory networks within the brain to produce and maintain upright posture under a wider variety of movement patterns (Huang et al., 2021). Decreased entropy values have been reported acutely following mTBI (Cavanaugh et al., 2006; Gao et al., 2011) and are shown here to persist in a subpopulation of symptomatic sufferers. The specific mechanisms driving these balance disruptions, however, require further investigation.

Mechanistic investigations may be limited to date, due to significant differences in the examination of balance in preclinical models compared to measures employed clinically. Relatively few preclinical studies incorporated gait analysis, which could be due to the technology required to perform detailed analysis. Surprisingly, the one consistent finding seen in more severe clinical TBI, a reduction in speed, was not replicated in preclinical studies. Indeed, minimal gait deficits in general were found in preclinical studies, with only a reduction in swing speed and stride length at 6 months following moderate focal injury (Daglas et al., 2019a; Ritzel et al., 2020), but no deficits at one month following a more severe injury (Schönfeld et al., 2017). This may reflect the differing mechanisms of injury and severity of preclinical compared to clinical models. Preclinical models are limited in their abilities to model more severe TBI, which are associated with prolonged stays within the intensive care unit and long periods of rehabilitation, which may impact upon motor function.(Dos Santos et al., 2016) Furthermore, the location of contusional injuries differs in preclinical compared to clinical models, typically found in the pre-frontal and temporal lobes clinically (Adatia et al., 2021) and the parietal lobes in preclinical models (Osier and Dixon, 2016). Key differences in gait are also obviously evident in biped versus quadrupeds, with center of mass higher in bipeds than quadrupeds (Zehr et al., 2016) and increased frequency of gait patterns at higher speeds, such as trotting and galloping, in rodents, which are generally not seen in bipedal human

(Mendes et al., 2015). However, there were some preclinical findings that were supported clinically, with a model of mTBI finding alterations in base of support to 3 months post-injury (Mountney et al., 2017), with clinical studies similarly showing an alteration in the equivalent double versus single-stance support at 6 years post-injury (Martini et al., 2011). Thus, incorporation of longitudinal gait analysis out to more chronic time-points in preclinical models of TBI would be useful.

Furthermore, there are no static tests of balance utilised in preclinical studies. Instead, balance assessment preclinically incorporates transitional movements utilizing tasks like the rotarod, balance beam, gridwalk and ladder walk, which are all scored with gross parameters, such as number of foot-faults, latency to cross and speed achieved on the rotarod. These measures may not be sensitive enough to detect subtle deficits, particularly in models of mTBI, especially given that the read out measures are relatively crude, a limitation that has previously been noted elsewhere (Shultz et al., 2020). Indeed. even in more severe models of diffuse injury, chronic (>6 months) impairments in motor performance were not seen, unlike focal or mixed injury models (Barrett et al., 2020; Daglas et al., 2019b; Dhillon et al., 2020b), where more widespread disruption of motor pathways may occur. It has previously been noted that the lack of functional deficits in preclinical models is surprising given the amount of histological damage (Turner et al., 2014). Refinement of motor tests is needed to discern whether this is because the damage is not sufficient to drive functional changes, or if the motor tests used are not sensitive enough. For example, utilising center of pressure measurements may be an option for future studies, with this successfully employed previously in models of vestibular injury in rodents (Rastoldo et al., 2020), given the sensitivity of the task in clinical work.

Another discrepancy between clinical and preclinical studies is the incorporation of fine-motor specific tasks. Although some of the balance tasks outlined above, like the grid-walk and rotarod, incorporate aspects of fine motor performance, the effects of injury

specifically on this domain cannot be discerned. Furthermore, tasks like the adhesive removal test may be complicated by the presence of sensory deficits (Mann and Chesselet, 2015). Notably, given that a history of repeated injury clinically appeared to be associated with poorer performance on dexterity tasks (Pearce et al., 2014a, 2018a), the need for greater inclusion of these within preclinical work is supported. Only two studies incorporated a fine-motor specific task in the pellet reaching (Adkins et al., 2015) and Montoya staircase tasks (Schönfeld et al., 2017). These were only utilised following moderate-severe and focal injury, noting deficits to six weeks post-injury, making comparisons with the clinical work, where moderate-severe injury led to deficits at 10 years post-injury (Burton et al., 2002), but repeated mild TBI at > 20 years (Pearce et al., 2018b, 2014b) difficult. Investigation of other forepaw dexterity-based tasks, like the vermicelli or cappelini handling tests (Mann and Chesselet, 2015), would also be useful to add to motor behavioural batteries post-TBI.

Alongside the need to utilise a wider variety of preclinical motor tasks, the field would also benefit from a broadening of the animals used. Preclinical rodent models have some limitations in the ability to fully model the types of white matter damage encountered in diffuse injury of any severity due to the relative lack of myelinated tracks (Vink, 2018). Indeed, single diffuse or mixed injury models did not produce long-term motor deficits (>6 months) (Arulsamy et al., 2019a; Komoltsev et al., 2021; Mouzon et al., 2018; Rowe et al., 2016; Sell et al., 2017) compared to purely focal injury (Daglas et al., 2019b; Ritzel et al., 2020), although there were obviously few studies that investigated these chronic timepoints. Promising gyrencephalic models with more extensive white matter tracts may provide a key bridge between preclinical and clinical work with development of porcine (Kinder et al., 2019), ferret (Schwerin et al., 2017), and sheep (Vink, 2018) TBI models that could be utilised for future investigation of longitudinal motor deficits. A number of motor tasks have already been

developed for these models including gait analysis (Shultz et al., 2020) which provide useful information about the longitudinal trajectory of motor impairment.

In addition, studies to date have failed to take into consideration what effect age at injury may have on long-term motor outcomes following TBI. Motor function is well known to decline substantially with advancing age, with changes at the level of the motor unit(Hunter et al., 2016), as well as at the neural level (for review, see Seidler et al. 2010 (Seidler et al., 2010)). For example, King and colleagues (2018) have demonstrated that age-related declines in motor performance are associated with stronger internetwork resting state connectivity, suggesting breakdown of organization of large-scale brain networks (King et al., 2018). Similar changes in resting state functional connectivity have been noted following TBI (Iraji et al., 2015). It is therefore reasonable to hypothesise that advanced age may exacerbate alterations in motor performance following injury. In line with this, older age is known to be associated with poorer outcome following TBI, with older adults having the highest rate of hospitalisation and death following TBI (Thompson et al., 2006). Older adults have also been shown to experience greater decline in Disability Rating Scale scores over the first five years following injury (Marquez de la Plata et al., 2008). Despite this, however, to date, no clinical studies have investigated the effect of age at injury on chronic motor outcomes. Preclinically, only one study incorporated rats injured at different time-points in adulthood, with no effect on overall long-term motor performance noted (Rowe et al., 2016). Future studies should therefore be designed to include assessment of how age at time of injury may influence motor response. Indeed, in the preclinical literature, there is a growing call to include aged animals into the modelling of neurological disease more generally (Sun et al., 2020).

Although the current paper was limited to chronic motor outcomes, it should be noted that these do not occur independent of effects on other functional domains, such as cognition. For example, following mTBI, it was found that there was a significant association between

cognitive and motor function following injury, but not prior (Sosnoff et al., 2008). A potential explanation is that the effects of injury on attentional networks, which are a driver of cognitive dysfunction (Halterman et al., 2006), but also impair performance on numerous motor tasks, including postural control (Woollacott and Shumway-Cook, 2002), gait (Yogev-Seligmann et al., 2008) and the ability to perform fine motor tasks (Dias da Silva and Postma, 2022). Executive function more broadly is also key for motor performance, with patients with normal measures of executive function following moderate-severe TBI demonstrating better balance and agility and increased speed of walking and running than those with executive function deficits (Sarajuuri et al., 2013). Further work should therefore examine chronic motor outcomes in the context of the effects on broader functional domains, including cognition, in order to better explore the bidirectional nature of these relationships. This is particularly important given that effects on cognitive function can affect ability to participate in physical rehabilitation programs, which can have detrimental consequences for functional motor recovery.

Finally, it should also be noted that a high risk of bias was noted for the majority of pre-clinical and clinical studies included here, which may have influenced the results. For the pre-clinical studies, key sources of bias included random outcome assessment and blinding of outcome assessment, as well as allocation concealment. For clinical studies, a key source of bias was blinding of outcome assessment, as well and blinding of participants and personnel. If researchers are not blinded, this will have implicit biases on the data recording process and potentially the randomisation of the study and random outcome detection, making it difficult to truly interpret results. Given the high risk of bias and high degree of heterogeneity between studies, it was not feasible to conduct a meta-analysis in the current study; however, in the future, it may be of interest to consider meta-analysis on specific data subsets.

In conclusion, despite the known relationship between mobility and quality of life following TBI (Kalpinski et al., 2013), chronic motor performance following injury is less well studied than either cognitive or affective outcomes. Additionally, across the few studies conducted, significant differences in experimental paradigms employed in both clinical and preclinical studies make it difficult to discern the pattern of motor deficits in the subacute and chronic phase following injury, with more standardised protocols required. Furthermore, a broadening of the motor batteries utilised within preclinical studies is warranted to more closely mirror the types of balance and fine motor deficits identified clinically. This review highlights the need for more consistent investigation and reporting of long-term motor deficits to allow an understanding of their evolution over time. An understanding is key to allow full insight into the recovery process, rehabilitation needs of those with TBI and how chronic motor changes post-TBI could provide a novel method for identifying risk of neurodegenerative movement disorders.

Table 1: Preclinical Neurological severity score evaluation

Authors	Years	Method	Severity	Type injury	Sample	Pre	<72h	1-3wks	1-2mo	3-5mo	6-11mo	12-24mo
Laurer	2001	CCI-CS	Mild	Diffuse	T:16-24;C:14-23		+	-	-			
Fehily	2019	WD	Mild	Diffuse	T:15; C:15					-		
Monteray	2017	PCS	Mild	Diffuse	T/C: 8-10					+		
Monteray	2017	PCS	Rep-Mild	Diffuse 4 x TBI/1hr	T/C: 8-10					+++		
Feng	2021	CHIMERA	Rep-Mild	Diffuse 3xTBI/day x 2 48 hrs	T:9-13; C:12-14		-	-	-			
Laurer	2001	CCI-CS	Rep-Mild	Diffuse 2xTBI 24 hrs	T:16-24;C:14-23		+++	+++	-			
Fehily	2019	WD	Rep-Mild	Diffuse 2xTBI 24hr	T:15; C:15					-		
			Rep-Mild	Diffuse 3xTBI 24hr	T:15; C:15					-		
Huynh	2020	CCI-CS	Rep-Mild	Left sided 5xTBI, 48hr	T:15; C:15					-	-	
Zhang	2021	CCI	Moderate	Focal	T:10; C:10		++	++	++			
Daglas	2019	CCI	Moderate	Focal	T:12, C:12		++++	++++	++++	++++	++++	
Sell	2017	LFP	Moderate	Mixed	T:11-14; C:12-14		+++			+++	-	-
Shear	2010	PBBI	Mod-Sev	Focal	T/C=10		+	+	+			
Wang	2019	Punch	Mod-Sev	Focal	T:8; C:8		++	++	++			
Segovia	2020	LFP	Severe	Mixed	T:7; C:7	-	+++		+++			
Nissinen	2017	LFP	Severe	Mixed	T:35;C:16	-	+++	+++	+++			
Zhau	2021	CCI	Severe	Focal	T:10; C:10		+++	+++	+++			
Zhang	2005	LFP	Severe	Mixed	T:14; C:24			++	++	++		
Pierce	1998	LFP	Severe	Mixed	T:12-16;C:11-15		+	+	+		+	-

+ = p<0.05; ++ = p<0.01; +++ = p<0.001; - = not significant; Grey = not evaluated. CCI-CS: controlled cortical impact- closed skull, WD: weight drop; CHIMERA: Closed-Head Impact Model of Engineered Rotational Acceleration; LFP: lateral fluid percussion, CCI: controlled cortical impact, PBBI: penetrating ballistic like impact

Table 2: Preclinical Gait evaluation

Authors	Years	Method	Severity	Type injury	Apparatus	Parameters examined	Sample	Pre	<72h	1-3wk	1-2mo	3-5mo	6-11mo	12-24mo
Namdar	2020	WD	Mild	Diffuse	CatWalk	Front base of support	T:15; C:13			+	+++			
						Standing on diagonal two				+	-			
						Standing on three				++	+			
						Hind base of support				-	-			
Mounterey	2017	PCI	Mild	Diffuse	CatWalk	Stand (sec)	T/C: 8-10		+	-	-			
						Stand index			+	-	-			
						Swing (sec)			-	-	-			
						Step cycle (sec)			-	-	-			
						Single stance			-	-	-			
						Stride length			-	-	+			
						Front base of support			++	+	+++			
						Hind base of support			-	-	+++			
						Three limb support			-	-	-			
						Cadence			-	-	-			
Bolton	2016	CCI-CS	Rep-Mild	5 x TBI 24 hrs	Digigait, treadmill 15cm/s	Gait symmetry	T/C: 10		-		-			
						Hindlimb shared stance								
						Paw contact area								
Bolton	2016	CCI-CS	Rep-Mild	5 x TBI 48 hrs	Digigait, treadmill 15cm/s	Gait symmetry			-		-			
						Hindlimb shared stance								
						Paw contact area								
Mounterey	2017	PCI	Mild	Diffuse 4 x TBI/1hr	CatWalk	Stand (sec)	T/C: 8-10		+++	+	-			
						Stand index			++	++	++			
						Swing (sec)			+++	+	+			
						Step cycle (sec)			+++	+++	+			
						Single stance			+++	+	+			
						Stride length			-	+	-			
						Front base of support			++	+	+++			
						Hind base of support			-	-	+++			
						Three limb support			++	+	-			

						Cadence			+++	+++	++			
Henry	2020	CCI	Moderate	Focal	CatWalk	Paw contact area Stance, swing Speed Interlimb coordination Base of support %Support time	T:12; C;12	-	-	-	-			
Ritzel	2020	CCI	Moderate	Focal	CatWalk	Step Sequence Stride length (RH) Swing speed (RF,RH) Print position Average speed, number of steps	T/C: 16-23						+	
Daglas	2019	CCI	Moderate	Focal	DigiGait 15cm/s	Swing duration (RH) Propulsion duration (RF)	T:12; C:12	-		+	+	+	++	
								-		-	++	+	+++	
Cline	2017	CCI	Severe	Focal	CatWalk	Cadence Average Speed Swing duration (RH) Average Swing speed (LF, RF, RH)	T:15; C:14		++ ++ ++ +		- - + -			
Schonfeld	2017	CCI	Severe	Focal	CatWalk	Stride length Base of support Three limb support; Speed Cadence	T:10; C:7	-		-	-			

+ = p<0.05; ++ = p<0.01; +++ = p<0.001; - = not significant; Grey = not evaluated. T= TBI; C= Control; RH = right hindlimb, RF = right forelimb, LF = left forelimb). CCI-CS: controlled cortical impact- closed skull, WD: weight drop, PCI = projectile concussive impact

Table 3: Preclinical sensorimotor evaluation

Authors	Years	Method	Severity	Type Injury	Test	Parameters examined	Sample	Pre	<72h	1-3wks	1-2mo	3-5 mo	6-11 mo	12-24mo
Hoffman	2003	CCI	Mod-Sev	Focal	Adhesive Test	3 x 2 min trials %sham	T:8; C:8			+	-			
Alwis	2012	WD	Mod-Sev	Diffuse	Adhesive Test	1 trial Latency	T:19; C: 12	-	+	-	-			
					Whisker-evoked forepaw placement	10 trials No. correct	T:4; C:4		+	+	+			

Schonfeld	2017	CCI	Severe	Focal	Adhesive Test	3 trials Paw difference	T:10; C: 6	-		+	+			
Zhang	2005	LFP	Severe	Mixed	Adhesive Test	6 trials Latency	T:14; C:13			++	++	++		

+ = p<0.05; ++ = p<0.01; +++ = p<0.001 - = not significant; Grey = not evaluated. T= TBI; C= Control/. CCI=Control cortical impact; WD= weight drop injury; LFP= Lateral fluid percussion injury.

Table 4: Preclinical grip strength and fine motor evaluation

Authors	Years	Method	Severity	Type Injury	Test	Parameters examined	Sample	Pre	<72h	1-3 wks	1-2mo	3-5mo	6-11mo	12-24mo
Evans	2014	CCI-CS	Mild	Diffuse	Grip strength meter	Average 10 trials	T9;C8		-	+	-	-		
Namdar	2020	WD	Mild	Diffuse	Grip strength meter	5 trials, average best 3	T:10; C:8			-	-			
Evans	2015	CCI-CS	Mild	Diffuse	Grip strength meter	Average 10 trials	T:12; C:11		-	-	-			
Tabet	2022	CCI-CS	Rep-Mild	3x TBI 24hrs	Grip strength meter	Average 3 trials relative to weight	T:11; C:11				-			
Dhillon	2020	CCI-CS	Rep-Mild	2xTBI (L+R) 5 x weekly	Grip strength meter	1 trial	T:10; C:8						+	
Rana	2020	WD	Moderate	Diffuse	Grip strength meter	1 trial	T:7; C:5			+	+			
Pruitt	2014	CCI	Moderate	Focal (motor cortex)	Isometric pull task	Maximal Force	T:15; C:11	-		+	+			
						% Successful Trials				+	+			
						Time to 120g threshold				+	-			
						Speed force generation				+	+			
						Total Trials				+	-			
Pruitt	2017	CCI	Moderate	Focal (motor cortex)	Isometric pull task	Maximal Force	T:6; C; 6	-		+	+			
						% Successful Trials				+	+			
Adkins	2015	CCI	Mod-Sev	Focal	Pellet reaching Test	% successful	T:41; C:31	-	+++	+++	+++			
Schonfeld	2017	CCI	Severe	Focal	Montoya staircase test	Pellets eaten	T:8, C:7	-		+++	++			

+ = p<0.05; ++ = p<0.01; +++ = p<0.001; - = not significant; Grey = Not evaluated. T= TBI; C= Control/Sham. CCI=Control cortical impact; CCI-CS=Closed skull-Control cortical impact; WD= weight drop injury.

Table 5: Preclinical locomotor activity evaluation

Authors	Year	Method	Severity	Type Injury	Test	Time	Size	Sample	Pre	<72hr	1-3wks	1-2 mo	3-5mo	6-11mo	12-24mo
Namdar	2020	WD	Mild	Diffuse	OF	10 mins	60 x 60 cm	T:15; c:13			-	-			
Homsi	2010	WD	Mild	Diffuse	OF	60 mins	19 x 11 cm	T/C: 10-12		+++	++	++	+		
Bajwa	2016	CCI-CS	Mild	Diffuse	OF	30 mins	49 x 36 cm	T:10; C:10		-	-		-		
McAteer	2016	WD	Mild	Diffuse	OF	5 mins	1 x 1 m	T:9; C; 9				+	-		
Arulsamy	2019	WD	Mild	Diffuse	OF	5 mins	1 x 1 m	T:14; T:14							-
Arun	2020	Blast	Mild	Blast	OF	60 mins	40 x 40 cm	T/C: 10-31		-	-	-	-	+	-
Feng	2021	Chimera	Rep-Mild	Diffuse 2x 3d	OF	Unknown	40 x 40 cm	T+C:81		-	-	-			
Bajwa	2016	CCI-CS	Rep-Mild	Diffuse 2x 3d	OF	30 mins	49 x 36 cm	T:10; C:10		-	-		-		
Corrigan	2017	WD	Rep-Mild	Diffuse 3x 5d	OF	5 mins	1 x 1 m	T/C: 8-10					+		
McAteer	2016	WD	Rep-Mild	Diffuse 3x 5d	OF	5 mins	1 x 1 m	T:7; C: 9				+	+		
Morriss	2021	WD	Rep-Mild	Diffuse 5x 24 hrs	OF	Unknown	Unknown	T:11; C;10				-	+	+	
Arulsamy	2019	WD	Rep-Mild	Diffuse 3x 5d	OF	5 mins	1 x 1 m	T:14; C:14							-
Arun	2020	Blast	Rep-Mild	Blast 2x 2mins	OF	60 mins	40 x 40 cm	T/C: 10-31		++	-	-	+	++	++
Tucker	2019	CCI-CS	Rep-Mild	Diffuse 3x 24 hrs	OF	20 mins	40 x 40 cm	T/C:17-21				-	+++	+++	+++
Bajwa	2016	CCI	Moderate	Focal	OF	30 mins	49 x 36 cm	T:10; C:10		-	+++		-		
Leconte	2020	CCI	Moderate	Focal	OF	9 mins	1 m x 1 m	T:15; C:13					+		
Rowe	2016	LFP	Moderate	Mixed	OF	5 mins	70 x 70 cm	T/C :11-12: 2M				-	-	-	
								T/C :11-12: 4M				-	-		
								T/C :11-12: 6M				-			
Arulsamy	2018	WD	Mod-Sev	Diffuse	OF	5 mins	1 x1 m	T:14^; C:13^				-	+		
Arulsamy	2019	WD	Mod-Sev	Diffuse	OF	5 mins	1 x1 m	T:12; C: 14							-
Islam	2021	CCI	Severe	Focal	OF	5 mins	54.5 x 54.5 cm	T,C: 9-13					+		
Komoltsev	2021	LFP	Severe	Mixed	OF	5 mins	1 x 1 m	T:13; C:7						-	

+ = $p<0.05$; ++ = $p<0.01$; +++ = $p<0.001$; - = not significant ; Grey = Not evaluated. T= TBI; C= Control; CCI=Control cortical impact; OF=Open field; WD= weight drop injury; LFP= Lateral fluid percussion injury

Table 6: Preclinical balance and coordination evaluation

Authors	Years	Method	Severity	Injury	Test	Parameters	Sample	Pre	<72h	1-3wk	1-2mo	3-5mo	6-11 mo	7-11mo	12-24mo
Evans	2015	CCI-CS	Mild	Diffuse	Rotarod (4-40prm)	Latency to fall	T:12; C: 11		+	+	-				
Namdar	2020	WD	Mild	Diffuse	Rotarod (4-40rpm)	Latency to fall	T:15; C:13			-	-				
					Eramus Ladder	Correct steps				-	+				
						Missteps				-	+				
						Time				-	-				
Lai	2019	WD	Mild	Diffuse	0.5 cm Beam	Traverse time	T:7; C:7	-		+++	+++				
Laurer	2001	CCI-CS	Mild	Diffuse	3 cm Rotating pole 1,3,5 rpm	Foot-faults	T:16-24;C:14-23		-	-	-				
Bajwa	2016	CCI-CS	Mild	Diffuse	0.65 cm Beam 2.5 cm Grid Walk Rotarod (	Traverse Time Foot faults Latency to fall	T:10; C:10		-	-		-			
Evans	2014	CCI-CS	Mild	Diffuse	Rotarod (4-40prm)	Latency to fall	T:9; C: 8			+	+	-			
Fehily	2019	WD	Mild	Diffuse	Ladder walk	% Stepping errors	T:15; C:15					-			
McAteer	2016	WD	Mild	Diffuse	Rotarod (3-30rpm)	Latency to fall	T:9 ;C:9		-	-	-	-			
Hou	2017	WD	Mild	Diffuse	Rotarod (3-30rpm)	Average 3 trials	T:8; C: 8	-		-	+	++			
Mouzon	2014	CCI-CS	Mild	Diffuse	Rotarod (5-50rpm)	Average 3 trials	T:12; C:12						-		
Mouzon	2018	CCI-CS	Mild	Diffuse	Rotarod (5-50rpm	Average 3 trials	T:7; C:8								-
Xu	2019	CCI	Mild	Diffuse	2 cm Beam	Traverse Time	T:10; C10		-	-	-				
Mountney	2017	PCS	Mild	Diffuse	Rotarod (0.1rpm/sec) Three sets x 5 with 2 min intertrial interval	Latency to fall	T/C: 8-10					-			
Mountney	2017	PCS	Rep-Mild	Diffuse 4xTBI 1hr apart	Rotarod (0.1rpm/sec) Three sets x 5 with 2 min intertrial interval	Latency to fall	T/C: 8-10					+			
Albayram	2017	WD			0.8cm beam	Score	T/C=9-10						++		

			Rep-Mild	Diffuse 7 in 9D	String Suspension (3 trials)	Score								++				
					Rotarod (4-40opm) 5 mins; 4x day for 2 days	Latency to fall Average 8 trials								+				
Feng	2021	Chimera	Rep-Mild	Diffuse 3xTBI/day x 2 48 hrs apart	Rotarod (5-40 rpm)	Latency to fall- average 3 trials	T:9; C:9					-						
Tabet	2022	CCI-CS	Rep-Mild	Diffuse 3x TBI 24hr	Ladder rung	% Foot faults to baseline	T:10; C:10					+						
					Pole climbing	Time (3 trials)	T:10; C:10					-						
Laurer	2001	CCI-CS	Rep-Mild	Diffuse 2xTBI 24 hrs	3 cm Rotating pole	(1, 3, 5 rpm)	T:49; C:36		++		-	++						
Bajwa	2016	CCI-CS	Rep-Mild	Diffuse 2x 3d	0.65 cm Beam 2.5 cm Grid Walk Rotarod (2x 5rpm, 2x 3rpm/5s, 2x 3rpm/3s	All measures Foot faults Average of trials	T:10; C:10		-		-			-				
Mannix	2014	WD	Rep-Mild	7inj/9D	Rotarod (0.1rpm/sec)	Average 4 trials	T:32;C:21		+					+				
Mannix	2017	WD	Rep-Mild	7inj/9D	Rotarod (0.1rpm/sec)	Average 4 trials	T:12;C:11		+					-				
McAteer	2016	WD	Rep-Mild	Diffuse 3 x 5d	Rotarod (3-30rpm)	Latency to fall	T:7 ;C:9		-		-	-		-				
Fehily	2019	WD	Rep-Mild	Diffuse 2xTBI 24hr	Ladder walk	% Stepping errors	T:15; C:15							-				
				Diffuse 3xTBI 24hr										-				
Mouzon	2014	CCI-CS	Rep-Mild	Diffuse 5xTBI 24hr	Rotarod (5-50rpm)	Latency to fall- average 3 trials	T:12; C:12							-				
Morriss	2021	WD	Rep-Mild	Diffuse 5xTBI 24hr	Rotarod	Latency to fall 4 trials	T:11; C:10									++		
Dhillon	2020	CCI-CS	Rep-Mild	Bilateral 5x TBI/5 weeks	Rotarod (3-30rpm)	Fall- 3 trials Hindlimb rating	T:10; C:8	-	-	+	+	+	+	+	+			
					2.5 cm beam									+				
Tucker	2019	CCI-CS	Rep-Mild	Diffuse 3xTBI 24hr	Rotarod (4-60rpm)	Latency to fall- average 3 trials	T:19-21; C17-19		+++		-	+	+		-			
Mouzon	2018	CCI-CS	Rep-Mild	Diffuse 5xTBI 24hr	Rotarod (5-50rpm)	Latency to fall- average 3 trials	T:7; C:7								-			

Hou	2017	WD	Moderate	Diffuse	Rotarod (3-30rpm)	Average 3 trials	T:8; C: 8	-		-	+	+++				
Toshkezi	2018	CCI	Moderate	Focal	Rotarod (2-20rpm)	Latency to fall	T:9; C: 5				+++					
Barrett	2020	CCI	Moderate	Focal	0.5cm Beam	Foot Faults	T/C: 8-13	-	+++	+++	+++					
Henry	2020	CCI	Moderate	Focal	0.5cm Beam	Foot Faults	T:11; C:12	-	+++	+++	+++					
					Rotarod (1-30rpm)	%Baseline		-	+++	-	+++					
Xie	2019	CCI	Moderate	Focal	0.6 cm Beam	Foot Faults	T:10; C:10	-	+++	+++	+++					
Xu	2019	CCI	Moderate	Focal	2 cm Beam	Traverse Time	T/C: 38		-	+	+					
Chen	2016	NY	Moderate	Focal	Ladder test	Errors	T:20; C:10	-	++	-	-					
Bajwa	2016	CCI	Moderate	Focal	0.65cm Beam	Time Active	T:10; C:10		+	-		-				
						Falls			++	-		-				
					2.5 cm Grid walk	Foot faults			+++	++		++				
					Rotarod (2x 5RPM, 2x 3rpm/5s, 2x 3rpm/3s	Average trials			+++	-		++				
Carron	2019	LFP	Moderate	Mixed	Rotarod (1.5rpm/3s)	% Baseline	T:10; C:10	-	+++	-	-					
					Tapered Beam	Ranking		-	+++	-	-					
					Tapered Beam	Foot faults		-	+++	-	-					
Tan	2020	LFP	Moderate	Mixed	Rotarod (4-40rpm)	Average 3 trials	T:18; C: 10			-		+				
Wright	2017	LFP	Moderate	Mixed	2cm Beam	Foot faults	T:10; C:10			+		+				
					2 cm Beam	Traverse time	T:10; C:10			+		+				
Rowe	2016	LFP	Moderate	Mixed	3 cm Beam	Foot faults	T/C :11-12 2M				+	-	-			
							T/C :11-12 4M				-	-				
							T/C :11-12 6M				-					
					3 cm Beam	Traverse Time	T/C :11-12 2M				-	-	-			
							T/C :11-12 4M				-	-				
							T/C :11-12 6M				-					
Sell	2017	LFP	Moderate	Mixed	2.5 cm Beam	Traverse Time	T:37; C:39	-	+			-	-			-
					1.75 cm Beam	Ranking		-	+			+[!]	-			-
Alwis	2012	WD	Mod-Sev	Diffuse	Rotarod (3-30rpm)	% Baseline	T:19; C: 12	-	+	+	+					
					2 cm Beam	Ranking	T:19; C: 12	-	+	+	+					
Arulsamy	2018	WD	Mod-Sev	Diffuse	Rotarod (3-30rpm)	Latency to fall	T:6; C; 6			+++	-	-				
Albayram	2017	WD	Mod-Sev		0.8cm beam	Score	T/C-9-10			+			+			

				Diffuse	String Suspension (3 trials)	Score				+			+	
Soblosky	1997	CCI	Mod-Sev	Focal	2.5 cm Beam	Ranking	T:10; C: 10	-	+	+	+	-		
					Pegged 2.5 cm Beam	Foot faults	T:10-13; C:10-14	-		+	+	-		
Wang	2019	Punch	Mod-Sev	Focal	2 cm Beam	Ranking	T:8; C:8		++	++	++			
Hanscom	2021	CCI	Mod-Sev	Focal	5 mm beam	Foot faults	T/C: 14-21	-	+++	+++	+++			
Vogel	2020	CCI	Mod-Sev	Focal	Rotarod 36rpm	Latency to fall	T:16; C:16			-			-	
					Rotarod:accelerating		T:20 C:10			-		-		
Cline	2017	CCI	Severe	Focal	2.5 cm Gridwalk	Foot faults	T:15; C:14	-	+		+			
Xu	2019	CCI	Severe	Focal	2 cm Beam	Traverse Time	T/C: 38		-	+	+			
He	2020	CCI	Severe	Focal	Rotarod (4-40rpm)	Average 3 trials	T:11; C: 7				+	+		
Nissinen	2017	LFP	Severe	Mixed	2 cm Beam	Ranking	T:23;C:10	-	+++	+++	++	-		
Zhang	2005	LFP	Severe	Mixed	Rotating pole (5 rpm)	Ranking	T:14; C: 24				++	++		
					2 cm Beam	Ranking					++	++		

+= p<0.05 ++ = p<0.01; +++ = p<0.001; - = not significant; Grey = Not evaluated. T= TBI; C= Control. CCI= controlled cortical impact; CCI-CS=Control cortical impact, closed skull; WD= weight drop injury, LFP= lateral fluid percussion.

Table 7: Clinical Neuroscore evaluation

Authors	Year	Severity	Population	Sample size	Age (mean)	Sex %male	Motor test	< 5 yrs	6-10 yrs	11-25 yrs	>25yrs
Gardner	2017	Rep-Mild	Military veterans	T:31-34 C:65-68	T: 79.4 C: 76.4	T:82.4% C:94.9%	mUPDRS Global Score				-
							mUPDRS Tremor Score				-
							mUPDRS Rigidity Score				-
							mUPDRS Bradykinesia Score				-
							mUPDRS Posture/Gait Score				-
		Moderate-Severe		T:20 C:65-68			**mUPDRS Global Score**				+
							mUPDRS Tremor Score				-
							mUPDRS Rigidity Score				-
							mUPDRS Bradykinesia Score				-
							mUPDRS Posture/Gait Score				+

+ = p<0.05. - = Not significant

Table 8: Clinical Fine Motor evaluation

Authors	Year	Severity	Population	Sample size	Age	Sex %male	Motor test	Parameters	< 5 yrs	6-10 yrs	11-25 yrs	>25 yrs
Burton	2002	Mild	Community	T:19; C:26	T:35.36 C: 32.77	T:78.9 C:46.15	Fine motor	Touch fingers to thumb		-		
							Grip strength	Dynamometer		-		
Walker	2018	Combination	Military veterans	T:380; C 73	T:36 C:40.5	T:88% C:79.5%	Fine motor	Grooved Pegboard		-		
DeBeaumont	2011	Combination	College Athletes	T:21; C:15	T/C: 22.3	T/C: 100%	Rapid alternating movement Wrist supination-pronation	Velocity	+ (↑)			
								Sharpness	-			
								Bimanual co-ordination	-			
DeBeaumont	2009	Combination	College Athletes	T:19; C:21	T:61; C:59	Not stated	Rapid alternating movement Wrist supination-pronation	Duration				-
								Range				-
								Sharpness				-
								Velocity				+
Pearce	2014	Rep-Mild	Professional Athletes	T:40; C:20	T:49.3 C:47.6	T/C: 100%	Fine motor	O'Connor Finger Dexterity Test			+	
Pearce	2018	Rep-Mild	Professional Athletes	T:25; C:25	T:48.4 C:48.8	T/C: 100%	Fine motor	O'Connor Finger Dexterity Test			+	
Burton	2002	Mod-Sev	Community	T:9; C:26	T:37.56 C: 32.77	T:66.67% C:46.15	Fine motor	Touch fingers to thumb			+	
							Grip strength	Dynamometer			--	

+ =p<0.05 - = Not Significant; Grey = not evaluated

Table 9: Clinical Gait studies

Authors	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	< 5 yrs	6-10 yrs	11-25 yrs	>25 yrs
Burton	2002	Mild	Community	T:19; C:26	T:35.36 C:32.77	T:78.9 C:46.15	Turn 360°	None	Speed		-		
							Timed 4 m walk						
Vandeploeg	2007	Mild	Military veterans	T:254; C:3214 MVA:539	T:37.8 C:38.5 MVA:37.8	100%	Heel toe walk	None	Abnormal/normal			3x	
Stuart	2020	Mild					13 m walkway, 2 mins		**Stride length**	**			
									Speed	***			
									Foot stride angle	**			
									Toe off angle	-			

			Community Self-reported balance instability > 3 months post-injury	T:52; C:59	T:39.6 C:37.0	T:30.8% C:42.4%		Five sensors strapped to feet, L5, sternum and head	**Single support time**	**			
									Double support time	**			
									Stride time	**			
									Turn duration	**			
									Turn step number	*			
									Turn velocity	***			
Martini	2021	Combination	Community Symptoms persisting > 3 months following TBI	T:65; C:57	T:39.6 C:36.9	T:64% C:55%	208 m walk	Five sensors strapped to feet, L5, sternum and head	**Speed**	+			-
									Variability	-			
									Rhythm	-			
									Turning	+			
							Dual task Above + auditory Stroop		**Speed**	+			
									Variability	-			
									Rhythm	+			
									Turning	+			
Walker	2018	Combination	Military veterans	T:258;C:47	T:36 C:40.5	T:88% C:79.5%	4 m walk	None	Speed			-	
Martini	2011	Combination	College students	T:25; C:25	T:21 C:20.7	T:60.7% C:50%	4 m walk	GAITr walkawy	**Speed**		+		
									Double Stance support		+		
							4 m walk + obstacle		Speed		-		
									Double Stance support		_		
							Brooks Spatial Memory task + 4 m walk		Speed		-		
									Double Stance support		-		
							Brooks Spatial Memory task + 4 m walk + obstacle		Speed		-		
									Double Stance support		+		
Pitt	2020	Combination	Military veterans with chronic symptoms	T:8; C:8	T:32.5 C:33.3	T:87.5% C:87.5%	10 m walk, 8-10 trials	Whole body retroeflective marker set with 12 motion analysis system	Speed	-			
									Step length	-			
									Step width	-			
									Medial-lateral COM displacement	+			
									Peak medial-lateral COM velocity	-			
							Above + auditory Stroop task		Speed	-			
									Step length	-			
									Step width	-			

									Medial-lateral COM displacement	+			
									Peak medial-lateral COM velocity	-			
Burton	2002	Mod-Sev	Community	T:9; C:26	T:37.56 C:32.77	T:66.67% C:46.15	Turn 360°	None	Speed		-		
							Timed 4 m walk						
Useros Olmo	2020	Mod-Sev	Hospital	T:20; C:19	T:36.1 C:38.2	T:85% C:89.5%	Treadmill 3 kms/hr,	Gait Trainer2	Cadence	-			
							Treadmill 3 kms/hr + cognitive task			-			
Vasudevan	2014	Mod-Sev	Community	T14; C:11	T:29.7 C:31.1	T:71.4% C:81.8%	Split treadmill: same speed	Markers on toe, ankle, knee, hip, pelvis, shoulder	Stride length	-			
									Stance time	-			
									Step symmetry	-			
									Center of oscillation	-			
									Temporal coordination	-			
							Split treadmill: different speed		Stride length	-			
									Stance time	-			
									Step symmetry	+			
									Center of oscillation	-			
									Temporal coordination	-			
							Split treadmill: same speed: Post-adaption		Stride length	-			
									Stance time	-			
									Symmetry	-			
									Center of oscillation	-			
									Temporal coordination	-			
Buster	2013	Severe	Community				Elliptical trainer, comfortable stride length, 3 mins	. Reflective markers on the pelvis, hip,	Speed		-		
									Cadence		-		
									Stride length		-		
									Motion profile		-		
									Joint angles		+		

				T:10; C:10	T:36: C:34	Not stated	Treadmill, comfortable speed 3 mins	knee, ankle and foot	Speed		-			
									Cadence		-			
									Stride length		-			
									Motion profile		-			
									Joint angles		-			
Williams	2009	Severe	Hospital Able to walk independently 20m	T:41; C:25	T:29.1 C:27.8	T:75.6% C:64%	12 m walk: Self-selected speed	25 reflective markers on pelvis and lower limbs	**Double support**		+			
									Speed		+			
									Cadence		+			
									Stride length		+			
									Stance duration		+			
									Base of support		+			
				T:41; C:15			12 m walk: Matched speed		**Trunk angle**		+			
									Pelvic angle		-			
									Hip angle		-			
									Knee angle		+			
									Ankle angle		-			
									Lateral COM displacement		+			
Williams	2010	Severe	Hospital Able to walk independently 20m	T:55; C:10	T:28.5 C:27.3	T:72.7% C:50%	12 m walk: Matched speed	25 reflective markers on pelvis and lower limbs	Speed		-			
									Cadence		-			
									Stride length		+			
									Stance time		-			
									Double support time		-			
									Lateral COM displacement		+			
									Peak ankle power		-			
									Peak hip power (initial)		+			
									Peak hip power (prewswing)		+			
				T:36;C:10	T:27.3 C:27.3	T:77.8% C:50%			Speed		-			
									Cadence		-			
									Stride length		-			
									Stance time		-			
									Double support time		-			

							12 m walk: Fastest speed		**Lateral COM displacement**		+			
									Peak ankle power		+			
									Peak hip power (initial)		+			
									Peak hip power (prewswing)		-			
Williams	2013	Severe	Hospital Able to run independently 20m	T:44; C:15	T:27.9 C:28.1	T:81.8% C:73.3%	15 m run: Matched speed	25 reflective markers on pelvis and lower limbs	Speed	-				
									Cadence	++				
									Stride length	+				
									Stance time	+				
									Flight phase	+				
									Base of support	-				
									Trunk flexion	-				
									Pelvic rotation	+				
									Hip extension/adduction	-				
									Knee flexion	+				
									Ankle flexion	-				
									Lateral COM displacement	+				
									Ankle power	+				
									Knee power	+				
									Hip power	+				
							15 m run: Fastest speed		**Speed**	+				
									Cadence	+				
									Stride length	+				
									Stance time	-				
									Flight phase	+				
									Base of support	+				
Williams	2016	Severe	Hospital Able to walk independently 20m	T:35; C:25	T:28.4 C:27.8	T:74.3% C:64%	12 m walkway: matched speed	25 reflective markers on pelvis and lower limbs	Hip work	-				
									Knee work	-				
									Ankle work	+				
									Hip power	-				
									Knee power	-				
									Ankle power	+				

*p<0.05 = significant, ***= strong power, **=medium power; *low power, - = non-significant, Grey=not assessed.

Table 10: Clinical postural stability studies

Authors	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test	Method	Parameters	< 5 yrs	6-10 yrs	11-25 yrs	>25 yrs
Geurts	1999	Mild	Hospital Persistent symptoms	T:15;C:20	T:35.9 C:35.4	T:53.3% C:60%	Postural control: Eyes open, closed or with simple cognitive task	Balance board 2x 30 secs per variable	**Anteroposterior sway**	+			
									Lateral sway	+			
									Weight shifting	+			
Wright	2018	Mild	Military veterans	T:9;C:19	T:25.95 C:33.57	T/C: 45%	Postural control Firm or foam, eyes open or closed, static or rolling scene	Virtual environment TBI screening 3 x 30secs per variable	Sway area	-			
Pan	2015	Mild	Military veterans Non-symptomatic	T:6, C:10	T:26.5 C: Not stated	T:100% C:Not stated	Postural control: On floor or foam; Eyes open or closed	Markers on sacrum, pelvis, C7 and shoulder.	Pelvic postural sway	-			
									Pelvic sway path length	-			
									Pitch trunk angle	-			
									Roll Trunk angle	-			
							Postural control+ perturbation: On floor or foam; Eyes open or closed		Upper trunk sway path length	-			
									Pelvic sway path length	-			
									Oscillations	-			
			Military veterans Symptomatic	T:8; C:10		T:87.5% C:Not stated	Postural control: On floor or foam; Eyes open or closed		**Pelvic postural sway**	+			
									Pelvic sway path length	+			
									Pitch trunk angle	+			
									Roll trunk angle	+			
							Postural control+ perturbation: On floor or foam; Eyes open or closed		**Upper trunk sway path length**	+			
									Pelvic sway path length	-			
									Oscillations	+			
Rosenblum	2020	Mild	College Athletes	T:91;C:129	T:18.9	T:56.6%	Sensory organization test	Smart Balance Master System	Anterior-posterior sway (equilibrium score)	-			
									Somatosensory sensory ratio	-			

					C:19.1	C:56.6%	Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding,	3 x 20secs per variable	Visual sensory ratio	-			
									Vestibular sensory ratio	-			
Ustinova	2017	Mild	College Athletes	T:13; C:13	T:34.9 C:33.8	T:30.8% C:38.4%	Functional reach Interception of targets via arm at 5 fixed location	3D video game 30 reflective body markers 10 x 90sec games	Postural angular displacement		-		
									Postural angular velocity		+		
									Arm angular displacement		-		
									Arm angular velocity		+		
Wright	**2018**	Combination	Military veterans	T:14; C:19	T:25.95 C:33.57	T/C: 45%	Postural control Firm or foam, eyes open or closed, static or rolling scene	Virtual environment TBI screening 3 x 30secs per variable	Sway area		+		
Helmich	2016	Combination	University students w/o symptoms	T:13; C:10	T:29 C:27	T:Not stated C:40%	Postural control Stable versus unstable surface, eyes open, closed or blurred	Force platform 10 x 10sec trials per variable	COP path		-		
									COP area		-		
									Effort of balance		-		
							Balance Error Scoring System		Score		-		
			University students with symptoms	T:7; C:10	T:26 C:26	T:Not stated C:40%	Postural control Stable versus unstable surface, eyes open, closed or blurred		COP path		-		
									COP area		-		
									Effort of balance		+		
							Balance Error Scoring System		**Score**		+		
Degani	2017	Combination	Community	T:11; C:11	T: 29.4 C: 26.8	T:45.5% C:36%	Postural control Natural stance vs crossed arms	Force platform 10 mins	**Body sway area**		+		
									Amplitude of COP displacement		+		
									Mean velocity of COP displacement		+		
									Frequency of COP displacement		+		
									Regularity of COP displacement		+		
De Beaumont	2011	Combination	Collegiate athletes	T:21; C:15	T/C: 22.3	T/C: 100%	Postural control	Force platform 2 x 30 sec trials	**Regularity of COP displacement**		+		
									Amplitude of COP displacement		-		
Johnston	2020	Combination					Y Balance test		Anterior	Reach distance	-		

			Collegiate athletes	T:30; C:90	T:20.3 C:20.2	T/C: 83.5%	(Stand on one leg, reach other in anterior, posteromedial and posterolateral direction)		Lumbar inertial sensor 3 trials		Regularity	-			
											Amplitude	-			
										Posteromedial	Reach distance	-			
											Regularity	-			
											Amplitude	-			
										Posterolateral	Reach distance	-			
											Regularity	-			
											Amplitude	-			
Ledwidge	2020	Combination	Collegiate athletes	T:21; C:24	T:20.17 C:20.03	T:90% C:79%	BESS balance test Feet together, non-dominant only, tandem on firm or foam surface		-	Score			-		
Lee	2020	Combination	College students	T:11; C:14	T:28.7 C:22	T:52% C:35.7	Postural stability		Force platform 120 sec	Body sway area			-		
										Amplitude			-		
										Mean velocity			-		
										Frequency			-		
										Regularity			-		
										Asynchrony AP and ML			-		
Reilly	2020	Combination	Community	T:27; C:27	T:26.1	T:44.4%	Postural stability	Bipedal only	Force platform	Mean velocity			-		
										Path length			-		
										AP sway			-		
										ML sway			-		
										Body sway area			-		
										Regularity (AP)			-		
										Regularity (ML)			-		
								Bipedal + cog task		Mean velocity			-		
										Path length			-		
										AP sway			+		
										ML sway			-		
										Body sway area			+		
										Regularity (AP)			+		
										Regularity (ML)			-		
								Unipedal only		Mean velocity			-		

					C:28.6	C:77.8%				Path length		-		
										AP sway		-		
										ML sway		-		
										Body sway area		-		
										Regularity (AP)		+		
										Regularity (ML)		+		
								Unipedal + cog task		Mean velocity		-		
										Path length		-		
										AP sway		-		
										ML sway		+		
										Body sway area		+		
										Regularity (AP)		+		
										Regularity (ML)		+		
Rosenblum	2020	Combination	College Athletes	T177;C:129	T:19.1 C:19.1	T:57.6% C:56.6%	Sensory organization test Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding,		Smart Balance Master System 3 x 20secs per variable	Anterior-posterior sway (equilibrium score)	-			
										Somatosensory sensory ratio	-			
										Visual sensory ratio	-			
										Vestibular sensory ratio	-			
Sosnoff	2011	Combination	College athletes	T=62; C=162	T/C: 20.04	T/C: 67.8%	Sensory organization test Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding		NeuroCom Smart Balance Master 3 x each test	Composite balance score	-			
										Somatosensory sensory ratio	-			
										Visual sensory ratio	-			
										Vestibular sensory ratio	-			
										Regularity (AP)	+			
										Regularity (ML)	+			
Walker	2018	Combination	Military veterans	T=248; C=47	T:36 C:40.5	T:88% C:79.5%	Sensory organization test	Eyes open/fixed surface		Equilibrium score		-		
								Eyes closed/fixed surface		**Equilibrium score**		+		
								Eyes open/fixed surface/sway surroundings		**Equilibrium score**		+		
								Eyes open, sway surface,		Equilibrium score		-		

								fixed surroundings	NeuroCom Smart Balance Master 3 x each test					
								Eyes closed, sway surrounding		Equilibrium score		-		
								Eyes open, sway surface, sway surrounding		Equilibrium score		-		
								Composite score		**Equilibrium score**		+		
Walker	2018	Combination	Military veterans	T=414; C=78	T:36 C:40.5	T:88.2% C:79.5%	Sensory organization test	Eyes open/fixed surface		Equilibrium score		-		
								Eyes closed/fixed surface		**Equilibrium score**		+		
								Eyes open/fixed surface/sway surroundings		**Equilibrium score**		+		
								Eyes open, sway surface, fixed surroundings		Equilibrium score				
								Eyes closed, sway surrounding		Equilibrium score				
								Eyes open, sway surface, sway surrounding		Equilibrium score				
Wright	2018	rmTBI	Military veterans	T:5;C:19	T:25.95 C:33.57	T/C: 45%	Postural control Firm or foam, eyes open or closed, static or rolling scene		Virtual environment TBI screening 3 x 30secs per variable	Sway area	+			
Rosenblum	2020	rmTBI					Sensory organization test		Smart Balance Master System	Anterior-posterior sway (equilibrium score)	-			

			College Athletes	2*TBI:52 3*TBI:34; C: 129	2*T:19.1 3*T:19.8 C:18.1	2*T:62% 3*T:52% C:56.6%	Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding,		3 x 20secs per variable	Somatosensory sensory ratio	-			
										Visual sensory ratio	-			
										Vestibular sensory ratio	-			
Walker	2018	rmTBI	Military veterans	T=248; C=47	T:41 C:46	T:88.3% C:78.7%	Sensory organization test	Eyes open/fixed surface	NeuroCom Smart Balance Master 3 x each test	Equilibrium score		-		
								Eyes closed/fixed surface		Equilibrium score		+		
								Eyes open/fixed surface/sway surroundings		Equilibrium score		+		
								Eyes open, sway surface, fixed surroundings		Equilibrium score		+		
								Eyes closed, sway surface		Equilibrium score		+		
								Eyes open, sway surface, sway surrounding		Equilibrium score		-		
								Composite score		Composite Equilibrium Score		+		
Useros Olmo	2020	Mod-sev	Hospital	T:20; C:19	T:36.1 C:38.2	T:85% C:89.5%	Postural control	Standing only	Force platform 2 mins recording	Displacement of COP	+			
								Standing + numerical cog task						
								Standing + spatial memory cog task						
Zhang	2002	Mod-sev	Rehabilitation	T:10; C:10	T:30.6 C:31.8	T:50% C:50%	Functional reach	Unpredictable	Force platform Board with two lights	Stability ratio	**			
								L predictable			**			
								R predictable			***			
Arce	2004	Severe	Community	T:7; C:10	T:26.4 C: 24.9	T:100% C:100%	Postural control:	No load x 3	Tetrax posturography	Stability score: standing	+			
								4kg x 6		Forward weight shift	-			

							Bimanual lifting	8kg x 6	system- two force platforms	% change vertical ground reaction force	-			
Buster	2013	Severe	Community	T;10: C:10	T:36; C:24	Not stated	Berg Balance test		None	Score		+		
							Dynamic posturography		Tilt platform 3 x 120 secs	Dynamic movement analysis score		+		

+=p<0.05, - = not significant, ***= strong power, **=medium power, Grey = not asses

An Alternate Approach to Investigate Chronic Consequences of Different Severity of Traumatic Brain Injury

In the systematic review presented in the previous chapter, it became evident that the long-term motor consequences of TBI remain unclear, revealing significant gaps in both the preclinical and clinical literature (Corrigan et al., 2023). While addressing this knowledge gap requires a consistent and thorough exploration of long-term motor function in future studies in order to better identify potential risks of neurodegenerative movement disorders, TBI can, in fact, induce a range of underlying neurobiological alterations that overlap with the pathology of PD (Delic et al., 2020b; Padmakumar et al., 2022), including, but not limited to, disruptions in dopamine and noradrenaline pathways (Jenkins et al., 2018, 2016), heightened neuroinflammatory responses (Acosta et al., 2015; Johnson et al., 2013), abnormal protein accumulation/ misfolding (Hutson et al., 2011; Uryu et al., 2007, 2003) and oxidative stress (Mackay et al., 2006; Webster et al., 2015) (refer to chapter 1). Investigation of chronic alterations in these shared mechanisms following injury could provide an alternative approach to identify brain mechanisms that may increase risk of developing PD.

Consequently, the next chapter will focus on understanding dopaminergic and noradrenergic changes, alongside chronic neuroinflammatory processes, at the 12-month timepoint, following three different severities of TBI: mild, repetitive mild and moderate-severe. These aspects were focused on due to their well-established associations both following TBI, as well as in the development of PD (Delaville et al., 2011; Impellizzeri et al., 2016; Jenkins et al., 2018; Tajiri et al., 2014). Specifically, the progressive degeneration of dopaminergic neurons in the substantia nigra, the hallmark pathological feature of PD, is notably impacted in individuals with a 4 to 12 months history of moderate to severe TBI according to several neuroimaging studies (Donnemiller et al., 2000; Wagner et al., 2014), with a 30% decrease noted up to 26 weeks after experimental moderate-severe TBI (Hutson et al.,

2011). Furthermore, changes in noradrenergic transmission, potentially accounting for both non-motor symptoms in PD and contributing to the degeneration of dopaminergic neurons in the disease (Delaville et al., 2011), are also reported following TBI (Jenkins et al., 2016), with a significant reduction in noradrenaline turnover rate persisting for up to 8 weeks after a moderate diffuse brain injury (Fujinaka et al., 2003). To date, however, a comprehensive analysis of long-lasting changes in these pathways has not been conducted, and questions remain about whether any effects are severity-dependent. Additionally, catecholamines have been shown to have a role as non-traditional cytokines, acting as important neuromodulators (for review, see Barnes et al. 2015)(Barnes et al., 2015). In turn, neuroinflammation can contribute to the degeneration of both dopaminergic (Koprich et al., 2008) and noradrenergic neurons (Evans et al., 2022). Despite neuroinflammation being a persistent part of the secondary injury following TBI (Xiong et al., 2018), however, how it may interact long-term with alterations in catecholamine signalling following injury remains largely unexplored. Thus, in the following chapter, we sought to address these noted gaps in the literature.

03

Characterisation of Persistent Changes in Neuroinflammation and Catecholaminergic Signalling at 12 Months Following Different Severities of Diffuse Traumatic Brain Injury

Chapter prepared in publication by **WEE, IC.**, ARULSAMY, A., CORRIGAN, F., COLLINS-PRAINO, L.E

Statement of Authorship

Title of Paper	Characterisation of persistent changes in neuroinflammation and catecholaminergic signalling at 12-month following different severities of moderate-severe diffuse traumatic brain injury.
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Principal Author

Name of Principal Author (Candidate)	Ing Chee Wee		
Contribution to the Paper	Performed analysis of molecular data, intepreted data and wrote manuscript		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	18/12/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Alina Arulsamy		
Contribution to the Paper	Generation of animal model		
Signature		Date	18/12/2023

Name of Co-Author	Frances Corrigan		
Contribution to the Paper	Conceptualised study, supervised completion of the work, assisted with statistic analysis and data interpretation, edited the manuscript.		
Signature		Date	18/12/2023

Please cut and paste additional co-author panels here as required.

Name of Co-Author	Lyndsey Collins-Praino		
Contribution to the Paper	Conceptualised study, supervised completion of the work, assisted with statistic analysis and data interpretation, edited the manuscript.		
Signature		Date	18/12/2023

Abstract

Traumatic brain injury (TBI) has been linked to an increased risk of developing Parkinson's disease (PD). While the exact mechanisms of this have not yet been elucidated, TBI triggers neuroinflammation and catecholamine dysfunction acutely after injury, with both processes implicated in the pathophysiology of PD. The long-term impact on these pathways following TBI, however, remains unclear. Therefore, this study aimed to investigate alterations in both catecholamines and neuroinflammation in key brain regions known to be implicated in PD up to 12-months following experimental TBI of different severities. Fifty-four male Sprague-Dawley rats (420-480g; 10-12 weeks) were either given a sham surgery (n =14) or subjected to Marmarou's impact acceleration model of diffuse TBI of one of three severities: a single mild TBI (mTBI; n = 12); repetitive mild TBI (rmTBI, n =14) or moderate-severe TBI (msTBI, n = 14). At 12-months post-injury, both astrocyte reactivity (GFAP) and microglial levels (IBA1) were assessed in the striatum (STR), substantia nigra (SN), and prefrontal cortex (PFC) using immunohistochemistry. Western blot was conducted to measure protein levels of key enzymes (TH, COMT, DβH) and receptors (DRD1, ADRA1a, ADRA2a, ADRB1) involved in catecholaminergic transmission within these same regions. Overall, minimal changes in both markers of catecholaminergic transmission and neuroinflammation were found in any brain region at 12 months post-injury, regardless of initial injury severity. Nevertheless, following mTBI, there were significantly elevated levels of dopamine D1 receptors (DRD1) in the PFC. Conversely, following msTBI, an increase in alpha-2A adrenoceptors (ADRA2A) was noted within the STR, with a concomitant decrease in dopamine beta-hydroxylase (DβH) in the SN. The only significant change in neuroinflammation noted in comparison to sham animals was a reduced number of GFAP+ve cells in the SN, although a non-significant elevation in total or percentage activated microglia was noted in the STR and PFC, respectively.

Taken together, the current work suggests that, while alterations in catecholaminergic and neuroinflammatory pathways may differ depending on initial injury and within brain regions, changes observed at this timepoint post-injury are subtle. Given that neurodegenerative outcomes can occur decades following injury, it may be that longer timepoints post-injury are necessary to observe changes that may be relevant for PD development.

1.0 INTRODUCTION

Traumatic brain injury (TBI) stands as a significant global cause of both mortality and disability(Dewan et al., 2019). In 2013, the United States alone recorded approximately 2.8 million such incidents, with about 1.7 million individuals experiencing a TBI annually (Faul et al., 2010; Taylor et al., 2017). While the conventional belief once held that the effects of TBI could resolve over time, emerging insights now underscore that TBI is, in fact, an ongoing neurological condition that may increase the risk of developing various neurodegenerative disorders, including Parkinson's disease (PD), in a dose-dependent manner. Following a moderate-severe TBI, the likelihood of developing PD is higher compared to mild TBI (hazard ratio 1.50 vs 1.24), and the risk of PD development increases further with the cumulative effect of multiple TBIs in comparison to a single TBI (hazard ratio 1.87 vs 1.45) (Gardner et al., 2015).

Despite this, the precise mechanisms underlying the relationship between TBI and the risk of PD remain a subject of ongoing exploration. Numerous reports have suggested that disruption in catecholamines may play a role in the progression of PD pathogenesis following TBI. The loss of dopaminergic (DA) neurons within the substantia nigra (SN) is the pathological hallmark of PD (for review, see (Zhou et al., 2023)) (Braak et al., 2003; Dauer and Przedborski, 2003; Ehringer and Hornykiewicz, 1998; Klein et al., 2019; Nandhagopal et al., 2009). This loss results in a disruption in the intricate balance of dopamine within the dorsal striatum (STR) - a region that receives DA projections directly from the SN (Booij et al., 1997; Kish et al., 1988; Ziebell et al., 2012)- subsequently leading to the manifestation of the cardinal motor symptoms observed in PD (Braak et al., 2003; Fearnley and Lees, 1991; Kamath et al., 2022; Otsuka et al., 1996; Rinne et al., 1999). However, PD also involves loss of noradrenergic (NA) neurons, with a 20-90% loss of NA neurons within the locus coeruleus (LC), a small brainstem structure that is the principal source of noradrenaline for the brain (Oertel et al., 2019;

Ohtsuka et al., 2013; Sitte et al., 2017; Zarow et al., 2003). This depletion of NA neurons disrupts supply of NA to regions such as the pre-frontal cortex (PFC), which is thought to contribute to various non-motor symptoms experienced by PD patients, including cognitive impairment, depression and anxiety (Abbott et al., 2005; Braak et al., 2003; Oliveira et al., 2017; Zhou et al., 2021).

Additionally, the depletion of dopamine and noradrenaline can trigger a cascade of events involving the immune system, driving microglial and astrocyte activation, and the concomitant release of pro-inflammatory molecules (Evans et al., 2022; Färber et al., 2005; Marinova-Mutafchieva et al., 2009; Mastroeni et al., 2009; Shao et al., 2013; T. Wang et al., 2018; Zhang et al., 2015). This neuroinflammatory process persists over time, in turn exacerbating the degeneration of DA and NA neurons and creating a self-perpetuating cycle of inflammation and neurodegeneration (Cebrián et al., 2014; Troncoso-Escudero et al., 2018). The evidence from *in vivo* studies and toxin-based animal models collectively underscores the vulnerability of DA-SN and NA-LC neurons in a chronic neuroinflammatory environment (Marinova-Mutafchieva et al., 2009; Mastroeni et al., 2009; Wang et al., 2020, 2022). There is also a growing body of neuropathological and biochemical evidence showing elevated levels of activated microglia and pro-inflammatory cytokines within the brains of PD individuals, hinting at the pivotal involvement of neuroinflammation in PD pathology (McGeer et al., 1988; Mogi et al., 1996, 1994b, 1994a).

Critically, these pathophysiological changes associated with PD are also present following TBI (Bales et al., 2010; Huger and Patrick, 1979; Mautes et al., 2001; McIntosh et al., 1989; van Bregt et al., 2012). Studies have shown that at 4 weeks following a moderate TBI in rat and mouse models, there are noticeable changes in dopamine metabolism, coupled with significant reductions in protein expression of the dopamine transporter (DAT), within both the STR and SN (Impellizzeri et al., 2016; van Bregt et al., 2012; Wagner et al., 2005).

Similarly, disruptions in NA turnover rates in the LC and frontal cortex were found at 30 minutes following a moderate unilateral contusion in the rat models (Levin et al., 1995). Notably, a significant increase in NA turnover (Levin et al., 1995) and intensity of NA neurons was also identified in LC, with this upregulation found to decrease from 1-8 weeks post moderate diffuse injury in the same model (Fujinaka et al., 2003). Clinically, among 10 patients who experienced severe TBI several months prior, Single Photon Emission Computed Tomography (SPECT) imaging demonstrated significant disturbances in nigrostriatal function, as evidenced by reduced striatal DAT and D2-like receptor binding(Donnemiller et al., 2000). While there has been limited clinical research exploring changes in noradrenaline levels following TBI, the utilisation of NA-targeting medications in TBI patients has shown promise with improved recovery and reduced mortality(Dunn-Meynell et al., 1998; Lloyd-Donald et al., 2020; Tschuor et al., 2008). Particularly noteworthy, a meta-analysis consisting of 17 randomised controlled trials investigating methylphenidate- a medication that enhances NA activity- suggested that methylphenidate administration led to improved processing speed, particularly with prolonged drug duration, implying that NA level may be compromised following TBI and promoting NA activity could potentially restore the disrupted NA balance(Chien et al., 2019).

In addition to the disruptions in catecholamine levels after TBI, upregulation and prolonged neuroinflammation across different brain regions is also one of the key consequential responses that the brain employs in response to the initial injury (Johnson et al., 2013; Loane et al., 2014; Rusiecki et al., 2020). For instance, in the TBI mouse model, a significant increase in proinflammatory cytokines, such as IL-1β, IL-6, TNF-α, was observed in the medial PFC up to 9 days following a diffuse mild injury (Chen et al., 2023). Furthermore, an increase in the transcription factor NF-κB, along with proinflammatory enzymes Cox-2 and iNOS, along with an upregulated total microglial population, was demonstrated within the SN

at 4 weeks following a focal moderate TBI (Impellizzeri et al., 2016). Similarly, in the TBI work utilising Sprague-Dawley rats, moderate midline fluid percussion injury demonstrated an elevation of proinflammatory cytokines, including IL-1β, IL-6, TNF-α and CXCL1 in the cortex, STR and SN, as early as 3-6 hours post-injury (Liu et al., 2017). This inflammatory response persists in the SN following a moderate diffuse TBI, indicated by upregulated TSPO and CD45 gene expression, indicative of microglial activation, up to 28 days post-injury (van Bregt et al., 2012). This continues up to 8 weeks post-severe TBI, with the injured STR exhibiting higher numbers of activated microglia compared to sham (Acosta et al., 2013). Clinically, in a study of 10 patients with moderate-severe TBI, microglial activation has been shown to persist in several relevant brain regions, including the putamen, thalamus and posterior limb of the internal capsule, even 17 years after the initial injury (Ramlackhansingh et al., 2011).

Despite the pathophysiological overlap between TBI and PD, however, our understanding of how these alterations could drive the later development of PD is limited. To date, only a limited number of preclinical studies have investigated the disruption of catecholamines following TBI, and the duration of observation often concludes at 6 months post-injury. The longest investigated timepoint is 28 weeks post-injury, where a 30% reduction in dopaminergic neurons was observed in animals subjected to moderate fluid percussion injury (Hutson et al., 2011). There is, however, some investigation of neuroinflammation up to 24 months after TBI, which shows persistent microglial activity (Loane et al., 2014; Mouzon et al., 2018, 2014; Nagamoto-Combs et al., 2007). For example, the work of Mouzon and colleagues demonstrated subtle but elevated neuroinflammation in the corpus callosum from 12 to 24 months following both single and repetitive mild closed-head diffuse injuries (Mouzon et al., 2018, 2014). Nevertheless, whether this effect is severity dependent, and how it may relate to alterations in the catecholamines, remains to be investigated.

As such, this study utilised archival brain tissue collected from animals at 12-months following a TBI model of diffuse axonal injury of varying severities, including single mild, repetitive mild and moderate-severe TBI, to investigate chronic alterations in markers related to catecholaminergic pathways and neuroinflammation in key regions related to PD (i.e. the STR, SN and PFC).

2.0 MATERIALS AND METHODS

2.1 Animals

This study utilized previously generated tissue from fifty-four adult male Sprague-Dawley rats (10–12 weeks, 420-480g), originally obtained from Laboratory Animal Services (The University of Adelaide, AU). Animals were housed under conventional laboratory conditions (2 animals per cage), with a 12-hour light-dark cycle and access to food and water *ad libitum*. This study was performed under the approval of the University of Adelaide Animal Ethics Committee (M-2015-187).

2.2 Experimental groups and study design

Animals were randomly allocated to receive either sham surgery (n=14), a single mild TBI (smTBI) (n=12), repetitive mild TBI (rmTBI) (n=14), or moderate-severe TBI (msTBI) (n=14). To control for anaesthesia and analgesia exposure, animals in the sham group received identical procedures excluding injury, with repetitive sham animals receiving the incision three times, with 5-day intervals between each incision. 12 months after the last TBI or sham procedure, each animal underwent a comprehensive functional battery assessing motor, neuropsychiatric and cognitive function as previously reported (Arulsamy et al., 2019a, 2019b). At the end of behavioural assessment, animals were randomly assigned for either (1) molecular analysis or

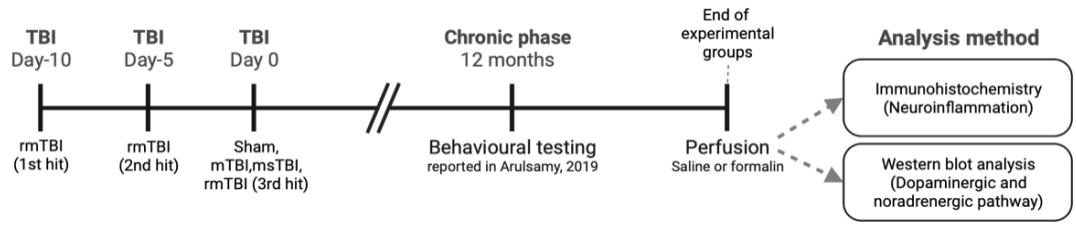

Figure 1. Experimental design of the study. During the chronic phase (12 months post injury), animals underwent neurological and behavioural testing. At the end of behavioural testing, the animals underwent perfusion using either saline or formalin fixative. This perfusion process was carried out for subsequent analyses-western blot and immunohistochemistry, respectively.

(2) immunohistochemistry using simple randomization procedures (computerized random numbers).

2.3 Injury protocol

Briefly, animals were injured with the Marmarou impact-acceleration injury method (Marmarou et al., 1994), an extensively validated diffuse injury model (Xiong et al., 2013). A 450g weight was released from either 2m (msTBI) or 0.75m (mTBI, rmTBI) onto a metal disc affixed centrally to the rat's skull. Contact was carefully monitored to ensure a single, direct impact without any subsequent rebound hits. The animals in the mTBI and msTBI groups experienced a single impact, while the rmTBI animals received 3 hits in 10 days (5-day intervals between each injury)(McAteer et al., 2016). This approach allowed for sufficient recovery time between each injury event and may have greater relevance for clinical scenarios, such as injuries due to contact sports or repeated falls, where there is an interval of time between injury events(Shultz et al., 2011).

In addition to the surgery procedure, the msTBI animals were exposed to 10 minutes hypoxic conditions (2L/min nitrogen; 0.2L/min ,oxygen) to mimic the clinical effects of more severe head trauma (Arulsamy et al., 2019a, 2019b; Xiong et al., 2013), as hypoxic conditions have been shown to exacerbate injury severity (Hellewell et al., 2010). It is important to note that hypoxia alone is unlikely to account for any disparities observed between the TBI groups, as demonstrated previously by Hellewell and colleagues (Hellewell et al., 2010). All remaining groups (sham, mTBI, rmTBI) were kept under normoxic ventilation via nose cone after the injury.

2.4 Immunohistochemistry

A subset of animals (6 sham, 6 mTBI, 7 rmTBI, and 8 msTBI) were transcardially perfused with saline, followed by formalin, and the brain was removed. Brains were exposed to 30% sucrose solution for cryoprotection and then segmented into 2-3 mm blocks. Blocks were embedded in Optimal Cutting Temperature compound (Tissue-Tek O.C.T. compound, Proscitech) and snap-frozen using freezing isopentane (2-methylbutane, Sigma Aldrich, M32631). 20µm coronal sections were obtained from the desired locations for each animal using a cryostat and a rat brain atlas (Paxinos and Watson, 2006)(Table 1). The frozen sections were mounted on slides and stored in a -20°C freezer.

For immunohistochemistry, slides were air-dried overnight at room temperature and rehydrated using ethanol. Endogenous peroxidases were blocked with 0.5% hydrogen peroxide in methanol (Thermo Fisher), and antigen retrieval was performed using citrate buffer. Two phosphate-buffered saline (PBS) washes of 3 minutes each were performed, followed by a 30-minute incubation in normal horse serum (1:30, Thermo Gibco) and an overnight incubation with the primary antibody (Table 2).

On the following day, the slides were washed with 0.1% triton-X-100 in PBS, followed by the application of the appropriate biotinylated secondary antibody (1:250, Vector) for 30 minutes. Three PBS washes of 3 minutes each were performed between the application of the secondary and tertiary antibodies. Subsequently, the tertiary antibody-streptavidin peroxidase conjugate (1:1000, Vector) was applied and incubated for one hour, and the bound antibody was detected using 3,3'-Diaminobenzidinetetrahydrochloride (1:50, Sigma Aldrich) for 7 minutes. After staining, the sections were mounted with coverslips using DPX mountant (Sigma Aldrich). The slides were air-dried in a fume hood for at least 2 days before being scanned with a Nanozoomer (Hamamatsu, Japan). The scanning process generated a total of 7 single layers, each taken 1µm

apart, with a spacing of 6μm apart between the top and bottom layers. The scanned images were viewed using the associated NDP view software (version 2).

2.5 Image analysis

Image analysis involved capturing 3 × 20x images from the clearest layer in the region of interest (Table 1), which were exported and stacked using Image J. GFAP immunoreactivity was assessed quantitatively by manually counting the reactive and immuno-positive cells per mm^2 within the same area. For IBA1 analysis, stacked images were exported and analysed using the HALO image analysis platform (Indica Labs, Albuquerque, New Mexico, USA), with settings based on the microglial activation module (v1.2) for automated counting. This analysis utilised images set to the scanning resolution, 20x mode, 0.46μm /pixel. Subsequently, the total microglia population and activation state were determined based on morphological parameters of $IBA1^{+ve}$ cells (Table 2). The resulting counts yielded the total number of immune-positive and activated cells from GFAP manual counting and IBA1 automated counting. The percentage of cell activation was calculated by dividing the total number of activated cells by the total number of immune-positive cells and expressing the result as a percentage. The experimenter was blinded to the experimental group throughout the analysis.

2.6 Western blot

Another subset of animals (8 sham, 6 mTBI, 7 rmTBI and 6 msTBI) were transcardially perfused with 9% cold saline. The brains were dissected and immediately frozen in liquid nitrogen and stored at -80°C. For further investigation, a 2-3mm region of interest was cut from each brain tissue sample (Table 1). These samples were sonicated in freshly prepared RIPA-buffer (20 mM Tris-HCl pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 140 mM 2-mercaptoethanol) supplemented with a protease inhibitor (cOmplete Mini, EDTA free, Sigma). Each sample

underwent three bursts of 10 seconds duration (at least 1 minute gap) using a sonicator probe. After sonication, the homogenized samples were centrifuged at 14,000 rpm and 4°C for 30 minutes, and the supernatant was collected. The protein concentration of each sample was determined using the Pierce BCA Protein Assay (Thermo Scientific, USA) by measuring the absorbance at 650 nm.

For western blot analysis, samples were prepared by adding sample buffer (Bolt™, 4x LDS Sample buffer) and reducing agent (Bolt™, 10x sample reducing agent) to achieve a concentration of 1mg/µl. A total of 20mg of protein was loaded in each well. Gel electrophoresis was performed using Bolt 4–12% Bis–Tris Plus gels (Invitrogen) to separate the protein samples, followed by transfer onto a PVDF membrane using the iBlot 2 Dry Blotting System (Invitrogen). The membranes were then incubated with 5% milk-Tris buffer saline-0.1% Tween 20 (TBST) for 2 hours and then with the appropriate primary antibody (Table 3) diluted in 2% bovine serum albumin (BSA, Sigma) overnight at 4°C. Afterward, the membranes were incubated with the corresponding secondary antibodies (donkey anti-rabbit, 1:10,000 and donkey anti-chicken, 1:10,000) for 2 hours at room temperature. The western blots were imaged using an Odyssey Infrared Imaging System (model 9120; software version 3.0.21) (LI-COR, Inc.) at a resolution of 169µm. Quantitative analysis of the band signals was performed using Image Studio Lite version 5.2.

2.7 Statistics

Data analysis was performed using Prism software (GraphPad v.9.0). Statistical outliers were identified and removed based on the interquartile range in a box plot in SPSS. Each outlier was evaluated individually, and exclusion was limited to the specific measurement affected. An ordinary one-way ANOVA (Analysis of Variance) with Tukey's multiple comparison post

hoc test was conducted to determine statistical significance. All values are presented as Mean ± SEM, and a significance level of $p<0.05$ was considered statistically significant.

Table 1. Region of Interest

Region	Coronal Coordinates (Bregma)(Paxinos and Watson, 2006)		Region of interest (both left and right)
Prefrontal cortex	3.7mm to 3.2mm		-Prelimbic Area -Anterior cingulate area -Infralimbic Area
Striatum	Early	1.0mm to 0.48mm	Caudoputamen
	Middle I	0.20mm to -0.40mm	
	Middle II	-0.80mm to -1.30mm	
	Late	-1.5mm to -2.10mm	
Substantia Nigra	Early	-4.5mm to -5.2mm	-Substantia nigra, compact part -Substantia nigra, reticular part
	Middle	-5.2mm to -5.8mm	
	Late	-5.8mm to -6.3mm	

Table 2. Antibodies investigated using immunohistochemistry

Primary Antibody	Species	Conc.	Catalogue#	Analysis Target	Analysis platform and parameters
Ionized Calcium binding adaptor molecule 1 (IBA1)	Rabbit	1: 20,000	Wako-019-19741	Microglial reactivity	Halo microglial activation module- ·Min cell body diameter-3.4µm ·Contrast threshold- 0.3 pixel ·Min process OD- 0.25 pixel ·Max process Radius- 12µm ·Max fragmentation length-2.5µm ·Activation process thickness-2.12µm
Glial fibrillary acidic protein (GFAP)	Rabbit	1: 40,000	Dako-Z0334	Astrocyte reactivity	Image J- ·Manual identification of astrocyte morphology

Table 3. Primary antibodies investigated via Western blot

Primary Antibody	Species	Conc.	Catalogue#	Analysis Target
Tyrosine Hydroxylase (TH)	Rabbit	1:1000	Abcam-ab112	Catalytic enzyme for conversion of tyrosine to DA
Dopamine Beta Hydroxylase (DβH)	Rabbit	1:500	Abcam-ab209487	Enzyme converts dopamine to norepinephrine
Dopamine receptor D1 (DrD1)	Rabbit	1:1000	Abcam-ab20066	Receptor from $D1_R$ family
Rabbit anti-Dopamine receptor D4 (DrD4)	Rabbit	1:1000	Abcam- ab20424	Receptor from $D2_R$ family
Rabbit anti-Catechol-O-methyltransferase (COMT)	Rabbit	1:1000	Abcam-ab226938	Enzyme that degrades catecholamines
Rabbit anti-alpha 1a Adrenergic receptor (ADRA1A)	Rabbit	1:1000	Abcam- ab137123	Alpha-1 adrenergic receptor subtypes
Rabbit anti-alpha 2a Adrenergic receptor (ADRA2A)	Rabbit	1:1000	Abcam-ab85570	Alpha-1 adrenergic receptor subtypes
Rabbit anti-beta 1 Adrenergic receptor (ADRB1)	Rabbit	1:1000	Abcam- ab3442	A beta-adrenergic receptor
Chicken anti-GAPDH	Chicken	1:10,000	Abcam-108162	Housekeeping protein

3.0 RESULTS

3.1 Subtle alterations in neuroinflammation observed following moderate-severe TBI at 12-months post injury

Analysis of the number of GFAP+ve cells at 12-months post-injury found no significant difference in the PFC ($F_{(3, 21)} = 0.57$, p=0.64) or the STR ($F_{(3, 20)} = 1.292$, p=0.3). However, an overall effect was observed in the SN ($F_{(3, 18)} = 4.293$, p=0.02) (Fig. 2a,c,e), with the msTBI group showing a significantly lower number of GFAP+ve cells/mm^2 compared to the sham group (326.41±22.17 cells/mm^2 vs 448.32±35.27 cells/mm^2, p=0.013), whereas no significant differences were observed in the mTBI (375.3±85.93 cells/mm^2) and rmTBI (395.8±32.44 cells/mm^2) groups. Additionally, no significant injury effect was found in any region for the percentage of reactive astrocytes (Table 4).

No significant difference in the number of IBA1+ve cells was noted in the PFC ($F_{(3, 21)} = 0.99$, p=0.41, Fig. 3a) or the SN ($F_{(3, 22)} = 0.61$, p=0.65, Fig. 3e) at 12-months post-injury, although a significant effect of injury was found in the STR ($F_{(3, 20)} = 4.2$, p=0.02, Fig, 3c). Post-hoc analysis found no significant differences relative to shams. However, sham, mTBI and rmTBI animals had similar numbers of IBA1+ve cells (142.9±15.13 cells/mm^2, 134.61±6.17 cells/mm^2, 130.5±8.37 cells/mm^2, respectively), while msTBI had a higher number with 166.8±34.6 IBA1+ve cells/mm^2 (Table 4).

No significant differences in the % activated microglia were noted in the STR ($F_{(3, 19)} = 2.32$, p=0.11) or the SN ($F_{(3, 20)} = 2.29$, p=0.11) at this time point following injury (Fig. 3f). However, a significant effect was found in the PFC ($F_{(3,19)}=4.11$, p=0.02), although post-hoc analysis found no significant differences relative to shams. Sham and msTBI animals had a similar

number of % activated microglia (11.34±0.46% and 11.88±3.30%), with lower numbers in the mTBI (8.30±1.75%) and rmTBI animals (6.70±1.38%) (Table 4).

Table 4. Summary of neuroinflammatory marker results

Marker Analysis	PFC		STR		SN	
	TBI effect	*Post-hoc*	TBI effect	*Post-hoc*	TBI effect	*Post-hoc*
Total GFAP+ cells	ns; p= 0.64	Δ^{mTBI} = -19.22 Δ^{rmTBI} = 12.85 Δ^{msTBI} = -6.70	ns; p= 0.3	Δ^{mTBI} = -44.45 Δ^{rmTBI} = -27.20 Δ^{msTBI} = -26.55	***p= 0.02**	Δ^{mTBI} = -73.03 Δ^{rmTBI} = 52.48 **Δ^{msTBI}= 121.9***
% of Reactive Astrocyte	ns; p=0.53	Δ^{mTBI} = -1.7 Δ^{rmTBI} = 0.71 Δ^{msTBI} = 0.13	ns; p= 0.08	Δ^{mTBI} = 0.25 Δ^{rmTBI} = 3.58 Δ^{msTBI} = 1.11	ns;p= 0.81	Δ^{mTBI} = -2.15 Δ^{rmTBI} = -0.75 Δ^{msTBI} = -1.18
Total IBA1+ cells	ns; p=0.42	Δ^{mTBI} = 21.90 Δ^{rmTBI} = 4.67 Δ^{msTBI} = 6.38	***p= 0.02**	Δ^{mTBI} = 8.32 Δ^{rmTBI} = 12.38 Δ^{msTBI} = -23.91 **$\Delta^{rmTBI-msTBI}$ = -36.29***	ns;p= 0.62	Δ^{mTBI} = 12.77 Δ^{rmTBI} = 24.57 Δ^{msTBI} = 9.06
% of Activated Microglial	***p= 0.02**	Δ^{mTBI} = 3.04 Δ^{rmTBI} = 4.64 Δ^{msTBI} = -0.54 **$\Delta^{rmTBI-msTBI}$ = -5.2***	ns; p= 0.11	Δ^{mTBI} = 8.94 Δ^{rmTBI} = 7.70 Δ^{msTBI} = 4.43	ns;p= 0.11	Δ^{mTBI} = -4.98 Δ^{rmTBI} = -2.05 Δ^{msTBI} = -5.46

Note: Δ= mean of sham- mean TBI (s), negative value= increase value in mean 2 when compared to mean 1, positive value= decrease value in mean 2 when compared to mean 1, ns=not significant, * = $p<0.05$. Bold text indicates statistical significance between treatment groups.

3.2 DRD1 elevation observed in PFC, but not STR or SN, following single mild TBI at 12-months post-injury

To assess potential alterations in the dopaminergic pathway at 12 months following TBI, we conducted Western blot analysis of relevant proteins involved in this pathway. These included TH (tyrosine hydroxylase), responsible for dopamine production (Daubner et al., 2011; Molinoff and Axelrod, 1971), and one of the dopamine D1-like receptors, DRD1. No alteration in the relative expression of TH was found in any of the regions examined, including PFC ($F_{(3, 19)} = 2.01$, $p=0.15$), STR ($F_{(3, 21)} = 0.08$, $p=0.97$) and SN ($F_{(3, 20)} = 0.34$, $p=0.8$) (Fig 4a,c,e). However, a significant change was observed for the relative expression of DRD1 in the PFC only ($F_{(3, 21)} = 7.56$, $p<0.01$), primarily driven by an increase in mTBI animals (1.95±0.53), such that they were significantly different from shams (1.09±0.39, $p=0.002$) and rmTBI

animals (1.09±0.17, p=0.003), but not msTBI rats (1.52±0.29, p=0.22) (Fig. 4b) (Table 5). To note, other players in the dopaminergic pathway, such as another member of the D1-like receptor family, DRD5, and D2-like receptors, including DRD2 and DRD4, as well as the dopamine transporter (DAT), were included in the experimental assessment. However, due to the poor antibody quality, further analysis of their expression levels and functional roles could not be reliably conducted for the purposes of this study.

Table 5. Summary of catecholamine pathway marker results

Marker Analysis	PFC		STR		SN	
	TBI effect	*Post-hoc*	TBI effect	*Post-hoc*	TBI effect	*Post-hoc*
TH	ns; p=0.15	$\Delta^{S\text{-}mTBI} = 0.19$ $\Delta^{S\text{-}rmTBI} = -0.19$ $\Delta^{S\text{-}msTBI} = -0.32$	ns; p= 0.38	$\Delta^{S\text{-}mTBI} = 0.23$ $\Delta^{S\text{-}rmTBI} = 0.13$ $\Delta^{S\text{-}msTBI} = 0.25$	ns; p=0.8	$\Delta^{S\text{-}mTBI} = -0.37$ $\Delta^{S\text{-}rmTBI} = -0.14$ $\Delta^{S\text{-}msTBI} = -0.13$
DRD1	**** p= 0.0013**	**$\Delta^{S\text{-}mTBI} = -0.86$**** $\Delta^{S\text{-}rmTBI} = -0.002$ $\Delta^{S\text{-}msTBI} = -0.43$ **$\Delta^{mTBI\text{-}rmTBI} = 0.85$****	ns; p= 0.97	$\Delta^{S\text{-}mTBI} = 0.008$ $\Delta^{S\text{-}rmTBI} = 0.04$ $\Delta^{S\text{-}msTBI} = 0.06$	ns; p=0.94	$\Delta^{S\text{-}mTBI} = 0.015$ $\Delta^{S\text{-}rmTBI} = -0.06$ $\Delta^{S\text{-}msTBI} = 0.02$
DβH	ns; p=0.16	$\Delta^{S\text{-}mTBI} = -0.4$ $\Delta^{S\text{-}rmTBI} = 0.26$ $\Delta^{S\text{-}msTBI} = 0.29$	ns; p= 0.29	$\Delta^{S\text{-}mTBI} = -0.27$ $\Delta^{S\text{-}rmTBI} = -0.42$ $\Delta^{S\text{-}msTBI} = -0.20$	*** p= 0.01**	$\Delta^{S\text{-}mTBI} = 0.24$ $\Delta^{S\text{-}rmTBI} = 0.14$ **$\Delta^{S\text{-}msTBI} = 0.89$**** **$\Delta^{rmTBI\text{-}msTBI} = 0.74$***
ADRA1a	ns; p= 0.63	$\Delta^{S\text{-}mTBI} = -0.12$ $\Delta^{S\text{-}rmTBI} = -0.11$ $\Delta^{S\text{-}msTBI} = -0.05$	ns; p= 0.86	$\Delta^{S\text{-}mTBI} = -0.03$ $\Delta^{S\text{-}rmTBI} = 0.08$ $\Delta^{S\text{-}msTBI} = 0.03$	ns; p= 0.09	$\Delta^{S\text{-}mTBI} = 0.16$ $\Delta^{S\text{-}rmTBI} = 0.20$ $\Delta^{S\text{-}msTBI} = 0.12$
ADRA2a	ns; p= 0.13	$\Delta^{S\text{-}mTBI} = -0.10$ $\Delta^{S\text{-}rmTBI} = 0.21$ $\Delta^{S\text{-}msTBI} = -0.44$	**** p= 0.02**	$\Delta^{S\text{-}mTBI} = -0.006$ $\Delta^{S\text{-}rmTBI} = -0.01$ **$\Delta^{S\text{-}msTBI} = -0.60$*** **$\Delta^{rmTBI\text{-}msTBI} = -0.59$***	ns; p= 0.68	$\Delta^{S\text{-}mTBI} = 0.33$ $\Delta^{S\text{-}rmTBI} = 0.21$ $\Delta^{S\text{-}msTBI} = 0.23$
ADRB1	ns; p= 0.74	$\Delta^{S\text{-}mTBI} = 0.01$ $\Delta^{S\text{-}rmTBI} = 0.01$ $\Delta^{S\text{-}msTBI} = -0.04$	ns; p= 0.09	$\Delta^{S\text{-}mTBI} = -0.1$ $\Delta^{S\text{-}rmTBI} = 0.27$ $\Delta^{S\text{-}msTBI} = 0.23$	ns; p= 0.17	$\Delta^{S\text{-}mTBI} = 0.12$ $\Delta^{S\text{-}rmTBI} = 0.03$ $\Delta^{S\text{-}msTBI} = 0.06$
MBCOMT	ns; p= 0.5	$\Delta^{S\text{-}mTBI} = 0.32$ $\Delta^{S\text{-}rmTBI} = 0.30$ $\Delta^{S\text{-}msTBI} = 0.80$	ns; p= 0.72	$\Delta^{S\text{-}mTBI} = 0.27$ $\Delta^{S\text{-}rmTBI} = -0.26$ $\Delta^{S\text{-}msTBI} = 0.09$	ns; p= 0.83	$\Delta^{S\text{-}mTBI} = -0.15$ $\Delta^{S\text{-}rmTBI} = -0.09$ $\Delta^{S\text{-}msTBI} = -0.13$
SCOMT	ns; p= 0.92	$\Delta^{S\text{-}mTBI} = 0.12$ $\Delta^{S\text{-}rmTBI} = 0.02$ $\Delta^{S\text{-}msTBI} = 0.13$	ns; p= 0.44	$\Delta^{S\text{-}mTBI} = -0.11$ $\Delta^{S\text{-}rmTBI} = -0.34$ $\Delta^{S\text{-}msTBI} = -0.18$	ns; p= 0.35	$\Delta^{S\text{-}mTBI} = -0.25$ $\Delta^{S\text{-}rmTBI} = -0.15$ $\Delta^{S\text{-}msTBI} = -0.41$

Note: Δ= mean 1- mean 2, negative value= increase value in mean 2 when compared to mean 1, positive value=decrease value in mean 2 when compared to mean 1, S=Sham, mTBI=single mild TBI, rmTBI=repetitive mild TBI, msTBI=moderate-severe TBI, ns=not significant, * = p<0.05, ** p<0.01. Bold text indicates statistical significance between treatment groups.

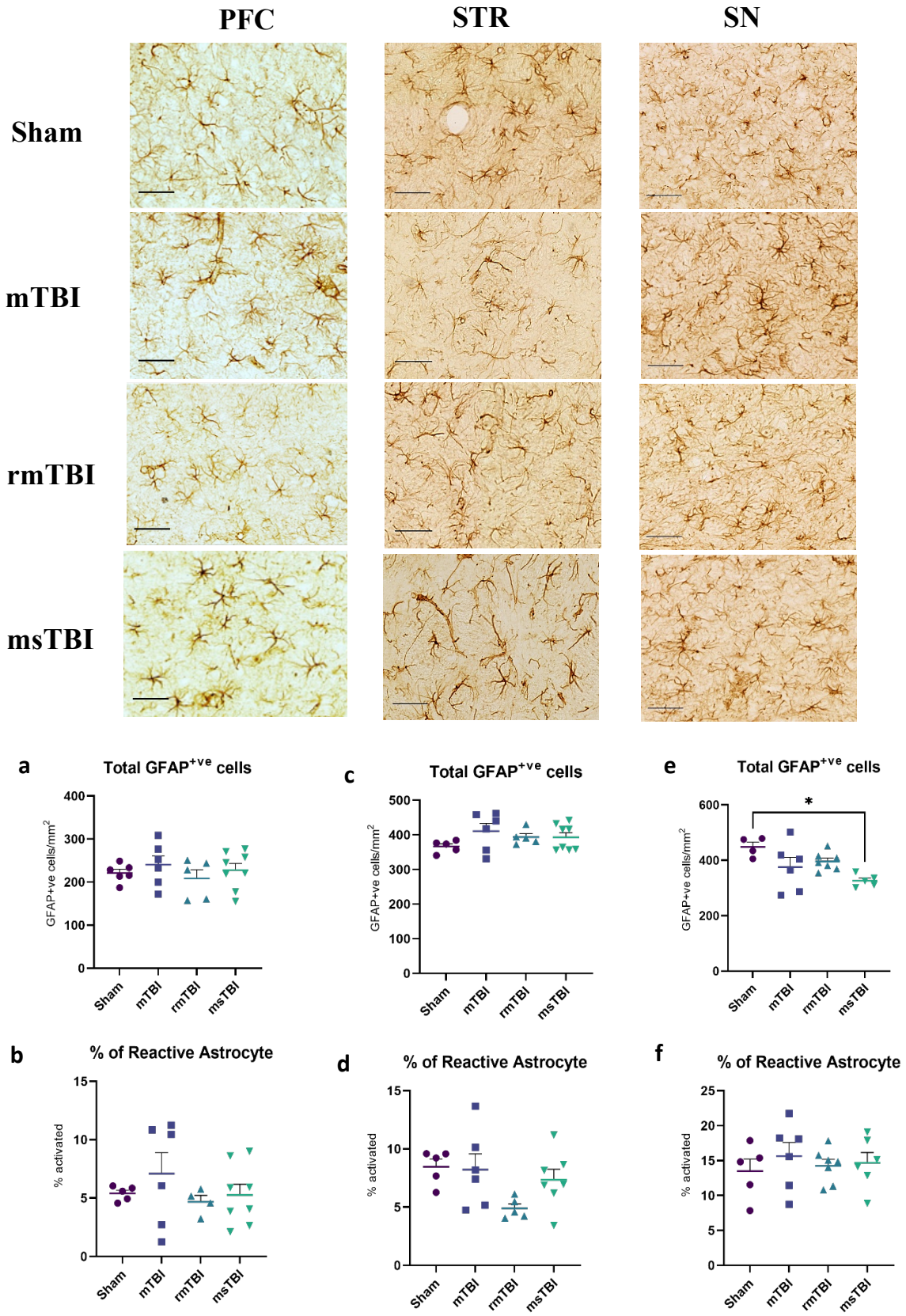

Figure 2. Neuroinflammation was assessed by Glial fibrillary acidic protein (GFAP) at 12 months post different severity of injury. Representative images and the corresponding analysis of GFAP are shown in the left column for the prefrontal cortex (PFC), in the middle column for the striatum (STR), and in the right column for the substantia nigra (SN). (a,b) Total GFAP^{+ve} cells per mm^2 and percentage of reactive astrocyte in PFC, (c,d) Total GFAP^{+ve} cells per mm^2 and percentage of reactive astrocyte in STR, (e,f) Total GFAP^{+ve} cells per mm^2 and percentage of reactive astrocyte in SN. Data are presented as mean±SEM, *p<0.05. Scale bar=50um.

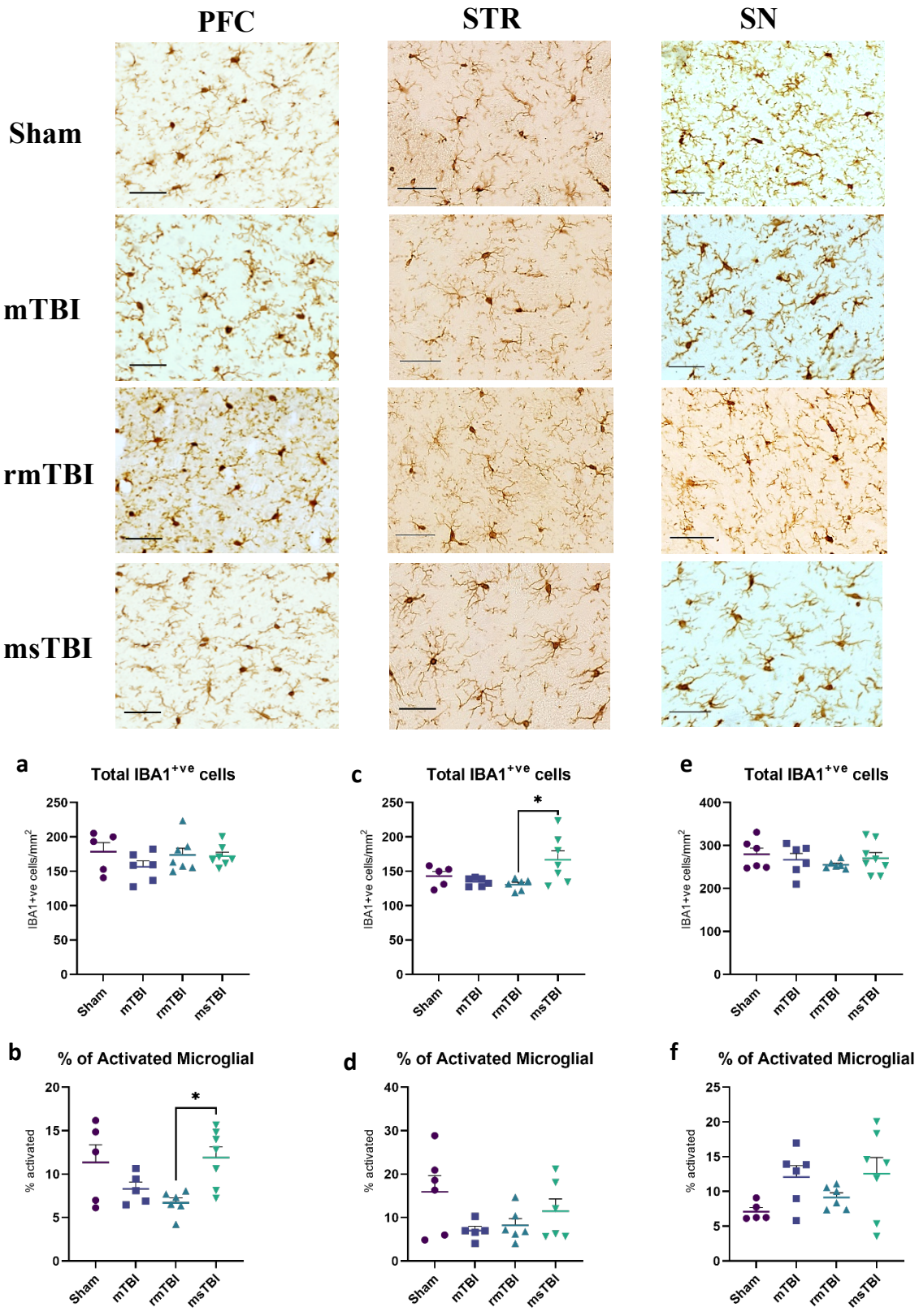

Figure 3. Neuroinflammation was assessed by Ionized calcium binding adaptor molecule 1 (Iba1) at 12 months post different severity of injury. Representative images and the corresponding analysis of IBA1 are shown in the left column for the prefrontal cortex (PFC), in the middle column for the striatum (STR), and in the right column for the substantia nigra (SN). (a, b) Total IBA1^{+ve} cells per mm^2 and percentage of activated microglial in PFC, (c, d) Total IBA1^{+ve} cells per mm^2 and percentage of activated microglial in STR, (e, f) Total IBA1^{+ve} cells per mm^2 and percentage of activated microglial in SN. Data are presented as mean±SEM, *p<0.05. Scale bar=50um

3.3 Moderate-severe TBI leads to chronic changes in STR ADRA2A and SN DβH levels at 12-months post-injury

The analysis of markers within the noradrenergic pathway included the examination of DβH (dopamine beta-hydroxylase), an enzyme responsible for noradrenaline production (Weinshilboum and Axelrod, 1971), as well as the noradrenergic receptors ADRA1a, ADRA2a, and ADRB1. Evaluation of noradrenaline synthesis using DβH showed no significant differences in the PFC ($F_{(3, 21)} = 1.89$, p=0.16) (Fig. 5a) or the STR ($F_{(3, 19)} = 1.35$, p=0.29) (Fig.5e). However, an injury effect was observed in the SN ($F_{(3, 19)} = 4.95$, p=0.01) (Fig.5i). Further post-hoc analysis revealed that the msTBI group exhibited significantly lower relative expression of DβH (0.57±0.39), compared to both the sham (1.46±0.51, p=0.009) and rmTBI (1.32±0.45, p=0.026) groups, while no significant difference was observed compared to the mTBI group (1.22±0.19, p=0.089). While the DβH bands are not visibly apparent on the grey-scale blot (Fig.5a,e,i), their position and intensity were cross-verified through the fluorescent intensity analysis tab in the Licor image studio.

Conversely, no changes were detected in ADRA1a levels in the PFC ($F_{(3, 21)} = 0.59$, p=0.63), STR ($F_{(3, 18)} = 1.07$, p=0.39) or SN ($F_{(3, 21)} = 2.44$, p=0.09) (Fig.5b,f,j). Similarly, for ADRB1, no changes were noted within any of these regions (PFC: $F_{(3, 20)} = 0.42$, p=0.74; STR: $F_{(3, 19)} = 2.48$, p=0.09; SN: $F_{(3, 20)} = 1.88$, p=0.17) (Fig.5d,h,l). While there did appear to be a trend towards reduction in this receptor in the STR following both rmTBI and msTBI, this failed to reach statistical significance. However, a significant effect of injury was observed in ADRA2a, specifically in the STR region ($F_{(3,19)} = 4.07$, p=0.02) (Figure 5g), with msTBI animals expressing significantly higher levels of ADRA2a (1.24 ± 0.47) compared to the sham (0.65 ± 0.28, p = 0.04) or rmTBI (0.66 ± 0.31, p = 0.03) groups. In contrast, no such effect was observed in either the PFC ($F_{(3,18)} = 2.21$, p=0.13) or SN ($F_{(3,20)} = 0.52$, p=0.68) (Figure 5c,k).

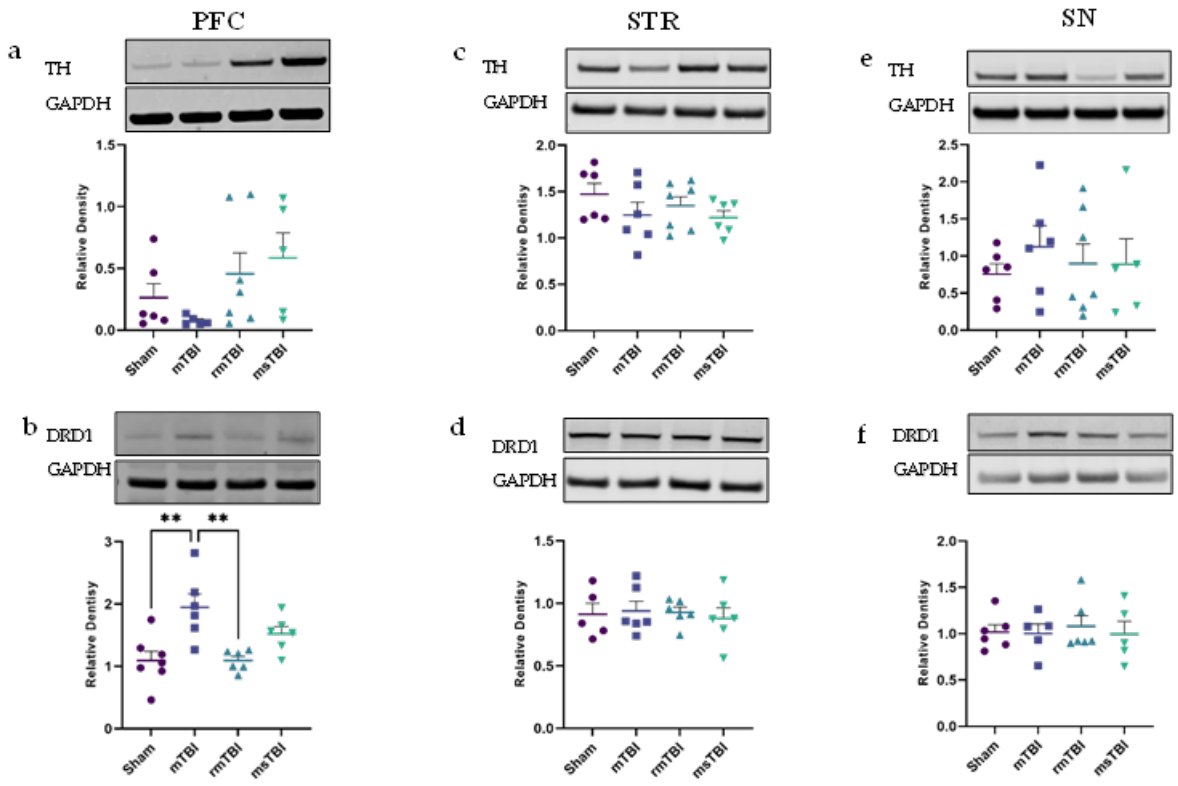

Figure 4. Dopaminergic pathway was assessed by TH and DRD1 at 12 months post different severity of injury within PFC (Left), STR (Middle) and SN (Right). (a,c,e)Relative density of TH; (b,d,f)Relative density of Drd1. GAPDH was used as a housekeeper protein for analysis. One-way ANOVA was performed. Data are presented as mean±SEM, **p<0.01. n=5-7 per group. Representative images of the western blots were extracted from Image Studio Lite.

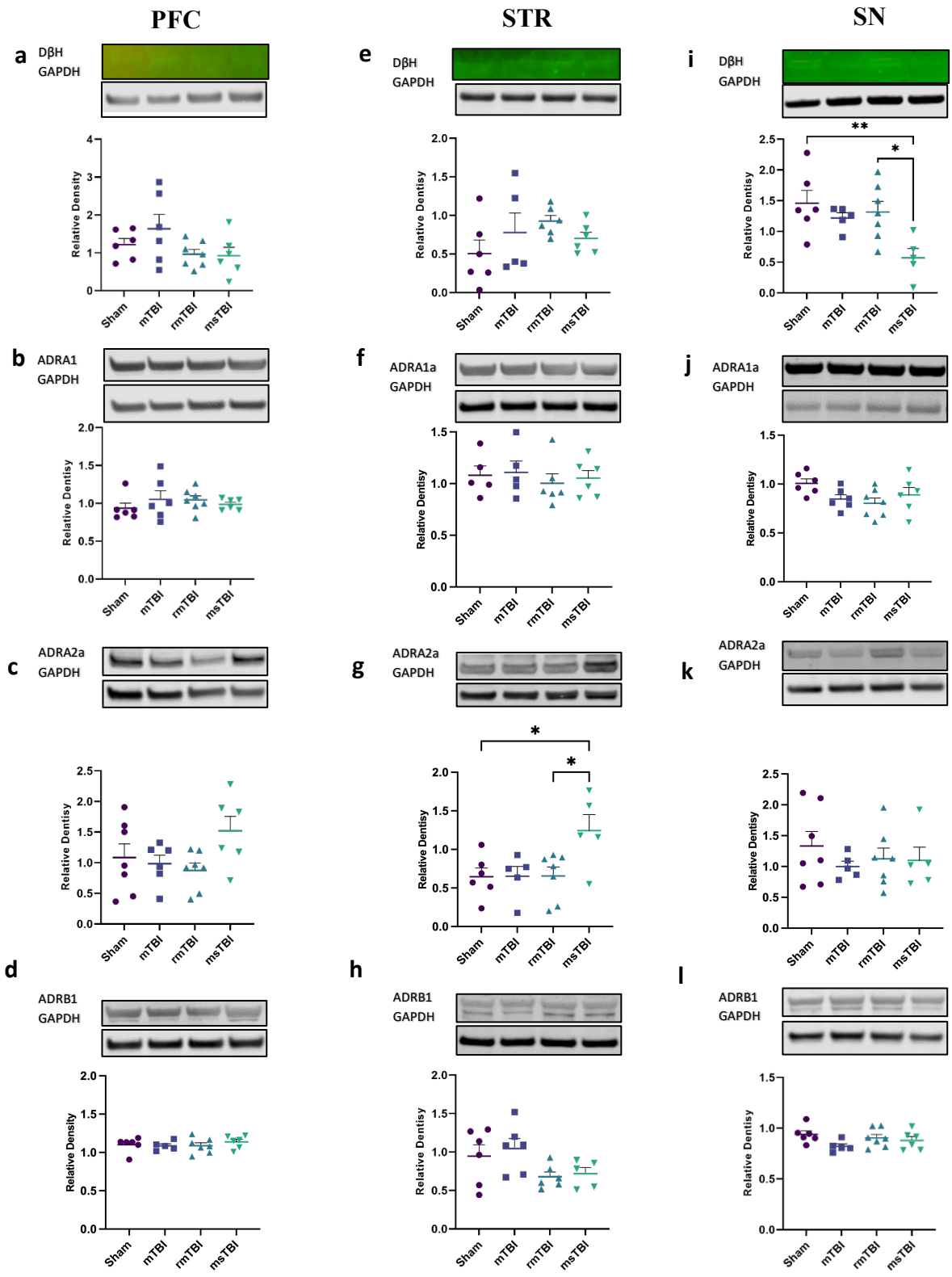

Figure 5. Noradrenergic pathway was assessed by DβH, ADRA1a, ADRA2a and ADRB1 at 12 months post different severity of injury within PFC (Left), STR (Middle) and SN (Right). (a, e, i)Relative density of DβH; (b, f, j)Relative density of ADRA1a; (c, g, k)Relative density of ADRA2a; (d, h, l)Relative density of ADRB1. GAPDH was used as a housekeeper protein for analysis. One-way ANOVA was performed. Data are presented as mean±SEM, **p<0.01,*p<0.05. n=5-7 per group. Representative images of the western blots were extracted from Image Studio Lite.

3.4 Expression of COMT was not altered by chronic TBI

At the 12-month post-injury mark, there were no changes in COMT, the enzyme that degrades dopamine and noradrenaline (Grossman et al., 1992), in any region, regardless of whether soluble (s-COMT) or membrane-bound (MB-COMT) was examined in the PFC (sCOMT: $F_{(3,22)}$=0.17, p=0.92; mbCOMT $F_{(3,22)}$=0.81, p=0.5, STR (sCOMT $F_{(3,21)}$=0.9323, p=0.44; mbCOMT: $F_{(3,20)}$=0.45, p=0.72), or SN (sCOMT $F_{(3,21)}$=1.15, p=0.35; mbCOMT $F_{(3,21)}$=0.3, p=0.83). A summary of the changes observed for all catecholaminergic markers is presented in Table 5.

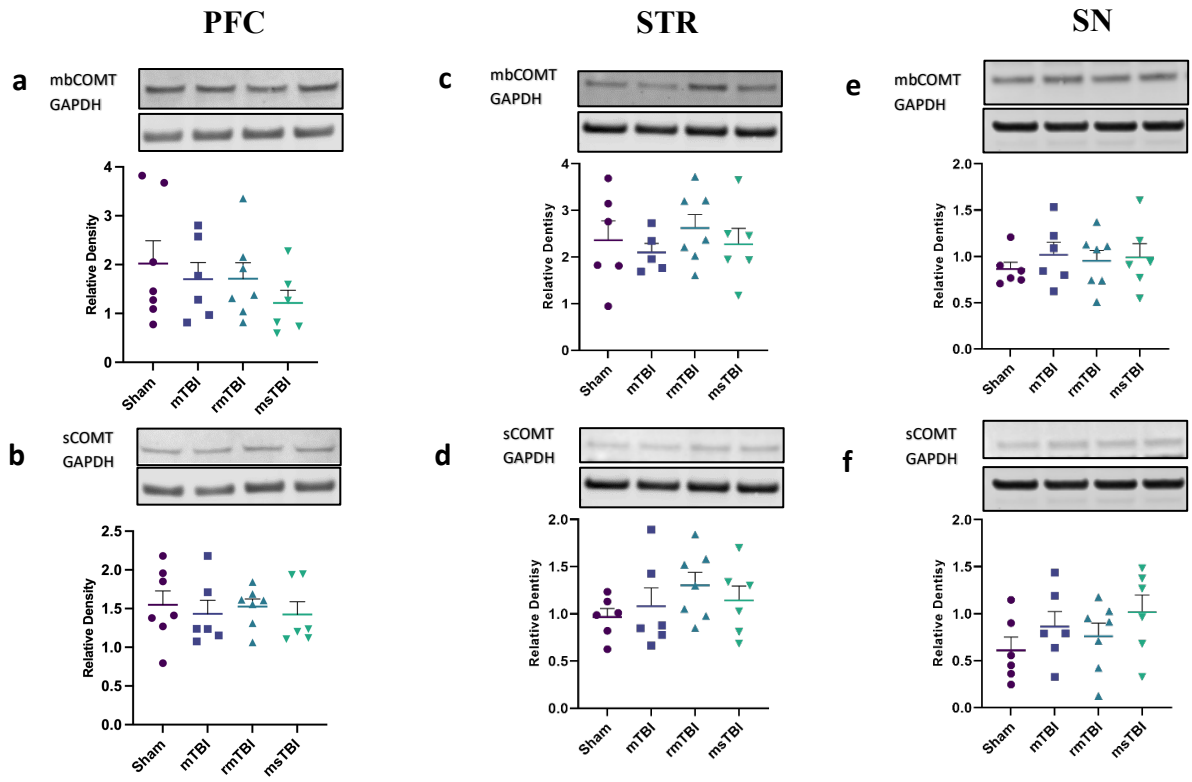

Figure 6. Catecholamine degradation rate was assessed by COMT at 12 months post different severity of injury within PFC (Left), STR (Middle) and SN (Right). (a,c,e) Relative density of mbCOMT. (b,d,f) Relative density of sCOMT. GAPDH was used as a housekeeper protein for analysis. Data are pre-sented as mean ± SEM. Outliers were removed (n = 0–2) and one-way ANOVA was performed. n = 5–7 per group. Representative images of the Western blots were extracted from Image Studio Lite.

4.0 DISCUSSION

Alterations in catecholamines, along with the persistent upregulation of neuroinflammatory processes, are acknowledged to play pivotal roles in the pathophysiology of PD, and are equally implicated in the aftermath of TBI (Delic et al., 2020). TBI is a known risk factor for the later development of PD, with risk varying based on injury severity (Bower et al., 2003; Gardner et al., 2018, 2015). Thus, to examine whether these alterations are responsible for driving the progression from TBI to PD, and exhibit a dose-dependent relationship, this study aimed to investigate markers of DA and NA signalling and microglial and astrocytic reactivity within key regions implicated in PD, the PFC, STR and SN, at 12 months following different severity of TBI.

Overall, the findings of this study indicate that, across the multiple DA and NA markers examined, following msTBI, only the NA pathway was disrupted with a decrease in DβH within the SN and an increase in the NA receptor ADR2A within the STR, an effect that is not noted in animals with a milder initial injury. In comparison, only mTBI animals showed alterations in dopaminergic signalling, and this was only associated with an increase in DRD1 in the PFC, an effect not seen in any other injury group. No other changes in any dopaminergic or noradrenergic marker were noted across the three brain regions examined following injury. Similarly, in the evaluation of the glial response, the only significant difference relative to shams was a decrease in astrocytes, as detected by GFAP in msTBI animals within the SN only. Taken together, the results of the current study seem to suggest that, 12 months following the initial insult, TBI has minimal effect on catecholamine and the neuroimmune response, with subtle differences seen in mild compared to moderate-severe TBI. Nevertheless, subtle alterations in NA within the nigrostriatal pathway may potentially set the stage for the later

emergence of more pronounced disruptions in neurotransmitters that are associated with the progression of PD.

The most notable finding in this study was that moderate-severe TBI led to an increase of ADRA2A within the STR and a decrease of DβH in SN at 12 months post-injury, while no such effects were seen in the PFC or any aspect of the DA pathway at this injury severity. DβH is an enzyme crucial for synthesising NA (Gonzalez-Lopez and Vrana, 2020; Robertson et al., 1986), with noradrenergic neurons known to project from the LC to the SN (Collingridge et al., 1979; Jones and Moore, 1977; Smits et al., 1990; Sugama et al., 2019), raising disruption of the LC as one potential explanation for this alteration. The LC is affected by TBI, with an acute increase in NA turnover in both focal and diffuse models (Fujinaka et al., 2003; Levin et al., 1995), followed by a decrease more chronically in the Marmarou weight drop model (Fujinaka et al., 2003). Consistent disruption in NA levels was also evident in clinical work; although it is not LC specific, plasma and cerebrospinal fluid (CSF) samples collected from severe TBI patients showed significantly increased NA levels from 1 to 14 days following the injury (Mautes et al., 2001). In a more chronic context, an investigation involving 52 veterans with or without a history of repetitive mild TBI found significantly elevated CSF NA levels in individuals with a trauma history (Hendrickson et al., 2018). However, how this disruption in NA signalling may affect the nigrostriatal pathway has yet to be explored in other studies.

It is noteworthy that prior research from our laboratory has identified mild cognitive deficits at 12 months post-moderate-severe TBI within the same animal cohort (Arulsamy et al., 2019a), suggesting a potential link between the observed alteration in NA levels following TBI and these cognitive impairments. In fact, NA is a neurotransmitter that plays a fundamental role in response to stress, mood regulation, attention, and cognitive functions, including executive function, cognitive flexibility, learning, and memory (Wassall et al., 2009). Clinical investigation using CSF NA measurement has demonstrated that alterations of NA levels

following TBI are associated with trauma-related mental health symptoms, such as post-traumatic stress disorder (PTSD), depression, insomnia, anxiety, and mood disorders (Hendrickson et al., 2018). More interestingly, prior research on Alpha-2A adrenergic agonists (which activate ADRA2A receptor and inhibit NA release) has consistently demonstrated their potential to enhance spatial working memory performance and cognitive flexibility across various species, including humans (Taylor and Russo, 2001), monkeys (Arnsten et al., 1988; Avery, 2000) and rodents (Carlson et al., 1992). While the outcomes may vary depending on the specific agonist and dosage used (Choi et al., 2006; Jäkälä, 1999; Rämä et al., 1996), these agonists have also been linked with neuroprotective effects, functional restoration, and notable improvement in working memory following TBI (McAllister et al., 2011; Sysoev et al., 2021; Wu et al., 2018). Conversely, studies involving the blockade of ADRA2A receptors by using Alpha-2A adrenergic antagonists have shown negative impacts on cognitive function. For instance, young adult monkeys exhibited impaired spatial working memory (Li and Mei, 1994) and aged rats displayed deficits in delayed alternation performance (Tanila et al., 1996). These collective findings suggest a pivotal role for ADRA2A receptors in cognitive function. Of note, the LC sends afferent projections to the SN and even scattered afferents to the STR(Ferrucci et al., 2013); (Smith and Kieval, 2000) thus, it is possible that the observed alterations within these regions may be a compensatory response to alterations in NA synthesis in the LC following msTBI. In line with this, striatal NA function plays important roles in cognition, with LC-striatal NA connections key for response inhibition (Grueschow et al., 2022) and cognitive flexibility (Hassani et al., 2020). However, the specific mechanisms via which TBI may subtly alter noradrenergic input to the nigrostriatal pathway from the LC and its downstream effects remain to be elucidated.

In fact, accumulating evidence suggests that LC-NA projections and NA itself play an important role in maintaining dopaminergic neurons in the SN region (af Bjerkén et al., 2019;

Carboni et al., 1990; Cragg et al., 1997). Studies demonstrate that disturbance of the NA system is correlated with both the onset and progression of DA neuronal loss in PD (Delaville et al., 2011; Hassani et al., 2020; Isaias et al., 2011; Tong et al., 2006). This is, however, contrary to our findings, where alterations in the NA system were noted, with no concomitant changes in the DA system. These discrepancies may be attributed to a delayed response within the DA system. In line with this, a post-mortem study by Zarow *et al.* investigating 19 idiopathic PD cases suggests that neuronal loss in the LC-NA system is greater than in the SN-DA system, which corroborates with Braak's theory, where degeneration of LC-NA neurons occurs before SN-DA neurons (Braak and Del Tredici, 2008; Zarow et al., 2003). Thus, it may be that the 12-month time point utilised in this study is insufficient to observe changes in the DA system, but rather earlier, more subtle changes in the NA system. This would also be consistent with the relatively mild cognitive changes observed in this cohort previously (Arulsamy et al., 2019a). It is also important to acknowledge that our analysis focused on a broad characterisation of DA and NA changes using Western blot analysis of total protein levels for each marker of interest. It is possible that a more detailed analysis using RNAseq or microdialysis might reveal subtle changes following TBI that are not readily apparent at the gross protein level. Future studies should also investigate longer timepoints following TBI to investigate progressive alteration in the DA system, particularly within the SN and STR, and to incorporate additional markers associated with these pathways, including repeating the dopamine transporter and the dopamine receptor superfamily (D2-D5).

Another notable finding of this study was the increase in DRD1 protein levels observed in the PFC of the mTBI group at 12 months post-injury compared to the sham and rmTBI groups. This increase in DRD1 expression might be associated with the previous findings in the elevated plus maze in this cohort of animals, with mTBI animals having reduced anxiety, as indicated by them spending more time in the open arms, and exhibiting a significantly higher

number of open arm entries and crossings when compared to msTBI animals (Arulsamy et al., 2019a; Walf and Frye, 2007). A similar phenotype of reduced anxiety and hyperactivity has also previously been reported following mTBI across multiple other studies (Budinich et al., 2013; Nolan et al., 2018; Shultz et al., 2011; Tucker et al., 2016). Elevated DRD1 expression could provide an explanation for this effect, as increased DRD1 activity has been linked to reduced anxiety (Hare et al., 2019). Both overexpression of DRD1 (Beyer et al., 2021) and optogenetic stimulation of DRD1, but not DRD2 (Hare et al., 2019), within the PFC have shown similar reductions in anxiety-like behaviour, as indicated by a higher number of open arm entries in the elevated plus maze (Beyer et al., 2021; Hare et al., 2019). DRD1 triggers a cascade of intracellular events through the adenylate cyclase/cyclic adenylate/protein kinase A (AC/cAMP/PKA) pathway (Jones-Tabah et al., 2021; Zhou et al., 2022), with the activity of PKA associated with anxiety (Keil et al., 2016). In support of this, a study utilising PKA inhibitor (H-89) demonstrated that injecting a high dosage of H-89 into medial PFC resulted in reduced anxiety symptoms (Miguel et al., 2014). Despite these findings, the reason why increased PFC DRD1 protein expression is present in the mTBI animals and not in the other injury groups remains unclear. It is possible that compensatory mechanisms are at play, where mTBI induces recovery through DRD1 signalling. Nevertheless, direct investigation into DRD1 expression and its specific effects on anxiety and hyperactivity following chronic TBI is required for a more comprehensive understanding.

Based on the findings of this study regarding the changes in the DA and NA systems after TBI, it is pertinent to also explore the protein levels of the catecholamine-metabolizing enzyme, COMT. The activity of COMT is associated with the degradation of catecholamines, where lower activity can potentially lead to elevated neurotransmitter levels, while higher activity can potentially result in decreased neurotransmitter levels (Kurowski et al., 2016; Lachman et al., 1996; Lotta et al., 1995). Indeed, COMT gene variants that alter dopamine

levels are associated with cognitive decline in PD (Lin et al., 2018; Tang et al., 2019) and similarly impaired cognitive flexibility following TBI (Kurowski et al., 2016; Lipsky et al., 2005). The investigation of COMT protein level is limited, with one study demonstrating that following focal TBI, both s and m-COMT were increased in the ipsilateral cortex at 3 and 14 days post-TBI (Redell and Dash, 2007), with longer time-points have not been assessed. Here, at 12-months post-injury, regardless of the TBI severity, no changes were seen. However, it is important to emphasise that a more comprehensive understanding of the alterations in catecholamine metabolism following TBI might be gained through the additional examination of other catecholamine-metabolizing enzymes, such as monoamine oxidase and phenylethanolamine N-methyltransferase, or the incorporation of high-performance liquid chromatography (HPLC), to obtain a clearer insight into catecholamine levels and levels of their metabolites in response to TBI.

Furthermore, despite the changes in catecholaminergic pathways discussed above, there was very little alteration in the glial response to injury in any of the brain regions examined, regardless of injury severity. In fact, the only significant finding relative to shams was a reduction in GFAP +ve cells in the SN of msTBI animals. Although it did not reach statistical significance relative to sham, there was also an increase in the total population of the microglial cells in the STR following msTBI compared to rmTBI, with a significant main effect of injury noted. Interestingly, these microglia appeared to be in a resting/ramified state, rather than an activated/ameboid state. The underlying mechanism driving this trend towards a higher resting microglia number within the STR at 12-months post-msTBI remains unclear; yet, it is plausible that these cells are returning to their ramified state in the STR. This notion could be supported by the concurrent increase in the levels of ADRA2A receptor in the STR, as ADRA2A is known to have anti-inflammatory effects, with administration of an agonist of this receptor shown to reduce the release of pro-inflammatory cytokines and downregulate pathways like

NF-kB and NLRP3 inflammasome in the acute phase following TBI (D. Wang et al., 2018; Wu et al., 2018). Additionally, a study investigating adrenergic receptor signalling in microglia suggests that the process of retraction is associated with ADRA2A, mainly in activated microglia rather than the resting microglia (Gyoneva and Traynelis, 2013). However, it is important to note that several studies have indicated that the role of microglia cannot be solely characterised by a morphological change from a ramified to an amoeboid shape, or vice versa (Li et al., 2019; Vidal-Itriago et al., 2022; Wake et al., 2009), as was done in the current work. It is thus crucial that future studies extend the analysis with additional markers, such as CD16/32 and CD26, and further quantification of cytokine release via ELISA, in order to identify the phenotype of microglia and their release of pro-inflammatory cytokines.

In addition to the increased population of microglia in the STR following msTBI, there was a notable reduction in the population of GFAP+ve astrocytes in the SN. Astrocytes can function in two primary states: resting and reactive. Under normal conditions, astrocytes play a crucial role in maintaining brain homeostasis by regulating the levels of reactive oxygen species, supporting neural development and survival, and providing structural support to mitochondria to maintain the energy level and integrity of neural circuits (Bélanger and Magistretti, 2009; Karve et al., 2016; Myer, 2006). Studies have shown that deficiency in astrocytes can lead to disruption of blood-brain-barrier seen in patients with PD. In a rat model genetically modified for astrocyte ablation, about 50% more loss of cortical tissue was seen compared to wild-type rats following a moderate TBI, highlighting the neuron-protective role of astrocytes in normal conditions (Myer, 2006). Most importantly, an *in vivo* study utilising human iPSCs derived astrocytes revealed that their responsibility for the clearance of extracellular α-syn and preventing cell-to-cell transfer between neurons (Tsunemi et al., 2020). Conversely, reactive astrocytes undergo morphological changes in response to injury or pathological conditions, which can exacerbate neuroinflammation and worsen secondary brain

injury following TBI (Valles et al., 2023). Interestingly, our analysis of astrocyte reactivity in the SN revealed that most of these cells were in a resting state, suggesting that they were not actively responding to injury or pathology and instead were likely providing support in maintaining the normal function of SN. In this context, the reduction in the astrocyte population in the SN may not be beneficial, potentially leading to a toxic environment that results in the degeneration of DA neurons and the progression of PD. This aligns with findings from a study by Frintrop *et al.*, demonstrating a correlation between the reduction of GFAP-positive astrocytes and GFAP expression, and volumetric changes observed in both the cerebral cortex and corpus callosum (Frintrop et al., 2018). Nevertheless, further investigation is imperative to comprehensively elucidate the underlying mechanisms driving this association and to ascertain whether it is influenced by distinct cellular responses compared to other TBI groups.

In conclusion, this study highlights a potentially subtle alteration in noradrenergic input to the nigrostriatal pathway following msTBI with alterations in ADRA2A in the STR and DβH in the SN. Conversely, at 12 months post-injury, the dopaminergic system was unaffected, with only an increase in DRD1 expression in the PFC following mTBI. It is important to acknowledge that the markers assessed in the current study provide only a partial view of the catecholamine signalling pathway and the intricate neuronal circuits involved. Similar to examining the neuroinflammatory pathway, future studies should incorporate in-depth analysis to provide a more comprehensive understanding of how the neuroinflammatory response contributes to the emergence of neuropsychiatric and cognitive deficits.

Taken together, this investigation provides the first comprehensive comparison of chronic alterations in catecholamine and the glial response within the SN, STR and PFC at 12 months post different severities of TBI. While the changes were subtle at this point, they appeared to be severity-dependent, with the most discernible effects observed in the moderate-severe TBI. This work also underscores the multifaceted nature of the brain's response to

different severities of diffuse axonal injury, emphasising the imperative need for further exploration to unravel the precise mechanisms and implications of the observed alterations in catecholamine systems and neurobehavioral outcomes. Nonetheless, it's essential to acknowledge that the neurodegenerative consequences of TBI can emerge many years after the initial injury event. Although minimal, these alterations might be indicative of early pathogenesis from TBI to PD. Therefore, extended periods following the injury may be necessary to detect subtle alterations that could contribute to the development of PD pathogenesis. This is particularly important given that these subtle alterations might be early indicators for diagnosing PD. Therefore, gaining insights into the temporal profiles of the changes of these molecular pathways following different severities of TBI holds clinical promise for assessing risk profiles.

Unravelling Heterogeneity and Synergistic Environmental Factors in a Parkinson's Disease Animal Model

The etiology of PD is complex and heterogeneous, involving a convergence of factors such as genetic predisposition, environmental influences and lifestyle elements (Baldereschi et al., 2003; Patrick et al., 2019; Sulzer, 2007). As elucidated previously in chapters 2 and 3, the focus on chronic alterations resulting from a singular risk factor, TBI, suggests that experiencing one or even multiple TBI events may not invariably lead to PD (Gardner et al., 2015; Lee et al., 2012). This understanding not only highlights the complex nature of PD etiology, but also emphasises the necessity for a comprehensive investigation into how different risk factors may interact and synergistically contribute to the onset and progression of PD, as it is important to note that risk doesn't occur in a vacuum. Indeed, it is likely that a given individual will experience exposure to several environmental risk factors in the course of a lifetime.

In the introductory chapter 1, we delved into the mechanistic overlap between TBI, pesticide exposure and PD. Considering this intricate interplay of factors, the next research efforts were directed towards developing an animal model that integrates two well-established PD risk factors, TBI and rotenone exposure, that aims to provide a more nuanced understanding of PD development, especially the intricate manifestation of symptoms and the potential synergistic effects of these risk factors.

04

Optimisation of Subcutaneous Rotenone Administration to Establish a Rodent Model of Underlying Vulnerability in the Nigrostriatal Pathway: A Pilot Study

Chapter prepared in publication format by **WEE, IC.**, CORRIGAN, F., COLLINS-PRAINO, L.E

ABSTRACT

Emerging evidence has highlighted traumatic brain injury (TBI) as a potential risk factor for Parkinson's disease (PD). However, the variable susceptibility to PD among individuals with TBI suggests the involvement of additional factors in disease progression. It is hypothesised that pre-existing vulnerability in dopaminergic neurons may enhance the impact of secondary risk factors, increasing the risk of PD. The combination of low-level pesticide exposure and TBI is a plausible scenario for such synergistic effects. Therefore, to investigate the potential synergistic effect of prior pesticide exposure on TBI outcomes, an appropriate model of pesticide exposure must first be developed. This model should involve chronic low-level rotenone exposure, resulting in mild inflammation and neurodegeneration within the nigrostriatal pathway, without the manifestation of overt PD motor symptoms. Initially, a pilot study was conducted using different doses of rotenone (0.5mg/kg/day or 1.0 mg/kg/day) for either 7 or 14 days in 18 male Sprague Dawley rats. Although a subtle decrease in dopamine levels was observed in the 14-day treatment group, it did not meet the requirements for the ongoing study. To explore higher doses, a second pilot study was conducted with an increased rotenone dose of 2mg/kg/day for 7 days. Unfortunately, after the 3rd injection, severe Parkinsonian symptoms developed in 66% of the animals, accompanied by significant weight loss (8-10%), leading to the cessation of the rotenone group. With a ~30% reduction in dopaminergic neurons in the substantia nigra (SN), the overt Parkinsonian symptoms were deemed unsuitable for the ongoing study, prompting a reduction in the rotenone dosage. In the third pilot study, a dose of 1.5mg/kg/48h rotenone was administered for either 3 or 6 injections. No adverse effects on body weight were observed after 6 injections, and the rats in this group consistently exhibited lower neuroscores after the 4th injection compared to the control group. Moreover, there was a ~20% loss of dopaminergic neurons in the SN and increased microglial activation in the striatum (STR). Based on these findings, we concluded that the administration

of six subcutaneous injections of 1.5 mg/kg rotenone every 48 hours in rats resulted in an underlying vulnerability within the nigrostriatal dopaminergic pathway. This model can now be utilised to investigate the synergistic effects of subthreshold pesticide exposure and TBI, potentially providing valuable insights into the development of PD following TBI.

1.0 INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders and is characterised by the progressive loss of dopaminergic neurons in the substantia nigra (SN), which subsequently leads to the depletion of dopaminergic projections to the striatum. The global number of PD patients has increased substantially, surpassing 6.2 million individuals between 1990 and 2015, and is projected to exceed 14 million individuals by 2040(Dorsey et al., 2018, 2007). Despite its high prevalence, the exact etiology of PD remains elusive, with multiple risk factors implicated in its development(Belvisi et al., 2020; Kalia and Lang, 2015). Traumatic brain injury (TBI) has been suggested as one of the environmental risk factors for the development of PD(de Lau and Breteler, 2006; Dick et al., 2007). A recent retrospective cohort study of veterans in the United States revealed that individuals with a history of TBI had a 71% increased risk of developing PD(Gardner et al., 2018). Risk was injury severity dependent, with mild TBI associated with a 56% increased risk, and moderate to severe TBI with an 83% increased risk. However, this has not been consistently reported, with a population-based cohort study in Denmark reporting a lower likelihood of developing PD following a previous severe head injury, with a morbidity ratio of 0.954(Spangenberg et al., 2009). Similarly, in the study of twin pairs discordant for PD, Bharucha *et al.* did not find an association between head injury and increased PD risk (Bharucha et al., 1986). The contradictory findings may suggest that TBI alone is insufficient to drive PD risk, but requires the presence of other risk factors.

Conceptually, pre-existing factors may render the dopaminergic system more susceptible to secondary damage, resulting in enhanced neurotoxicity and increased risk of PD manifestation (i.e. a "two-hit" model)(Cory-Slechta et al., 2005a, 2005b). In fact, numerous studies have investigated the combination of TBI and another known risk factor for PD development: pesticide exposure. In an epidemiological investigation by Lee *et al.,* involving 357 cases of

idiopathic PD and 754 controls in central California, individuals reporting a TBI with loss of consciousness exceeding 5 mins exhibited a 2-fold increase in risk (adjusted odd ratio (AOR) 2.0, 95% confidence interval (CI) 1.28-3.14) of developing PD. While ambient pesticide exposure had a weaker association (AOR 1.36, 95% CI 1.02-1.81), the risk escalated significantly for individuals exposed to both pesticides and TBI, indicating a 3-fold higher risk (AOR 3.01, 95% CI 1.51-6.01) compared to the control group (Lee et al., 2012). Supporting this finding, an *in vivo* study investigating the effect of moderate TBI and paraquat injection revealed that TBI alone induced a 15% loss of dopaminergic neurons in SN, and this loss escalated to 30% with the subsequent injection of paraquat (Hutson et al., 2011). This synergistic effect was similarly observed in an *in vitro* study where both the moderate stretch and paraquat exposure exacerbated intracellular damage in SHSY-5Y neurons (Wang et al., 2014).

These findings emphasise that TBI and the presence of other risk factors, in this case, pesticide exposure, exacerbate the risk of developing PD. However, a significant gap persists in our understanding of the underlying mechanisms propelling this complex process. To unravel these intricacies, which is essential for the understanding of interactions and molecular cascades that contribute to the heightened susceptibility to PD observed in the context of TBI, the development of a preclinical model replicating the underlying vulnerability in the nigrostriatal dopaminergic pathway before introducing TBI is necessary.

One approach to achieve this involves chronic low-level pesticide exposure, such as rotenone (Pan-Montojo et al., 2010), which has been linked to an increased risk of developing PD (Dhillon et al., 2008; Tanner et al., 2011) and was more prominently used than paraquat. However, in pre-clinical models, rotenone is often administered at high doses to replicate PD-like pathology (Betarbet et al., 2002; Cannon et al., 2009; Sherer et al., 2003; Zhang et al., 2017). For instance, Zhang *et al.* and Sherer *et al.* Reported that subcutaneous administration

of rotenone (dosages ranging from 1.5-3 mg/kg/day) over periods ranging from 7 days to 5 weeks (Sherer et al., 2003; Zhang et al., 2017) induced PD-like motor impairments, including bradykinesia, hypokinesia, and postural instability. This was accompanied by selective degeneration of nigrostriatal dopaminergic neurons and significantly increased levels of α-synuclein in SN (Sherer et al., 2003; Zhang et al., 2017). These models, however, were created using high doses of rotenone and represent a mature PD model that exhibits complete PD hallmarks. Thus, these models are unsuitable for investigating the synergistic effects of rotenone exposure with TBI, as they do not reflect the early stages of PD progression.

To address this gap in the field, this study aims to develop a rodent model that accurately represents the underlying vulnerability by finding a low-level rotenone exposure regime that results in mild dopaminergic neuron loss (<20%) and neuroinflammation within the nigrostriatal pathway, before introducing TBI. In this chapter, we present the results of three pilot studies aimed at optimising the dosage of rotenone for this purpose. Following rotenone injections, behavioural and histological features were examined, including dopaminergic loss (via tyrosine hydroxylase) and neuroinflammation (via microglial staining). By carefully selecting the rotenone dosage, our aim was to create a preclinical model that induces underlying vulnerability within the nigrostriatal pathway without the manifestation of overt PD motor symptoms, thus allowing for the investigation of how a subsequent TBI could enhance PD-like neuropathology and the possibility of developing PD prodromal symptoms.

2.0 MATERIAL AND METHODS

2.1 Animal Handling

For all pilot studies, adult male Sprague-Dawley rats (8-9 weeks old) were used under the approval of the University of Adelaide Animal Ethics Committee (M-2022-004). Animals were housed in groups of three per cage, and raised under conventional laboratory conditions (12 h light/dark cycle, temperature 22±2 °C, humidity 60±5%), with food and water provided *ad libitum*. Animals were permitted to acclimatise for 1 week before starting the study. Daily monitoring of body weight was conducted throughout the study to track potential changes in the animals' overall health and well-being. Baseline weight measurements were obtained before the initiation of the experimental procedures, specifically before the first rotenone injection. The weight range during the first injection varied between 350-450g. The percentage of weight changes was calculated using the formula:

$$\text{Percentage of Weight Change} = \frac{weight\ on\ given\ day - baseline\ weight}{Baseline\ weight}\ x\ 100$$

This calculation allowed for a quantitative assessment of weight fluctuations, providing insights into the impact of rotenone exposure on the animals' body weight over the course of the experiment.

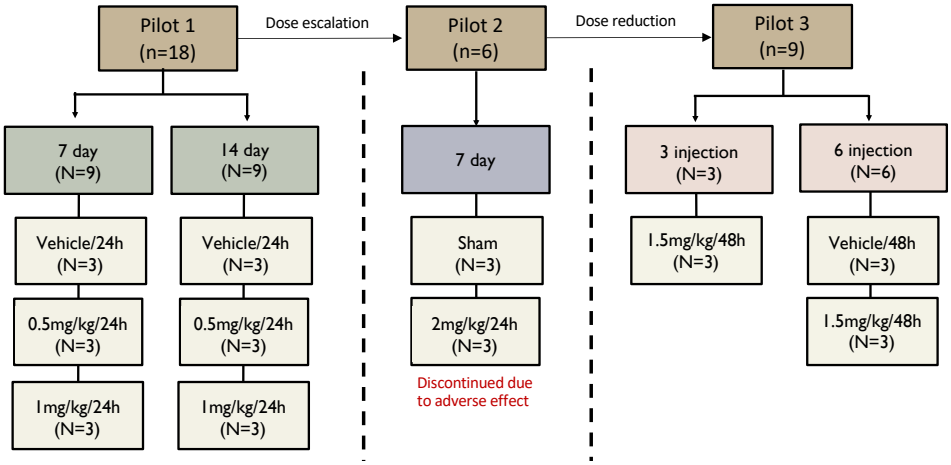

Figure 1. *Study design.* Figure depicted the experimental flow chart and sample size. Total 33 male Sprague-Dawley rats were used for this study.

2.2 Pilot Study 1

18 rats were randomly assigned to 6 groups, with each group receiving daily subcutaneous injections for either 7 or 14 days, with the following dosing regimen: 1) vehicle, consisting of a mixture of 2% DMSO and 98% sunflower oil; 2) 0.5mg/kg rotenone; 3) 1.0mg/kg rotenone (Fig. 1). Rotenone (Sigma Aldrich, R8875) was prepared in a 2% DMSO:98% sunflower mixture at either 0.5 or 1mg/mL Animals were weighed daily for a period of 14 days before each injection. 24 hours after the final injection, the animals were transcardially perfused with 10% formalin, and their brain tissues were collected for further analysis using immunohistochemistry (IHC) (Fig. 2a).

2.2.1 Tissue collection and processing

The brains were post-fixed in formalin for 7 days to ensure thorough fixation and preservation of tissue structure, then underwent cryoprotection by gradually transitioning them from 10% to 30% sucrose at 4°C over the course of a week. Once the brains had sunk to the bottom of the sucrose solution, they were sectioned into 2-3mm coronal slices and embedded in an optimum cutting temperature compound (OCT, ProSciTech). For the examination of the striatum, 10μm coronal sections were taken starting at +0.7 mm from Bregma, representing the early STR, for each animal. Similarly, for the SN, 10μm coronal sections were taken starting at -4.8 mm from Bregma for each animal. The tissue-mounted slides were then stored at -20°C until further analysis.

2.2.2 Histological analysis

For immunohistochemistry (IHC), the following primary antibodies were used: rabbit anti-IBA1 (1:20,000; WAKO 019-19741) to label microglia and rabbit anti-tyrosine hydroxylase (TH, 1:1000; Abcam 112) to label dopaminergic neurons. Prior to IHC, the tissue-mounted

slides were allowed to acclimate to room temperature overnight. Subsequently, the slides were rehydrated in 100% ethanol, followed by treatment with diluted hydrogen peroxide in methanol for 30 minutes to remove non-specific background staining. After the washes with phosphate-buffered saline (PBS), antigen retrieval was performed using citrate buffer. The slides were washed again, then incubated in 5% normal horse serum in PBS for 1 hour to block non-specific binding. The primary antibodies, diluted in 5% normal horse serum in PBS, were applied to the slides, which were then incubated overnight at room temperature. The following day, the slides were washed with 10% Triton X-100 in PBS and incubated with the secondary antibody (Vector labs, anti-Rabbit, BA1100, 1:1000) for 1 hour at room temperature. After another wash with PBS, the slides were incubated with streptavidin peroxidase conjugate (SPC, 1:1000) for 1 hour. Following a final wash in PBS, the slides were incubated with 3,3'-Diaminobenzidine tetrahydrochloride (DAB) for 7 minutes. The slides were then rinsed with ethanol and, subsequently histolene before mounting coverslips.

Images were generated by scanning the slide with the Nanozoomer slide-scanner (Hamamatsu, Japan), and images were viewed on NDPview (version 2). TH and IBA1 immunoreactivity were assessed by using the HALO image analysis platform (Indica Labs), with the modules area quantification and microglial activation quantification (parameters as per Table 1), respectively. The experimenter was blinded to the experimental group until the end of the analysis.

Table 1. Halo microglial activation module v1.2

Min cell body diameter	3.4 μm
Contrast threshold	0.2-0.3 pixel
Min process OD	0-0.15 pixel
Max process Radius	15 μm
Max fragmentation length	5 μm

Activation process thickness	2.3 μm

Contrast threshold and minimum process OD were modified individually in each section to reach 70-80% accuracy.

2.3 Pilot Study 2

Given the negligible loss of dopaminergic immunoreactivity within the nigrostriatal pathway observed in the study, a second study was conducted with a higher dose of rotenone. The decision was based on previous findings by Sherer *et al.*, who demonstrated that administration of 2.0mg/kg for 15 days resulted in diffuse loss of dopaminergic neurons in the STR, and a dose of 2.7mg/kg for 7 days reproduced approximately 10-15% of dopaminergic loss in the striatum while maintaining SN integrity(Sherer et al., 2003). However, it is worth noting that a dosage of 2.7mg/kg for 7 days had a high mortality rate of 64%(Sherer et al., 2003). Therefore, to strike the balance, a dosage of 2.0mg/kg for 7 days was chosen for the second study, with the inclusion of a sham group as comparator.

2.3.1 Experimental design

6 rats were randomly assigned to 2 groups for this pilot study: (1) Sham and (2) 2.0mg/kg/day rotenone for 7 days (Fig. 1). Subsequently, the animals were weighed daily for a period of 7 days before each injection. At the end of the experiment, the animals were transcardially perfused with 10% formalin 24 hours after the last injection, and their brains were collected for further analysis using IHC, as previously described in 2.2.1 and 2.2.2.

2.4 Pilot Study 3

Given the adverse effects seen in study 2, experimentation was ceased, and the rotenone dosage regimen was modified again based on previous work by Fathalla *et al.*(Fathalla et al., 2016),

where the rotenone injections were conducted every 48 hours with a dosage higher than 1.0mg/kg and lower than 2.0mg/kg(Fathalla et al., 2016).

2.4.1 Experimental design

9 rats were randomly allocated to receive subcutaneous injections of 1.5mg/kg rotenone every 48h, with one group receiving 3 doses and the other group receiving 6 doses. A vehicle group was included that received 6 injections in total, 48h apart (Fig. 1).

Unlike the previous pilots, behavioral tests were conducted on the day without the injection to assess motor function (adjusting step test) and grip strength. In addition, the composite neuroscore and constipation were evaluated daily, commencing from day 7 (Fig. 6a). The experimenter was blinded to the treatment groups during the tests until the analysis was completed. At the end of the experiment, the animals were transcardially perfused with 10% formalin 24 hours after the last injection, and their brains were collected for further analysis using IHC as previously described in 2.2.1 and 2.2.2.

2.4.2 Forelimb adjusting step test

The forelimb adjusting step test was used to assess forelimb akinesia(Olsson et al., 1995). Previous work has suggested that the forepaw adjusting step test is particularly useful for predicting striatal dopamine levels and correlates well with decreased body weight gain, making it particularly useful for this model(Miyanishi et al., 2019). In brief, the animal's body and hindlimbs were wrapped in a surgical drape and held in one hand by the experimenter, slightly raising it above the surface. The other hand fixed the forelimb not to be monitored in the drape. The rat was then held with one paw touching the table and moved slowly sideways (1m) by the experimenter, first in the left-to-right and then in the right-to-left direction. The number of adjusting steps were counted for both paws in each direction of movement.

2.4.3 Grip strength test

Forelimb muscle strength was determined by measuring peak force (in gram-force) using the Digital Grip Strength meter equipped with a Hind Limb Pull Bar Assembly (Ugo Basile, Italy)(El-Saiy et al., 2022). Animals were allowed to grip the metal grids connected to a force traducer with their forepaws, and gently pulled backwards by the body and the base of the tail until they could no longer hold the grids. Each animal was given five consecutive trials, and the average value was taken with the three highest peak force.

2.4.4Composite Neuroscore

Neurological function was evaluated using the 28-point composite neuroscore, which was adapted from previous studies on motor function impairment following TBI in the rat(McIntosh et al., 1989; Nissinen et al., 2017; Zhang et al., 2005). The neuroscore was comprised of 11 tests with a cumulative maximum score of 28, including (1) circling (maximum four points); (2) motility (maximum three points); (3) general condition (maximum three points); (4) righting reflex (maximum one point); (5) paw placement (maximum four points); (6) horizontal bar (maximum three points); (7) inclined platform (maximum three points); (8) grip strength (maximum two points); (9) contralateral reflex (maximum one point); (10) contralateral rotation when held by the base of tail (maximum two points); and (11) visual forepaw reaching (maximum two points). A neuroscore of 0 indicated significant neurological impairment, whereas a cumulative score of 28 indicated healthy functioning.

2.4.5 Constipation test

Constipation is a well-established non-motor symptom in PD, and is often used to monitor individuals with PD or those at risk for developing the disease(Abbott et al., 2005; Wüllner et al., 2007). Fecal material of each animal was collected during a 15-minute handling session.

The total weight of fecal material was then measured and recorded. Animals that failed to produce feces were excluded from the analysis.

2.5 Statistics

Data for all pilot studies was graphed using GraphPad Prism software (GraphPad v.9). Due to the small sample sizes, statistical analyses were not performed; however, we report all data to help inform future studies. All values are displayed as median (range).

3.0 RESULTS

3.1 PILOT 1

3.1.1 Body weight

The body weight was measured everyday before the injection. The Over 7 days, the vehicle rats exhibited weight gain ranging from 7 to 14% from their starting weight. The lower 0.5mg/kg dose of rotenone had minimal effect on the weight gain trajectory (5 to 9%), whilst the 1.0mg/kg reduced weight gain (-1 to 6%). Similarly, the 14- day vehicle animals had an increase of 14 to 18% with little change with the 0.5mg/kg rotenone group (12 to 14%), whereas minimal weight gain was observed in the 1.0mg/kg rotenone group (-4 to 4%) (Fig. 2b)

3.1.2 TH immunohistochemistry

Analysis of the TH+ area in the STR region revealed minimal changes compared to vehicle animals with rotenone exposure (Fig.2c). In the 0.5mg/kg rotenone groups, there was a median reduction of 7.89% (-2 to 27%) in the 7-day group and 11.8% (3 to 17%) in the 14-day group. Similarly, in the 1.0mg/kg rotenone groups, there was a median reduction of 1.5% (-1 to 8.6%) in the 7-day group and 5.2% (5 to 10%) in the 14-day group (Fig.2e).

In the SN region, the median TH+ cell count in the 7-day vehicle group was 114 (82-152) cells, with similar numbers in the 0.5mg/kg rotenone animals [101 (94-143) cells] and 1.0mg/kg rotenone animals [104 (103-131) cells]. There was more variation at the 14-day time-point, with no overall effect of rotenone exposure [vehicle: 127 (105-136); 0.5mg/kg: 146 (102-153); 1.0mg/kg: 116 (54-156) cells] (Fig.2f).

3.1.3 IBA1 analysis

The microglial response was evaluated using an automated microglial activation module in the HALO imaging analysis platform to assess the number of IBA1+ cells and the percentage of activated microglia within both the STR and SN regions.

In the STR region (Fig.3a), minimal changes were observed in the population of IBA1+ cells/mm^2, regardless of the rotenone dosage regimen. After 7-days, the median count of IBA1+ cells in the vehicle group was 60.33 (55-76) cells/mm^2, which closely resembled the count in the 1.0mg/kg rotenone-treated animals [65.66 (56-77) cells/mm^2], with a slight reduction noted in the 0.5mg/kg rotenone group [47.45 (39-65) cells/mm^2]. At the 14-day time-point, vehicle animals had a similar number of IBA1+ cells/mm^2 [52.61 (49 -55) cells/mm^2] compared to both the 0.5mg/kg rotenone group [48.95 (46-53) cells/mm^2] and the 1mg/kg rotenone group [65.27 (65-74) cells/mm^2] (Fig.3b). Conversely, the median count of IBA1+ cells in the SN exhibited a dose-dependent pattern in response to rotenone dosage (Fig.3d). After 7-day injections, the vehicle group had the lowest population of IBA1+ cells [56.58 (47-90) cells/mm^2], followed by the 0.5mg/kg [71.77 (62-197) cells/mm^2] and 1.0mg/kg groups [88.74 (64-96) cells/mm^2]. A similar pattern was observed after 14-days, with the vehicle group showing a median count of 84.16 (55-98) cells/mm^2 and the 0.5mg/kg 90.99 (58-115) cell/mm^2 and the 1.0mg/kg groups 109.28 (97-189) cells/mm^2 (Fig.3e).

High variability was observed in the activation status of microglia within these regions. Specifically, in the STR, a higher median percentage of microglial activation was evident in the 0.5mg/kg rotenone group following both the 7-day [22.86 (22-28)%] and 14-day regimens [22.57(10-26)%] when compared to the vehicle group [7-day: 11.48 (8-18)%; 14-day: 7.93 (6-18)%]. In contrast, the 1.0mg/kg rotenone group did not exhibit a noticeable change in the

microglial activation percentage following either the 7-day regimen [11.62 (9-21)%], or 14 days of treatment [14.71(14-40)%] (Fig.3c).

A different pattern emerged in the SN. In the 0.5mg/kg rotenone group, there were minimal changes in the percentage of microglial activation [16.88 (8-24)%] compared to the vehicle group [13.33 (13-20)%] were noted. However, in the 1.0mg/kg rotenone group, there was an increase in the percentage of microglial activation after 7 days of treatment [26.46 (14-19)%], which reduced to levels comparable to the vehicle group after 14 days of treatment [vehicle: 10.08 (6 -16)% vs 1.0mg/kg: 11.97 (2-18)%] (Fig.3f).

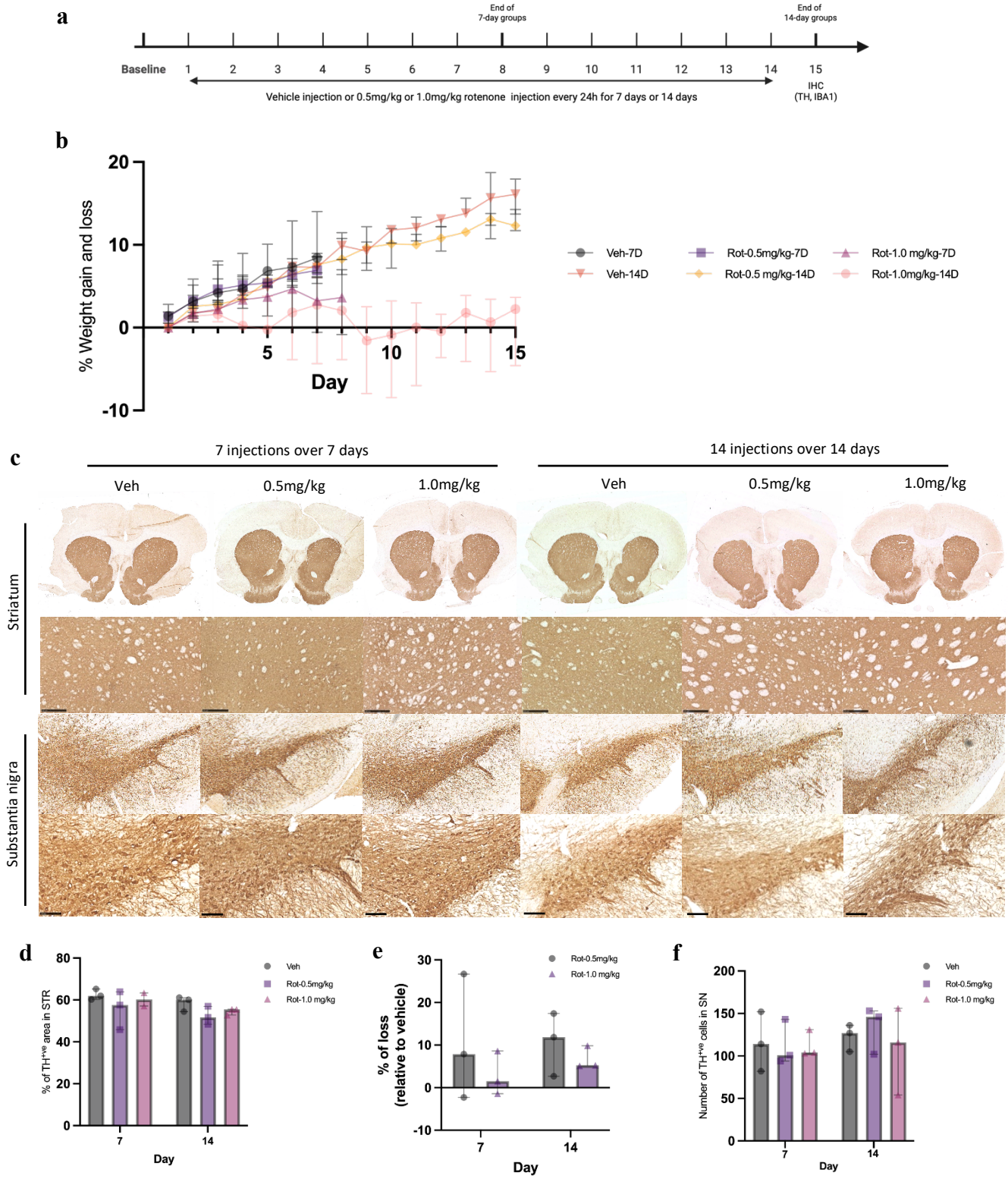

Figure 2. Assessment of Dopaminergic Function and Weight Changes Following Rotenone Administration in Pilot 1. (a) Study timeline. A total of 18 rats were enrolled in pilot 1, divided into 6 groups consisting of 3 rats per group: vehicle,0.5 or 1.0 mg/kg/day rotenone for either 7 days or 14 days. (b) Percentage of weight gain and loss overtime. Animal weight was measured daily before the injection. (c) Representative images of tyrosine hydroxylase (TH) reactivity. Top: striatum; bottom: substantia nigra. Magnification of substantia nigra x 100; scale bar=250nm. (d-f) Quantification of TH level through staining intensity analysis. (d) Percentage of TH-positive area in striatum; (e) Percentage of TH-positive area loss relative to vehicle groups; (f) Number of TH-positive cells in the substantia nigra. Data are presented in median±range. (n=3/group).

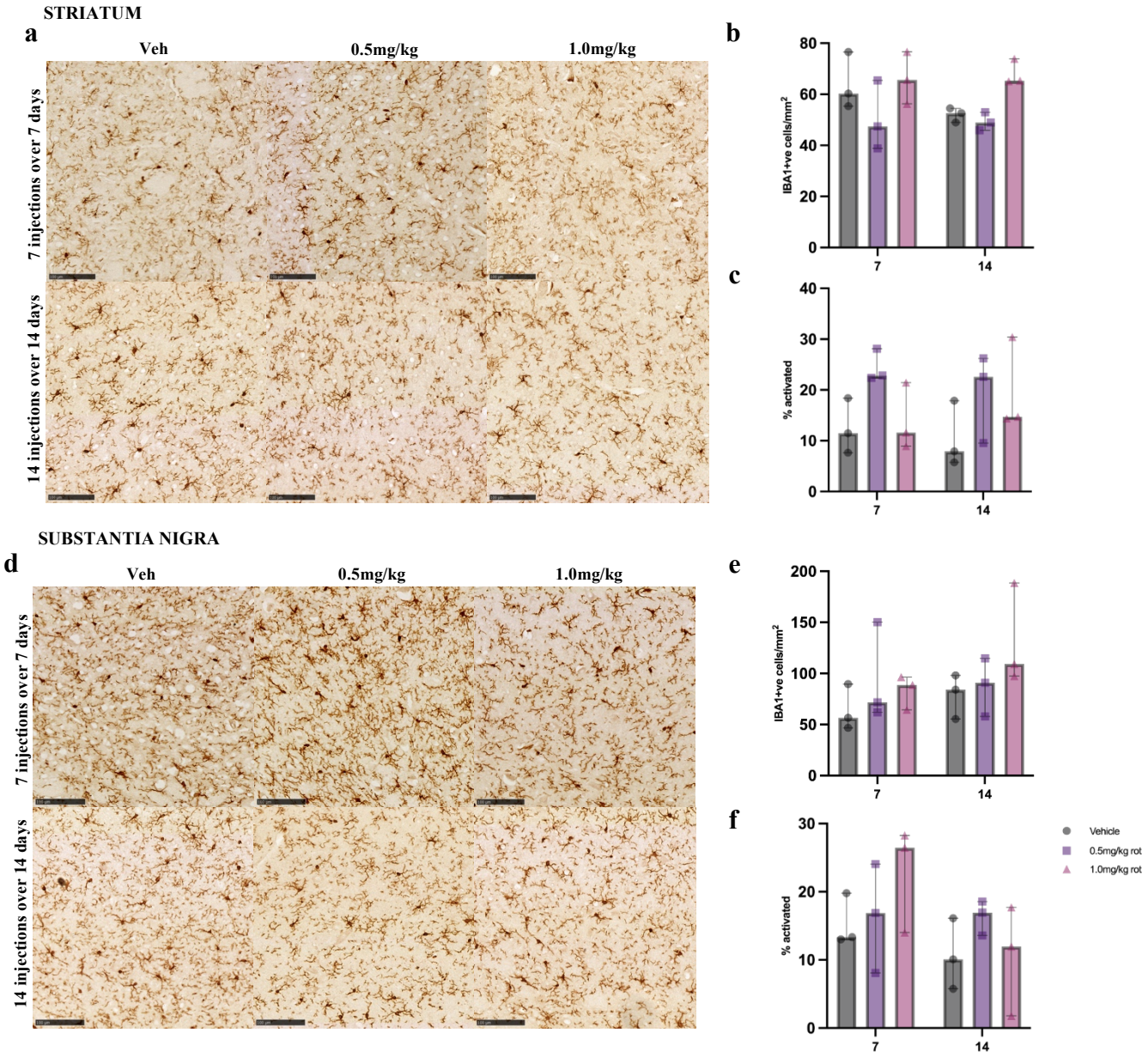

Figure 3. Analysis of Neuroinflammation using Ionized Calcium Binding Adaptor Molecule 1 (IBA1) in Striatum and Substantia Nigra in Pilot 1. (a) Representative images depicting each experimental group and IBA1 staining in the striatum. These images highlight regions with minimal IBA1 activity. (b) Striatum: Total count of IBA1-positive cells per mm^2; (c) Striatum: Percentage of microglial activation. (d) Representative images illustrating each experimental group and the IBA1 staining in the substantia nigra. (e) Substantia Nigra: Total count of IBA1-positive cells per mm^2; (f) Substantia Nigra: Percentage of microglial activation. Scale bar=100nm. Data are presented as median±range. (n=3/group).

3.2 PILOT 2

3.2.1 Physiological presentation and body weight

After administering the 2.0mg/kg rotenone injection for 3 days, two out of three animals exhibited pronounced and debilitating parkinsonian-like symptoms. One rat suffered from partial paralysis, rendering the right-side of its body immobile. The second rat displayed prominent tremors and experienced difficulty in moving. These overt symptoms were accompanied by a noticeable decrease in body weight, ranging from 7 to 12%, in the rotenone-treated animals. In contrast, the control group (shams) showed weight increases ranging from 0 to 3%, indicating a stark contrast in the physiological effects of rotenone administration (Fig. 4a).

3.2.2 TH immunoreactivity

The analysis of the TH+ area within the STR region (Fig. 4c) revealed no differences in the median values between the 2.0mg/kg rotenone group [65.0 (60.5-71.5)%] and the sham group [64.4 (62.1-71.4)%] (Fig. 4d). However, in the SN, there was a notable decrease in the number of TH+ cells in the rotenone-treated animals [68 (38-118) cells] compared to the sham group [105 (104-109) cells], although there was considerable variability in the cell count (Fig. 4e).

3.2.3 IBA1 analysis

As seen in Figure 5a, it is evident that the administration of three injections of 2.0mg/kg/day of rotenone resulted in an increase in the median count of IBA1+ within the STR [69.67 (45-81) vs sham: 52.95 (48-55) cells/mm^2] (Fig.5b). Additionally, the median of the percentage of activated microglia were doubled in the rotenone-treated group compared to the sham group within the STR [26.27 (16-31) vs sham: 11.48 (10-17)%] (Fig.5c). In contrast, when comparing the median values of total IBA1+ cells in SN, no differences were observed between the

rotenone-treated [92.18 (43-103) cells/mm^2] and sham groups [93.80 (76-163) cells/mm^2] (Fig.5d). Similarly, the analysis of the percentage of activated microglia revealed only a minimal increase in the rotenone group [29.29 (10-33)%] compared to the sham group [20 (11-31)%] (Fig.5e).

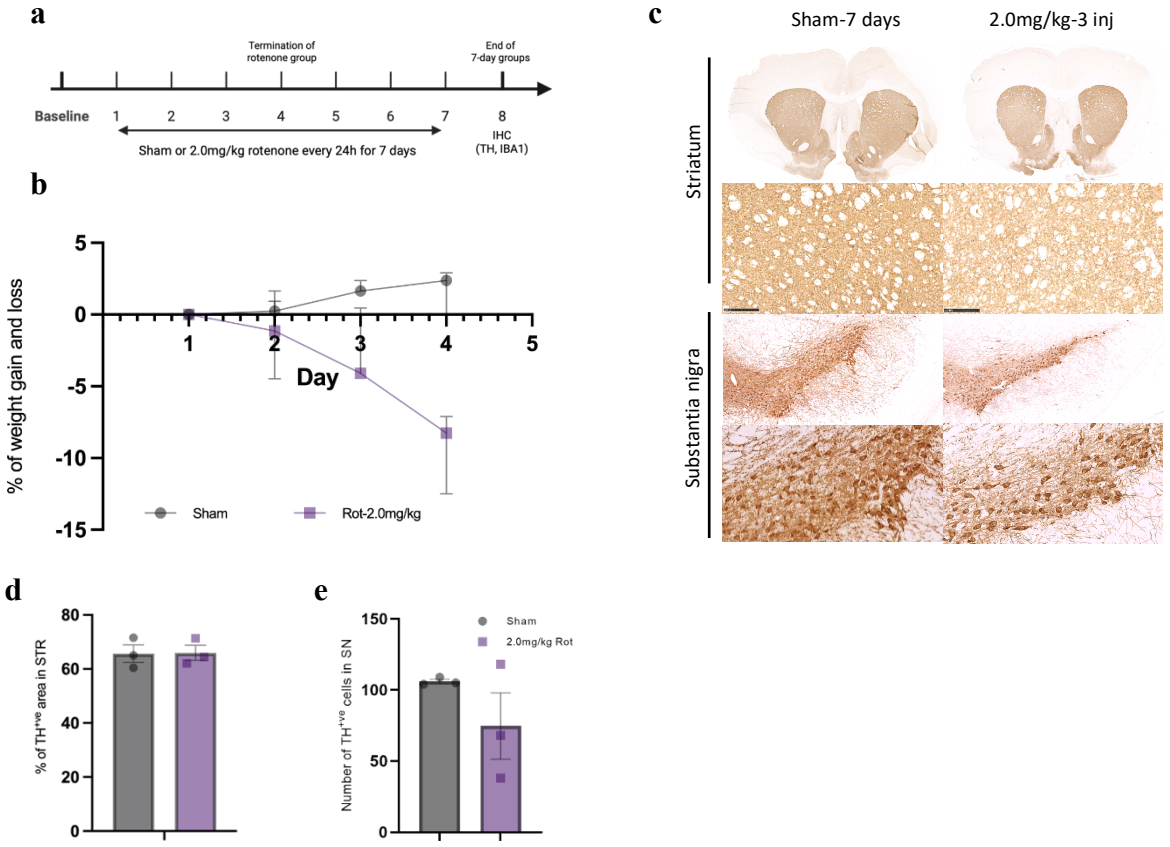

Figure 4. Investigation of Dopaminergic Function and Weight Alterations in Response to Rotenone Administration in Pilot 2. (a) Study design. Timeline illustrating the experimental setup. A total of 6 rats were included in pilot 2, with 3 rats in the sham group and another 3 rats in 2.0mg/kg rotenone group. (b) Percentage of weight gain and loss. Escalation in rotenone dosage lead to significant decrease in male Sprague Dawley's body weight after the 3rd injection. (b) Representative images of tyrosine hydroxylase (TH) reactivity. Top: striatum; bottom: substantia nigra. Magnification of substantia nigra x 100; scale bar=250um. (c-d) Quantification of TH levels using staining intensity analysis. (c) Number of TH-positive cells in substantia nigra; (d) Percentage of TH-positive area in striatum. Data are presented in median±range. (n=3/group).

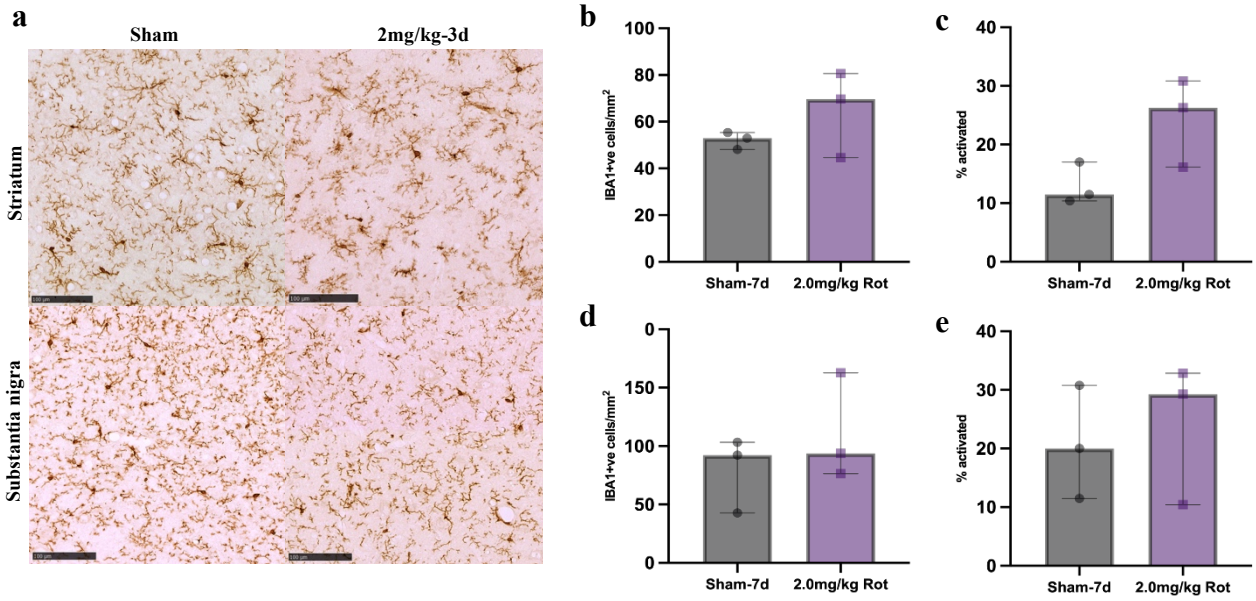

Figure 5. Evaluation of Neuroinflammation using Ionized Calcium Binding Adaptor Molecule 1 (IBA1) in Striatum and Substantia Nigra in Pilot 2. (a) Representative images illustrating each experimental group and the IBA1 staining in the striatum and substantia nigra. (b)Striatum: Total count of IBA1-positive cells per mm2; (c) Striatum: Percentage of microglial activation. (d) Substantia Nigra: Total count of IBA1-positive cells per mm2; (e) Substantia Nigra: Percentage of microglial activation. Scale bar: 100um. The data are presented as median±range. (n=3/group).

3.3 PILOT 3

3.3.1 Body weight

Administration of rotenone every 48 hours resulted in a distinct weight fluctuation pattern compared to the daily rotenone injection regime. After each injection, there was a decrease in body weight, followed by an increase on the day without injection (Fig. 6b). Upon the third injection, the vehicle group exhibited weight gain ranging from 3 to 6%, and had further gained 5-10% of weight by the sixth injection. The rotenone group showed weight changes ranging from -4 to 1% after the third injection and by the sixth injection, weight changes remained steady ranging from -4 to 2%.

3.3.2 Sensorimotor outcome

Sensorimotor function was evaluated using the forepaw adjusting step test at baseline, day 2, 4, 6, 8, 10, and 12 following the first injection (Fig. 6a, c). At baseline, no differences in the median number of adjusting steps were observed among the groups [Vehicle: 6.17 (5.33-8.5), 3*1.5mg/kg:6.08 (4.67-7.5), 6*1.5mg/kg: 6.17 (5.75-6.42) steps]. No changes were seen in the vehicle animals over the testing period with the median varying from 4.75 (3.33-6.58) to 6.5 (5.75-8.08) steps. Following the initiation of rotenone injections, the 3-injection group demonstrated variable results, with adjusting steps ranging from 3.92 (2.5-4.75) to 6.5 (3.17-6.5 steps) on days 2-6 post-first injection. The 6-injection group showed greater variability, with a lower median of adjusting steps [2.83 (2.5-6.25) steps] recorded on days 2 and 8 post-first injection, with the highest median of 5.38 (0.75-6.42) steps recorded on days 6 and 10 post-injection.

3.3.3 Grip strength outcome

On the same day as the forepaw adjusting step test (Fig. 6a), forelimb grip strength was assessed by requiring the animals to pull the handle for 5 consecutive trials, with the average calculated. As shown in Figure 4d, the assessment of grip strength revealed no evident effect of rotenone, as similar median values in the average peak force were observed across all groups (Fig. 6d). The vehicle group exhibited a median grip strength of 401.3 (242.1-557.8)gf from baseline to day 12 following the first injection. Similarly, the rotenone group exhibited a median grip strength of 419.6 (217.2-557.6)gf during the experimental period.

3.3.4 Neuroscore outcome

Neuroscore was only evaluated in the 6*1.5mg/kg rotenone group, as the assessment was conducted on days 7-12 post-injection (Fig. 6a). Throughout the testing period, the vehicle group consistently exhibited a median neuroscore of 27 (25-28) pts on days 7-11 and a median score of 28 (25-28) pts on day 12. In contrast, the rotenone group demonstrated slightly lower neuroscores across all evaluated days. The lowest neuroscore of 23 (21-25) pts was observed on days 8 and 11, while the highest neuroscore of 26 (24-26) pts was recorded on day 9 (Fig. 6e). The reduced neuroscore on days 8 and 11 was primarily attributed to challenges encountered during the horizontal bar and inclined platform tasks.

3.3.5 Constipation

Constipation was assessed by collecting fecal material during a 15-minute handling session and was similarly conducted on days 7-12 in the vehicle and 6*1.5mg/kg rotenone groups (Fig. 4a). Total fecal weight in the vehicle animals was consistent across the days assessed ranging from 0.91 (0.67-1.34)g to 1.34 (1.28-1.4)g. Fecal weight was more variable in the 6*1.5mg/kg animals and was higher than vehicles at all time-points examined, with a low of 1.38 (0.89-2.8)g and high of 2.80 (1.23-3.39)g fecal weight (Fig. 6f).

3.3.6 TH immunoreactivity

Minimal changes in the TH+ area in the STR were observed in both rotenone-treated groups. This was reflected by the similarity in median TH+ area in the vehicle [65.0 (62.4-65.4)%], 3*1.5mg/kg group [63.3 (61.7-64.5)%] and 6*1.5mg/kg group [63.1 (61.0-63.9)%] (Fig. 7b). Likewise, the median reduction in the TH+ area relative to the vehicle was 1.48 (0.48-4.0)% in the 3*1.5mg/kg group and 1.8 (0.47-5.1)% in the 6*1.5mg/kg group, further indicating minimal changes in dopaminergic markers within the STR (Fig. 7d).

In contrast, the analysis of TH+ neuron numbers in the SN region revealed more pronounced effects in 6*1.5mg/kg group [166(140-192) cells], compared to vehicle [210(197-216) cells] and 3*1.5mg/kg group [217(159-242) cells] (Fig. 7c). Specifically, 6*1.5mg/kg rotenone group exhibited a median reduction of 20 (7.5-32.6)% in the number of TH+ neurons, with a minimal decrease of -4 (-16.5-23.4)% observed in the 3*1.5mg/kg rotenone group compared to the vehicle (Fig. 7e).

3.3.7 IBA1 analysis

The analysis of IBA1+ cells in both the STR [Veh: 68.14 (52-71) vs 3*1.5mg/kg injection: 57.04 (54-57) vs 6*1.5mg/kg injection: 63.83 (45-82) cells/mm^2] and SN regions [Veh: 84.92 (77-95) vs 3*1.5mg/kg injection: 82.58 (31-86) vs 6*1.5mg/kg injection: 79.6 (69-85) cells/mm^2] exhibited a similar distribution among all groups (Fig. 8b,d). However, within the STR, a distinctive activation status of microglia was evident in the 6*1.5mg/kg rotenone group [9.73 (7.7-10.5)%] in comparison to both the 3*1.5mg/kg rotenone [5.99 (5.4-6.5)%] and vehicle groups [5.21 (4.5-7.3)%] (Fig. 8c). This activation pattern was not replicated in the SN region, as the percentage of activated microglia remained comparable between vehicle [9.39 (9.2-15)%] and the 6*1.5mg/kg rotenone group [10.5 (8.2-10.8)%], with the 3*1.5mg/kg rotenone group exhibiting a slight decrease in microglial activation [6.82 (4.9-8.2)%] (Fig. 8e).

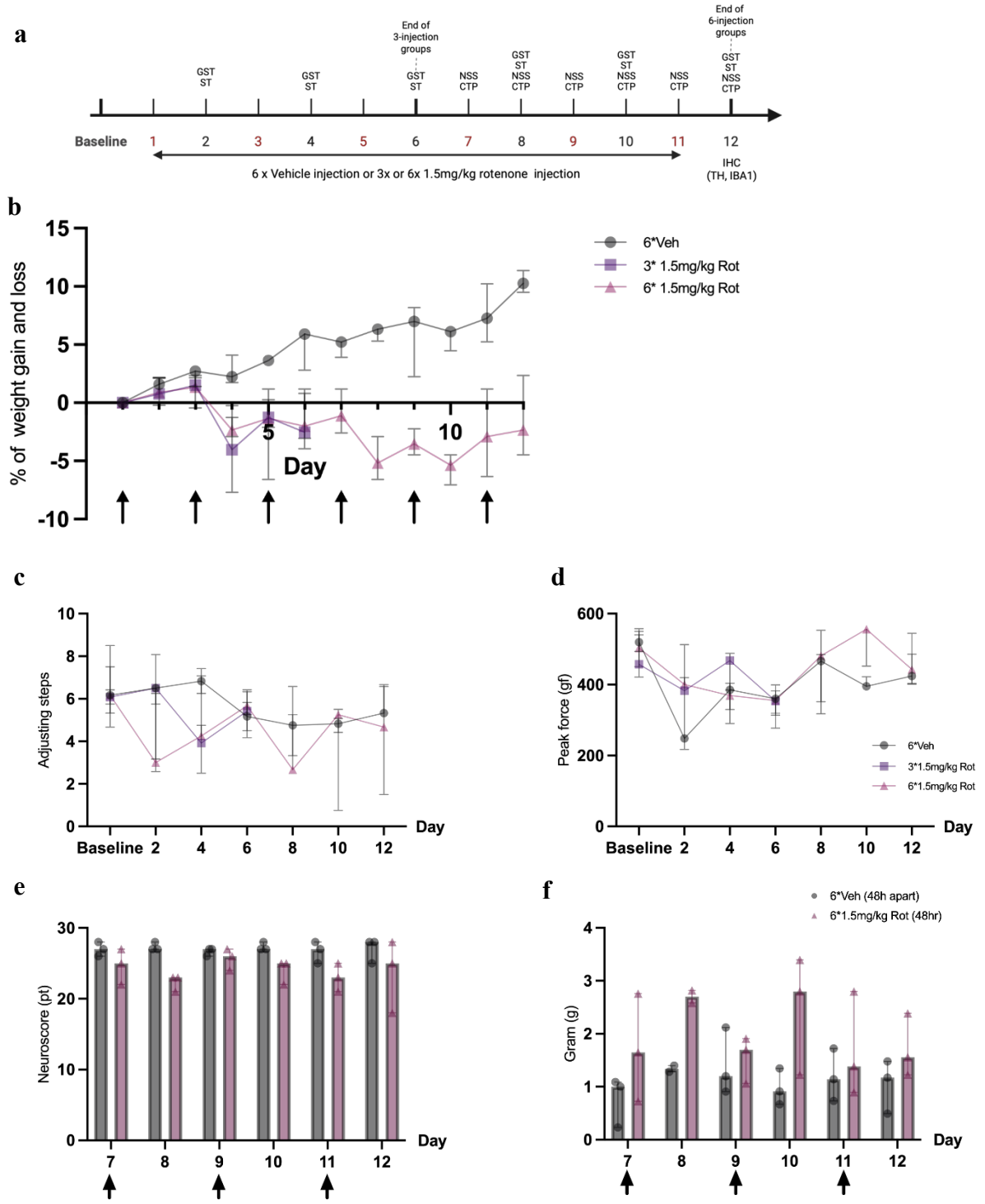

Figure 6. The Effects of Rotenone Administration on Dopaminergic Function, Weight Changes, and Behavioural Measures in Pilot 3. (a) Study design. Timeline depicting the experimental design in pilot 3. A total of 9 rats were used for this study, 3in 6* vehicle injection group, 3 in 3* 1.5mg/kg rotenone injection group, and 3 in 6*1.5mg/kg rotenone injection group. The days of injection were indicated by red fonts. The behavioural tests including grip strength test (GST), forelimb adjusting step test (ST), neuroscore (NSS), and constipation test (CTP). (b) Weight changes in the treatment groups. Rotenone injection every 48 hours prevent significant weight lost in animals. Black arrows indicate the days of injection. Animal weight was measured daily before the injection or behavioural tests. (c-f) Functional changes. (c) adjusting step test and (d) grip strength test at day 2,4,6,8,10 and 12 of regimen; (e) 28-points composite neuroscore and (f) constipation test from day 7 to 12 during the regimen. Data are presented in median±range (n=3/group).

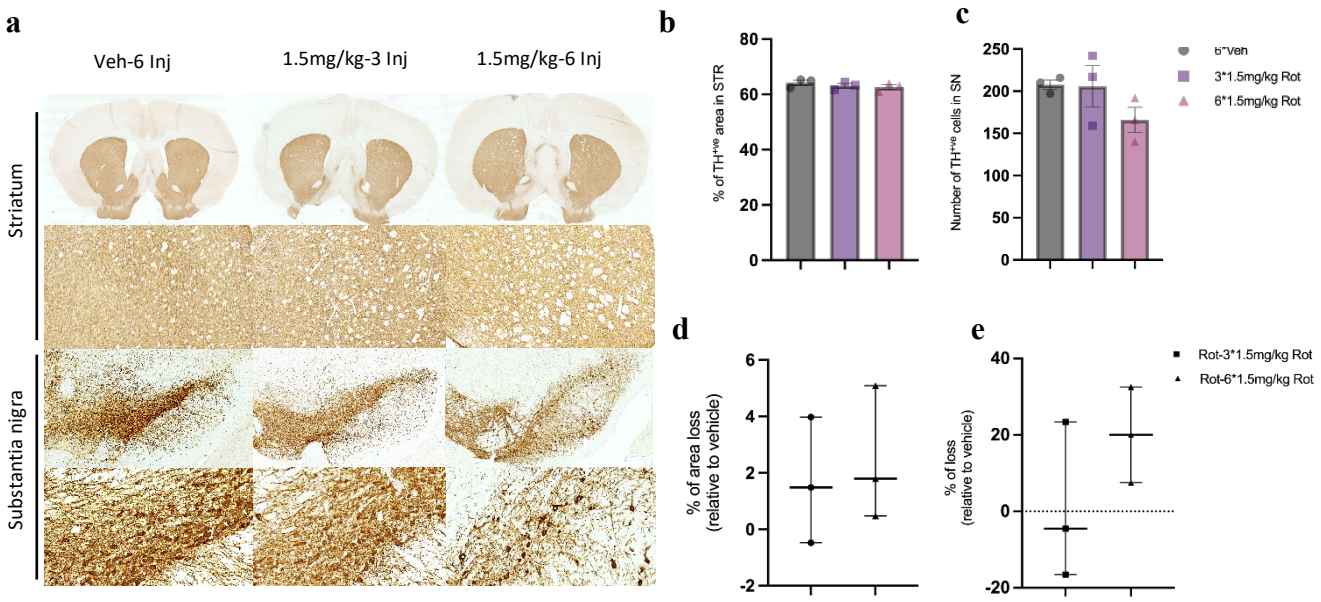

Figure 7. Pilot 3-Dopamine level quantification through TH staining in animals received 1.5mg/kg every 48h for either 3 or 6 injections. **(a) Representative images of tyrosine hydroxylase (TH) reactivity.** Top: striatum; bottom: substantia nigra. Magnification of substantia nigra x 100; scale bar=250nm. **(b-e) Quantification of TH level through staining intensity analysis.** (b) Percentage of TH-positive area in striatum; (c) Number of TH-positive cells in substantia nigra; (d) Percentage of TH-positive area loss relative to vehicle groups in striatum; (e) Percentage of TH-positive cells loss relative to vehicle groups in substantia nigra. Data are presented in median±range (n=3/group).

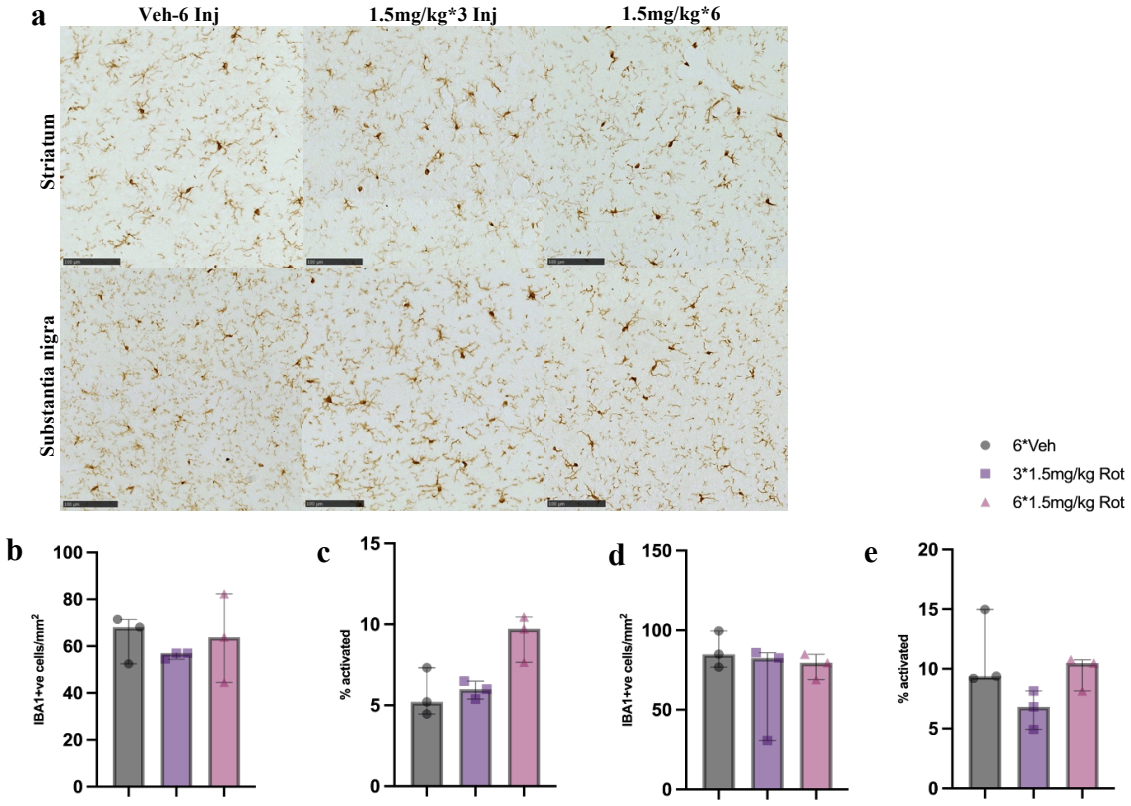

Figure 8. Evaluation of Neuroinflammation using Ionized Calcium Binding Adaptor Molecule 1 (IBA1) in Striatum and Substantia Nigra in Pilot 3. (a) Representative images illustrating each experimental group and the IBA1 staining in the striatum and substantia nigra. (b)Striatum: Total count of IBA1-positive cells per mm2; (b) Striatum: Percentage of microglial activation. (c) Substantia Nigra: Total count of IBA1-positive cells per mm2; (d) Substantia Nigra: Percentage of microglial activation. Scale bar: 100um. The data are presented as median±range. *

4.0 DISCUSSION

The purpose of this study was to determine the optimal rotenone dosage to induce vulnerability in the nigrostriatal dopaminergic system, as determined by TH loss and microglial reactivity, without overt motor symptoms. Three pilot studies were conducted to adjust the dosage based on the findings of the previous literature (Fathalla et al., 2016; Sherer et al., 2003; Zhang et al., 2017).

The initial dosage regime of 0.5 or 1mg/kg of rotenone daily for 7 or 14 days had minimal effects on the nigrostriatal pathway with no loss of TH staining or in degree of neuroinflammation. In response, the dosage was increased to 2.0mg/kg, but animals developed severe parkinsonian-like symptoms by 3 days of the regimen, which was reflected by a loss of TH+ cells within the SN and increased neuroinflammation within the striatum. The dosage regime was then further titrated to investigate the effects of 1.5mg/kg/48h rotenone for either 3 or 6 injections. The 6-injection group exhibited mild TH loss in the SN and evidence of microglial activation in the STR, with only minor motor deficits in the neuroscore test, but not in grip strength or the adjusting step test.

Weight Changes and Toxicity Effect in Response to Various Rotenone Dosing Regimen

Our findings suggest a clear dose-dependent relationship between rotenone administration and weight loss in the experimental animals. The lowest dose of 0.5mg/kg of rotenone had minimal effects on weight, whereas the 2.0mg/kg even after three days led to dramatic reductions in weight. In the final pilot, a 48hr interval between injections was introduced with a 1.5mg/kg dose, producing weight fluctuations with decreases on the days following injection followed by an increase, which prevented a dramatic loss in weight. While the studies reporting weight changes and acute toxicity/performance following rotenone injection remain relatively scarce, certain studies have reported weight alterations consistent with our observations. Sherer *et al.*

and Cannon *et al.*, for example, documented significant weight loss following injections ranging from 2.0 to 3.0mg/kg rotenone, accompanied by acute severe systemic toxicity in the animals, which aligned with our findings at the 2.0mg/kg dose (Cannon et al., 2009; Sherer et al., 2003). Additionally, Zhang and colleagues observed similar dose-dependent weight patterns across rotenone concentration of 1.5, 2.0, and 2.5mg/kg, demonstrating weight loss at higher rotenone concentration that reinforced our observations with various rotenone dosing regimens (Zhang et al., 2017). These effects could be induced by rotenone due to the disruption of mitochondrial function, primarily through the inhibition of complex I of the mitochondrial electron transport chain (ETC) responsible for the production of adenosine triphosphate (ATP). Direct inhibition in the ETC leads to reduced energy production, increased reactive oxygen species (ROS) levels, and induced oxidative stress(Greenamyre et al., 2003, 1999). These factors can indirectly influence various pathological processes, including disruption of dopamine levels crucial for motor control and affecting the enteric neuron system in the gastrointestinal tract(Greene et al., 2009; Testa et al., 2005), which is relevant with what we observed in this study.

Assessment of Dopamine Levels in STR and SN Following Rotenone Administration

The 0.5mg/kg and 1.0mg/kg dosing regimens were insufficient to lead to changes with TH expression within the nigrostriatal pathway. This is in line with a previous study that found no significant difference in the number of TH+ cells in the SN region, even after 16 weeks of 1.0mg/kg of rotenone (Bai et al., 2016). Increasing the dose to 2.0mg/kg resulted in a pronounced decrease in the number of TH+ neurons in the SN, but no changes were observed in the TH-positive area in the STR after only 3 days. Previous reports have similarly reported dopaminergic loss within the SN after 15 days to 5 weeks (Sherer et al., 2003; Zhang et al., 2018, 2017), although this was accompanied by changes within the STR as well (Sherer et al.,

2003; Zhang et al., 2017). This may reflect early compensatory changes within the STR in this study that are lost with more extended treatment. A 6*1.5mg/kg/48h rotenone injection regimen was able to replicate the loss of TH+ staining within the SN, without the overt motor symptoms and dramatic weight loss. This finding is consistent with a study by Fathalla *et al.*, which also reported a significant reduction in dopamine levels measured by high-performance liquid chromatography (HPLC) in SN following the same dosing regimen (Fathalla et al., 2016). It is possible that in the animals treated at lower doses with a 48-hour interval between injections and relatively short treatment period, terminal sprouting occurred from surviving dopamine neurons as a response to rotenone exposure that likely help in maintaining some level of functions. Indeed, dopaminergic neurons are highly susceptible to oxidative stress due to their inherent characteristics-high dopamine oxidation rates and low antioxidant defences (Barlow et al., 2005). This dosing regimen could potentially offer a window for mitochondria to repair energy metabolism, enhance mitochondrial integrity, and eventually restore function (Nicolson, 2014).

Neuroinflammatory Responses in the STR and SN Following Rotenone Administration

An increase in the percentage of activated microglia was detected within the STR with doses of 0.5mg/kg and 2.0mg/kg for 7-14 days and 6*1.5mg/kg/48h of rotenone, but not 1.0mg/kg or 3*1.5/mg/kg/48h rotenone doses, indicating a general increase in activation status with increasing rotenone exposure. It is unclear why microglia activation was detected in the 0.5mg/kg group, but not the 1.0mg/kg, which may just reflect the low number of animals in this pilot study. Notably, only the daily dose of 2.0mg/kg led to an increase in IBA1+ cells in the STR, aligning with findings from prior studies (Abdel-Salam et al., 2020; Arab et al., 2021; Singh and Chauhan, 2022). For instance, Abdel-Salam *et al.,* employing a mouse model and subcutaneous injection of 1.5mg/kg rotenone every other day for two weeks, demonstrated

increased NF-kB expression in the STR (Abdel-Salam et al., 2020). Similarly, in a study by Singh *et al.* utilising a rat model and intraperitoneal injection of 1.5mg/kg rotenone every day for 40 days, significantly elevated levels of proinflammatory markers such as IL-6, IL-1β and TNF-α in the STR, were observed with rotenone treatment as compared to the control (Singh and Chauhan, 2022).

Within the SN, an increase in microglial activation was seen with the 1.0mg/kg dose, but only after 7, but not 14 days, as well as with the 2.0mg/kg dose. No change was seen with the 0.5mg/kg dose or when a 1.5mg/kg dose was administered every second day. A similar increase in inflammation in the SN has been found with a 2.0mg/kg rotenone daily injection for 5 weeks, which led to an increase in the number of activated astrocytes and enhanced expression of pro-inflammatory cytokines, including TNF-α, INF-γ, IL-1β, and IL-6 (Thakur and Nehru, 2015; Zhang et al., 2018). The differential response between the SN and STR suggests that the threshold for microglia activation in the SN is higher than in the STR. This regionally distinct microglial response is supported by previous studies that have reported increased microglial reactivity peaked in the STR prior to the SN in the 2.5mg/kg rotenone intraperitoneal injection mice model (Rocha et al., 2022). Conversely, the emergence of reactive astrocytes showed an opposite pattern, with an initial peak in the SN, followed by the STR (Rocha et al., 2022). The reasons underlying these distinct regional responses of glial cells to rotenone exposure require further exploration. However, considering the results from TH staining, it is conceivable that rotenone-induced mitochondrial dysfunction occurs initially in the SN, subsequently triggering an innate immune response and loss of dopaminergic neurons within this region.

Functional assessment following rotenone administration in Pilot 3

Despite observing a decrease in TH+ cells in the SN and an increase in neuroinflammation in the STR following the 6*1.5mg/kg/48h rotenone administration, the impact on various behavioural assessments was inconsistent. Interestingly, only the neuroscore showed ccnsistent impairment in the rotenone-treated group, which could primarily be attributed to difficulties in motor coordination, balance, and muscle strength, as assessed by the inclined platform task and the horizontal bar task. These findings are in line with previous studies that reported impaired motor coordination and weakened muscle strength following rotenone administration (Arab et al., 2021; Singh and Chauhan, 2022; Zhang et al., 2018). However, there are discrepancies between our study and previous research, such as the lack of significant grip strength changes observed in our study compared to others, which could be attributed to differences in treatment duration, injection intervals and delivery route, as the previous study employed 40 days daily intraperitoneal injection of 1.5mg/kg rotenone compared to our 6 subcutaneous injections in 48 hours interval (Singh and Chauhan, 2022).

Previous studies have suggested that chronic exposure to rotenone can lead to gastrointestinal dysfunction seen in PD (Drolet et al., 2009). In our study, the constipation assessment revealed that fecal weight was more variable in the rotenone-treated group compared to the vehicle group. Consistently, the rotenone group exhibited higher fecal weight across all time points examined. However, it is important to interpret these results with caution due to the limitation of not examining the dry weight of the fecal material. Assessing constipation based solely on total fecal weight may not provide a precise measure of gastrointestinal function. To obtain a more accurate evaluation of constipation, it is necessary to consider additional parameters such as stool consistency, frequency of defecation, and examination of the dry weight of the fecal matter. Further study is needed to fully probe these effects.

In conclusion, our findings suggest that the optimum dosage for establishing a rat model with underlying vulnerability in the dopaminergic system, ongoing neuroinflammation, and the absence of overt parkinsonian motor symptoms is 6*1.5mg/kg/48h injection of rotenone in male Sprague-Dawley rats. Importantly, this dosing regimen did not result in significant adverse effects, and weight loss remained within 0-5% without worsening over the dosing period. Furthermore, this dosing regimen successfully replicated subtle motor deficits, induced approximately 20% dopaminergic cell loss in the substantia nigra, and triggered ongoing inflammatory responses in the local region, particularly in the striatum. This pilot study provides a valuable tool for studying the early stages of PD and offers a unique opportunity to investigate the synergistic effects of subthreshold pesticide exposure and TBI, thus potentially providing valuable insights into the development of PD following TBI.

05

Modelling the "Two-Hit" Hypothesis: Characterisation of the Synergistic Effect of Low-Level Rotenone Exposure and Moderate-Severe Diffuse Traumatic Brain Injury at One-Month Post-Injury

Chapter prepared by **WEE, IC.**, CHEUNG, M., EDWARD, S.E., CORRIGAN, F., COLLINS-PRAINO, L.E

Statement of Authorship

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Publication Details	NA

Principal Author

Name of Principal Author (Candidate)	Ing Chee Wee		
Contribution to the Paper	Conducted behavioural tests, performed analysis of behavioural data, intepreted data and wrote manuscript		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	18/12/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Marissa Wing Ki Cheung		
Contribution to the Paper	Conducted behavioural tests, helped in animal model generation		
Signature		Date	

Name of Co-Author	Samantha Elizabeth Edwards		
Contribution to the Paper	Conducted behavioural tests, helped in animal model generation		
Signature		Date	18/12/23

Please cut and paste additional co-author panels here as required.

Name of Co-Author	Frances Corrigan		
Contribution to the Paper	Supervised the work, provided assistance with data interpretation and statistical analysis, edited the manuscript.		
Signature		Date	18/12/2023

Name of Co-Author	Lyndsey Collins-Praino		
Contribution to the Paper	Conceptualised and supervised the study, provided assistance with data interpretation, edited the manuscript		
Signature		Date	18/12/23

Please cut and paste additional co-author panels here as required.

ABSTRACT

Traumatic Brain Injury (TBI) has been associated with an increased risk of developing Parkinson's Disease (PD) in later life. However, the varying susceptibility of individuals to develop PD following TBI has prompted investigations into potential modifying factors. Previous research in preclinical models and clinical settings has indicated that the combination of TBI with subthreshold paraquat exposure can induce dopaminergic neurodegeneration and exacerbate the risk of developing PD. Despite that, the progression of functional deficits from these risk factors to PD remains unclear. Therefore, this study aimed to examine the functional changes following low-dose rotenone exposure and TBI, both independently and in combination, at 1 month following injury. Forty-seven male Sprague-Dawley rats were administered six injections of either vehicle or rotenone (1.5mg/kg/48h, s.c) before being subjected to either sham surgery or diffuse moderate-severe TBI using Marmarou's impact acceleration model. At 4 weeks post-injury, they were tested on a functional battery encompassing motor, anxiety, cognition and prodromal-PD related symptoms. Results revealed that TBI animals previously exposed to rotenone exhibited significant impairments in motor coordination during the beam walking test ($p<0.001$) and demonstrated learning deficits ($p<0.05$) in the Barnes maze acquisition phase. Moreover, observations of prodromal symptoms showed this dual-exposure animal model exhibited lower defecation frequency (Chi-square test, $p<0.0001$). Some main effects of injury or rotenone were observed, but they were restricted to the motor subsets. These findings underscore the potential synergistic effects of TBI and rotenone exposure on functional outcomes related to PD pathophysiology. Further investigation is warranted to elucidate the underlying mechanisms and long-term implications of these findings, particularly in relation to the development of PD.

1.0 INTRODUCTION

Traumatic brain injury (TBI) is a complex and multifaceted condition resulting from rapid movement of the head leading to brain displacement within the skull, such as that seen in sports injuries, falls, motor vehicle accidents, violence, and combat injuries (Prins et al., 2013). It is an increasingly common health concern and socioeconomic problem, which affects more than 69 million people worldwide and accounts for approximately 52,000 deaths a year in the United States (Dewan et al., 2019; Faul et al., 2010). Following a TBI, individuals can experience a wide range of symptoms that vary in severity and duration (Khan et al., 2003). These symptoms can affect different aspects of a person's functioning, including motor, cognitive, emotional, and behavioural domains (Draper and Ponsford, 2008; Jorge and Robinson, 2003; Till et al., 2008; Walker, 2007). Once thought of as an acute event, emerging evidence suggests that TBI is not simply an isolated event, but rather an ongoing disease process that may contribute to the development of neurodegenerative disorders, such as Parkinson's disease (PD) (Brett et al., 2022; Gardner et al., 2015; Gardner and Yaffe, 2015).

Over the past 35 years, at least 10 epidemiologic studies have consistently reported associations between head injury and an elevated risk of PD (Bower et al., 2003; Gardner et al., 2018; Gardner and Yaffe, 2015; Goldman et al., 2012, 2006; Seidler et al., 1996; Smargiassi et al., 1998; Stern, 1991; Taylor et al., 1999; Tsai et al., 2002). A meta-analysis conducted in 2013, which included 22 studies, revealed a higher risk (hazard ratio =1.57, 95% CI=1.35-1.83) of developing PD among individuals with a history of TBI (Jafari et al., 2013). This contrasts with a later meta-analysis conducted in 2018, encompassing 18 studies, which found much smaller effect size (Odds ratio=1.19, 95% CI=0.5-2.84) of increased PD risk in individuals with previous TBI compared to those without TBI (Huang et al., 2018). Such contradictory findings could be attributed to differences in the definition of head injury, the inclusion of study participants, or other methodological considerations. Alternatively, they could reflect the

presence of differential PD susceptibility among individuals with TBI, underscoring the likelihood of interactions between a history of TBI and other risk factors in the disease progression, which aligns with the "multiple hit hypothesis" of neurodegenerative disease (Patrick et al., 2019; Sulzer, 2007).

Pesticide exposure, including substances like rotenone and paraquat, has also garnered considerable attention for its association with PD (Dick, 2006; Tanner and Goldman, 1996). Individuals who reported rotenone use had a 2.5 times higher risk of developing PD compared to non-users (Tanner et al., 2011). Similarly, a case-control study conducted in the Central Valley of California revealed that individuals residing within 500 meters of pesticide-sprayed fields had a 75% higher risk of developing PD (Costello et al., 2009). Most importantly, a population-based control study conducted in predominantly rural agricultural regions demonstrated that the risk of developing PD was nearly tripled when TBI was accompanied by ambient paraquat exposure (adjusted odd ratio=3.01, 95% CI=1.51-6.01) (Lee et al., 2012). Preclinical work has further demonstrated exacerbated loss of dopaminergic neurons within the substantia nigra (SN), the pathognomonic hallmark of PD, in TBI animals exposed to paraquat compared to those with TBI alone (Hutson et al., 2011).

Thus, could these two variables synergistically contribute to an increased risk of developing PD? Preliminary evidence from the literature supports this hypothesis, revealing an elevated risk of PD and greater loss of dopaminergic neurons in the SN (Hutson et al., 2011; Lee et al., 2012). However, notable gaps persist in the literature, particularly in understanding how the ambient pesticide could influence the pattern of functional disorders post-injury and the precise interplay and specific events that occur following these combined environmental exposures, shaping the progression of PD symptoms.

Given these gaps, in this study, we sought to examine both the individual and synergistic effects of head trauma and subthreshold pesticide exposure using the rotenone rat model established

in the previous chapter. A comprehensive behavioural battery encompassing various domains, including both motor function and cognitive ability, was employed at 1 month following the injury to investigate the impact of TBI+rotenone, in comparison to either TBI or rotenone exposure alone, on the progression of key symptoms associated with PD. The choice of a one-month time point was influenced by prior research, particularly the work conducted by De Oliveira *et al.*, where they investigated increased susceptibility to motor and neurological functional deficits 1 month after moderate TBI and 6-OHDA inductions (De Oliveira et al., 2017). Of particular note, the current battery also assessed the presentation of some of the key prodromal symptoms associated with PD; namely, olfactory dysfunction (Ponsen et al., 2004), constipation (Abbott et al., 2001) and anxiety (Postuma and Berg, 2019). By delving into these aspects, we aim to contribute valuable insights into the potential interplay between head trauma, pesticide exposure, and the emergence of symptoms related to PD, paving the way for a more comprehensive understanding of these complex interactions.

2.0 MATERIALS AND METHOD

2.1 Animals

Forty-eight male Sprague Dawley rats (9-10 weeks old, 400-450g) were used under the approval of the University of Adelaide Animal Ethics Committee (M-2022-004). Animals were housed in groups of three per cage and raised under conventional laboratory conditions (12 h light/dark cycle, temperature 22±2 °C, humidity 60±5%), with food and water provided ad libitum. Animals were acclimatised for 1 week before starting the study. After acclimatisation, animals were randomly allocated into 4 groups: (1) vehicle injection and sham surgery (Veh+Sham, n=12), (2) vehicle injection and TBI surgery (Veh+TBI, n=12), (3) rotenone injection and sham surgery (Rot+Sham, n=12), and (4) rotenone injection and TBI surgery (Rot+TBI, n=12) (Fig.1). Animals underwent a comprehensive functional battery assessing motor, cognitive, neuropsychiatric function at 1-month post-injury.

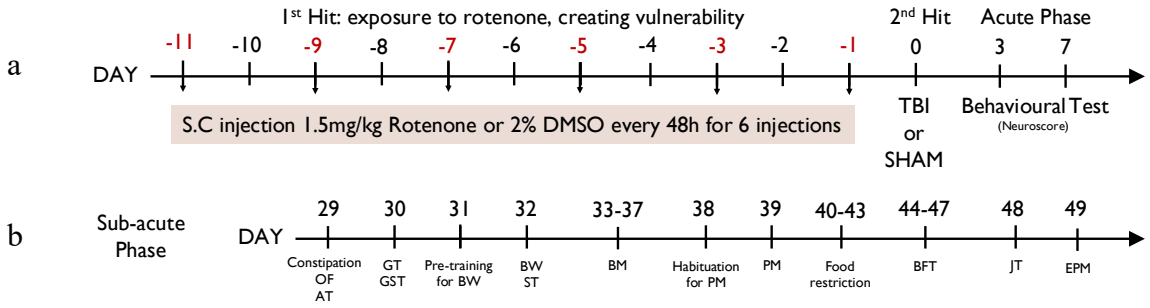


Figure 1. A scheme describing the study design. (a) Rats were divided into groups and received either subcutaneous vehicle or 1.5 mg/kg rotenone injections every 48 hours for a total of 6 injections. The days of injection were indicated by arrows and red fonts. On day 0, the rats underwent either TBI or Sham surgery, depending on their assigned group. Following the injury, the rats underwent neuroscore testing during the acute phase at 3- and 7-days post-injury (dpi). (b) Subsequently, a comprehensive behavioral test was conducted during the sub-acute phase from 29 to 45 dpi. (b) The testing battery included various assessments such as the adhesive removal test (AT), pasta manipulation test (PMT), forelimb adjusting step test (ST), grip strength test (GST), gait test (GT), elevated plus maze (EPM), open field (OF), beam walking (BW), Barnes maze (BM), buried food test (BFT), constipation test, and jaw tremor test (JT).

2.2 Subcutaneous administration of rotenone

75mg rotenone (Sigma Aldrich, R8875) was suspended in 1ml of Dimethyl Sulfoxide (DMSO, Chem-supply, DA013), then diluted 1:50 in 100% pure sunflower oil (Crisco, Australia) to make up the 1.5mg/ml rotenone dosage in 2% DMSO sunflower oil. Vehicle was made by

mixing 1mL of DMSO with 49mL sunflower oil. Solutions were vortexed thoroughly before the injection to ensure a uniform suspension. This rotenone regimen consisted of six subcutaneous injections of rotenone (1.5mg/kg/48h) in a volume of 1ml/kg, with the dosing regimen based on the extensive piloting presented in Chapter 4. Animals received the first rotenone injection 11 days before the brain injury and every 48 hours following the first rotenone injection (Fig. 1). This regimen did not result in any mortality over the course of rotenone administration.

2.3 Moderate-severe diffuse weight drop injury

Briefly, animals were injured with the Marmarou impact-acceleration injury method (Marmarou et al., 1994), an injury protocol extensively validated for the diffuse injury model (C.Marmarou et al., 2009). Animals underwent anesthetic induction via inhalation of 2-3% isoflurane under normoxic conditions for 5 minutes. They were subsequently intubated, mechanically ventilated and maintained on 1-2% isoflurane throughout. A midline incision was made to facilitate the placement of a metal disc centrally between lambda and bregma. Animals assigned to TBI surgery were then transiently taken off ventilation and strapped onto a foam bed, with injury induced by releasing a 450 g brass weight from a height of 2 m down a clear tube onto the centre of the metal disc. Contact was observed to ensure a single, direct impact without a rebound hit. Following the impact, animals were promptly returned to the surgical stage for manual resuscitation using a resuscitation balloon if independent breathing was hindered. No mortality was noted in the Veh+TBI, Veh+sham or rotenone+sham groups, with two animals lost from the Rot+TBI group over the course of the study.

2.4 Body weight

The body weight of each animal was measured every day prior to the rotenone/vehicle injection from day -11 to day -1. On the surgery day (day 0), the weight was measured before performing the surgery, as well as on days 1-7, and 14,21,28. As rotenone produced weight loss, animal weights ranged from 441 to 612g in vehicle-administered animals compared to 364 to 528g in rotenone-administered animals at the time of TBI. While variations in weight can influence factors like metabolism and physical impact tolerance (List et al., 2013; Marques Miranda et al., 2021), it's worth noting that following the cessation of rotenone administration, rotenone-exposed animals showed a gradual weight increase. Despite initial differences in weight between rotenone-exposed and vehicle-exposed animals at the time of TBI, there was no significant weight disparity observed in animals exposed solely to rotenone compared to sham group on day 14 post-rotenone cessation. This suggests a rapid recovery from the initial weight loss induced by rotenone, indicating that the variability in study outcomes is unlikely to be solely attributed to rotenone-induced weight-loss. It’s possible that larger animals may experience less injury than smaller animals, however, this pattern aligns with the nature of rotenone exposure, where prior exposure could potentially render individuals weaker and more vulnerable to injury. Percentage changes in body weight at these time points were calculated based on their weight at day –11.

2.5 Behavioural battery

Composite neuroscore was tested acutely at 3- and 7-days post injury (dpi), and a thorough functional battery assessing motor function, cognition, anxiety and prodromal PD symptoms was performed during the subacute phase (Fig.1). All tests at each time point were carried out within the 12-hr-light cycle. Behavioural tests administered between 29-49 dpi were completed in the following order: constipation test, open field (OF), adhesive tape removal test (AT), grip strength test (GST), gait test (GT), forelimb adjusting step test (ST), beam walking (BW),

Barnes maze (BM), pasta manipulation test (PMT), buried food test (BFT), jaw tremor test (JT) and elevated plus maze (EPM) (Fig. 1b). Notably, functional data of OF, BM and EPM were recorded and analysed through the ANY-maze Video Tracking System version 4.99m (Stoelting Co.). On the other hand, the analysis of the recorded video in the PMT and BW was conducted by using Cowlog 3. The experimenter was blinded to the experimental groups of each animal throughout the duration of the study, with unblinding only occurring after completing the analysis of the tests.

2.5.1 Composite Neuroscore

Neurological function was evaluated using the 28-point composite neuroscore, which was adapted from previous studies on motor function impairment following TBI in rat (McIntosh et al., 1989; Nissinen et al., 2017; Zhang et al., 2005). The neuroscore was comprised of 11 tests with a cumulative maximum score of 28, including (1) circling (maximum four points); (2) motility (maximum three points); (3) general condition (maximum three points); (4) righting reflex (maximum one point); (5) paw placement (maximum four points); (6) horizontal bar (maximum three points); (7) inclined platform (maximum three points); (8) grip strength (maximum two points); (9) contralateral reflex (maximum one point); (10) contralateral rotation when held by the base of tail (maximum two points); and (11) visual forepaw reaching (maximum two points). A neuroscore of 0 indicated significant neurological impairment, whereas a cumulative score of 28 indicated healthy functioning.

2.5.2 Jaw Tremor Test

The jaw tremor test (JT) assesses tremors or abnormal movements in rodents' jaw regions (Salamone et al., 2013). Animals were placed individually on a mesh stage enclosed within a transparent box to allow for unobstructed observation of their behavior. A total of three 5-

minute trials were conducted for each animal. During these trials, trained assessors visually observed and recorded the frequency of tremors in the jaw area using a handheld tally counter. The mean number of jaw tremors was obtained by averaging across the three observation periods.

2.5.3 Forelimb Adjusting Step Test

The forelimb adjusting step test (FST) was used to assess motor coordination and sensorimotor integration. In brief, the animal's body and hindlimbs were wrapped in a surgical drape and held in one hand by the experimenter, slightly raising it above the surface. The other hand fixed the forelimb that was not to be monitored in the drape. The rat was then held with one paw touching the table and moved slowly sideways (1m) by the experimenter, first in the left-to-right and then in the right-to-left direction. The number of adjusting steps was counted for both paws in each direction of movement.

2.5.4 Beam Walking

The BW task was conducted to assess balance and motor coordination in rodents by evaluating their ability to navigate a black acrylic beam 1.6m in length and tapering in width from 6cm to 1.5cm, with underhanging ledges of 1cm width on both sides The beam was suspended approximately 60cm above the ground and positioned at a 30^{o} angle, such that the narrow end was at the highest point of the incline. Animals were placed on the wider end of the beam and required to walk up the incline to the narrow end, where an enclosed box was positioned. Prior to the actual test, animals underwent training to ensure they could successfully traverse the beam without stopping in the middle. On the test day, rats performed three consecutive trials, spaced 1 minute apart, and were recorded using video cameras positioned on both sides of the

beam. The latency to transverse the beam, the number of steps taken, and the number of foot faults, including slips and misses, were analysed.

2.5.5 Gait Test

The GT was utilised to examine and evaluate the animals' walking patterns and coordination. The test was performed in a transparent plexiglass walkway, measuring 90cm in length, 8 cm in width, and 12cm in height, with a white paper line at the bottom. During the trial, the walkway was exposed to bright light, and a dark box was placed at one end as a target. To facilitate tracking and analysis, the animals' paws were first coated with non-toxic acrylic paint, with a different colour for each paw. The animals were then placed at the starting point of the walkway and allowed to walk towards the dark box. Stride length, stance length, stance wide, forelimb base of support (BOS) and hindlimb BOS were calculated.

2.5.6 Adhesive Removal Test

The AT was used to evaluate sensorimotor function and fine motor control. It involved measuring the latency-to-contact, latency-to-remove, and latency-to-remove-after-contact of a piece of adhesive tape (8mm x 8mm) attached to the animal's forepaw. Each trial had a maximum duration of 2 minutes, and if the tape remained on the paw, the trial was terminated. The test was repeated three times for each paw, resulting in a total of six trials, and the average latency times were calculated.

2.5.7 Pasta Manipulation Test

The PMT was conducted to assess forepaw movements, following the protocol outlined by Tennant et al. (2010). Capellini pieces (7 cm long, marked at 1.75 cm intervals) were provided to the animals in their home cage for up to 7 days before the testing phase. The afternoon prior

to the test, the animals were habituated to the Plexiglass testing chamber for at least 30 minutes, and food was removed from their home cage overnight to increase their motivation for eating the pasta. On the test day, the animals were placed in a Plexiglass cage positioned on top and in front of a bi-folded mirror with dimmed lighting. They were given an additional 10 minutes to habituate to the environment before the pasta exposure began. During each trial, a pasta piece was dropped into the cage, and recording commenced until the pasta was consumed. This process was repeated for a total of 3 trials. The time to consume each pasta and the number of adjustments made during pasta handling were recorded.

2.5.8 Grip Strength Test

Forelimb muscle strength was determined by measuring peak force (in gram-force) using the digital grip strength meter equipped with a hind limb pull bar assembly (Ugo Basile, Italy). Animals were allowed to grip the metal grids connected to a force transducer with their forepaws and were gently pulled backwards by the body and the base of the tail until they could no longer hold the grids. Each animal was given five consecutive trials, and the average value was taken from the three highest peak force readings for further analysis.

2.5.9 Open Field Test

The OF is a standard test of locomotor activity. Animals were positioned in the central area of a spacious square box measuring 95cm x 95cm, with walls at a height of 44.5cm. The distance travelled by the animals during a 5-minute period was quantified to assess locomotor function. Additionally, the duration spent in the centre of the field was measured as an indicator of anxiety-like behaviour.

2.5.10 Elevated Plus Maze

The elevated plus maze (EPM) is a common test in anxiety-related studies(Kraeuter et al., 2019; Walf and Frye, 2007). Animals were positioned in the centre of an elevated (50cm from the floor) cross-shaped maze consisting of two open arms and two closed arms, each measuring 50cm long and enclosed by walls 40cm tall. The animals were allowed to explore the maze for a duration of 5 minutes. The total distance travelled, the time spent in the open arms, and the number of open arms entries determined by the centre point of the animal's body were recorded.

2.5.11 Barnes Maze

The BM evaluates spatial learning and memory in rodents. The maze is elevated ~10m from the floor and consists of a circular black platform (1.2m in diameter) with 18 evenly spaced holes along its edges. One hole is pre-determined as the escape hole, with a black escape box positioned beneath it. The Barnes maze test spanned five days, including three acquisition days, a rest day, and a probe day. During the acquisition days, rats underwent two trials spaced 15 minutes apart. They were placed in the centre of the maze, which was exposed to strong light, and the time it took for the animals to find and enter the escape box was recorded. On the fifth day, the escape box was relocated to a different hole, and two trials were conducted 30 minutes apart. In the first trial, the time taken for the rats to reach the previous location of the escape hole was measured. In both trials, the time to locate and enter the newly relocated escape box was recorded as an indicator of cognitive flexibility. Additional cognitive parameters were assessed during the second trial of the probe day; these include the number of revisits to the previous escape hole location, working memory errors (measured by revisiting the same hole after exploring less than three different holes), and reference memory errors (measured by visiting any hole other than the escape hole).

2.5.12 Constipation Test

Animals were temporarily placed in clean plastic cages without access to food or water for a period of one hour. Stools were promptly collected after expulsion and sealed in tubes for analysis. Following the trial, the total wet weight of the collected stools was measured and recorded. Subsequently, the stools were left to dry overnight in a 65°C oven and weighed again to determine the dry weight. The fecal moisture percentage was calculated by subtracting the dry weight from the wet weight, dividing it by the wet weight, and multiplying the result by 100. Animals that failed to produce fecal matter during the test period were excluded from the analysis.

2.5.13 Buried Food Test

The BFT was employed to evaluate the animals' ability to retrieve a buried food reward, which relied on their intact sense of smell. Prior to the test, the animals underwent a gradual food restriction over the course of 4 days until they reached ~85% of their free-feeding weight. On the testing day, a peanut cookie (~10g) was buried 0.5 cm below the fluffy bedding to make it invisible. The time taken for the animal to uncover the pellet and begin consuming it was recorded, with a maximum time limit of 5 minutes. The task was performed daily for three consecutive days. On the fourth testing day, a surface food trial was conducted to assess the animals' motivation to retrieve the food reward.

2.6 Statistical analysis

Behavioural data were primarily analysed using two-way ANOVA to compare the mean values among the injury vs no injury groups under two different treatment conditions (vehicle or rotenone exposure). A three-way ANOVA or mixed effect analysis was conducted in cases where evaluations were needed for different time points (such as percentage weight gain and loss, neuroscore, buried food test, and Barnes maze). Pairwise comparisons between groups

were carried out using Tukey's multiple comparisons. A significance value of $p \leq 0.05$ was set for all statistical tests, and all results are presented as mean±SEM (standard error of the mean). Outlier removals were performed using IBM SPSS® Statistics (version 23.0), and data analysis and graphical representation were conducted using GraphPad Prism (version 9.0.0).

3.0 RESULTS

3.1 Exposure to rotenone affects normal weight gain

Significant main effects for rotenone ($F_{1,462}$=1305, p<0.0001), TBI ($F_{1, 484}$ = 6.737, p=0.0097) and time ($F_{21,484}$ = 107.1, p<0.0001), as well as significant interactions between time x rotenone ($F_{21,462}$ = 15.87, p<0.0001), time x TBI ($F_{21,484}$ = 2.175, p=0.002), TBI x rotenone ($F_{1,462}$ = 17.43, p<0.0001) were found for percentage of weight gain (Fig. 2a). There was no three-way interaction time x TBI x rotenone ($F_{21,462}$ = 0.9868, p=0.48). Vehicle animals showed 8.90±2.17% weight gain during the pre-TBI treatment period (day -11 to day -1). In contrast, rotenone-injected animals did not gain weight (1.67±4.7%), with significant differences observed from day -7 (p<0.01) to day -1 (p<0.0001) compared to vehicle groups (Fig.2a). Following TBI, both Veh+TBI and Rot+TBI animals demonstrated a 4-7% loss compared to their previous day's weight, although both groups were no longer significantly different from their pre-injury baseline by day 7 following injury (Veh+TBI- pre-injury:10.36±2.7%; 7dpi:11.53±3.7%) (Rot+TBI-pre-injury: -1.25±4.1%; 7dpi: -1.19±3.95%). Although rotenone animals showed weight gain following cessation of the rotenone injections, as the Rot+TBI animals were injured at a lower weight than the Veh+TBI animals, they still showed significantly less weight gain until 28dpi (18.96±6.94% vs 26.34±6.71%, p<0.001). In contrast, Rot+sham animals were no longer significantly different from Veh+sham animals by day 14 (10.43±5.95% vs 15.77±3.27%, p=0.199) (Fig. 2A).

3.2 Prior rotenone exposure aggravates post-TBI neurological impairment during the acute phase

To determine whether prior rotenone exposure in our diffuse injury model of TBI induced neurological impairment during the acute phase, we first examined neurological motor function with neuroscore at 3 and 7 dpi (Fig.2B). A main effect of pre-exposure to rotenone ($F_{1,38}$=24.26,

p<0.0001), TBI ($F_{1,\ 44}$ = 90.98 p<0.001) and time ($F_{1,44}$ = 7.66, p=0.008) was found. Additionally, notable interactions emerged between TBI and rotenone ($F_{1,38}$ = 24.26, p<0.0001). However, no discernible effects were found for time x TBI ($F_{1,44}$ = 3.5, p=0.068), time x rotenone ($F_{1,38}$ = 1.31, p=0.26), or time x TBI x rotenone ($F_{1,38}$ = 2.48, p=0.124) (Fig. 2B). Further post-hoc analysis revealed significant differences in the Veh+Sham animals (26(23-28) pt) compared to the Veh+TBI groups [23(16-26) pt, p<0.001] and Rot+TBI [19 (12-28)pt, p<0.0001] groups. Similarly, significant distinctions were apparent between Rot+Sham [26(23-28)pts] and the two TBI groups (Veh+TBI, p<0.01, Rot+TBI, p<0.00010). Furthermore, the combination of Rot+TBI made the animals perform worse in comparison to Veh+TBI animals (p<0.0001), with this effect not observed between shams (Fig. 2B).

3.3 Behavioural Outcomes at One-Month Post-Injury-Motor function

Motor function was assessed using the beam walking task for balance and motor coordination, jaw tremor test and forelimb adjusting step test for voluntary movement, distance travelled in OF and EPM for locomotor activity, and gait test for walking pattern (Fig. 3-6).

3.3.1 Gross motor function

Locomotor activity was assessed via distance travelled, which showed no main effect of TBI ($F_{1,\ 43}$= 0.06, p=0.81) or rotenone ($F_{1,\ 43}$= 0.029, p=0.87), nor an interaction ($F_{1,\ 43}$= 1.44, p=0.24) on the OF (Fig.3A) or in the EPM (rotenone effect: $F_{1,\ 43}$= 0.092, p=0.76, TBI effect: $F_{1,\ 43}$= 0.023, p=0.88; interaction: $F_{1,\ 43}$= 3.79, p=0.058) (Fig.3B).

3.3.2 Parkinsonian symptoms

In the evaluation of parkinsonian symptoms through the jaw tremor test, a significant main effect of rotenone ($F_{1,42}$= 5.525, p=0.023), but not TBI ($F_{1,42}$=3.858, p=0.056), was found, with no interaction of rotenone x TBI ($F_{1,42}$=0.015, p=0.903). This was driven by higher jaw tremor values in animals pre-exposed to rotenone compared to vehicle animals (47.32±24.3 vs

32.6±20.49) (Fig. 4A). In contrast, for the forelimb adjusting step test, a measurement of akinesia, a significant main effect of TBI was noted ($F_{1, 41}$= 8.154, p=0.0067), but not rotenone ($F_{1, 41}$= 1.495, p=0.2284), with no interaction ($F_{1, 41}$= 2.956, p=0.093). This was driven by a lower number of adjusting steps in TBI compared to sham animals (5.71±1.45 vs 6.63±0.73 steps) (Fig. 4B).

3.3.3 Balance, motor coordination and gait

At 29 days following the injury, the latency to transverse the beam did not exhibit any significant main effects attributed to TBI ($F_{1,43}$ = 0.415, p=0.523), rotenone ($F_{1,43}$ = 0.4236, p=0.519), or rotenone x TBI interaction ($F_{1,43}$ = 0.356, p=0.554) (Fig. 5A), but there was a significant difference in the percentage of foot slips (Fig. 5B). A two-way ANOVA revealed a main effect of pre-exposure to rotenone ($F_{1,43}$ = 13.03, p=0.0008), TBI ($F_{1,43}$ = 23.47, p<0.0001) and rotenone x TBI interaction ($F_{1,43}$ = 12.27, p=0.0011). Subsequent post-hoc analysis indicated that animals in the Rot+TBI group produced a significantly higher percentage of errors on the beam compared to other groups (32.88±6.06% vs 15.44±5.09%, 15.66±8.17% and 18.21±8.1%, all p<0.0001 vs Veh+Sham, Rot+Sham and Veh+TBI, respectively).

In contrast, in the gait test, the forelimb BOS demonstrated a significant TBI effect ($F_{1, 40}$= 7.032, p=0.01), but no significant rotenone effect ($F_{1, 40}$= 0.17, p=0.69), nor interaction between the two ($F_{1, 40}$= 0.004, p=0.95) (Fig. 5G). TBI animals showed narrower forelimb BOS than sham animals (3.08±0.7cm vs 3.57±0.44cm). No significant main effects of TBI, rotenone or interaction were noted for any other gait parameter, including stride length, stance length, stance width or hindlimb base of support (Fig. 5C-E, H, Supplementary Table 1).

3.3.4 Fine motor control and grip

To measure fine motor control, the AT and PMT were employed to evaluate fine movement and dexterity. Notably, no significant main effects or an interaction were observed in the AT test, encompassing latency to contact (rotenone effect: $F_{1, 37}$= 1.94, p=0.17; TBI effect: $F_{1, 37}$=

0.624, p=0.43; interaction: $F_{1, 37}$= 0.54, p=0.47) (Fig. 6A), latency to remove (rotenone effect: $F_{1, 43}$= 0.41, p=0.52; TBI effect: $F_{1, 43}$= 1.56, p=0.22; interaction: $F_{1, 43}$= 0.21, p=0.65) (Fig. 6B) and latency to remove after contact (rotenone effect: $F_{1, 42}$= 1.0, p=0.32; TBI effect: $F_{1, 42}$= 0.77, p=0.39; interaction: $F_{1, 43}$= 0.32, p=0.57) (Fig. 6C).

Similarly, within the PMT, the time taken to consume the pasta and atypical handling patterns revealed no significant main effects or interaction (Fig. 6D, F, Supplementary Table 1). However, a significant main effect of TBI was found for the number of adjustments ($F_{1, 34}$ = 8.28, p = 0.007), whereas rotenone did not show a significant effect ($F_{1, 34}$ = 0.26, p = 0.62), with no significant interaction between the two ($F_{1, 34}$ = 2.42, p = 0.13). TBI-subjected animals exhibited fewer adjustments compared to the sham groups (13±6.99 vs 19.47±7.44 adjustments) (Fig. 6E).

Furthermore, in the grip strength analysis, which evaluated grip and muscle strength based on the three highest values, a significant main effect of TBI was observed ($F_{1, 43}$= 10.38, p=0.002), whereas no substantial effect was observed due to rotenone ($F_{1, 43}$= 3.17, p=0.08), and no significant rotenone x TBI interaction ($F_{1, 43}$= 0.46, p=0.50) was detected. Notably, animals subjected to TBI exhibited reduced strength compared to those in the sham group (327.53±106.88 vs 414.24±81.15gf) (Fig. 6G).

3.4 Behavioural Outcomes at One-Month Post-Injury -Non-motor symptoms

Non-motor symptoms examined included anxiety-like behaviour in the OF and EPM, cognitive function through BM analysis, olfaction via the BFT and constipation with fecal water content.

3.4.1Anxiety-like behaviour

The OF and EPM assess different anxiety stimuli in the animal, with the OF assessing anxiety over open spaces and the EPM assessing anxiety over open spaces and height (Walf and Frye, 2007). No main effects of TBI or rotenone nor an interaction between the two were found for

time in the centre of the OF, time in open arms of the EF, or number of arm entries (Fig 7, Supplementary Table 1)

3.4.2 Cognition

Cognitive outcomes were evaluated using the BM for spatial learning, memory (reference and working memory), and cognitive flexibility (ability to reprogram previously learned tasks) (Barnes, 1979). Throughout the acquisition phase, all experimental groups exhibited significant learning (Trial effect: $F_{2,111} = 7.79$, $p=0.0007$), as evidenced by the decrease in escape latency from day 1 to 2 ($p < 0.001$) and from day 1 to 3 ($p < 0.0001$). The three-way ANOVA analysis revealed no significant main effect in terms of rotenone ($F_{1,111} = 2.74$, $p=0.1$), TBI ($F_{1,111} = 0.69$, $p=0.41$), rotenone x trial ($F_{2, 111}= 0.85$, $p=0.43$), TBI x trial ($F_{2, 111}= 2.27$, $p=0.11$) or trial x TBI x rotenone ($F_{2, 111}= 0.69$, $p=0.51$). However, interestingly, a significant interaction between rotenone x TBI ($F_{1,111} = 9.2$, $p=0.003$) was observed. This was driven by an overall significant difference in performance in the Veh+TBI vs Rot+TBI animals ($p<0.05$), with no other significant differences between groups (Fig. 8a). This pattern is further elucidated in the heat map, wherein the sham groups demonstrated a faster and more accurate spatial memory, evidenced by their direct navigation towards the escape box on days 2 and 3 compared to the more exploratory pattern on day 1. Conversely, the Rot+TBI group demonstrated a consistent exploratory pattern across all three acquisition days, indicative of limited improvement in their spatial memory (Fig. 9).

By trial 1 of the probe day, all animals were able to find the old escape box successfully, with no main effect of rotenone ($F_{1, 43}= 0.17$, $p=0.68$) or TBI ($F_{1, 43}= 0.61$, $p=0.44$), nor interaction between two ($F_{1, 43}= 0.19$, $p=0.67$) (Fig. 8B). Similarly, for cognitive flexibility, indicated by the time taken to find the new escape box on the probe day. there was no significant main effect of either TBI ($F_{1, 78}= 0.07$, $p=0.8$) or rotenone exposure ($F_{1, 78}= 0.59$, $p=0.45$), nor were there any significant interactions (trial x TBI x rotenone ($F_{1, 78}= 0.252$, $p=0.87$); trial x TBI ($F_{1, 78}=$

0.4, p=0.53); trial x rotenone ($F_{1, 78}$= 0.03, p=0.87); TBI x rotenone ($F_{1, 78}$= 0.49, p=0.87). As would be expected, however, a significant main effect of trials ($F_{1,78}$=14.39, p<0.001) was observed, with animals improving their escape latency from trial1 to trial 2 (Fig. 8C). No further effects were found for revisits to the old escape box location, reference memory error or working memory error during trial 2 on the probe day (Fig 8D-F, Supplementary table 1). This is further illustrated by the construction of a heat map, where all treatment groups showed similar navigation patterns and average time spent in each part of the Barnes maze across the two trials (Fig. 10).

3.4.3 Prodromal PD symptoms

Constipation was evaluated by analysing the weight of fecal samples, which were subsequently dried overnight to determine water content—an indicator of constipation. Throughout the trial, a subset of rats failed to defecate within the 1-hour test period. Specifically, this included three out of twelve Veh+Sham rats (25%), two out of twelve Veh+TBI rats (17%), and four out of eleven Rot+TBI rats (36%). A chi-square test revealed a significant association between frequency of defecation and treatments (Chi-square, df= 43.89, 3; p<0.0001) (Fig. 11A). In the measurement of total fecal weight, no discernible changes were observed among the groups, nor any main effects of rotenone ($F_{1, 35}$= 0.008, p=0.93), TBI ($F_{1, 35}$= 0.47, p=0.5) and interaction ($F_{1, 35}$= 0.21, p=0.65) (Fig.11B). However, a significant interaction was found for rotenone x injury ($F_{1, 32}$= 4.19, p=0.049) during the assessment of the percentage of water content, although posthoc analysis failed to show any significant differences between the groups (Fig. 11C).

Olfactory function was evaluated using an odor-guided food-seeking paradigm, wherein rats were tasked with locating a buried cookie underneath the bedding. The latency to find the cookie served as a measure of olfactory performance. Overall, the three-way ANOVA analysis of the BFT did not demonstrate any significant interactions of trial x TBI x rotenone ($F_{3, 160}$=

0.224, p=0.88), trial x TBI ($F_{3, 160}$= 0.08, p=0.97), trial x rotenone ($F_{3, 160}$= 0.17, p=0.91) or TBI x rotenone ($F_{1, 160}$= 0.71, p=0.4), nor was there an effect of either TBI ($F_{1, 160}$= 0.26, p=0.61) or rotenone exposure ($F_{1, 160}$= 0.002, p=0.96). All animals exhibited similar latencies in locating the cookie, with the only notable main effect observed for time ($F_{3, 160}$= 13.9, p<0.0001), as all groups located the visible, unburied cookie faster on trial 4 (Fig. 11D).

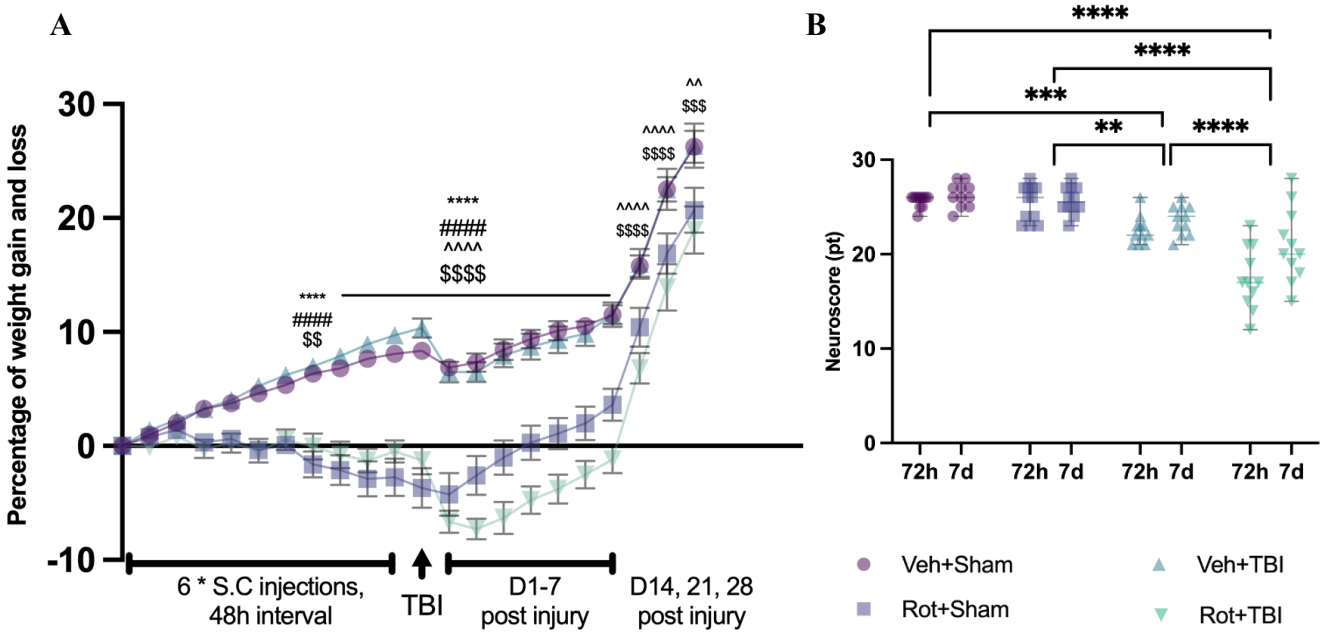


Figure 2. Response to rotenone administration and TBI as assessed by weight gain/loss and composite neuroscore. (A) Time course of the percentage of body weight gain or loss over an 11-day rotenone regimen and 28 days post-injury. Data are presented as mean ± SEM. Statistical significance levels are denoted as follows: *** $p < 0.001$, **** $p < 0.0001$: Statistically significant difference between the Veh+Sham and Rot+Sham groups. #### $p < 0.0001$: Statistically significant difference between the Rot+Sham and Veh+TBI groups. ^^ $p < 0.01$, ^^^^ $p < 0.0001$: Statistically significant difference between the Veh+Sham and Rot+TBI groups. $$ $p < 0.01$, $$$ $p < 0.001$, $$$$ $p < 0.0001$: Statistically significant difference between the Veh+TBI and Rot+TBI groups. Data are presented as mean ± SEM. (B) Interleaved scatter plot depicting neurological motor function determined using a composite neuroscore during the acute phase (Day 3 and 7 post-injury). Rats subjected to TBI exhibited impairment in the neuroscore when compared with sham-injured rats at 3 and 7 dpi. The rats previously exposed to rotenone showed a more pronounced deficit, as detected by significantly lower composite neuroscores. Data are presented as median ± range. **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ between treatment groups. n = 12 per group, except for n = 11 in the Rot+TBI group.

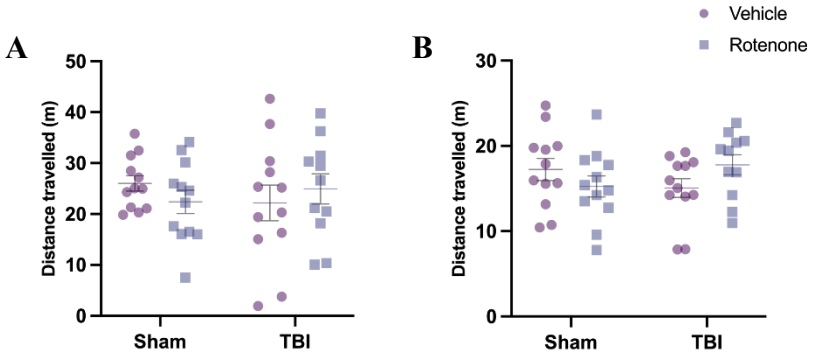


Figure 3. Exposure to rotenone and TBI, either individually or in combination, did not lead to changes in gross motor function at the 1-month post-injury. The distance travelled during (A) open field and (B) elevated plus maze was measured as an indicator of locomotor activity and general motor function. Data are presented as means ± SEM, n = 11-12 per group.

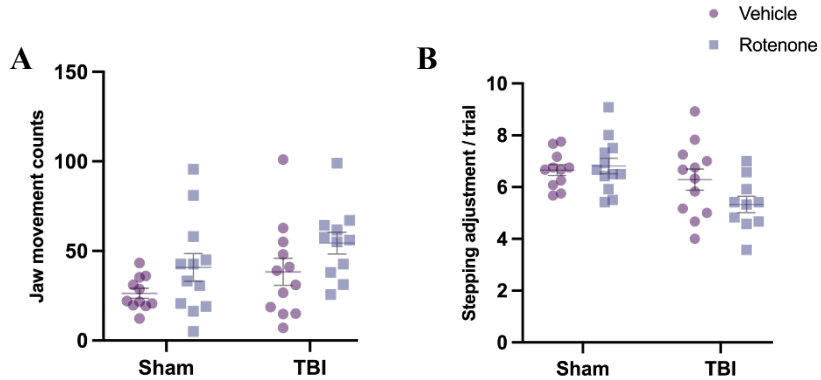


Figure 4. Subtle individual effects of injury and rotenone on Parkinsonian-like symptoms. (A) The tremor was quantified by counting involuntary oscillations or movements in the jaw region. (B) Akinesia was assessed by determining the number of stepping adjustments per trial in the forelimb adjusting step test. Data are presented as means ± SEM, n = 10-12 per group.

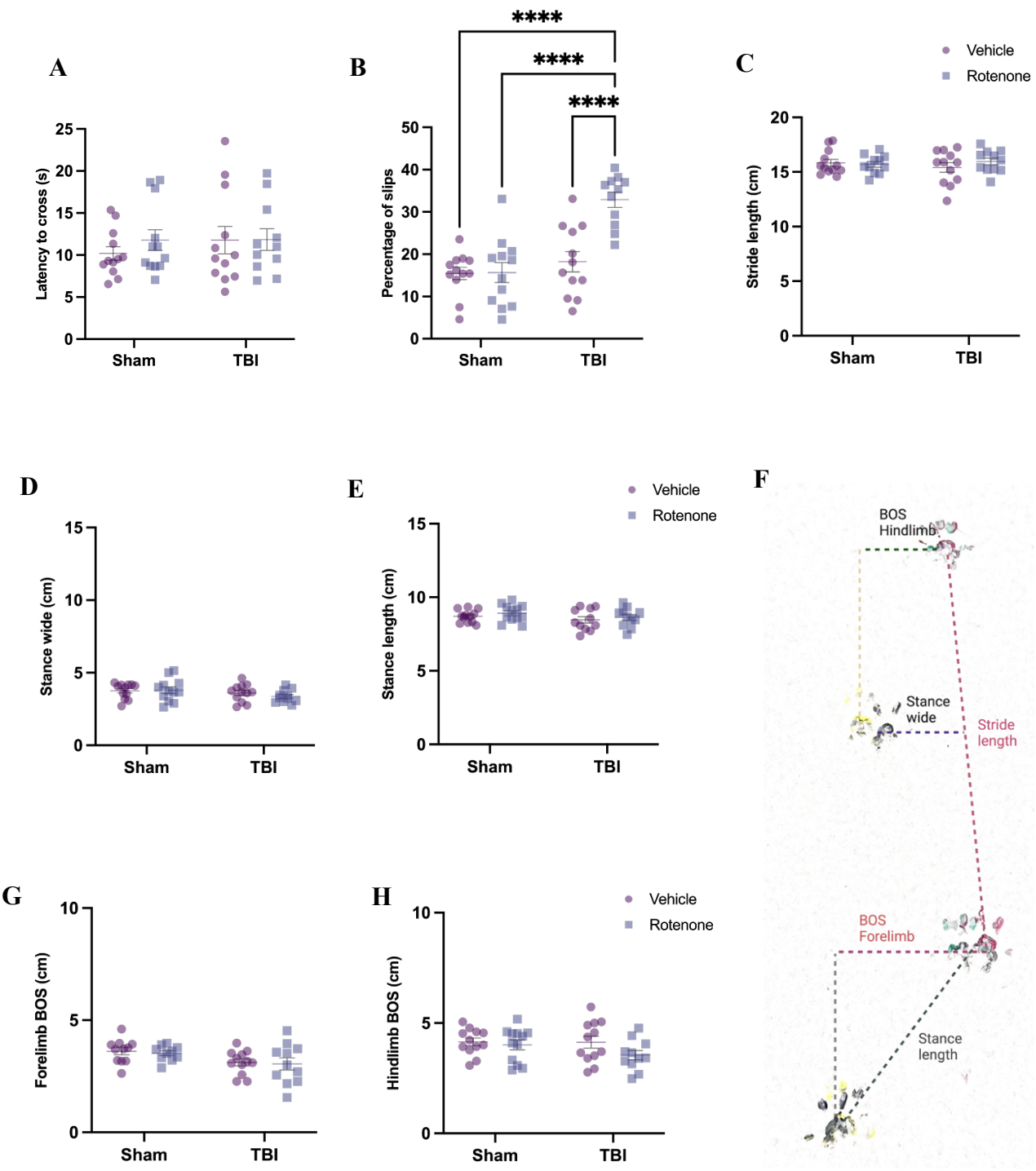


Figure 5. Rotenone exposure prior to TBI induces significant deficits in motor coordination with beam walking but not gait at 1-month post-injury. (A-B) Balance and motor coordination. Beam walking performance for each animal was expressed as (A) the average latency to cross the beam and (B) the percentage of slips/missteps. (C-H) Gait analysis. The gait test was assessed by 4-colour paws printing, the walking pattern, measured as (C) stride length, (D) stance wide, (E) stance length, (F)representative trances of footprint image collected, (G) forelimb base of support (BOS) and (H) hindlimb BOS. Data are presented as means ± SEM, n = 10-12 per group. ****$p < 0.0001$ between treatment groups.

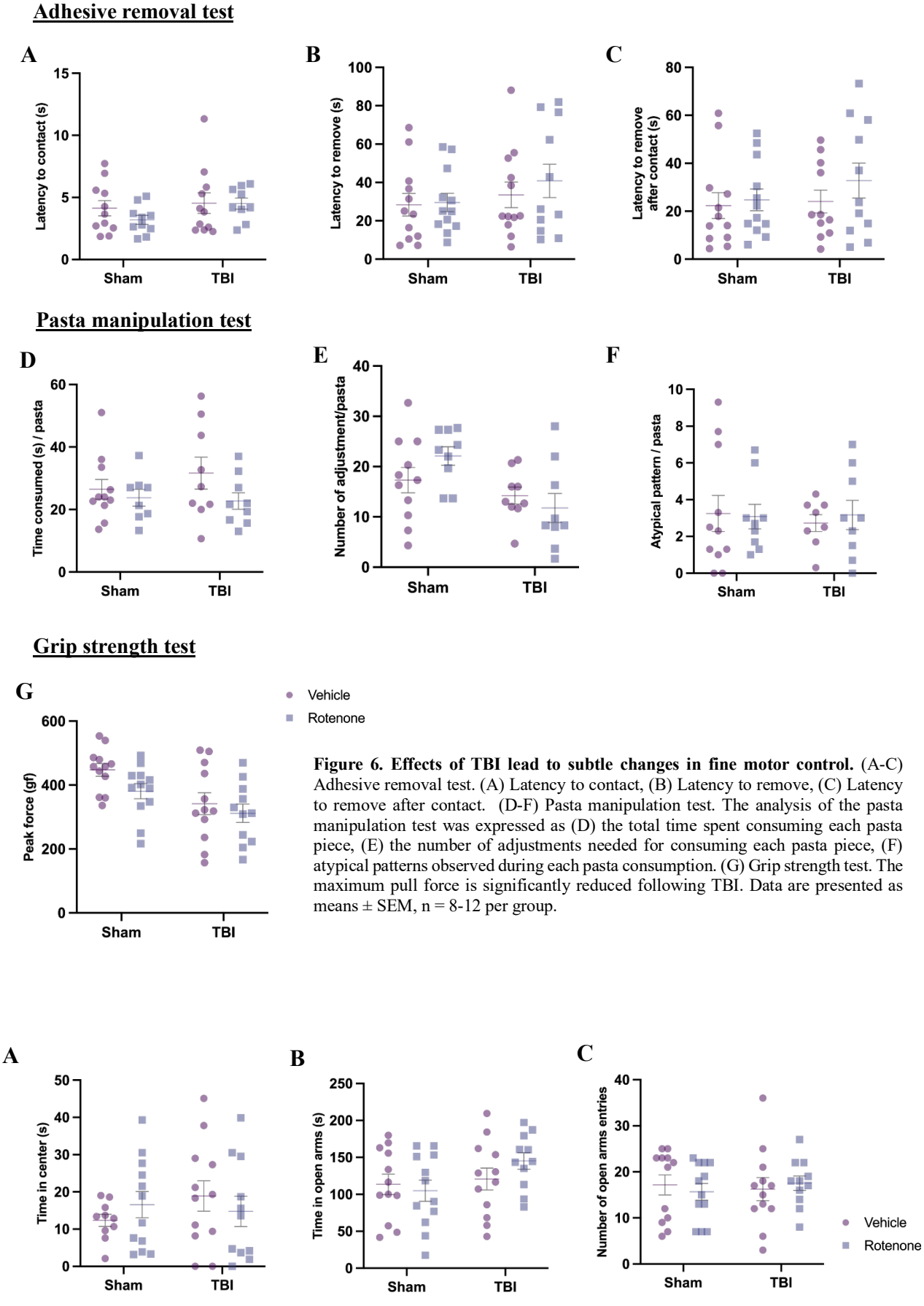


Figure 6. Effects of TBI lead to subtle changes in fine motor control. (A-C) Adhesive removal test. (A) Latency to contact, (B) Latency to remove, (C) Latency to remove after contact. (D-F) Pasta manipulation test. The analysis of the pasta manipulation test was expressed as (D) the total time spent consuming each pasta piece, (E) the number of adjustments needed for consuming each pasta piece, (F) atypical patterns observed during each pasta consumption. (G) Grip strength test. The maximum pull force is significantly reduced following TBI. Data are presented as means ± SEM, n = 8-12 per group.

Figure 7. Neither rotenone nor TBI exposure led to anxiety-like phenotype in rats at 1-month post-injury. Anxiety-like behaviour was measured by (A) time spent in the centre in the open field as well as evaluated by (B) time in open arms and (C) number of entries into the open arms in the elevated plus maze. Data are presented as means±SEM, n=10-12/group.

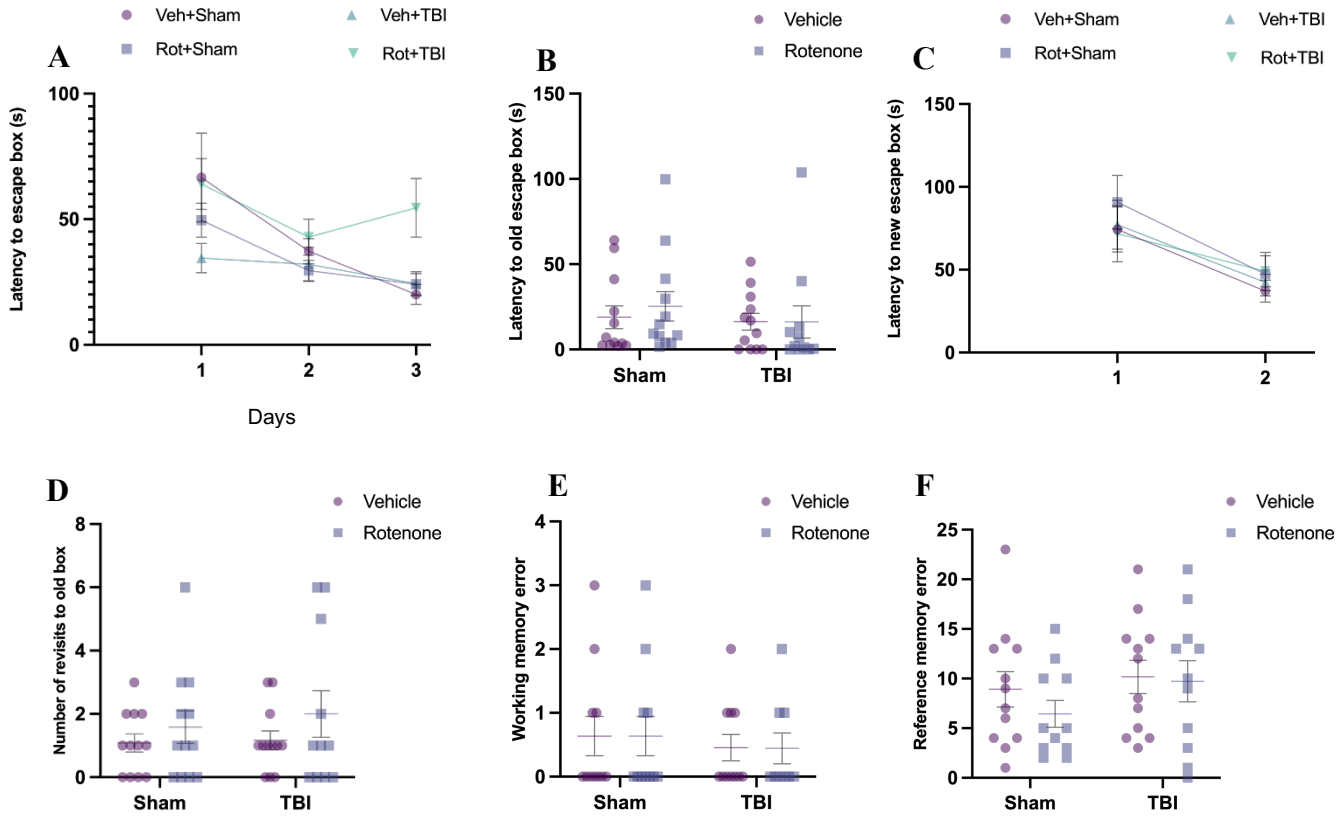


Figure 8. Subtle learning deficits, but not memory and cognitive flexibility were observed using the Barnes maze. Spatial learning was evaluated by measuring the (A) latency to escape to the dark box during the acquisition phase (day 1-3). Memory performance was assessed on trial 1 probe day by measuring the (B) latency to the old box location, and on trial 2 probe day by measuring the (D) number of revisits to the old box, (E) reference memory error, and (F) working memory error. Cognitive flexibility was examined by measuring the (C) latency to the new escape box on the probe day. Data are presented as means ± SEM, n = 10-12 per group.

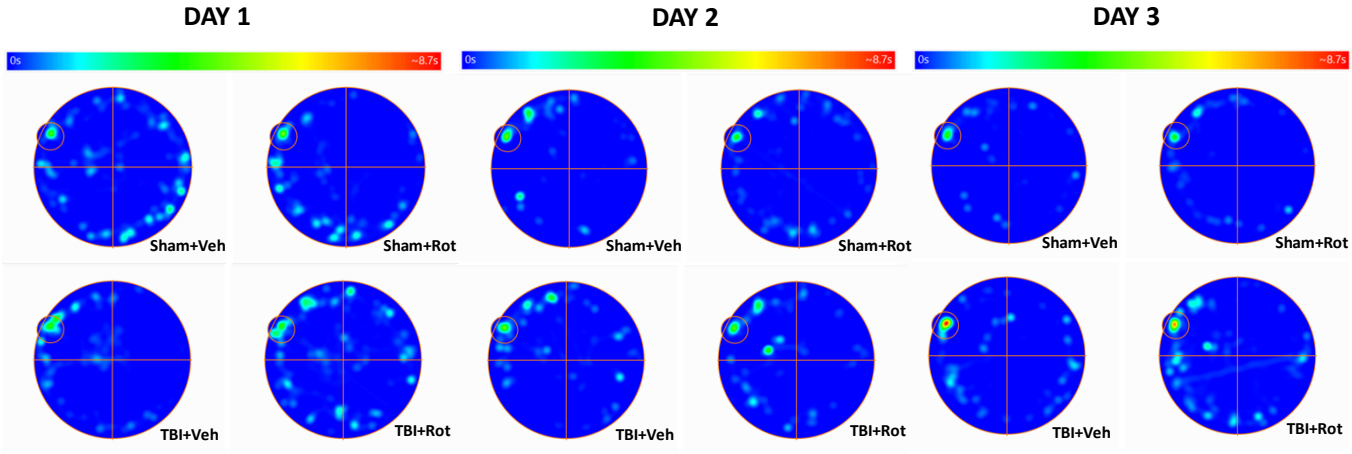


Figure 9. Heat map analysis was conducted on the Barnes maze during the acquisition phase following the injury. The heat maps represent the average exploration patterns of the animals' centre point within the Barnes maze throughout the entire duration of the test. The color scale displayed at the top indicates the intensity of exploration, with warmer colours indicating higher exploratory activity. The orange circle indicates the location of the escape box. Each heat map in the figure represents a composite of data from four animals per group (n=4/group).

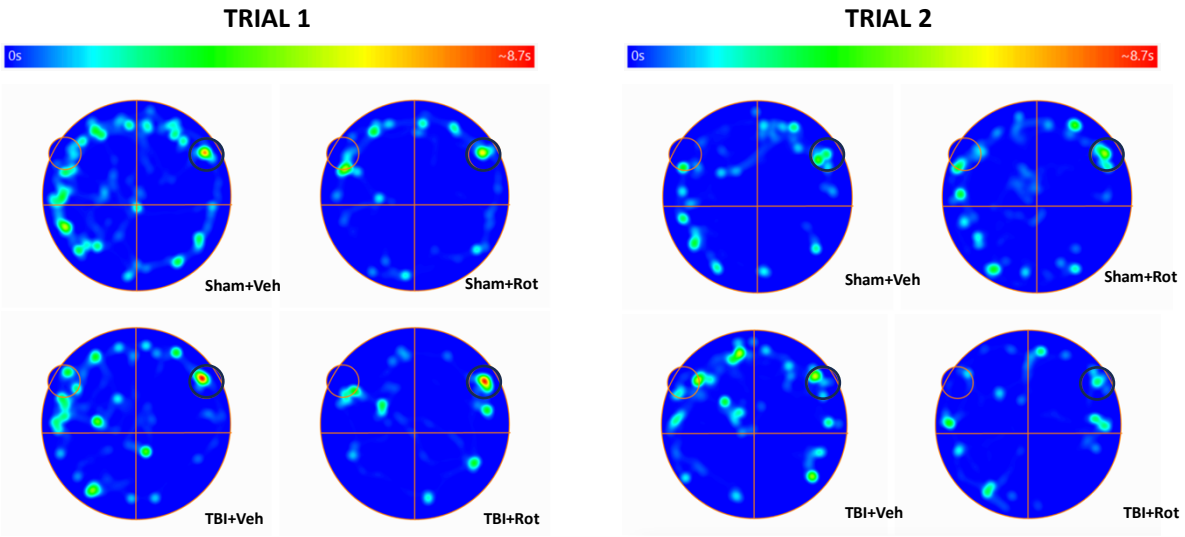


Figure 10. Heat map analysis was conducted on the Barnes maze during trials 1 and 2 of the probe day. The heat maps display the average exploration patterns of the animals' centre point within the Barnes maze throughout the entire duration of the test. The colour scale presented at the top represents the intensity of exploration, with warmer colours indicating higher exploratory activity. The orange circles indicate the location of the old escape box, while the black circles indicate the new escape box location. Each heat map in the figure represents a composite of data from four animals per group (n=4/group).

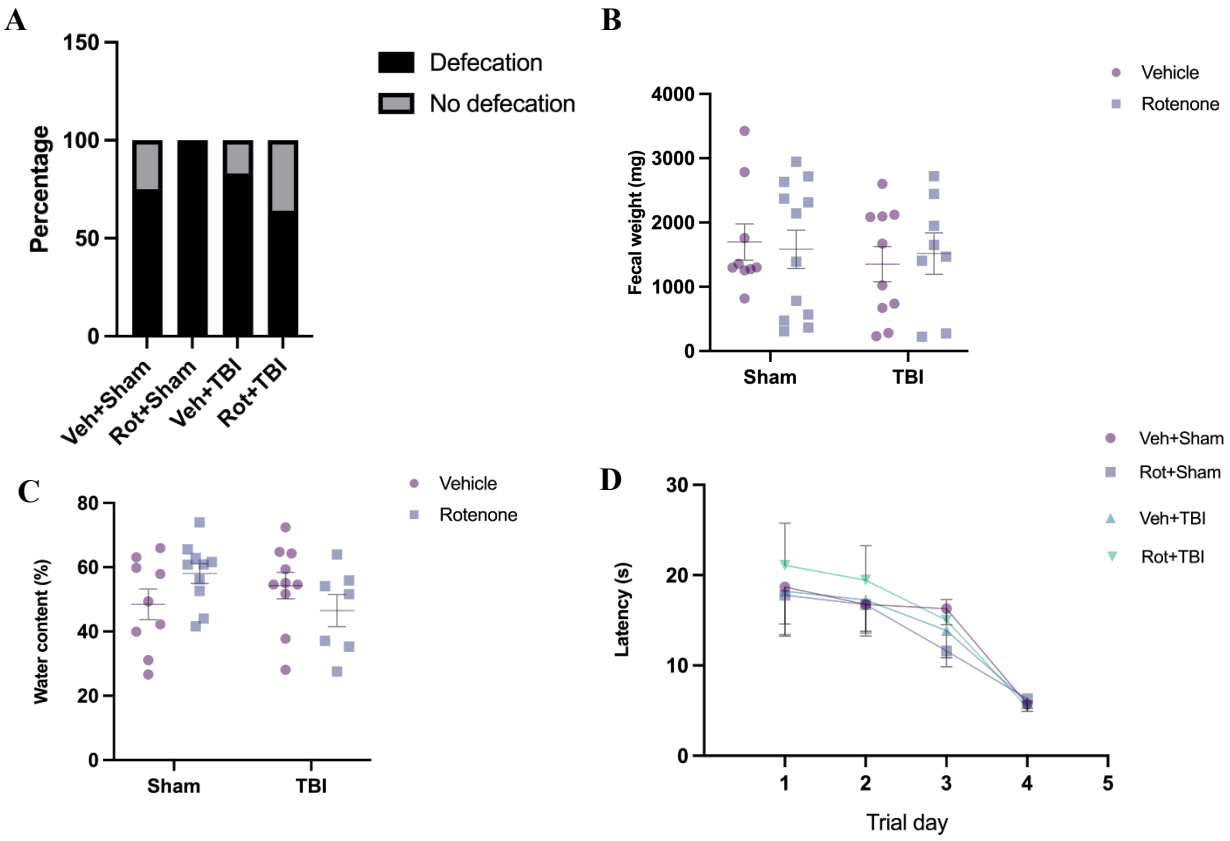


Figure 11. Assessment of common prodromal PD symptoms: constipation and hyposmia. (A-C) Constipation rate. Constipation was evaluated using (A) the percentage of defecation vs. no defecation during a 1-hour trial of the constipation test (B) the total weight of the fecal sample (C) the percentage of water content. **(D) The integrity of olfactory function** was assessed using the buried food test. The latency to find the buried cookie was measured on days 1-3. On trial day 4, the cookie was placed on top to test for motivation and any motor deficits. Data are presented as means ± SEM. n=10-12/group in A,D; n=7-10/group in B,C.

Table 1

Summary of behavioural results; P-value of rotenone, TBI and interaction effect

Test Paradigm	Behaviour measurement	P-Value		
		Rotenone effect	**TBI effect**	**Interaction**
Neuroscore	28-point composite neuroscore	<0.0001	<0.0001	=0.0003
General Motor Function				
Open Field Test (OF)	Distance travelled (m)	ns	ns	ns
Elevated Plus Maze (EPM)	Distance travelled (m)	ns	ns	ns
Parkinsonian Symptoms				
Jaw Tremor Test (JT)	Jaw movement counts	=0.0235	ns	ns
Forelimb Adjusting Step Test (ST)	Stepping adjustment/ trial	ns	=0.0067	ns
Balance Coordination and Gait				
Beam Walk Test (BW)	Latency to cross (s)	ns	ns	ns
	Percentage of slips (%)	=0.0008	<0.0001	=0.0011
Gait Test (GT)	Stride length (cm)	ns	ns	ns
	Stance wide (cm)	ns	ns	ns
	Stance length (cm)	ns	ns	ns
	Forelimb base of support (cm)	ns	=0.0114	ns
	Hindlimb base of support (cm)	ns	ns	ns
Fine motor control and Grip				
Adhesive Removal Test (AT)	Latency to contact (s)	ns	ns	ns
	Latency to remove (s)	ns	ns	ns
	Latency to remove after contact (s)	ns	ns	ns
Pasta Manipulation Test (PMT)	Time consumed (s)/pasta	ns	ns	ns
	Number of adjustments/pastas	ns	=0.0069	ns
	Atypical pattern/pasta	ns	ns	ns
Grip Strength Test (GST)	Peak force (gf)	ns	=0.0024	ns
Anxiety				
Open Field Test (OF)	Time in center (s)	ns	ns	ns
Elevated Plus Maze (EPM)	Time in open arms (s)	ns	ns	ns
Cognition				
Barnes Maze	Training: Escape latency to box (s)	ns	ns	=0.0030
	Probe: Latency to old box (s)	ns	ns	ns
	Probe: Latency to new box (s)	ns	ns	ns
	Probe: Number of revisits to old box	ns	ns	ns
	Probe: Working memory error	ns	ns	ns
	Probe: Reference memory error	ns	ns	ns
PD Prodromal Symptoms				
Constipation Test	Rate of defecation (%)	Chi-square test, p<0.0001		
	Total fecal weight (mg)	ns	ns	ns
	Water content (%)	ns	ns	=0.0491
Buried Food Test	Latency to find cookies (s)	ns	ns	ns

4.0 DISCUSSION

The etiology of PD is complex, likely involving a multifaceted interplay of diverse genetic and environmental factors (Gorell et al., 2004). PD encompasses more than just its prominent motor symptoms, as emerging evidence indicates an extended prodromal phase marked by non-motor symptoms such as cognitive impairment, anxiety, and autonomic irregularities (Gaenslen et al., 2011; Postuma and Berg, 2019). Various animal models, including TBI models (Acosta et al., 2015), toxin-based models (Betarbet et al., 2000; Simola et al., 2007), an α-synuclein transgenic model (Giasson et al., 2002; Lee et al., 2002), and an α-synuclein propagation model (Kim et al., 2019; Luk et al., 2012), have been developed to better understand the pathogenesis underlying PD. However, none have accurately reproduced the progression of PD seen in clinical populations (Taguchi et al., 2020). Herein, this study focused on two established PD risk factors, TBI and rotenone exposure, to generate a new preclinical model, with the aim of understanding their individual and synergistic impacts on functional changes at 1-month post-injury. Such a model may have utility for understanding the pathophysiological mechanisms that lead to PD emergence. This study is not the first to explore the link between two environmental risk factors and PD. Prior studies, including those by Hutson *et al.*(Hutson et al., 2011) and de Oliveira *et al.* (De Oliveira et al., 2017), have investigated these combined effects, with injury implemented prior to high dosage of either the neurotoxin 6-hydroxydopamine (6-OHDA) or the pesticide paraquat, leading to significant motor impairments and substantial dopaminergic neurodegeneration in SN. Importantly, however, none of the previous work in this area focused on generating a mild underlying vulnerability in the dopaminergic system prior to the TBI, which is arguably the more clinically relevant scenario. Further, the current study went beyond the study of motor function alone, instead incorporating a more comprehensive battery including not only an extensive assessment

of motor function, but also cognition, anxiety and aspects of the prodromal symptoms of PD, including both constipation and olfactory function.

The overall results from the present study demonstrated that, at 1-month post-injury, no discernible impairments in general motor function, anxiety-like behaviour, prodromal PD symptom (hyposmia), or cognitive functions, such as cognitive flexibility, working memory, and recognition memory, were observed when compared to Veh+Sham animals, irrespective of the treatment. However, exposure to a low systemic dose of rotenone-induced subtle motor dysfunction, particularly in tremors, balance, and motor coordination. Similarly, animals given a moderate-severe TBI exhibited a more pronounced impairment in motor function, including akinesia-like symptoms, as well as deficits in fine motor control, deficit, grip, gait, balance and motor coordination. Of note, rotenone pre-exposure prior to moderate-severe TBI showed synergistic effects, exacerbating balance and motor coordination dysfunction, as evidenced by a significantly higher percentage of foot slips in beam walking compared to other treatment groups. A synergistic effect was also observed in spatial learning during the acquisition phase of the Barnes maze, with Rot+TBI animals demonstrating a consistent exploratory pattern across all three acquisition days, an effect absent in animals in either the rotenone or TBI alone groups. This synergistic effect extended to defecation frequency, with Rot+TBI animals exhibiting a lower defecation rate than the other treatment groups. These findings suggest that, while TBI and rotenone may induce relatively subtle motor impairments individually, their combination results in an exacerbated impact that extends to deficits beyond motor function alone, which could potentially set the stage for the later emergence of PD.

The initial significant finding in this study was that, although low-dose rotenone had an effect on the neuroscore test, it did not lead to significant acute neurological impairments compared to Veh+Sham, suggesting rotenone alone may not cause such impairments. While the investigations using the same dosing regimen of this study may not be available, the

cumulative effect observed on neuroscore following 2-3 weeks of continuous 4mg/kg rotenone injections implies that noticeable effects on neurological function indeed require a longer exposure duration or a higher dosing regimen (Troshev et al., 2022). In the current study, at 1-month following the last injection, subtle rotenone effects manifested in impairment in balance/motor coordination and increased tremor-like symptoms, with no discernible impacts on other motor functions, cognition, or any of the prodromal-related symptoms assessed. This implies that transient, low-dose exposure to rotenone can result in delayed and mild behavioural changes over a more extended timeframe. This observation seems to align with the findings from Van Laar *et al.*, where Lewis rats injected with intraperitoneal 2.8mg/kg rotenone for 5 consecutive days experienced a rapid recovery from the rotenone effect within 9 days of cessation and remained without behavioural deficits for 3 to 4 months, before subsequently developing progressive motor abnormalities in the following months (Van Laar et al., 2023).

This delayed emergence of symptoms may be attributed to progressive loss in dopaminergic neurons within the SN, considering the critical role of this region in motor function and its vulnerability to rotenone exposure (Fan et al., 2022; Guilarte et al., 2006; Sherer et al., 2003b). In support of this, in our previous pilot study, it was found that, 24- hours after 6 injections of 1.5mg/kg/48h rotenone injection, approximately 20% of dopaminergic neurons were lost in the SN [Chapter 4]. While the examination of DA neurons at a 1-month time point is not covered in this study, it is plausible that there could be a progressive loss of neurons following rotenone exposure. Supporting this, a study by Rocha and colleagues reported an approximate 35% gradual decline in the number of DA neurons within the SN over a two-week period following the cessation of a 14-day intraperitoneal injection of 2.5mg/kg rotenone in mice (Rocha et al., 2022). In a more chronic timeframe, Van Laar *et al.* demonstrated a progressive loss of DA neurons in the SN, with a 24% loss at 3 months that

increased to a loss of 32% at 9 months following the cessation of rotenone exposure (Van Laar et al., 2023).

It is important to note that the SN serves as an important source of dopamine in the brain, projecting to the striatum (STR), forming the nigrostriatal pathway (Haber et al., 2000). Loss of DA neurons in the SN could subsequently reduce dopamine transmission to the STR (Sherer et al., 2003b; Zhang et al., 2017), profoundly impacting motor function and potentially giving rise to the symptoms seen. The absence of rotenone effects in other behavioural tests at this particular dose, however, raises the possibility that this dosing regimen might not have surpassed the threshold necessary to induce profound motor impairment (Engelender and Isacson, 2017). In support of this, a single photon emission computed tomography (SPECT) imaging study, utilising the [123I]-FP-CIT ligand that labels the dopamine transporter, demonstrated that tremor was associated with 43% loss of striatal DA content (Schwartz et al., 2004). Similarly, the presentation of the classic cardinal motor symptoms of PD famously requires a 40-60% loss of DA neurons within the SN (Kordower et al., 2013) and a considerably more profound (i.e. >80%) loss of striatal DA (Bernheimer et al., 1973; Scherman et al., 1989). Therefore, it is imperative for future studies to investigate the correlation between the subtle motor deficits detected one month after low-dose rotenone exposure and DA levels in the nigrostriatal pathway. Such a comprehensive examination is currently ongoing in our lab using tissue generated from this study.

In contrast, animals subjected to a moderate-severe diffuse TBI demonstrated notable and acute impairment in neurological function, as measured by neuroscore, compared to the sham groups. This observation aligns with outcomes from severe TBI animal models induced through different injury methods, including lateral fluid percussion injury and controlled cortical impact, which consistently demonstrate acute decreases in neurosocre following injury (Nissinen et al., 2017; Pierce et al., 1998; Song et al., 2019; Thau-Zuchman et al., 2021).

Similarly, clinical work by Dahdah *et al.* further supports this notion, revealing that patients with moderate-severe TBI exhibit deficits in functional outcomes even upon discharge from a rehabilitation centre (Dahdah et al., 2014). This acute impairment indicates the immediate and profound impact of TBI on the nervous system, affecting various aspects of neurological function (McAllister, 2011).

Furthermore, the impact induced by moderate-severe diffuse TBI appears to persist, and perhaps even worsen, sub-acutely at 1-month post-injury, affecting various motor functions such as forelimb akinesia, reduced grip strength, compromised fine motor control, impaired balance and motor coordination, and altered forelimb base of support in gait. These findings are consistent with our systematic review, demonstrating the sub-acute persistence of these motor dysfunctions after moderate-severe diffuse TBI (Corrigan et al., 2023). Indeed, mounting evidence indicates that diffuse TBI leads to altered gait during sub-acute time points, even following a mild injury. Specifically, studies by Mountney *et al.* and Namdat *et al.* revealed significant deficits in the front base of support that closely align with our observation (Mountney et al., 2017; Namdar et al., 2020). Dysfunctions in the forelimb, assessed by forepaw placement and grip strength, are also prominent in animals with moderate/severe diffuse TBI (Alwis et al., 2012; Rana et al., 2020). However, the results for balance and motor coordination are more mixed, with our previous work on rotarod showing no significant differences in latency to fall at either 1- or 3-months following the same TBI severity and injury method utilised in this study (Arulsamy et al., 2018). Conversely, in line with the current study, previous work by Alwis *et al.* and Albayram *et al.* found worse performance in beam walking using a diffuse weight drop injury at 1- and, more chronically, at 6 months following a moderate-severe diffuse TBI (Albayram et al., 2017; Alwis et al., 2012).

While this differential effect could be attributed to the motor tasks used, it is important to note that the impact of moderate-severe diffuse TBI did not extend to certain other motor

symptoms, including sensorimotor/fine motor control and gross motor function. Parameters like latency to remove adhesive tape, traversal time on the beam, and locomotor activity in both the open field and elevated plus maze showed no significant alterations, which, intriguingly, aligns with the previous findings that TBI does not significantly affect sensorimotor or gross motor function at 1-month following the injury (Alwis et al., 2012; Arulsamy et al., 2018; Özen et al., 2022). This suggests that moderate-severe diffuse TBI may have a differential effect on motor function. However, it is worth mentioning that limited work has explored motor functions using the moderate-severe diffuse TBI model employed in this study. Among the 65 preclinical studies included in our systematic review investigating chronic motor performance, 27 examined moderate and moderate-severe TBI, with just 5 studies utilising the diffuse TBI model (Albayram et al., 2017; Alwis et al., 2012; Arulsamy et al., 2018; Hou et al., 2017; Rana et al., 2020), with the rest employing mixed and focal TBI models (Corrigan et al., 2023). Thus, further studies will be needed to assess alterations in sensorimotor and gross motor function following diffuse axonal injury. Nonetheless, the motor impairments observed in this study highlight the enduring nature of the damage caused by moderate-severe diffuse TBI, extending well beyond the initial acute phase (Bramlett and Dietrich, 2015).

Interestingly, within this study, the deficits in motor function following moderate-severe diffuse TBI predominantly affected the forelimb, with a main effect found for tests such as the forelimb adjusting step test, forelimb base of stance and the number of forepaw adjustments made during the pasta manipulation task. Notably, motor impairments, especially in the upper limb and hand, often mark the initial manifestation in PD cases (Monje et al., 2021). Given that dopamine loss in the nigrostriatal pathway is a key pathological hallmark of PD, it is plausible to suggest that these motor impairments could be attributed to TBI-induced alterations in dopaminergic function, possibly contributing to a motor phenotype reminiscent of early PD. In fact, TBI has been consistently identified as factor that leads to impaired striatal

dopaminergic transmission, including reduced synthesis and dopamine release in the STR (Chen et al., 2017; Donnemiller et al., 2000; Jenkins et al., 2018; Wagner et al., 2005b, 2005a). SPECT imaging studies have reported a 20% reduction in binding ratios for the dopamine transporter in the STR of patients with moderate-severe TBI (Jenkins et al., 2018). Preclinical work has also shown that TBI alone can result in a 15% loss of dopaminergic neurons in the STR 11 days after a moderate fluid percussion injury (Hutson et al., 2011).

However, not all studies have been consistent, with a previous study reporting that dopaminergic neurons in both the STR and SN were not impacted at 1-month following a diffuse weight drop injury, the same type of injury induced in the current study (De Oliveira et al., 2017). This raises the possibility that the motor deficits observed following TBI in our study might be due to effects in other brain regions or on other mechanisms responsible for motor control beyond the nigrostriatal pathway (Graybiel, 1991). It remains to be investigated whether these motor impairments were associated with the alterations of the nigrostriatal pathway in this study. Additionally, exploring other brain regions and mechanisms related to motor control will be important for unraveling the dynamic contributions of other neurotransmitters and neuromodulators beyond dopamine to the complex motor profile seen following injury.

In addition to the effects seen for rotenone and TBI alone, a significant synergistic effect of the two was observed during acute neurological assessment using neuroscore. Specifically, the Rot+TBI animals exhibited significantly lower neuroscores than the other treatment groups, suggesting that pre-exposure to rotenone, while not impactful on its own, exacerbated the effects of TBI. This underlines the notion that the combined impact of rotenone and TBI is more than the sum of their individual effects on neurological assessment (Lee et al., 2012). When extending the investigation to other motor tests at 1-month post-injury, a significant interaction was seen during the beam walk test, with no such interaction observed in other

motor tests. It's noteworthy that the motor deficits identified in the beam walk test in this study predominantly manifested as alterations in balance and coordination, indicative of underlying disruptions in motor function commonly observed in both TBI and PD. This finding is comparable to a study by de Oliveira *et al.*, where a moderate diffuse weight drop injury, followed by an intraperitoneal administration of 100mg/kg 6-hydroxydopamine (6-OHDA) five hours post-injury, significantly induced impairment in rotarod motor coordination at 4 weeks post-injury, an effect that wasn't statistically significant for animals given either the TBI or 6-OHDA alone (De Oliveira et al., 2017). It is also in line with a similar study by Hutson and colleagues (2011), in which dual exposure could lead to exacerbated effects compared to a single risk factor alone (Hutson et al., 2011).

The likely origin of this synergistic effect— indicated by the increased number of foot-slip errors detected in the beam walking test, signifying a loss of balance and motor coordination—lies in the observed ability of rotenone to induce subtle underlying vulnerability in the dopaminergic system (Chapter 4). In line with our expectation, this vulnerability might have subsequently allowed moderate-severe TBI to cause more pronounced damage. This is also supported by the only two preclinical studies to date that have explored the synergistic effect of TBI and neurotoxin, revealing an exacerbated reduction in dopaminergic neurons than with TBI or exposure to either the neurotoxin 6-OHDA (De Oliveira et al., 2017) or the pesticide paraquat (Hutson et al., 2011). However, it remains plausible that the synergistic effect between rotenone and TBI could also stem from their impact on other pathways relevant to the pathogenesis of PD, including their shared ability to induce oxidative stress, mitochondrial dysfunction, neuroinflammation and axonal injury (McAllister, 2011; Sherer et al., 2003a). Consequently, a definitive conclusion regarding the precise mechanisms of this synergistic effect cannot be reached without further investigation.

Interestingly, the synergistic effect between rotenone and TBI extends beyond motor function alone. In line with this, animals subjected to both factors demonstrated impaired learning during the acquisition phase of the Barnes Maze, with no effect seen on cognitive flexibility, reference memory or working memory. This effect could be due to impacts on dopamine within the nigrostriatal pathway. In line with this, 8-week-old transgenic MitoPark mice showed similar deficits in learning during the acquisition phase of the Barnes Maze as those observed in the current study, which were not confounded by motor impairment. Similarly, as observed in the current study, performance of these MitoPark mice on the Barnes maze did not differ from that of wild-type littermates by day 4 of the protocol, nor did they display differences in probe trial performance. Thus, while the Barnes maze is often thought of as a hippocampal-dependent spatial learning and memory task, given the selective vulnerability of the nigrostriatal dopamine pathway seen in MitoPark mice(Ekstrand et al., 2007), it seems likely that this pathway also contributes to normal performance on this task. Further, it provides support for potential impairment of this pathway within our ROT+TBI animals, although, as stated above, this remains to be formally assessed (Li et al., 2013).

However, it's also crucial to carefully consider the experimental conditions and potential confounding factors that could influence the results obtained from the Barnes Maze (Gawel et al., 2019; Rosenfeld and Ferguson, 2014). Odour cues, for instance, can be one of the factors influencing the investigation of cognitive function, as rodents commonly rely on odour cues for navigation and localization (Liu et al., 2020). Indeed, this behaviour was notably observed in Veh+TBI animals during the acquisition phase, where the learning curve appears flat, rather than displaying the downward trajectory observed in Sham animals (Figure 8a), suggesting that rats in this group may be using odour cues to find the escape box. This is further supported by the heat map on Day 1 of the acquisition phase, illustrating how Veh+TBI animals tend to head directly to the escape box, compared to the more exploratory patterns observed in

the other treatment groups (Figure 9). This could impact the ability to fully assess the impact of injury on performance. Additionally, the mildly aversive stimuli used in the Barnes maze, such as the bright lights in this study, might not sufficiently motivate rodents to locate the escape box. This lack of motivation could result in increased exploration, prolong the latency to locate the escape box and path length, and consequently result in slower learning curves (Kennard, 2011). Another confounding factor affecting the learning curve is the number of trails trained per day. In a study comparing animals trained in 2, 3, and 4 trials per day in the Barnes Maze acquisition phase, animals trained with only 2 trials per day exhibited significantly slower latency to reach the escape box, covered a longer distance, and showed lower path efficiency compared to the 3 and 4 trial groups (Illouz et al., 2016). Collectively, these factors may contribute to the observed fluctuating learning performance in Rot+TBI animals. This is evident in both Figure 6a and the heat map, where Rot+TBI animals display worse latency to escape and exploratory patterns on Day 3 than on Day 2 of the acquisition phase. Thus, a more comprehensive measurement utilising additional measures of learning and cognitive function, particularly prefrontal-dependent cognitive function, are needed in order to tease apart these effects.

Finally, the synergistic effect between TBI and rotenone was further supported by our data on constipation, one of the prodromal symptoms of PD (Gaenslen et al., 2011). Specifically, we found a decrease in defecation frequency and a decrease in the percentage of water content in the fecal samples collected from the Rot+TBI group, indicating a potential impact on bowel movement. This observation aligns with the known association between constipation and PD, as slow bowel movement frequency is frequently reported in individuals with PD (Ashraf et al., 1997; Verbaan et al., 2007). Research has indicated that constipation correlates with the duration and severity of PD, with both the frequency and severity of constipation tend to increase as the disease progresses (Edwards et al., 1993; Krogh et al., 2007). Additionally, a

study highlighted indicators of constipation, including bowel dysfunction and prolonged colon transit time, in a cohort of 55 TBI patients 1 year post-injury (Lim et al., 2012). Furthermore, in a rat model, a 26-day subcutaneous injection of 2mg/kg rotenone significantly reduced fecal dry weight, water content, and length 1 month after rotenone cessation (Song et al., 2023). Collectively, the observed decrease in defecation frequency and relative dry fecal samples from the Rot+TBI may, therefore, indicate the presence of the autonomic dysfunction associated with prodromal PD. Future investigations, such as colonic transit time using marker substances, should be pursued for a more detailed examination of the frequency, severity, and other characteristics of constipation (Lin et al., 2015). It is also important to note that this study did not show any synergistic effects between TBI and rotenone for other prodromal symptoms associated with PD, such as olfactory dysfunction (Bang et al., 2021), as tested by the buried food test, or anxiety-like behaviour (Gaenslen et al., 2011), as assessed using the elevated plus maze and open field, indicative of the intact of the frontal region, however, future investigation is necessary to substantiate this hypothesis. Further, other key prodromal symptoms, such as REM-sleep behaviour disorder (Postuma, 2014) or depression (Bareeqa et al., 2022) were not assessed in the current study. Thus, a more comprehensive assessment of prodromal symptom presentation in this model must be performed.

Collectively, our findings suggest that TBI alone can result in a range of impairments involving motor functions, and that prior exposure to rotenone potentiates this effect, manifesting as subtle motor impairments, along with mild cognitive deficits and the presence of constipation symptoms commonly observed in the early-stage PD. This observed synergistic effect of rotenone exposure and TBI in the experimental model expands on the concept of the "two-hit" hypothesis of PD and is consistent with the epidemiological study by Lee *et al*.(Lee et al., 2012), where TBI and pesticide exposure individually moderately increased PD risk, while the exposure to both factors significantly amplified the PD risk. Most importantly, for

the first time to date, these results demonstrate the potential of the Rot+TBI model in unravelling the intricate mechanisms underlying PD progression following injury. Future research should build upon these findings to further elucidate the molecular, cellular, and circuit-level changes associated with the combined impact of TBI and rotenone exposure. Additionally, greater attention should also be given to the investigation of the precise time span and symptoms progression between head trauma, rotenone exposure and the onset of PD. Such investigations may provide insight into which individuals are at particular risk of PD development, a critical first step in early identification and, consequently, potentially more effective therapeutic intervention.

06

Discussion, Future Directions and Conclusion

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease following Alzheimer's disease. Although advances in medical science and biotechnology, such as the invention of deep brain stimulation—offering a 51% chance of surviving for at least 10 years after the procedure—have prolonged the survival rates for PD patients (Hitti et al., 2020), a cure remains elusive. Existing treatments, such as levodopa and the previously mentioned deep brain stimulation, primarily focus on managing symptoms rather than halting or reversing disease progression and also come with side effects associated with their long-term use (Combs et al., 2015; Marsden, 1994). With the growing recognition of the prodromal PD stage, current research seeks to unravel the complexities of early PD progression, aiming to develop disease-modifying therapies (Kalia et al., 2015; Vijiaratnam et al., 2021). While the exact etiology of PD remains largely unknown, epidemiological and clinical studies have identified a significant correlation between environmental exposures—such as pesticide exposures and traumatic brain injury (TBI)—and an elevated risk of developing PD in later life (Gardner et al., 2018; Tanner et al., 2011). The literature review presented in the first chapter of this thesis highlights a considerable overlap in pathology and mechanisms between TBI and exposure to pesticides and PD. Despite extensive discussion and investigation of this overlap by the research community, however, there remains a gap in understanding the stepwise progression of the disease, especially regarding the development and evolution of symptoms.

To address this knowledge gap, Chapter 2 conducts a systematic review of chronic motor symptoms arising from various severities of TBI across both preclinical and clinical studies, aiming to elucidate the intricate details of how motor symptoms may evolve following injury, and may eventually progress to neurodegenerative motor disorders, such as PD (Corrigan et al., 2023). Despite significant gaps and inconsistencies in the literature, a discernible pattern nevertheless emerges, showing that greater injury severity is correlated with more persistent motor deficits, with subtle fine motor deficits also observed clinically following

repeated injury. In domain-specific analyses, there was evidence for an association between postural control and gait and levels of injury severity, particularly in those who had suffered the injury in the last ten years. Speed reduction was also observed in cases of more severe clinical TBI. However, reaching a consensus on generalising findings about the specific motor dysfunction triggered by varying levels of severity, and the progression of these deficits over time, remains challenging. This is primarily attributed to variations in methodologies across studies and discrepancies in the parameters used for reporting. It is also uncommon for studies, including only a handful of preclinical (n = 3) (Arulsamy et al., 2019; Bajwa et al., 2016; Xu et al., 2019) and clinical (n = 2) ones (Burton et al., 2002; Gardner et al., 2017), to report on the impact of different severities of TBI on long-term motor functional outcomes within the same study. With only limited studies adopting such an approach, confounding variables between studies, including, but not restricted to, differences in animal species, type of injuries, source of participants and injury parameters, add complexity to the interpretation of results.

Another contributing factor to the difficulty in generalising findings is the scarcity of longitudinal studies on this topic. Despite a meta-analysis by Zapparoli *et al.* concluding that the degradation of motor functions is associated with the process of aging (Zapparoli et al., 2022), the predominant focus of motor functional outcomes following TBI in preclinical research has been on the acute to early chronic phases (1 to 3 months post-injury), with a relatively smaller number of studies (n = 8) extending their follow-up period to 12 months post-injury (Arulsamy et al., 2019; Arun et al., 2020; Daglas et al., 2019; Dhillon et al., 2020; Mouzon et al., 2018; Pierce et al., 1998; Sell et al., 2017; Tucker et al., 2019). This trend is similar in clinical research, with only six studies identified in this review investigating motor outcomes beyond ten years post-injury (De Beaumont et al., 2009; Martini et al., 2021; Pearce et al., 2018, 2014; Vanderploeg et al., 2007; Walker et al., 2018) , and the rest of the studies inadequately considering the effect of aging on motor functional outcomes. In this context,

conducting further longitudinal studies that explore various TBI severities within a single study is needed to elucidate how a history of TBI interacts with normal aging, impacting motor functional alterations and potentially setting the stage for PD development or other neurodegenerative movement disorders.

As the disparities and limitations identified in this systematic review (Chapter 2) did not lead to much consensus on the long-term progression of motor symptoms following TBI and its relationship to the development of PD, particularly regarding long-term motor deficit indicators of later-onset PD, the focus of this thesis's first experimental chapter (Chapter 3) shifted towards investigating underlying neuropathological changes. Building upon the insights discussed in Chapter 1, where we delved into the long-term persistence of neuroinflammation following TBI and the complex pathology of PD, our hypothesis posited that neuroinflammation persists chronically following diffuse axonal injury, and might be concomitant with changes in key neurotransmitter pathways (i.e. dopaminergic and noradrenergic pathways) known to be affected in PD. Indeed, the loss of dopaminergic neurons in the substantia nigra (SN) (Fabbri et al., 2017) is the well-characterised pathological hallmark of PD. The striatum, on the other hand, which serves as an important site for receiving dopamine projections from the SN, demonstrated a notable 35-45% loss in striatal dopamine transporter activity during the early stage of PD (Heng et al., 2023). The striatum, together with the SN, forms the nigrostriatal pathway that plays a critical role in regulating voluntary movements and coordinating motor control (Crossman, 2000; Kordower et al., 2013; Ungerstedt, 1976). PD is also associated with a reduction in noradrenergic neurons in the locus coeruleus (LC), a pivotal brainstem nucleus (Zarow et al., 2003a), accompanied by a disruption in the noradrenergic network projecting to the prefrontal cortex (PFC), which together, are associated with the cognitive decline observed during the disease's progression (Delaville et al., 2011; Sampaio et al., 2020; Zarow et al., 2003b). By investigating changes in these

neurotransmitter pathways following TBI, we hoped to understand how TBI and neuroinflammation may initiate or worsen PD-related neurodegenerative processes. Moreover, we proposed that this effect is severity-dependent (Hellmich et al., 2005), supported by the differential effects on motor symptoms and PD risk following different severity of TBI (Corrigan et al., 2023; Gardner et al., 2018). Therefore, in Chapter 3, archival tissue from a group of rats with a 12-month history of different TBI severities (single mild, repetitive mild, and moderate to severe) was used for comprehensive analysis, including neuroinflammation, dopaminergic (DA), and noradrenergic (NA) assessments via immunohistochemistry and Western blotting. The regions of interest, encompassing the SN, striatum and PFC, were selected based on the known associations with these neurotransmitter pathways and both motor and non-motor aspects of PD pathology (Assad et al., 2022; Fazl and Fleisher, 2018).

The investigation into the neuroinflammatory pathway revealed a significant impact of TBI on the astrocyte population within the SN, the microglial population within the striatum, and the percentage of activated microglia within the PFC at 12 months following the injury. However, *post-hoc* analysis revealed that the only significant difference compared to sham was observed in a decreased astrocyte population in animals with moderate-severe TBI, with no significant alterations noted in other neuroinflammatory aspects assessed in this study at this severity level. Similarly, there were no changes observed in the population or activation state of microglia and astrocytes in the other TBI severities within the regions examined. These findings contrast with the previous discussion in Chapter 1, where neuroinflammation was noted to persist long-term following TBI, especially in the clinical literature, with activated microglia have been reported up to 17 years post-TBI in multiple brain regions (Ramlackhansingh et al., 2011). This observation, however, could alternatively imply a temporary resolution of chronic inflammation in rodents during this "middle-age" phase, which might re-emerge with aging.

Indeed, a growing body of evidence supports inflammaging—a phenomenon where aging is correlated with chronic low-grade inflammation (Frank et al., 2006; Godbout et al., 2005; Norden and Godbout, 2013). This is particularly evident in a study consisting of 582 individuals aged 65 years or older, without risk factors or clinically active disease, presenting high levels of proinflammatory markers, including IL-6 and C-reactive protein (Ferrucci et al., 2010). In a more detailed comparison, *in vitro* work utilising mononuclear cells collected from healthy young and aged donors demonstrated that mitogen-stimulated cultures from aged donors produce significantly higher levels of IL-6, TNF-α and IL-1β than those from young donors, suggesting that with age, neuroinflammatory responses induced by stimuli are heightened compared to the young (Fagiolo et al., 1993). This is further supported by Ritzel *et al.*'s findings, where microglial activation is amplified and prolonged in the aged brain compared to young-adult rats following a moderate TBI (Ritzel et al., 2022, 2019). These observed heightened neuroinflammatory responses associated with aging may contribute to an increased susceptibility to prolonged and intensified neuroinflammation following TBI, but more chronic time points post-injury, or investigations in older rodents, may be necessary to see this effect.

Nevertheless, it is crucial to note that this assumption is drawn solely from examining microglial and astrocyte morphology in their activation states. A substantial aspect of the neuroinflammatory pathway remained uninvestigated, particularly whether there are alterations in levels of pro-inflammatory cytokines (IL-1, TNF-α, IL-18), chemokines (IL-8, CXC family), and interferons (IFNα, β), which are known to play a pivotal role in sustaining neuroinflammatory responses and consequent neuronal damage (Sordillo et al., 2016; Ziebell and Morganti-Kossmann, 2010). Hence, employing a more extended time-course post-injury and incorporating more comprehensive analyses of the neuroinflammatory response is needed

to fully probe the alterations in neuroinflammation that occur within the nigrostriatal pathway and associated regions post-TBI.

Considering the robust association between TBI and PD, we hypothesised that there might still be an onset of neuropathological changes occurring in relevant brain areas at this time point. In the investigation of catecholamine pathways, the Western blot results revealed disruptions in the NA system, specifically decreased dopamine beta-hydroxylase (DβH) in the SN and increased adrenoreceptor alpha 2A (ADRA2A) in the striatum, within the nigrostriatal pathway following moderate-severe TBI, with no such alterations observed in the PFC or any aspect of the DA pathway at this severity. Previous studies on the effects of TBI on the noradrenergic system in animals have been minimal. Several have demonstrated that noradrenergic turnover consistently increased acutely within 30 minutes around the injury site (Dunn-Meynell et al., 1998; Levin et al., 1995), followed by a reduction throughout the brain over a subacute timescale (Dunn-Meynell et al., 1998; Fujinaka et al., 2003). Therefore, chronic alterations of noradrenaline signalling following TBI remain largely unknown. Nevertheless, it is interesting that functional assessments conducted previously in this animal cohort indicated mild cognitive flexibility deficits, specifically in this moderate-severe TBI group (Arulsamy et al., 2019). Although it remains to be explored, these findings might suggest that moderate-severe TBI induces subtle alterations in neuroinflammation and disruptions in NA signalling, particularly in the SN and striatum, which, in turn, are associated with mild impairments in cognitive flexibility at 12 months post-injury.

This hypothesis, while intriguing, leaves a significant portion of this complex interplay unknown due to the limitations of this investigation, including the insufficient neuroinflammatory exploration mentioned earlier. Further, the study relied on Western blot analysis to determine the total protein levels of a subset of markers in the catecholamine pathway, rather than directly assessing neurotransmitter levels using microdialysis or probing

receptor expression profiles using immunohistochemistry. Additionally, we did not investigate the locus coeruleus (LC)—the primary source of noradrenaline in the brain (Dahlstroem and Fuxe, 1964). Thus, this study is more of a preliminary exploration and a more comprehensive investigation is needed. This could include assessment of the expression of these markers in the LC or probing alterations in LC-nigrostriatal connectivity using neuroimaging, which may reveal not only their impact on cognitive performance (Aston-Jones et al., 2000; Evans et al., 2022) but also the beginning of more subtle changes that could potentially set the stage for a key non-motor symptom associated with PD (Ohtsuka et al., 2013; Zarow et al., 2003a). In line with this, focal lesions of the basal ganglia have been shown to be associated with impairments in cognitive flexibility (Dubois et al., 2005) and individuals with PD are known to have impairments in cognitive flexibility that impact their daily life (Siquier and Andrés, 2021). Moreover, mounting evidence suggests that LC-NA system loss occurs before SN-DA (Braak and Del Tredici, 2008; Zarow et al., 2003a), and its projections play an essential role in regulating balance and maintaining DA neurons in the SN region (Jovanovic et al., 2022). It is crucial to elucidate the mechanisms by which the LC influences SN and, consequently, impacts cognitive performance. However, such an extensive examination was beyond the scope of the current thesis.

Contrary to the severity-dependent hypothesis, the dopamine pathway, particularly the dopamine receptor D1 (DRD1), exhibited upregulation solely in the PFC following a single mild TBI. This effect was attenuated in animals with repeated mild TBI or more severe TBI and was not observed in other brain regions. While the reason for this differential effect remains unclear, the single mild TBI animals from the same cohort also demonstrated increased time spent in and higher entries to the open arms of the elevated plus maze at 12 months post-injury, indicating reduced anxiety, a behaviour that was not seen in animals subjected to other TBI severities (Arulsamy et al., 2019). This suggests that DRD1 upregulation in the PFC may be

associated with reduced anxiety behaviour, specifically following a single mild TBI. In support of this, a growing body of preclinical research links DRD1 activity to anxiety and risk-taking behaviour (Beyer et al., 2021; Hare et al., 2019; Sonntag et al., 2014). However, previous work from our lab refutes this assumption by demonstrating intact structural integrity in the PFC in animals subjected to single mild TBI via Western blot, diffuse tensor imaging and immunohistochemistry (Supplementary Figure 1-3). While this discrepancy warrants further investigation, these findings provide further evidence for the association between prefrontal DRD1 and anxiety, and support that behavioural changes may be sustained long-term following a single mild TBI. Nonetheless, investigating anxiety-related regions, such as the hippocampus and amygdala, is crucial to gaining a more comprehensive understanding of this link, particularly the effect of a single mild TBI on chronic anti-anxiety behaviour (Shin, 2006; Shin and Liberzon, 2010).

Overall, the investigation in both catecholamine markers and neuroinflammation in the SN, striatum and PFC demonstrated minimal changes at 12 months following diffuse axonal injury. It is important to note that analysis of alpha-synuclein levels using Western blot in the same cohort also did not reveal changes in either the SN or striatum at this time point (Alina Arulsamy's unpublished 2019 thesis). Although examining earlier regions of Lewy body deposition, such as the LC and the olfactory bulb (Braak et al., 2003), may be more relevant in these still relatively young (~15-month-old animals), these findings nevertheless demonstrate that detecting the brain mechanisms that may set the stage for PD development following a TBI alone is challenging. This notion is further supported by the largely preserved motor function, as indicated by normal locomotor activity, among animals in this cohort at the 12-month timepoint following injury (Arulsamy et al., 2019), although it is important to note that a comprehensive analysis of motor function was not conducted in this cohort. Nevertheless, this is also supported by a study by Gardner and colleagues (Gardner et al., 2015). Despite the

increased likelihood of individuals with a history of TBI being diagnosed with PD (hazard ratio 44%, 95% confidence interval = 1.31-1.58), the vast majority did not develop PD (Gardner et al., 2015). This suggests there must be additional factors that either increase or protect against susceptibility to post-TBI neurodegeneration (Pang et al., 2019).

As detailed in Chapter 1, pesticide exposure represents another robust environmental factor contributing to an elevated risk of PD (Noyce et al., 2012). Previous epidemiological work by Lee and colleagues (2012) revealed that while both TBI and paraquat exposure individually moderately increased PD risk, combining these factors tripled the risk (adjusted odd ratio 3.01, 95% CI 1.51-6.01) (Lee et al., 2012). This synergistic effect is further examined in animal work, where administering twice the 10mg/kg intraperitoneal paraquat injections after a moderate TBI resulted in a 30% loss of DA neurons in the SN, compared to the 15% loss observed with TBI alone (Hutson et al., 2011). This suggests that pesticide exposure induces pathological vulnerability within key brain circuits, such as the nigrostriatal pathway, and in the presence of TBI, they could synergistically exacerbate the risk of PD.

While acknowledging this connection, the mechanisms that may drive disease progression following the combination of these risk factors remain largely unclear. To probe this further, the development of a preclinical model exposed to chronic, "ambient-level" pesticides before introducing TBI is needed. Pesticide, particularly rotenone, has been commonly employed to generate a PD model, characterised by a substantial loss of DA neurons and highly upregulated neuroinflammation in the nigrostriatal pathway. However, the dosage previously generates a mature PD model that consists of the cardinal motor symptoms and pathological hallmarks (Li et al., 2012; Sherer et al., 2003; Zhang et al., 2017), without capturing the onset and progression of the disease. Thus, to use rotenone to establish a model reflecting underlying vulnerability, featuring mild DA loss (below 20%) and

neuroinflammatory responses, optimisation of the current dosage is needed before introducing TBI (Chapter 4).

Since this was the first time that subcutaneous rotenone was used to create a model with mild dopaminergic vulnerability in the nigrostriatal pathway, much lower rotenone dosages of 0.5mg/kg/day and 1.0/kg/day for 7 and 14 injections were applied in the first pilot study to investigate whether the dose and duration were sufficient to induce the desired vulnerability. The results revealed that the percentage of weight gain was significantly lower in 1mg/kg/day rotenone rats, particularly those with 14 injections, compared to the vehicles and animals subjected to 0.5mg/kg/day rotenone. However, the analysis of dopamine and neuroinflammatory markers demonstrated minimal changes in both striatum and SN that did not meet our expectations. Therefore, in the second pilot, the rotenone dosage was increased to 2mg/kg/day for 7 injections based on Sherer *et al.,* where 2.7mg/kg rotenone for 7 days reproduced the desired DA loss in the striatum but had a high systemic toxicity rate of 36% (Sherer et al., 2003). In our hands, at 24 hours after the 3rd injection, 2 out of 3 rotenone-injected animals exhibited significant parkinsonian symptoms, the adverse effect we sought to avoid. As a result, the rotenone dosage regimen was modified again in the third pilot study to involve 1.5mg/kg rotenone administered every 48 hours for either 3 or 6 injections, based on the work by Fathalla *et al.* (Fathalla et al., 2016). A battery of physiological and motor evaluations was performed. Across the twelve days of the injection period, no adverse effects on body weight or Parkinsonian-like symptoms were observed. Additionally, the animals receiving 6 injections consistently displayed lower neuroscores after the 4th injection, with no specific motor deficits observed in forelimb function. Histopathological evaluation revealed these animals exhibited a ~20% loss of dopaminergic neurons in the SN and increased microglial activation in the striatum. Collectively, these piloting works indicate that the optimum dosage for establishing a rat model with underlying vulnerability is a regime of 6 * 1.5mg/kg/48h subcutaneous

injection of rotenone. This outcome lays a solid foundation for the upcoming investigation into the synergistic effects of rotenone and TBI on the onset of PD.

Since symptom progression is important for identifying biomarkers associated with different disease stages and is crucial for early diagnosis, a specific focus was then on the functional changes following low-dose rotenone exposure and moderate-severe TBI, both independently and in combination at one month following the injury. Test results revealed a synergistic effect, mainly in motor impairments, first observed in composite neuroscore at 1-week post-injury and remaining on balance and motor coordination at 1-month post-injury. This impact, however, was not evident in other aspects of motor function, such as general motor function as assessed by the locomotor activity, parkinsonian-like symptoms, fine motor control and grip. Although a main effect of injury was observed in some of these functions, the effects were relatively subtle and inconsistent, aligning with our findings in the systematic review (Corrigan et al., 2023). In fact, motor symptoms during the prodromal PD stage are often subtle and may not significantly impact daily functioning (Gaenslen et al., 2011), similar to the patterns observed in this dual-hit animal model. While these findings should be interpreted with caution, interestingly, the presence of these subtle motor symptoms nevertheless appears to predict later conversion to PD. In line with this, a retro-prospective study with 93 PD patients indicated that 63.4% and 77.4% experienced general bradykinesia and reduced am swing—an indicator of the underlying issue with balance or motor coordination- 2.9 and 2.2 years before the PD diagnosis, respectively (Gaenslen et al., 2011). Similarly, a population-based cohort study involving 6038 participants without dementia and 100% free of any Parkinsonian signs found that consistent complaints of body stiffness and postural imbalance significantly increased the risk of developing PD during a 5.8-year follow-up, with stiffness showing a hazard ratio of 2.11 and imbalance a hazard ratio of 3.47 (De Lau et al., 2006). Importantly, changes in balance and motor coordination observed in tests such as the beam walk test are the

key hallmark of PD (Jessop et al., 2006). Thus, future studies should probe this further to investigate whether these subtle motor symptoms observed in this dual-hit animal model can serve as predictors for the later conversion to PD.

Interestingly, however, upon the examination of non-motor symptoms, no discernible effect on either anxiety or prodromal PD-related symptoms was observed. It is worth noting, however, that there was a subtle synergistic effect on both defecation rate and faecal water content percentage noted, indicating potential constipation in animals with a combination of rotenone exposure and TBI. However, further investigation, such as gastrointestinal transit ratio or colon tissue examination, is needed to confirm these findings (Camilleri and Linden, 2016). Importantly, a more pronounced synergistic effect was observed for cognition. While neither rotenone exposure nor TBI alone was sufficient to lead to impairment, the combination of rotenone and TBI resulted in learning-like deficits, although this effect did not extend to impacts on memory or cognitive flexibility. This is in line with literature supporting the idea that mild cognitive deficits manifest in the prodromal phase of PD (Muslimovic et al., 2005; Shibuya et al., 2001). A recent meta-analysis, incorporating findings from 9 prospective observational articles investigating cognitive performance in individuals free of PD and their subsequent development of PD, suggests that studies relying on cognitive screening measures did not discover a significant association between cognitive performance and the onset of idiopathic PD (Speelberg et al., 2022). In contrast, studies employing comprehensive cognitive test batteries, consisting of investigation of specific domains, including executive function, processing speed/attention, verbal memory, and language, provided a different perspective (Darweesh et al., 2017; Pausch et al., 2016; Weintraub et al., 2017). They revealed that specific cognitive deficits in global and executive functions were linked to an increased risk of developing PD, implying that cognitive impairment that appeared during prodromal PD is relatively subtle and domain-specific (Speelberg et al., 2022). Collectively, the observed mild

motor impairments and subtle cognitive deficits, coupled with some changes indicative of constipation, could potentially indicate that this dual-hit animal model may be useful for assessing the progressive symptomatology typically observed in prodromal-stage PD patients.

It is imperative to note, however, that it remains uncertain whether this observed synergistic impact on motor function and learning, as well as potentially constipation, at 1-month post-injury is a temporary outcome influenced by the acute effects of both rotenone toxicity and TBI, due to the relatively short duration of our study. Due to time constraints, our only exploration into the neuropathological alterations in this model to date was acutely following rotenone exposure in the pilot work (Chapter 4), where 6*1.5mg/kg/48h exhibited ~20% loss of DA neurons in SN and an increase of microglia activation in the striatum, and chronic impact of rotenone itself, as well as its effect in combination with moderate-severe diffuse axonal injury, remains elusive. Much work still needs to be done to delineate the progression of behavioural and neuropathological changes. This includes the extension of the temporal scope to unravel the trajectory of these synergistic effects, as well as the examination of molecular and cellular mechanisms, such as the dynamics of α-synuclein aggregation, degeneration of DA and NA neurons, and the involvement of mechanisms such as neuroinflammation and oxidative stress, in critical regions such as the olfactory bulb, LC, striatum and SN. This comprehensive approach would provide a deeper understanding of the intricate interplay between chronic low-dose rotenone exposure, moderate-severe TBI, and the emergence of Parkinson's disease-related pathology and could provide an exciting new animal model in which to further probe these effects, as well as potential treatment options.

Collectively, this thesis outlines the challenges in studying PD progression following TBI, particularly in chronic development of motor symptoms. While further assessment of this question is clearly needed, this thesis, nevertheless, provides evidence suggesting an association between TBI severity and persistent motor deficits, manifesting in a dose-

dependent pattern, in balance, gait, and overall speed over 10 years post-injury. The exploration of underlying neuropathology aligns with this pattern, with moderate-severe TBI having the most apparent impact on long-term neuropathogenesis, affecting the neuroinflammatory processes and noradrenaline signalling in the nigrostriatal pathway, accompanied by subtle impairments in cognitive flexibility at 12 months post-injury. An exception is noted in the single mild TBI model, where altered dopamine signalling in the prefrontal cortex was associated with a reduction in anxiety, an effect not found in the repeated mild injury. The reason for this differential effect remains largely unclear, emphasizing the need for future studies to expand the exploration across various facets and conditions, including oxidative stress and mitochondrial dysfunction, as well as investigations in regions like the olfactory bulb and LC, to rule out the possibility that the injury may have induced more subtle cellular consequences that are not readily observed by the approaches used in this thesis.

Furthermore, by introducing additional PD risk factor—rotenone exposure- prior to the TBI, this thesis provides support to existing literature (Hutson et al., 2011), revealing that dual environmental risk factors indeed generate more pronounced functional deficits than individual risk alone. In this case, exacerbated deficits in balance, motor coordination, and learning ability are observed. The subtle functional deficits induced by these dual PD risk factors, coupled with the preservation of other functional tests assessing motor, cognition, and prodromal symptoms (with the possible exception of constipation), may indicate heightened risk for the future development of PD. However, a comprehensive understanding of this phenomenon remains elusive without insights into the temporal profile of these functional deficits and the concomitant neuropathological changes occurring in the months following this dual-hit exposure. An in-depth analysis is imperative for unravelling the intricate relationship between dual environmental risk factors and the subsequent manifestation of functional impairments, contributing to a more comprehensive understanding of early PD progression.

While acknowledging the preliminary nature of this work, the thesis contributes significant insights into the mechanisms and symptomatology of TBI and explores the potential synergistic effect of low-level rotenone exposure and TBI. These findings offer valuable preliminary insights into the predictability of PD following such exposures, enriching our understanding of the early stages of PD and emphasising the need for further investigations into the intricate relationship between dual environmental risk factors and functional impairments.

Bibliography

Aarsland, D., Kurz, M.W., 2010. The epidemiology of dementia associated with Parkinson disease. J. Neurol. Sci. 289, 18–22. https://doi.org/10.1016/j.jns.2009.08.034

Abbott, R.D., Petrovitch, H., White, L.R., Masaki, K.H., Tanner, C.M., Curb, J.D., Grandinetti, A., Blanchette, P.L., Popper, J.S., Ross, G.W., 2001. Frequency of bowel movements and the future risk of Parkinson's disease. Neurology 57, 456–462. https://doi.org/10.1212/WNL.57.3.456

Abbott, R.D., Ross, G.W., White, L.R., Tanner, C.M., Masaki, K.H., Nelson, J.S., Curb, J.D., Petrovitch, H., 2005. Excessive daytime sleepiness and subsequent development of Parkinson disease. Neurology 65, 1442–1446. https://doi.org/10.1212/01.wnl.0000183056.89590.0d

Abdel-Salam, O.M.E., Morsy, S.M.Y., Youness, E.R., Yassen, N.N., Sleem, A.A., 2020. The effect of low dose amphetamine in rotenone-induced toxicity in a mice model of Parkinson's disease. Iran. J. Basic Med. Sci. https://doi.org/10.22038/ijbms.2020.45175.10524

Acosta, G., Race, N., Herr, S., Fernandez, J., Tang, J., Rogers, E., Shi, R., 2019. Acrolein-mediated alpha-synuclein pathology involvement in the early post-injury pathogenesis of mild blast-induced Parkinsonian neurodegeneration. Mol. Cell. Neurosci. 98, 140–154. https://doi.org/10.1016/j.mcn.2019.06.004

Acosta, S.A., Tajiri, N., de la Pena, I., Bastawrous, M., Sanberg, P.R., Kaneko, Y., Borlongan, C.V., 2015. Alpha-Synuclein as a Pathological Link Between Chronic Traumatic Brain Injury and Parkinson's Disease: PATHOLOGICAL LINK BETWEEN TBI AND PD. J. Cell. Physiol. 230, 1024–1032. https://doi.org/10.1002/jcp.24830

Acosta, S.A., Tajiri, N., Shinozuka, K., Ishikawa, H., Grimmig, B., Diamond, D., Sanberg, P.R., Bickford, P.C., Kaneko, Y., Borlongan, C.V., 2013. Long-Term Upregulation of Inflammation and Suppression of Cell Proliferation in the Brain of Adult Rats Exposed to Traumatic Brain Injury Using the Controlled Cortical Impact Model. PLoS ONE 8, e53376. https://doi.org/10.1371/journal.pone.0053376

Adatia, K., Newcombe, V.F.J., Menon, D.K., 2021. Contusion Progression Following Traumatic Brain Injury: A Review of Clinical and Radiological Predictors, and Influence on Outcome. Neurocrit. Care 34, 312–324. https://doi.org/10.1007/s12028-020-00994-4

Adkins, D.L., Ferguson, L., Lance, S., Pevtsov, A., McDonough, K., Stamschror, J., Jones, T.A., Kozlowski, D.A., 2015. Combining Multiple Types of Motor Rehabilitation Enhances Skilled Forelimb Use Following Experimental Traumatic Brain Injury in Rats. Neurorehabil. Neural Repair 29, 989–1000. https://doi.org/10.1177/1545968315576577

Adnan, A., Crawley, A., Mikulis, D., Moscovitch, M., Colella, B., Green, R., 2013. Moderate–severe traumatic brain injury causes delayed loss of white matter integrity: Evidence of fornix deterioration in the chronic stage of injury. Brain Inj. 27, 1415–1422. https://doi.org/10.3109/02699052.2013.823659

af Bjerkén, S., Stenmark Persson, R., Barkander, A., Karalija, N., Pelegrina-Hidalgo, N., Gerhardt, G.A., Virel, A., Strömberg, I., 2019. Noradrenaline is crucial for the substantia nigra dopaminergic cell maintenance. Neurochem. Int. 131, 104551. https://doi.org/10.1016/j.neuint.2019.104551

Agrawal, M., Joshi, M., 2014. Impact of rehabilitation on functional outcome during the first year of moderate and severe traumatic brain injury. Brain Inj. 28, 292–297. https://doi.org/10.3109/02699052.2013.865266

Al-Hassani, A., Strandvik, G.F., El-Menyar, A., Dhumale, A.R., Asim, M., Ajaj, A., Al-Yazeedi, W., Al-Thani, H., 2018. Functional Outcomes in Moderate-to-Severe Traumatic Brain Injury Survivors. J. Emerg. Trauma Shock 11, 197–204. https://doi.org/10.4103/JETS.JETS_6_18

Al-Kader, D.A., Onyechi, C.I., Ikedum, I.V., Fattah, A., Zafar, S., Bhat, S., Malik, M.A., Bheesham, N., Qadar, L.T., Sajjad Cheema, M., 2022. Depression and Anxiety in Patients With a History of Traumatic Brain Injury: A Case-Control Study. Cureus 14, e27971. https://doi.org/10.7759/cureus.27971

Albayram, O., Kondo, A., Mannix, R., Smith, C., Tsai, C.-Y., Li, C., Herbert, M.K., Qiu, J., Monuteaux, M., Driver, J., Yan, S., Gormley, W., Puccio, A.M., Okonkwo, D.O., Lucke-Wold, B., Bailes, J., Meehan, W., Zeidel, M., Lu, K.P., Zhou, X.Z., 2017a. Cis P-tau is induced in clinical and preclinical brain injury and contributes to post-injury sequelae. Nat. Commun. 8, 1000. https://doi.org/10.1038/s41467-017-01068-4

Albrecht, F., Poulakis, K., Freidle, M., Johansson, H., Ekman, U., Volpe, G., Westman, E., Pereira, J.B., Franzén, E., 2022. Unraveling Parkinson's disease heterogeneity using subtypes based on multimodal data. Parkinsonism Relat. Disord. 102, 19–29. https://doi.org/10.1016/j.parkreldis.2022.07.014

Algarni, M.A., Stoessl, A.J., 2016. The role of biomarkers and imaging in Parkinson's disease. Expert Rev. Neurother. 16, 187–203. https://doi.org/10.1586/14737175.2016.1135056

Alwis, D.S., Yan, E.B., Morganti-Kossmann, M.-C., Rajan, R., 2012. Sensory Cortex Underpinnings of Traumatic Brain Injury Deficits. PLoS ONE 7, e52169. https://doi.org/10.1371/journal.pone.0052169

Andriessen, T.M.J.C., Jacobs, B., Vos, P.E., 2010. Clinical characteristics and pathophysiological mechanisms of focal and diffuse traumatic brain injury. J. Cell. Mol. Med. 14, 2381–2392. https://doi.org/10.1111/j.1582-4934.2010.01164.x

Arab, H.H., Safar, M.M., Shahin, N.N., 2021. Targeting ROS-Dependent AKT/GSK-3β/NF-κB and DJ-1/Nrf2 Pathways by Dapagliflozin Attenuates Neuronal Injury and Motor Dysfunction in Rotenone-Induced Parkinson's Disease Rat Model. ACS Chem. Neurosci. 12, 689–703. https://doi.org/10.1021/acschemneuro.0c00722

Arce, F.I., Katz, N., Sugarman, H., 2004. The scaling of postural adjustments during bimanual load-lifting in traumatic brain-injured adults. Hum. Mov. Sci. 22, 749–768. https://doi.org/10.1016/j.humov.2003.12.002

Arnsten, A., Cai, J., Goldman-Rakic, P., 1988. The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: evidence for alpha-2 receptor subtypes. J. Neurosci. 8, 4287–4298. https://doi.org/10.1523/JNEUROSCI.08-11-04287.1988

Arulsamy, A., Corrigan, F., Collins-Praino, L.E., 2019a. Cognitive and neuropsychiatric impairments vary as a function of injury severity at 12 months post-experimental diffuse traumatic brain injury: Implications for dementia development. Behav. Brain Res. 365, 66–76. https://doi.org/10.1016/j.bbr.2019.02.045

Arulsamy, A., Corrigan, F., Collins-Praino, L.E., 2019b. Age, but not severity of injury, mediates decline in executive function: Validation of the rodent touchscreen paradigm for preclinical models of traumatic brain injury. Behav. Brain Res. 368, 111912. https://doi.org/10.1016/j.bbr.2019.111912

Arulsamy, A., Teng, J., Colton, H., Corrigan, F., Collins-Praino, L., 2018. Evaluation of early chronic functional outcomes and their relationship to pre-frontal cortex and hippocampal pathology following moderate-severe traumatic brain injury. Behav. Brain Res. 348, 127–138. https://doi.org/10.1016/j.bbr.2018.04.009

Arun, P., Wilder, D.M., Eken, O., Urioste, R., Batuure, A., Sajja, S., Van Albert, S., Wang, Y., Gist, I.D., Long, J.B., 2020. Long-Term Effects of Blast Exposure: A Functional Study in Rats Using an Advanced Blast Simulator. J. Neurotrauma 37, 647–655. https://doi.org/10.1089/neu.2019.6591

Ashraf, W., Pfeiffer, R.F., Park, F., Lof, J., Quigley, E.M.M., 1997. Constipation in parkinson's disease: Objective assessment and response to psyllium. Mov. Disord. 12, 946–951. https://doi.org/10.1002/mds.870120617

Assad, M., Galperin, I., Giladi, N., Mirelman, A., Hausdorff, J.M., Maidan, I., 2022. Disease severity and prefrontal cortex activation during obstacle negotiation among patients with Parkinson's disease: Is it all as expected? Parkinsonism Relat. Disord. 101, 20–26. https://doi.org/10.1016/j.parkreldis.2022.06.006

Aston-Jones, G., Rajkowski, J., Cohen, J., 2000. Locus coeruleus and regulation of behavioral flexibility and attention. Prog. Brain Res. 126, 165–182. https://doi.org/10.1016/S0079-6123(00)26013-5

Atias, M., Tevet, Y., Sun, J., Stavsky, A., Tal, S., Kahn, J., Roy, S., Gitler, D., 2019. Synapsins regulate α-synuclein functions. Proc. Natl. Acad. Sci. 116, 11116–11118. https://doi.org/10.1073/pnas.1903054116

Avery, R., 2000. The Alpha-2A-Adrenoceptor Agonist, Guanfacine, Increases Regional Cerebral Blood Flow in Dorsolateral Prefrontal Cortex of Monkeys Performing a Spatial Working Memory Task. Neuropsychopharmacology 23, 240–249. https://doi.org/10.1016/S0893-133X(00)00111-1

Azimullah, S., Jayaraj, R.L., Meeran, M.Fizur.N., Jalal, F.Y., Adem, A., Ojha, S., Beiram, R., 2023. Myrcene Salvages Rotenone-Induced Loss of Dopaminergic Neurons by Inhibiting Oxidative Stress, Inflammation, Apoptosis, and Autophagy. Molecules 28, 685. https://doi.org/10.3390/molecules28020685

Bae, E.-J., Yang, N.-Y., Song, M., Lee, C.S., Lee, J.S., Jung, B.C., Lee, H.-J., Kim, S., Masliah, E., Sardi, S.P., Lee, S.-J., 2014. Glucocerebrosidase depletion enhances cell-to-cell transmission of α-synuclein. Nat. Commun. 5, 4755. https://doi.org/10.1038/ncomms5755

Bai, Q., He, J., Tang, Y., Wang, S., Qiu, J., Wang, Y., Yu, C., 2016. Rotenone-induced energy stress decompensated in ventral mesocerebrum is associated with Parkinsonism progression in rats. Exp. Ther. Med. 12, 1060–1066. https://doi.org/10.3892/etm.2016.3352

Bajwa, N.M., Halavi, S., Hamer, M., Semple, B.D., Noble-Haeusslein, L.J., Baghchechi, M., Hiroto, A., Hartman, R.E., Obenaus, A., 2016. Mild Concussion, but Not Moderate Traumatic Brain Injury, Is Associated with Long-Term Depression-Like Phenotype in Mice. PLOS ONE 11, e0146886. https://doi.org/10.1371/journal.pone.0146886

Balabandian, M., Noori, M., Lak, B., Karimizadeh, Z., Nabizadeh, F., 2023. Traumatic brain injury and risk of Parkinson's disease: a meta-analysis. Acta Neurol. Belg. 123, 1225–1239. https://doi.org/10.1007/s13760-023-02209-x

Baldereschi, M., Di Carlo, A., Vanni, P., Ghetti, A., Carbonin, P., Amaducci, L., Inzitari, D., for the Italian Longitudinal Study on Aging Working Group, 2003. Lifestyle-related risk factors for Parkinson's disease: a population-based study: Risk factors for Parkinson's disease. Acta Neurol. Scand. 108, 239–244. https://doi.org/10.1034/j.1600-0404.2003.00128.x

Bales, J.W., Kline, A.E., Wagner, A.K., Dixon, C.E., 2010. Targeting Dopamine in Acute Traumatic Brain Injury. Open Drug Discov. J. 2, 119–128. https://doi.org/10.2174/1877381801002010119

Ball, N., Teo, W.-P., Chandra, S., Chapman, J., 2019. Parkinson's Disease and the Environment. Front. Neurol. 10, 218. https://doi.org/10.3389/fneur.2019.00218

Bang, Y., Lim, J., Choi, H.J., 2021. Recent advances in the pathology of prodromal non-motor symptoms olfactory deficit and depression in Parkinson's disease: clues to early diagnosis and effective treatment. Arch. Pharm. Res. 44, 588–604. https://doi.org/10.1007/s12272-021-01337-3

Bareeqa, S.B., Samar, S.S., Kamal, S., Masood, Y., Allahyar, Ahmed, S.I., Hayat, G., 2022. Prodromal depression and subsequent risk of developing Parkinson's disease: a systematic review with meta-analysis. Neurodegener. Dis. Manag. 12, 155–164. https://doi.org/10.2217/nmt-2022-0001

Barlow, B.K., Lee, D.W., Cory-Slechta, D.A., Opanashuk, L.A., 2005. Modulation of Antioxidant Defense Systems by the Environmental Pesticide Maneb in Dopaminergic Cells. NeuroToxicology 26, 63–75. https://doi.org/10.1016/j.neuro.2004.07.004

Barnes, C.A., 1979. Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. J. Comp. Physiol. Psychol. 93, 74–104. https://doi.org/10.1037/h0077579

Barnes, M.A., Carson, M.J., Nair, M.G., 2015. Non-traditional cytokines: How catecholamines and adipokines influence macrophages in immunity, metabolism and the central nervous system. Cytokine 72, 210–219. https://doi.org/10.1016/j.cyto.2015.01.008

Barone, P., 2010. Neurotransmission in Parkinson's disease: beyond dopamine: Neurotransmission in Parkinson's disease. Eur. J. Neurol. 17, 364–376. https://doi.org/10.1111/j.1468-1331.2009.02900.x

Barrett, J.P., Henry, R.J., Shirey, K.A., Doran, S.J., Makarevich, O.D., Ritzel, R.M., Meadows, V.A., Vogel, S.N., Faden, A.I., Stoica, B.A., Loane, D.J., 2020. Interferon-β Plays a Detrimental Role in Experimental Traumatic Brain Injury by Enhancing Neuroinflammation That Drives Chronic Neurodegeneration. J. Neurosci. 40, 2357–2370. https://doi.org/10.1523/JNEUROSCI.2516-19.2020

Bélanger, M., Magistretti, P.J., 2009. The role of astroglia in neuroprotection. Dialogues Clin. Neurosci. 11, 281–295. https://doi.org/10.31887/DCNS.2009.11.3/mbelanger

Belli, S., Vanacore, N., 2005. Proportionate mortality of Italian soccer players: is amyotrophic lateral sclerosis an occupational disease? Eur. J. Epidemiol. 20, 237–242. https://doi.org/10.1007/s10654-004-6879-7

Belvisi, D., Pellicciari, R., Fabbrini, A., Costanzo, M., Pietracupa, S., De Lucia, M., Modugno, N., Magrinelli, F., Dallocchio, C., Ercoli, T., Terravecchia, C., Nicoletti, A., Solla, P., Fabbrini, G., Tinazzi, M., Berardelli, A., Defazio, G., 2020. Risk factors of Parkinson disease: Simultaneous assessment, interactions, and etiologic subtypes. Neurology 95, e2500–e2508. https://doi.org/10.1212/WNL.0000000000010813

Bendlin, B.B., Ries, M.L., Lazar, M., Alexander, A.L., Dempsey, R.J., Rowley, H.A., Sherman, J.E., Johnson, S.C., 2008. Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. NeuroImage 42, 503–514. https://doi.org/10.1016/j.neuroimage.2008.04.254

Berg, D., Postuma, R.B., Adler, C.H., Bloem, B.R., Chan, P., Dubois, B., Gasser, T., Goetz, C.G., Halliday, G., Joseph, L., Lang, A.E., Liepelt-Scarfone, I., Litvan, I., Marek, K., Obeso, J., Oertel, W., Olanow, C.W., Poewe, W., Stern, M., Deuschl, G., 2015. MDS research criteria for prodromal Parkinson's disease: MDS Criteria for Prodromal PD. Mov. Disord. 30, 1600–1611. https://doi.org/10.1002/mds.26431

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., Seitelberger, F., 1973. Brain dopamine and the syndromes of Parkinson and Huntington Clinical, morphological and neurochemical correlations. J. Neurol. Sci. 20, 415–455. https://doi.org/10.1016/0022-510X(73)90175-5

Betarbet, R., Canet-Aviles, R.M., Sherer, T.B., Mastroberardino, P.G., McLendon, C., Kim, J.-H., Lund, S., Na, H.-M., Taylor, G., Bence, N.F., Kopito, R., Seo, B.B., Yagi, T., Yagi, A., Klinefelter, G., Cookson, M.R., Greenamyre, J.T., 2006. Intersecting pathways to neurodegeneration in Parkinson's disease: Effects of the pesticide rotenone on DJ-1, α-synuclein, and the ubiquitin–proteasome system. Neurobiol. Dis. 22, 404–420. https://doi.org/10.1016/j.nbd.2005.12.003

Betarbet, R., Sherer, T.B., Greenamyre, J.T., 2002. Animal models of Parkinson's disease. BioEssays 24, 308–318. https://doi.org/10.1002/bies.10067

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. 3, 1301–1306. https://doi.org/10.1038/81834

Beyer, D.K.E., Mattukat, A., Freund, N., 2021. Prefrontal dopamine D1 receptor manipulation influences anxiety behavior and induces neuroinflammation within the hippocampus. Int. J. Bipolar Disord. 9, 9. https://doi.org/10.1186/s40345-020-00212-2

Bharucha, N.E., Stokes, L., Schoenberg, B.S., Ward, C., Ince, S., Nutt, J.G., Eldridge, R., Calne, D.B., Mantel, N., Duvoisin, R., 1986. A case-control study of twin pairs discordant for Parkinson's disease: A search for environmental risk factors. Neurology 36, 284–284. https://doi.org/10.1212/WNL.36.2.284

Blum-Degena, D., Müller, T., Kuhn, W., Gerlach, M., Przuntek, H., Riederer, P., 1995. Interleukin-1β and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci. Lett. 202, 17–20. https://doi.org/10.1016/0304-3940(95)12192-7

Bolton Hall, A.N., Joseph, B., Brelsfoard, J.M., Saatman, K.E., 2016. Repeated Closed Head Injury in Mice Results in Sustained Motor and Memory Deficits and Chronic Cellular Changes. PLOS ONE 11, e0159442. https://doi.org/10.1371/journal.pone.0159442

Bonifati, V., Rizzu, P., Van Baren, M.J., Schaap, O., Breedveld, G.J., Krieger, E., Dekker, M.C.J., Squitieri, F., Ibanez, P., Joosse, M., Van Dongen, J.W., Vanacore, N., Van Swieten, J.C., Brice, A., Meco, G., Van Duijn, C.M., Oostra, B.A., Heutink, P., 2003. Mutations in the

DJ-1 Gene Associated with Autosomal Recessive Early-Onset Parkinsonism. Science 299, 256–259. https://doi.org/10.1126/science.1077209

Booij, J., Tissingh, G., Boer, G.J., Speelman, J.D., Stoof, J.C., Janssen, A.G., Wolters, E.C., Van Royen, E.A., 1997. [123I]FP-CIT SPECT shows a pronounced decline of striatal dopamine transporter labelling in early and advanced Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 62, 133–140. https://doi.org/10.1136/jnnp.62.2.133

Bouet, V., Boulouard, M., Toutain, J., Divoux, D., Bernaudin, M., Schumann-Bard, P., Freret, T., 2009. The adhesive removal test: a sensitive method to assess sensorimotor deficits in mice. Nat. Protoc. 4, 1560–1564. https://doi.org/10.1038/nprot.2009.125

Bower, J.H., Maraganore, D.M., Peterson, B.J., McDonnell, S.K., Ahlskog, J.E., Rocca, W.A., 2003. Head trauma preceding PD: a case-control study. Neurology 60, 1610–1615. https://doi.org/10.1212/01.wnl.0000068008.78394.2c

Braak, H., Del Tredici, K., 2008. Invited Article: Nervous system pathology in sporadic Parkinson disease. Neurology 70, 1916–1925. https://doi.org/10.1212/01.wnl.0000312279.49272.9f

Braak, H., Rb, U., Gai, W.P., Del Tredici, K., 2003. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J. Neural Transm. 110, 517–536. https://doi.org/10.1007/s00702-002-0808-2

Braak, Heiko, Tredici, K.D., Rüb, U., de Vos, R.A.I., Jansen Steur, E.N.H., Braak, E., 2003. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 24, 197–211. https://doi.org/10.1016/S0197-4580(02)00065-9

Bramlett, H.M., Dietrich, W.D., 2015. Long-Term Consequences of Traumatic Brain Injury: Current Status of Potential Mechanisms of Injury and Neurological Outcomes. J. Neurotrauma 32, 1834–1848. https://doi.org/10.1089/neu.2014.3352

Brett, B.L., Gardner, R.C., Godbout, J., Dams-O'Connor, K., Keene, C.D., 2022. Traumatic Brain Injury and Risk of Neurodegenerative Disorder. Trauma. Brain Inj. Ment. Health 91, 498–507. https://doi.org/10.1016/j.biopsych.2021.05.025

Budinich, C.S., Tucker, L.B., Lowe, D., Rosenberger, J.G., McCabe, J.T., 2013. Short and long-term motor and behavioral effects of diazoxide and dimethyl sulfoxide administration in the mouse after traumatic brain injury. Pharmacol. Biochem. Behav. 108, 66–73. https://doi.org/10.1016/j.pbb.2013.04.001

Burton, C.L., Hultsch, D.F., Strauss, E., Hunter, M.A., 2002. Intraindividual Variability in Physical and Emotional Functioning: Comparison of Adults With Traumatic Brain Injuries and Healthy Adults. Clin. Neuropsychol. 16, 264–279. https://doi.org/10.1076/clin.16.3.264.13854

Buster, T., Burnfield, J., Taylor, A.P., Stergiou, N., 2013. Lower Extremity Kinematics During Walking and Elliptical Training in Individuals With and Without Traumatic Brain Injury: J. Neurol. Phys. Ther. 37, 176–186. https://doi.org/10.1097/NPT.0000000000000022

Caccese, J.B., Santos, F.V., Yamaguchi, F.K., Buckley, T.A., Jeka, J.J., 2021. Persistent Visual and Vestibular Impairments for Postural Control Following Concussion: A Cross-Sectional Study in University Students. Sports Med. Auckl. NZ 51, 2209–2220. https://doi.org/10.1007/s40279-021-01472-3

Camilleri, M., Linden, D.R., 2016. Measurement of Gastrointestinal and Colonic Motor Functions in Humans and Animals. Cell. Mol. Gastroenterol. Hepatol. 2, 412–428. https://doi.org/10.1016/j.jcmgh.2016.04.003

Cannon, J.R., Tapias, V., Na, H.M., Honick, A.S., Drolet, R.E., Greenamyre, J.T., 2009. A highly reproducible rotenone model of Parkinson's disease. Neurobiol. Dis. 34, 279–290. https://doi.org/10.1016/j.nbd.2009.01.016

Carboni, E., Tanda, G.L., Frau, R., Chiara, G.D., 1990. Blockade of the Noradrenaline Carrier Increases Extracellular Dopamine Concentrations in the Prefrontal Cortex: Evidence that Dopamine Is Taken up In Vivo by Noradrenergic Terminals. J. Neurochem. 55, 1067–1070. https://doi.org/10.1111/j.1471-4159.1990.tb04599.x

Carlson, S., Tanila, H., Rama, P., Mecke, E., Pertovaara, A., 1992. Effects of medetomidine, an α-2 adrenoceptor agonist, and atipamezole, an α-2 antagonist, on spatial memory performance in adult and aged rats. Behav. Neural Biol. 58, 113–119. https://doi.org/10.1016/0163-1047(92)90327-Z

Castellani, R.J., Perry, G., Siedlak, S.L., Nunomura, A., Shimohama, S., Zhang, J., Montine, T., Sayre, L.M., Smith, M.A., 2002. Hydroxynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem Lewy bodies in humans. Neurosci. Lett. 319, 25–28. https://doi.org/10.1016/S0304-3940(01)02514-9

Cavanaugh, J.T., Guskiewicz, K.M., Giuliani, C., Marshall, S., Mercer, V.S., Stergiou, N., 2006. Recovery of postural control after cerebral concussion: new insights using approximate entropy. J. Athl. Train. 41, 305–313.

Cebrián, C., Loike, J.D., Sulzer, D., 2014. Neuroinflammation in Parkinson's Disease Animal Models: A Cell Stress Response or a Step in Neurodegeneration?, in: Nguyen, H.H.P., Cenci, M.A. (Eds.), Behavioral Neurobiology of Huntington's Disease and Parkinson's Disease, Current Topics in Behavioral Neurosciences. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 237–270. https://doi.org/10.1007/7854_2014_356

Cernak, I., Savic, V.J., Kotur, J., Prokic, V., Veljovic, M., Grbovic, D., 2000. Characterization of Plasma Magnesium Concentration and Oxidative Stress Following Graded Traumatic Brain Injury in Humans. J. Neurotrauma 17, 53–68. https://doi.org/10.1089/neu.2000.17.53

Chandler, J.H., Marking, L.L., 1982. Toxicity of Rotenone to Selected Aquatic Invertebrates and Frog Larvae. Progress. Fish-Cult. 44, 78–80. https://doi.org/10.1577/1548-8659(1982)44[78:TORTSA]2.0.CO;2

Chen, A.-D., Cao, J.-X., Chen, H.-C., Du, H.-L., Xi, X.-X., Sun, J., Yin, J., Jing, Y.-H., Gao, L.-P., 2022. Rotenone aggravates PD-like pathology in A53T mutant human α-synuclein transgenic mice in an age-dependent manner. Front. Aging Neurosci. 14, 842380. https://doi.org/10.3389/fnagi.2022.842380

Chen, H., Chan, Y.L., Nguyen, L.T., Mao, Y., de Rosa, A., Beh, I.T., Chee, C., Oliver, B., Herok, G., Saad, S., Gorrie, C., 2016. Moderate traumatic brain injury is linked to acute behaviour deficits and long term mitochondrial alterations. Clin. Exp. Pharmacol. Physiol. 43, 1107–1114. https://doi.org/10.1111/1440-1681.12650

Chen, H., Richard, M., Sandler, D.P., Umbach, D.M., Kamel, F., 2007. Head injury and amyotrophic lateral sclerosis. Am. J. Epidemiol. 166, 810–816. https://doi.org/10.1093/aje/kwm153

Chen, H., Ritz, B., 2018. The Search for Environmental Causes of Parkinson's Disease: Moving Forward. J. Park. Dis. 8, S9–S17. https://doi.org/10.3233/JPD-181493

Chen, W., Man, X., Zhang, Y., Yao, G., Chen, J., 2023. Medial prefrontal cortex oxytocin mitigates epilepsy and cognitive impairments induced by traumatic brain injury through reducing neuroinflammation in mice. Sci. Rep. 13, 5214. https://doi.org/10.1038/s41598-023-32351-8

Chen, Y.-H., Huang, E.Y.-K., Kuo, T.-T., Miller, J., Chiang, Y.-H., Hoffer, B.J., 2017. Impact of Traumatic Brain Injury on Dopaminergic Transmission. Cell Transplant. 26, 1156–1168. https://doi.org/10.1177/0963689717714105

Cheng, H., Ulane, C.M., Burke, R.E., 2010. Clinical progression in Parkinson disease and the neurobiology of axons. Ann. Neurol. 67, 715–725. https://doi.org/10.1002/ana.21995

Cherian, L., Hlatky, R., Robertson, C.S., 2004. Nitric Oxide in Traumatic Brain Injury. Brain Pathol. 14, 195–201. https://doi.org/10.1111/j.1750-3639.2004.tb00053.x

Chien, Chien, Liu, Wu, Chang, Wu, 2019. Effects of Methylphenidate on Cognitive Function in Adults with Traumatic Brain Injury: A Meta-Analysis. Brain Sci. 9, 291. https://doi.org/10.3390/brainsci9110291

Chiò, A., Benzi, G., Dossena, M., Mutani, R., Mora, G., 2005. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. Brain J. Neurol. 128, 472–476. https://doi.org/10.1093/brain/awh373

Chmielewski, T.L., Tatman, J., Suzuki, S., Horodyski, M., Reisman, D.S., Bauer, R.M., Clugston, J.R., Herman, D.C., 2021. Impaired motor control after sport-related concussion

could increase risk for musculoskeletal injury: Implications for clinical management and rehabilitation. J. Sport Health Sci. 10, 154–161. https://doi.org/10.1016/j.jshs.2020.11.005

Choi, Y., Kim, E.Y., Sun, J., Kim, H.-K., Lee, Y.S., Oh, B.-M., Park, H.Y., Leigh, J.-H., 2022. Incidence of Depression after Traumatic Brain Injury: A Nationwide Longitudinal Study of 2.2 Million Adults. J. Neurotrauma 39, 390–397. https://doi.org/10.1089/neu.2021.0111

Choi, Y., Novak, J.C., Hillier, A., Votolato, N.A., Beversdorf, D.Q., 2006. The Effect of α-2 Adrenergic Agonists on Memory and Cognitive Flexibility. Cogn. Behav. Neurol. 19, 204–207. https://doi.org/10.1097/01.wnn.0000213919.95266.0d

Cline, M.M., Yumul, J.C., Hysa, L., Murra, D., Garwin, G.G., Cook, D.G., Ladiges, W.C., Minoshima, S., Cross, D.J., 2017. Novel application of a Radial Water Tread maze can distinguish cognitive deficits in mice with traumatic brain injury. Brain Res. 1657, 140–147. https://doi.org/10.1016/j.brainres.2016.11.027

Collingridge, G.L., James, T.A., MacLeod, N.K., 1979. Neurochemical and electrophysiological evidence for a projection from the locus coeruleus to the substantia nigra [proceedings]. J. Physiol. 290, 44P.

Combs, H.L., Folley, B.S., Berry, D.T.R., Segerstrom, S.C., Han, D.Y., Anderson-Mooney, A.J., Walls, B.D., Van Horne, C., 2015. Cognition and Depression Following Deep Brain Stimulation of the Subthalamic Nucleus and Globus Pallidus Pars Internus in Parkinson's Disease: A Meta-Analysis. Neuropsychol. Rev. 25, 439–454. https://doi.org/10.1007/s11065-015-9302-0

Corrigan, F., Arulsamy, A., Collins-Praino, L.E., Holmes, J.L., Vink, R., 2017a. Toll like receptor 4 activation can be either detrimental or beneficial following mild repetitive traumatic brain injury depending on timing of activation. Brain. Behav. Immun. 64, 124–139. https://doi.org/10.1016/j.bbi.2017.04.006

Corrigan, F., Arulsamy, A., Teng, J., Collins-Praino, L.E., 2017b. Pumping the Brakes: Neurotrophic Factors for the Prevention of Cognitive Impairment and Dementia after Traumatic Brain Injury. J. Neurotrauma 34, 971–986. https://doi.org/10.1089/neu.2016.4589

Corrigan, F., Wee, I.C., Collins-Praino, L.E., 2023. Chronic motor performance following different traumatic brain injury severity—A systematic review. Front. Neurol. 14, 1180353. https://doi.org/10.3389/fneur.2023.1180353

Corrigan, J.D., Hammond, F.M., 2013. Traumatic Brain Injury as a Chronic Health Condition. Arch. Phys. Med. Rehabil. 94, 1199–1201. https://doi.org/10.1016/j.apmr.2013.01.023

Cory-Slechta, D.A., Thiruchelvam, M., Barlow, B.K., Richfield, E.K., 2005. Developmental Pesticide Models of the Parkinson Disease Phenotype. Environ. Health Perspect. 113, 1263–1270. https://doi.org/10.1289/ehp.7570

Costello, S., Cockburn, M., Bronstein, J., Zhang, X., Ritz, B., 2009. Parkinson's Disease and Residential Exposure to Maneb and Paraquat From Agricultural Applications in the Central Valley of California. Am. J. Epidemiol. 169, 919–926. https://doi.org/10.1093/aje/kwp006

Cragg, S.J., Rice, M.E., Greenfield, S.A., 1997. Heterogeneity of Electrically Evoked Dopamine Release and Reuptake in Substantia Nigra, Ventral Tegmental Area, and Striatum. J. Neurophysiol. 77, 863–873. https://doi.org/10.1152/jn.1997.77.2.863

Crane, P.K., Gibbons, L.E., Dams-O'Connor, K., Trittschuh, E., Leverenz, J.B., Keene, C.D., Sonnen, J., Montine, T.J., Bennett, D.A., Leurgans, S., Schneider, J.A., Larson, E.B., 2016. Association of Traumatic Brain Injury With Late-Life Neurodegenerative Conditions and Neuropathologic Findings. JAMA Neurol. 73, 1062. https://doi.org/10.1001/jamaneurol.2016.1948

Crossman, A.R., 2000. Functional anatomy of movement disorders. J. Anat. 196, 519–525. https://doi.org/10.1046/j.1469-7580.2000.19640519.x

Daglas, M., Draxler, D.F., Ho, H., McCutcheon, F., Galle, A., Au, A.E., Larsson, P., Gregory, J., Alderuccio, F., Sashindranath, M., Medcalf, R.L., 2019. Activated CD8(+) T Cells Cause Long-Term Neurological Impairment after Traumatic Brain Injury in Mice. Cell Rep. 29, 1178-1191.e6. https://doi.org/10.1016/j.celrep.2019.09.046

Dahdah, M.N., Barisa, M.T., Schmidt, K., Barnes, S.A., Dubiel, R., Dunklin, C., Harper, C., Callender, L., Wilson, A., Diaz-Arrastia, R., Shafi, S., 2014. Comparative Effectiveness of Traumatic Brain Injury Rehabilitation: Differential Outcomes Across TBI Model Systems Centers. J. Head Trauma Rehabil. 29, 451–459. https://doi.org/10.1097/HTR.0b013e3182a61983

Dahlstroem, A., Fuxe, K., 1964. EVIDENCE FOR THE EXISTENCE OF MONOAMINE-CONTAINING NEURONS IN THE CENTRAL NERVOUS SYSTEM. I. DEMONSTRATION OF MONOAMINES IN THE CELL BODIES OF BRAIN STEM NEURONS. Acta Physiol. Scand. Suppl. SUPPL 232:1-55.

Damier, P., Hirsch, E.C., Zhang, P., Agid, Y., Javoy-Agid, F., 1993. Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 52, 1–6. https://doi.org/10.1016/0306-4522(93)90175-F

Daneshvar, D.H., Mez, J., Alosco, M.L., Baucom, Z.H., Mahar, I., Baugh, C.M., Valle, J.P., Weuve, J., Paganoni, S., Cantu, R.C., Zafonte, R.D., Stern, R.A., Stein, T.D., Tripodis, Y., Nowinski, C.J., McKee, A.C., 2021. Incidence of and Mortality From Amyotrophic Lateral Sclerosis in National Football League Athletes. JAMA Netw. Open 4, e2138801. https://doi.org/10.1001/jamanetworkopen.2021.38801

Daneshvar, D.H., Nowinski, C.J., McKee, A.C., Cantu, R.C., 2011. The epidemiology of sport-related concussion. Clin. Sports Med. 30, 1–17, vii. https://doi.org/10.1016/j.csm.2010.08.006

Danzer, K.M., Krebs, S.K., Wolff, M., Birk, G., Hengerer, B., 2009. Seeding induced by alpha-synuclein oligomers provides evidence for spreading of alpha-synuclein pathology. J. Neurochem. 111, 192–203. https://doi.org/10.1111/j.1471-4159.2009.06324.x

Darweesh, S.K.L., Wolters, F.J., Postuma, R.B., Stricker, B.H., Hofman, A., Koudstaal, P.J., Ikram, M.K., Ikram, M.A., 2017. Association Between Poor Cognitive Functioning and Risk of Incident Parkinsonism: The Rotterdam Study. JAMA Neurol. 74, 1431. https://doi.org/10.1001/jamaneurol.2017.2248

Daubner, S.C., Le, T., Wang, S., 2011. Tyrosine hydroxylase and regulation of dopamine synthesis. Arch. Biochem. Biophys. 508, 1–12. https://doi.org/10.1016/j.abb.2010.12.017

Dauer, W., Przedborski, S., 2003. Parkinson's disease: mechanisms and models. Neuron 39, 889–909.

De Beaumont, L., Mongeon, D., Tremblay, S., Messier, J., Prince, F., Leclerc, S., Lassonde, M., Théoret, H., 2011. Persistent motor system abnormalities in formerly concussed athletes. J. Athl. Train. 46, 234–240. https://doi.org/10.4085/1062-6050-46.3.234

De Beaumont, L., Theoret, H., Mongeon, D., Messier, J., Leclerc, S., Tremblay, S., Ellemberg, D., Lassonde, M., 2009. Brain function decline in healthy retired athletes who sustained their last sports concussion in early adulthood. Brain 132, 695–708. https://doi.org/10.1093/brain/awn347

de la Fuente-Fernández, R., 2013. Imaging of Dopamine in PD and Implications for Motor and Neuropsychiatric Manifestations of PD. Front. Neurol. 4, 90. https://doi.org/10.3389/fneur.2013.00090

de Lau, L.M.L., Breteler, M.M.B., 2006. Epidemiology of Parkinson's disease. Lancet Neurol. 5, 525–535. https://doi.org/10.1016/S1474-4422(06)70471-9

De Lau, L.M.L., Koudstaal, P.J., Hofman, A., Breteler, M.M.B., 2006. Subjective Complaints Precede Parkinson Disease: The Rotterdam Study. Arch. Neurol. 63, 362. https://doi.org/10.1001/archneur.63.3.noc50312

De Oliveira, P.A., Ben, J., Matheus, F.C., Schwarzbold, M.L., Moreira, E.L.G., Rial, D., Walz, R., Prediger, R.D., 2017. Moderate traumatic brain injury increases the vulnerability to neurotoxicity induced by systemic administration of 6-hydroxydopamine in mice. Brain Res. 1663, 78–86. https://doi.org/10.1016/j.brainres.2017.03.002

Degani, A.M., Santos, M.M., Leonard, C.T., Rau, T.F., Patel, S.A., Mohapatra, S., Danna-dos-Santos, A., 2017. The effects of mild traumatic brain injury on postural control. Brain Inj. 31, 49–56. https://doi.org/10.1080/02699052.2016.1225982

Delaville, C., Deurwaerdère, P.D., Benazzouz, A., 2011. Noradrenaline and Parkinson's Disease. Front. Syst. Neurosci. 5. https://doi.org/10.3389/fnsys.2011.00031

Delic, V., Beck, K.D., Pang, K.C.H., Citron, B.A., 2020. Biological links between traumatic brain injury and Parkinson's disease. Acta Neuropathol. Commun. 8, 45. https://doi.org/10.1186/s40478-020-00924-7

Deloitte, 2015. Living with Parkinson's Disease: Challenges and positive steps for the future. Available: https://www2.deloitte.com/au/en/pages/economics/articles/living-with-parkinsons-disease.html [Accessed 08 Oct 2023].

Deng, H., Wang, P., Jankovic, J., 2018. The genetics of Parkinson disease. Ageing Res. Rev. 42, 72–85. https://doi.org/10.1016/j.arr.2017.12.007

Dewan, M.C., Rattani, A., Gupta, S., Baticulon, R.E., Hung, Y.-C., Punchak, M., Agrawal, A., Adeleye, A.O., Shrime, M.G., Rubiano, A.M., Rosenfeld, J.V., Park, K.B., 2019. Estimating the global incidence of traumatic brain injury. J. Neurosurg. 130, 1080–1097. https://doi.org/10.3171/2017.10.JNS17352

Dhillon, A.S., Tarbutton, G.L., Levin, J.L., Plotkin, G.M., Lowry, L.K., Nalbone, J.T., Shepherd, S., 2008. Pesticide/Environmental Exposures and Parkinson's Disease in East Texas. J. Agromedicine 13, 37–48. https://doi.org/10.1080/10599240801986215

Dhillon, N.K., Linaval, N.T., O'Rourke, J., Barmparas, G., Yang, A., Cho, N., Shelest, O., Ley, E.J., 2020a. How repetitive traumatic injury alters long-term brain function. J. Trauma Acute Care Surg. 89, 955–961. https://doi.org/10.1097/TA.0000000000002811

Di Nottia, M., Masciullo, M., Verrigni, D., Petrillo, S., Modoni, A., Rizzo, V., Di Giuda, D., Rizza, T., Niceta, M., Torraco, A., Bianchi, M., Santoro, M., Bentivoglio, A.R., Bertini, E., Piemonte, F., Carrozzo, R., Silvestri, G., 2017. DJ-1 modulates mitochondrial response to oxidative stress: clues from a novel diagnosis of PARK7. Clin. Genet. 92, 18–25. https://doi.org/10.1111/cge.12841

Dias da Silva, M.R., Postma, M., 2022. Straying Off Course: The Negative Impact of Mind Wandering on Fine Motor Movements. J. Mot. Behav. 54, 186–202. https://doi.org/10.1080/00222895.2021.1937032

Dias, V., Junn, E., Mouradian, M.M., 2013. The Role of Oxidative Stress in Parkinson's Disease. J. Park. Dis. 3, 461–491. https://doi.org/10.3233/JPD-130230

Diaz, K., Kohut, M.L., Russell, D.W., Stegemöller, E.L., 2022. Peripheral inflammatory cytokines and motor symptoms in persons with Parkinson's disease. Brain Behav. Immun. - Health 21, 100442. https://doi.org/10.1016/j.bbih.2022.100442

Dick, F.D., 2006. Parkinson's disease and pesticide exposures. Br. Med. Bull. 79–80, 219–231. https://doi.org/10.1093/bmb/ldl018

Dick, F.D., De Palma, G., Ahmadi, A., Scott, N.W., Prescott, G.J., Bennett, J., Semple, S., Dick, S., Counsell, C., Mozzoni, P., Haites, N., Wettinger, S.B., Mutti, A., Otelea, M., Seaton,

A., Soderkvist, P., Felice, A., on behalf of the Geoparkinson study group, 2007. Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study. Occup. Environ. Med. 64, 666–672. https://doi.org/10.1136/oem.2006.027003

Dikmen, S., Machamer, J., Temkin, N., 1993. Psychosocial outcome in patients with moderate to severe head injury: 2-year follow-up. Brain Inj. 7, 113–124. https://doi.org/10.3109/02699059309008165

Donnemiller, E., Brenneis, C., Wissel, J., Scherfler, C., Poewe, W., Riccabona, G., Wenning, G.K., 2000. Impaired dopaminergic neurotransmission in patients with traumatic brain injury: a SPET study using 123I-β-CIT and 123I-IBZM. Eur. J. Nucl. Med. 27, 1410–1414. https://doi.org/10.1007/s002590000308

Dorsey, E.R., Bloem, B.R., 2018. The Parkinson Pandemic—A Call to Action. JAMA Neurol. 75, 9. https://doi.org/10.1001/jamaneurol.2017.3299

Dorsey, E.R., Constantinescu, R., Thompson, J.P., Biglan, K.M., Holloway, R.G., Kieburtz, K., Marshall, F.J., Ravina, B.M., Schifitto, G., Siderowf, A., Tanner, C.M., 2007. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology 68, 384–386. https://doi.org/10.1212/01.wnl.0000247740.47667.03

Dorsey, E.R., Elbaz, A., Nichols, E., Abbasi, N., Abd-Allah, F., Abdelalim, A., Adsuar, J.C., Ansha, M.G., Brayne, C., Choi, J.-Y.J., Collado-Mateo, D., Dahodwala, N., Do, H.P., Edessa, D., Endres, M., Fereshtehnejad, S.-M., Foreman, K.J., Gankpe, F.G., Gupta, R., Hamidi, S., Hankey, G.J., Hay, S.I., Hegazy, M.I., Hibstu, D.T., Kasaeian, A., Khader, Y., Khalil, I., Khang, Y.-H., Kim, Y.J., Kokubo, Y., Logroscino, G., Massano, J., Mohamed Ibrahim, N., Mohammed, M.A., Mohammadi, A., Moradi-Lakeh, M., Naghavi, M., Nguyen, B.T., Nirayo, Y.L., Ogbo, F.A., Owolabi, M.O., Pereira, D.M., Postma, M.J., Qorbani, M., Rahman, M.A., Roba, K.T., Safari, H., Safiri, S., Satpathy, M., Sawhney, M., Shafieesabet, A., Shiferaw, M.S., Smith, M., Szoeke, C.E.I., Tabarés-Seisdedos, R., Truong, N.T., Ukwaja, K.N., Venketasubramanian, N., Villafaina, S., weldegwergs, K. gidey, Westerman, R., Wijeratne, T., Winkler, A.S., Xuan, B.T., Yonemoto, N., Feigin, V.L., Vos, T., Murray, C.J.L., 2018. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 17, 939–953. https://doi.org/10.1016/S1474-4422(18)30295-3

Dos Santos, C., Hussain, S.N.A., Mathur, S., Picard, M., Herridge, M., Correa, J., Bain, A., Guo, Y., Advani, A., Advani, S.L., Tomlinson, G., Katzberg, H., Streutker, C.J., Cameron, J.I., Schols, A., Gosker, H.R., Batt, J., 2016. Mechanisms of Chronic Muscle Wasting and Dysfunction after an Intensive Care Unit Stay. A Pilot Study. Am. J. Respir. Crit. Care Med. 194, 821–830. https://doi.org/10.1164/rccm.201512-2344OC

Draper, K., Ponsford, J., 2008. Cognitive functioning ten years following traumatic brain injury and rehabilitation. Neuropsychology 22, 618–625. https://doi.org/10.1037/0894-4105.22.5.618

Drolet, R.E., Cannon, J.R., Montero, L., Greenamyre, J.T., 2009. Chronic rotenone exposure reproduces Parkinson's disease gastrointestinal neuropathology. Neurobiol. Dis. 36, 96–102. https://doi.org/10.1016/j.nbd.2009.06.017

Du, L., Xu, L., Liang, T., Wing, Y.-K., Ke, Y., Yung, W.-H., 2021. Progressive Pontine-Medullary Dysfunction Leads to REM Sleep Behavior Disorder Symptoms in a Chronic Model of Parkinson's Disease. Nat. Sci. Sleep 13, 1723–1736. https://doi.org/10.2147/NSS.S328365

Dubois, B., Funkiewiez, A., Pillon, B., 2005. Behavioral changes in patients with focal lesions of the basal ganglia. Adv. Neurol. 96, 17–25.

Dunn-Meynell, A.A., Hassanain, M., Levin, B.E., 1998. Norepinephrine and traumatic brain injury: a possible role in post-traumatic edema. Brain Res. 800, 245–252. https://doi.org/10.1016/S0006-8993(98)00524-1

Edwards, L., Quigley, E.M.M., Hofman, R., Pfeiffer, R.F., 1993. Gastrointestinal symptoms in parkinson disease: 18-month follow-up study. Mov. Disord. 8, 83–86. https://doi.org/10.1002/mds.870080115

Ehringer, H., Hornykiewicz, O., 1998. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. Parkinsonism Relat. Disord. 4, 53–57. https://doi.org/10.1016/S1353-8020(98)00012-1

Ekstrand, M.I., Terzioglu, M., Galter, D., Zhu, S., Hofstetter, C., Lindqvist, E., Thams, S., Bergstrand, A., Hansson, F.S., Trifunovic, A., Hoffer, B., Cullheim, S., Mohammed, A.H., Olson, L., Larsson, N.-G., 2007. Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Proc. Natl. Acad. Sci. 104, 1325–1330. https://doi.org/10.1073/pnas.0605208103

El-Kattan, M.M., Rashed, L.A., Shazly, S.R., Ismail, R.S., 2022. Relation of serum level of tumor necrosis factor-alpha to cognitive functions in patients with Parkinson's disease. Egypt. J. Neurol. Psychiatry Neurosurg. 58, 25. https://doi.org/10.1186/s41983-022-00460-2

El-Saiy, K.A., Sayed, R.H., El-Sahar, A.E., Kandil, E.A., 2022. Modulation of histone deacetylase, the ubiquitin proteasome system, and autophagy underlies the neuroprotective effects of venlafaxine in a rotenone-induced Parkinson's disease model in rats. Chem. Biol. Interact. 354, 109841. https://doi.org/10.1016/j.cbi.2022.109841

Engelender, S., Isacson, O., 2017. The Threshold Theory for Parkinson's Disease. Trends Neurosci. 40, 4–14. https://doi.org/10.1016/j.tins.2016.10.008

Esselman, P.C., Uomoto, J.M., 1995. Classification of the Spectrum of Mild Traumatic Brain Injury. Brain Inj. 9, 417–424. https://doi.org/10.3109/02699059509005782

Evans, A.K., Defensor, E., Shamloo, M., 2022. Selective Vulnerability of the Locus Coeruleus Noradrenergic System and its Role in Modulation of Neuroinflammation, Cognition, and Neurodegeneration. Front. Pharmacol. 13, 1030609. https://doi.org/10.3389/fphar.2022.1030609

Evans, T.M., Jaramillo, C.A., Sataranatarajan, K., Watts, L., Sabia, M., Qi, W., Van Remmen, H., 2015. The effect of mild traumatic brain injury on peripheral nervous system pathology in wild-type mice and the G93A mutant mouse model of motor neuron disease. Neuroscience 298, 410–423. https://doi.org/10.1016/j.neuroscience.2015.04.041

Evans, T.M., Remmen, H.V., Purkar, A., Mahesula, S., Gelfond, J.A.L., Sabia, M., Qi, W., Lin, A.-L., Jaramillo, C.A., Haskins, W.E., 2014. Microwave and magnetic (M2) proteomics of a mouse model of mild traumatic brain injury. Transl. Proteomics 3, 10–21. https://doi.org/10.1016/j.trprot.2014.03.002

Fabbri, M., Reimão, S., Carvalho, M., Nunes, R.G., Abreu, D., Guedes, L.C., Bouça, R., Lobo, P.P., Godinho, C., Coelho, M., Gonçalves, N.C., Rosa, M.M., Antonini, A., Ferreira, J.J., 2017. Substantia Nigra Neuromelanin as an Imaging Biomarker of Disease Progression in Parkinson's Disease. J. Park. Dis. 7, 491–501. https://doi.org/10.3233/JPD-171135

Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D., Franceschi, C., Paganelli, R., 1993. Increased cytokine production in mononuclear cells of healthy elderly people. Eur. J. Immunol. 23, 2375–2378. https://doi.org/10.1002/eji.1830230950

Fan, J., Geng, H., Ji, Y., Jia, T., Treweek, J.B., Li, A., Zhou, C., Gradinaru, V., Xiao, C., 2022. Age-dependent alterations in key components of the nigrostriatal dopaminergic system and distinct motor phenotypes. Acta Pharmacol. Sin. 43, 862–875. https://doi.org/10.1038/s41401-021-00713-5

Färber, K., Pannasch, U., Kettenmann, H., 2005. Dopamine and noradrenaline control distinct functions in rodent microglial cells. Mol. Cell. Neurosci. 29, 128–138. https://doi.org/10.1016/j.mcn.2005.01.003

Fathalla, A.M., Soliman, A.M., Ali, M.H., Moustafa, A.A., 2016. Adenosine A2A Receptor Blockade Prevents Rotenone-Induced Motor Impairment in a Rat Model of Parkinsonism. Front. Behav. Neurosci. 10. https://doi.org/10.3389/fnbeh.2016.00035

Fato, R., Bergamini, C., Bortolus, M., Maniero, A.L., Leoni, S., Ohnishi, T., Lenaz, G., 2009. Differential effects of mitochondrial Complex I inhibitors on production of reactive oxygen species. Biochim. Biophys. Acta 1787, 384–392. https://doi.org/10.1016/j.bbabio.2008.11.003

Faul, M., Wald, M.M., Xu, L., Coronado, V.G., 2010. Traumatic brain injury in the United States: emergency department visits, hospitalizations, and deaths, 2002-2006.

Fazl, A., Fleisher, J., 2018. Anatomy, Physiology, and Clinical Syndromes of the Basal Ganglia: A Brief Review. Semin. Pediatr. Neurol. 25, 2–9. https://doi.org/10.1016/j.spen.2017.12.005

Fearnley, J.M., Lees, A.J., 1991. AGEING AND PARKINSON'S DISEASE: SUBSTANTIA NIGRA REGIONAL SELECTIVITY. Brain 114, 2283–2301. https://doi.org/10.1093/brain/114.5.2283

Fehily, B., Bartlett, C.A., Lydiard, S., Archer, M., Milbourn, H., Majimbi, M., Hemmi, J.M., Dunlop, S.A., Yates, N.J., Fitzgerald, M., 2019. Differential responses to increasing numbers of mild traumatic brain injury in a rodent closed-head injury model. J. Neurochem. 149, 660–678. https://doi.org/10.1111/jnc.14673

Feigin, V.L., Vos, T., Alahdab, F., Amit, A.M.L., Bärnighausen, T.W., Beghi, E., Beheshti, M., Chavan, P.P., Criqui, M.H., Desai, R., Dhamminda Dharmaratne, S., Dorsey, E.R., Wilder Eagan, A., Elgendy, I.Y., Filip, I., Giampaoli, S., Giussani, G., Hafezi-Nejad, N., Hole, M.K., Ikeda, T., Owens Johnson, C., Kalani, R., Khatab, K., Khubchandani, J., Kim, D., Koroshetz, W.J., Krishnamoorthy, V., Krishnamurthi, R.V., Liu, X., Lo, W.D., Logroscino, G., Mensah, G.A., Miller, T.R., Mohammed, S., Mokdad, A.H., Moradi-Lakeh, M., Morrison, S.D., Shivamurthy, V.K.N., Naghavi, M., Nichols, E., Norrving, B., Odell, C.M., Pupillo, E., Radfar, A., Roth, G.A., Shafieesabet, A., Sheikh, A., Sheikhbahaei, S., Shin, J.I., Singh, J.A., Steiner, T.J., Stovner, L.J., Wallin, M.T., Weiss, J., Wu, C., Zunt, J.R., Adelson, J.D., Murray, C.J.L., 2021. Burden of Neurological Disorders across the US from 1990-2017: A Global Burden of Disease Study. JAMA Neurol. 78, 165–176. https://doi.org/10.1001/jamaneurol.2020.4152

Feng, Y., Li, K., Roth, E., Chao, D., Mecca, C.M., Hogan, Q.H., Pawela, C., Kwok, W.-M., Camara, A.K.S., Pan, B., 2021. Repetitive Mild Traumatic Brain Injury in Rats Impairs Cognition, Enhances Prefrontal Cortex Neuronal Activity, and Reduces Pre-synaptic Mitochondrial Function. Front. Cell. Neurosci. 15, 689334. https://doi.org/10.3389/fncel.2021.689334

Ferrucci, L., Semba, R.D., Guralnik, J.M., Ershler, W.B., Bandinelli, S., Patel, K.V., Sun, K., Woodman, R.C., Andrews, N.C., Cotter, R.J., Ganz, T., Nemeth, E., Longo, D.L., 2010. Proinflammatory state, hepcidin, and anemia in older persons. Blood 115, 3810–3816. https://doi.org/10.1182/blood-2009-02-201087

Ferrucci, M., S. Giorgi, F., Bartalucci, A., L. Busceti, C., Fornai, F., 2013. The Effects of Locus Coeruleus and Norepinephrine in Methamphetamine Toxicity. Curr. Neuropharmacol. 11, 80–94. https://doi.org/10.2174/157015913804999522

Fesharaki-Zadeh, A., 2022. Oxidative Stress in Traumatic Brain Injury. Int. J. Mol. Sci. 23, 13000. https://doi.org/10.3390/ijms232113000

Finnie, J.W., Blumbergs, P.C., 2002. Traumatic Brain Injury. Vet. Pathol. 39, 679–689. https://doi.org/10.1354/vp.39-6-679

Fleming, S., 2004. Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. Exp. Neurol. 187, 418–429. https://doi.org/10.1016/j.expneurol.2004.01.023

Frank, M.G., Barrientos, R.M., Biedenkapp, J.C., Rudy, J.W., Watkins, L.R., Maier, S.F., 2006. mRNA up-regulation of MHC II and pivotal pro-inflammatory genes in normal brain aging. Neurobiol. Aging 27, 717–722. https://doi.org/10.1016/j.neurobiolaging.2005.03.013

Frintrop, L., Liesbrock, J., Paulukat, L., Johann, S., Kas, M.J., Tolba, R., Heussen, N., Neulen, J., Konrad, K., Herpertz-Dahlmann, B., Beyer, C., Seitz, J., 2018. Reduced astrocyte density underlying brain volume reduction in activity-based anorexia rats. World J. Biol. Psychiatry 19, 225–235. https://doi.org/10.1080/15622975.2016.1273552

Froula, J.M., Castellana-Cruz, M., Anabtawi, N.M., Camino, J.D., Chen, S.W., Thrasher, D.R., Freire, J., Yazdi, A.A., Fleming, S., Dobson, C.M., Kumita, J.R., Cremades, N., Volpicelli-Daley, L.A., 2019. Defining α-synuclein species responsible for Parkinson's disease phenotypes in mice. J. Biol. Chem. 294, 10392–10406. https://doi.org/10.1074/jbc.RA119.007743

Fujinaka, T., Kohmura, E., Yuguchi, T., Yoshimine, T., 2003. The morphological and neurochemical effects of diffuse brain injury on rat central noradrenergic system. Neurol. Res. 25, 35–41. https://doi.org/10.1179/016164103101201094

Furlong, M., Tanner, C.M., Goldman, S.M., Bhudhikanok, G.S., Blair, A., Chade, A., Comyns, K., Hoppin, J.A., Kasten, M., Korell, M., Langston, J.W., Marras, C., Meng, C., Richards, M., Ross, G.W., Umbach, D.M., Sandler, D.P., Kamel, F., 2015. Protective glove use and hygiene habits modify the associations of specific pesticides with Parkinson's disease. Environ. Int. 75, 144–150. https://doi.org/10.1016/j.envint.2014.11.002

Gaenslen, A., Swid, I., Liepelt-Scarfone, I., Godau, J., Berg, D., 2011. The patients' perception of prodromal symptoms before the initial diagnosis of Parkinson's disease: Perception of Prodromal Symptoms in PD. Mov. Disord. 26, 653–658. https://doi.org/10.1002/mds.23499

Galluzzi, L., Kepp, O., Kroemer, G., 2012. Mitochondria: master regulators of danger signalling. Nat. Rev. Mol. Cell Biol. 13, 780–788. https://doi.org/10.1038/nrm3479

Gao, F., Chen, D., Hu, Q., Wang, G., 2013. Rotenone Directly Induces BV2 Cell Activation via the p38 MAPK Pathway. PLoS ONE 8, e72046. https://doi.org/10.1371/journal.pone.0072046

Gao, H.-M., Hong, J.-S., Zhang, W., Liu, B., 2002. Distinct Role for Microglia in Rotenone-Induced Degeneration of Dopaminergic Neurons. J. Neurosci. 22, 782–790. https://doi.org/10.1523/JNEUROSCI.22-03-00782.2002

Gao, H.-M., Liu, B., Hong, J.-S., 2003. Critical Role for Microglial NADPH Oxidase in Rotenone-Induced Degeneration of Dopaminergic Neurons. J. Neurosci. 23, 6181–6187. https://doi.org/10.1523/JNEUROSCI.23-15-06181.2003

Gao, J., Hu, J., Buckley, T., White, K., Hass, C., 2011. Shannon and Renyi entropies to classify effects of Mild Traumatic Brain Injury on postural sway. PloS One 6, e24446. https://doi.org/10.1371/journal.pone.0024446

Gao, X., Chen, H., Schwarzschild, M.A., Ascherio, A., 2011. A Prospective Study of Bowel Movement Frequency and Risk of Parkinson's Disease. Am. J. Epidemiol. 174, 546–551. https://doi.org/10.1093/aje/kwr119

García-García, F., Ponce, S., Brown, R., Cussen, V., Krueger, J.M., 2005. Sleep disturbances in the rotenone animal model of Parkinson disease. Brain Res. 1042, 160–168. https://doi.org/10.1016/j.brainres.2005.02.036

Gardner, R.C., Burke, J.F., Nettiksimmons, J., Goldman, S., Tanner, C.M., Yaffe, K., 2015. Traumatic brain injury in later life increases risk for Parkinson disease: TBI Increases Risk for PD. Ann. Neurol. 77, 987–995. https://doi.org/10.1002/ana.24396

Gardner, R.C., Byers, A.L., Barnes, D.E., Li, Y., Boscardin, J., Yaffe, K., 2018. Mild TBI and risk of Parkinson disease: A Chronic Effects of Neurotrauma Consortium Study. Neurology 90, e1771–e1779. https://doi.org/10.1212/WNL.0000000000005522

Gardner, R.C., Peltz, C.B., Kenney, K., Covinsky, K.E., Diaz-Arrastia, R., Yaffe, K., 2017. Remote Traumatic Brain Injury Is Associated with Motor Dysfunction in Older Military Veterans. J. Gerontol. Ser. A 72, 1233–1238. https://doi.org/10.1093/gerona/glw341

Gardner, R.C., Yaffe, K., 2015. Epidemiology of mild traumatic brain injury and neurodegenerative disease. Mol. Cell. Neurosci. 66, 75–80. https://doi.org/10.1016/j.mcn.2015.03.001

Gawel, K., Gibula, E., Marszalek-Grabska, M., Filarowska, J., Kotlinska, J.H., 2019. Assessment of spatial learning and memory in the Barnes maze task in rodents—methodological consideration. Naunyn. Schmiedebergs Arch. Pharmacol. 392, 1–18. https://doi.org/10.1007/s00210-018-1589-y

George, S., Rey, N.L., Tyson, T., Esquibel, C., Meyerdirk, L., Schulz, E., Pierce, S., Burmeister, A.R., Madaj, Z., Steiner, J.A., Escobar Galvis, M.L., Brundin, L., Brundin, P., 2019. Microglia affect α-synuclein cell-to-cell transfer in a mouse model of Parkinson's disease. Mol. Neurodegener. 14, 34. https://doi.org/10.1186/s13024-019-0335-3

Gera, G., Chesnutt, J., Mancini, M., Horak, F.B., King, L.A., 2018. Inertial Sensor-Based Assessment of Central Sensory Integration for Balance After Mild Traumatic Brain Injury. Mil. Med. 183, 327–332. https://doi.org/10.1093/milmed/usx162

Gerhard, A., Pavese, N., Hotton, G., Turkheimer, F., Es, M., Hammers, A., Eggert, K., Oertel, W., Banati, R.B., Brooks, D.J., 2006. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. Neurobiol. Dis. 21, 404–412. https://doi.org/10.1016/j.nbd.2005.08.002

Geurts, A.C.H., Knoop, J.A., van Limbeek, J., 1999. Is postural control associated with mental functioning in the persistent postconcussion syndrome? Arch. Phys. Med. Rehabil. 80, 144–149. https://doi.org/10.1016/S0003-9993(99)90111-9

Geurts, A.C.H., Ribbers, G.M., Knoop, J.A., Van Limbeek, J., 1996. Identification of static and dynamic postural instability following traumatic brain injury. Arch. Phys. Med. Rehabil. 77, 639–644. https://doi.org/10.1016/S0003-9993(96)90001-5

Giasson, B.I., Duda, J.E., Quinn, S.M., Zhang, B., Trojanowski, J.Q., Lee, V.M.-Y., 2002. Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. Neuron 34, 521–533. https://doi.org/10.1016/s0896-6273(02)00682-7

Giguère, N., Burke Nanni, S., Trudeau, L.-E., 2018. On Cell Loss and Selective Vulnerability of Neuronal Populations in Parkinson's Disease. Front. Neurol. 9, 455. https://doi.org/10.3389/fneur.2018.00455

Glaser, C.B., Yamin, G., Uversky, V.N., Fink, A.L., 2005. Methionine oxidation, α-synuclein and Parkinson's disease. Biochim. Biophys. Acta BBA - Proteins Proteomics 1703, 157–169. https://doi.org/10.1016/j.bbapap.2004.10.008

Glass, C.K., Saijo, K., Winner, B., Marchetto, M.C., Gage, F.H., 2010. Mechanisms Underlying Inflammation in Neurodegeneration. Cell 140, 918–934. https://doi.org/10.1016/j.cell.2010.02.016

Godbout, J.P., Chen, J., Abraham, J., Richwine, A.F., Berg, B.M., Kelley, K.W., Johnson, R.W., 2005. Exaggerated neuroinflammation and sickness behavior in aged mice after activation of the peripheral innate immune system. FASEB J. 19, 1329–1331. https://doi.org/10.1096/fj.05-3776fje

Goldberg, M.S., Pisani, A., Haburcak, M., Vortherms, T.A., Kitada, T., Costa, C., Tong, Y., Martella, G., Tscherter, A., Martins, A., 2005. Nigrostriatal dopaminergic deficits and hypokinesia caused by inactivation of the familial Parkinsonism-linked gene DJ-1. Neuron 45, 489–496.

Goldman, S.M., Kamel, F., Ross, G.W., Jewell, S.A., Bhudhikanok, G.S., Umbach, D., Marras, C., Hauser, R.A., Jankovic, J., Factor, S.A., Bressman, S., Lyons, K.E., Meng, C., Korell, M., Roucoux, D.F., Hoppin, J.A., Sandler, D.P., Langston, J.W., Tanner, C.M., 2012. Head injury, alpha-synuclein Rep1, and Parkinson's disease. Ann. Neurol. 71, 40–48. https://doi.org/10.1002/ana.22499

Goldman, S.M., Tanner, C.M., Oakes, D., Bhudhikanok, G.S., Gupta, A., Langston, J.W., 2006. Head injury and Parkinson's disease risk in twins. Ann. Neurol. 60, 65–72. https://doi.org/10.1002/ana.20882

Gonzalez-Lopez, E., Vrana, K.E., 2020. Dopamine beta-hydroxylase and its genetic variants in human health and disease. J. Neurochem. 152, 157–181. https://doi.org/10.1111/jnc.14893

Gorell, J.M., Peterson, E.L., Rybicki, B.A., Johnson, C.C., 2004. Multiple risk factors for Parkinson's disease. J. Neurol. Sci. 217, 169–174. https://doi.org/10.1016/j.jns.2003.09.014

Grandhi, R., Tavakoli, S., Ortega, C., Simmonds, M., 2017. A Review of Chronic Pain and Cognitive, Mood, and Motor Dysfunction Following Mild Traumatic Brain Injury: Complex, Comorbid, and/or Overlapping Conditions? Brain Sci. 7, 160. https://doi.org/10.3390/brainsci7120160

Graybiel, A., 1991. Basal ganglia —input, neural activity, and relation to the cortex. Curr. Opin. Neurobiol. 1, 644–651. https://doi.org/10.1016/S0959-4388(05)80043-1

Green, R., Colella, B., Maller, J., Bayley, M., Glazer, J., Mikulis, D., n.d.

Greenamyre, J.T., Betarbet, R., Sherer, T.B., 2003. The rotenone model of Parkinson's disease: genes, environment and mitochondria. Parkinsonism Relat. Disord. 9, 59–64. https://doi.org/10.1016/S1353-8020(03)00023-3

Greenamyre, J.T., MacKenzie, G., Peng, T.-I., Stephans, S.E., 1999. Mitochondrial dysfunction in Parkinson's disease. Biochem. Soc. Symp. 66, 85–97. https://doi.org/10.1042/bss0660085

Greenberg, G., Mikulis, D.J., Ng, K., DeSouza, D., Green, R.E., 2008. Use of diffusion tensor imaging to examine subacute white matter injury progression in moderate to severe traumatic brain injury. Arch. Phys. Med. Rehabil. 89, S45-50. https://doi.org/10.1016/j.apmr.2008.08.211

Greene, J.G., Noorian, A.R., Srinivasan, S., 2009. Delayed gastric emptying and enteric nervous system dysfunction in the rotenone model of Parkinson's disease. Exp. Neurol. 218, 154–161. https://doi.org/10.1016/j.expneurol.2009.04.023

Greffard, S., Verny, M., Bonnet, A.-M., Beinis, J.-Y., Gallinari, C., Meaume, S., Piette, F., Hauw, J.-J., Duyckaerts, C., 2006. Motor Score of the Unified Parkinson Disease Rating Scale as a Good Predictor of Lewy Body–Associated Neuronal Loss in the Substantia Nigra. Arch. Neurol. 63, 584. https://doi.org/10.1001/archneur.63.4.584

Grossman, M.H., Emanuel, B.S., Budarf, M.L., 1992. Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11.1→q11.2. Genomics 12, 822–825. https://doi.org/10.1016/0888-7543(92)90316-K

Grueschow, M., Kleim, B., Ruff, C.C., 2022. Functional Coupling of the Locus Coeruleus Is Linked to Successful Cognitive Control. Brain Sci. 12, 305. https://doi.org/10.3390/brainsci12030305

Gu, D., Ou, S., Tang, M., Yin, Z., Wang, Z., Liu, G., 2021. Trauma and amyotrophic lateral sclerosis: a systematic review and meta-analysis. Amyotroph. Lateral Scler. Front. Degener. 22, 170–185. https://doi.org/10.1080/21678421.2020.1861024

Guilarte, T., Chen, M., Mcglothan, J., Verina, T., Wong, D., Zhou, Y., Alexander, M., Rohde, C., Syversen, T., Decamp, E., 2006. Nigrostriatal dopamine system dysfunction and subtle motor deficits in manganese-exposed non-human primates. Exp. Neurol. 202, 381–390. https://doi.org/10.1016/j.expneurol.2006.06.015

Guo, Z., Ruan, Z., Zhang, D., Liu, X., Hou, L., Wang, Q., 2022. Rotenone impairs learning and memory in mice through microglia-mediated blood brain barrier disruption and neuronal apoptosis. Chemosphere 291, 132982. https://doi.org/10.1016/j.chemosphere.2021.132982

Gupta, R.C., 2014. Rotenone, in: Encyclopedia of Toxicology. Elsevier, pp. 185–187. https://doi.org/10.1016/B978-0-12-386454-3.00194-9

Guzman, J.N., Sanchez-Padilla, J., Wokosin, D., Kondapalli, J., Ilijic, E., Schumacker, P.T., Surmeier, D.J., 2010. Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature 468, 696–700. https://doi.org/10.1038/nature09536

Gyoneva, S., Traynelis, S.F., 2013. Norepinephrine modulates the motility of resting and activated microglia via different adrenergic receptors. J. Biol. Chem. 288, 15291–15302. https://doi.org/10.1074/jbc.M113.458901

Haber, S.N., Fudge, J.L., McFarland, N.R., 2000. Striatonigrostriatal Pathways in Primates Form an Ascending Spiral from the Shell to the Dorsolateral Striatum. J. Neurosci. 20, 2369–2382. https://doi.org/10.1523/JNEUROSCI.20-06-02369.2000

Hall, E.D., Kupina, N.C., Althaus, J.S., 1999. Peroxynitrite Scavengers for the Acute Treatment of Traumatic Brain Injury. Ann. N. Y. Acad. Sci. 890, 462–468. https://doi.org/10.1111/j.1749-6632.1999.tb08025.x

Halterman, C.I., Langan, J., Drew, A., Rodriguez, E., Osternig, L.R., Chou, L.-S., van Donkelaar, P., 2006. Tracking the recovery of visuospatial attention deficits in mild traumatic brain injury. Brain J. Neurol. 129, 747–753. https://doi.org/10.1093/brain/awh705

Han, G., Casson, R.J., Chidlow, G., Wood, J.P.M., 2014. The Mitochondrial Complex I Inhibitor Rotenone Induces Endoplasmic Reticulum Stress and Activation of GSK-3β in Cultured Rat Retinal Cells. Investig. Opthalmology Vis. Sci. 55, 5616. https://doi.org/10.1167/iovs.14-14371

Hanscom, M., Loane, D.J., Aubretch, T., Leser, J., Molesworth, K., Hedgekar, N., Ritzel, R.M., Abulwerdi, G., Shea-Donohue, T., Faden, A.I., 2021. Acute colitis during chronic experimental traumatic brain injury in mice induces dysautonomia and persistent extraintestinal, systemic, and CNS inflammation with exacerbated neurological deficits. J. Neuroinflammation 18, 24. https://doi.org/10.1186/s12974-020-02067-x

Hare, B.D., Shinohara, R., Liu, R.J., Pothula, S., DiLeone, R.J., Duman, R.S., 2019. Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. Nat. Commun. 10, 223. https://doi.org/10.1038/s41467-018-08168-9

Harris, T.C., de Rooij, R., Kuhl, E., 2019. The Shrinking Brain: Cerebral Atrophy Following Traumatic Brain Injury. Ann. Biomed. Eng. 47, 1941–1959. https://doi.org/10.1007/s10439-018-02148-2

Hart, T., Kozlowski, A.J., Whyte, J., Poulsen, I., Kristensen, K., Nordenbo, A., Heinemann, A.W., 2014. Functional recovery after severe traumatic brain injury: an individual growth curve approach. Arch. Phys. Med. Rehabil. 95, 2103–2110. https://doi.org/10.1016/j.apmr.2014.07.001

Hassani, O.K., Rymar, V.V., Nguyen, K.Q., Huo, L., Cloutier, J.-F., Miller, F.D., Sadikot, A.F., 2020. The noradrenergic system is necessary for survival of vulnerable midbrain dopaminergic neurons: implications for development and Parkinson's disease. Neurobiol. Aging 85, 22–37. https://doi.org/10.1016/j.neurobiolaging.2019.09.014

Hauser, D.N., Hastings, T.G., 2013. Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. Neurobiol. Dis. 51, 35–42. https://doi.org/10.1016/j.nbd.2012.10.011

He, J., Russell, T., Qiu, X., Hao, F., Kyle, M., Chin, L., Zhao, L.-R., 2020. The contribution of stem cell factor and granulocyte colony-stimulating factor in reducing neurodegeneration and promoting neurostructure network reorganization after traumatic brain injury. Brain Res.

Heikkila, R.E., Nicklas, W.J., Vyas, I., Duvoisin, R.C., 1985. Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: Implication for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. Neurosci. Lett. 62, 389–394. https://doi.org/10.1016/0304-3940(85)90580-4

Heinz, S., Freyberger, A., Lawrenz, B., Schladt, L., Schmuck, G., Ellinger-Ziegelbauer, H., 2017. Mechanistic Investigations of the Mitochondrial Complex I Inhibitor Rotenone in the Context of Pharmacological and Safety Evaluation. Sci. Rep. 7, 45465. https://doi.org/10.1038/srep45465

Hellewell, S.C., Yan, E.B., Agyapomaa, D.A., Bye, N., Morganti-Kossmann, M.C., 2010. Post-Traumatic Hypoxia Exacerbates Brain Tissue Damage: Analysis of Axonal Injury and Glial Responses. J. Neurotrauma 27, 1997–2010. https://doi.org/10.1089/neu.2009.1245

Hellmich, H.L., Capra, B., Eidson, K., Garcia, J., Kennedy, D., Uchida, T., Parsley, M., Cowart, J., DeWitt, D.S., Prough, D.S., 2005. Dose-dependent neuronal injury after traumatic brain injury. Brain Res. 1044, 144–154. https://doi.org/10.1016/j.brainres.2005.02.054

Helmich, I., Berger, A., Lausberg, H., 2016. Neural Control of Posture in Individuals with Persisting Postconcussion Symptoms: Med. Sci. Sports Exerc. 48, 2362–2369. https://doi.org/10.1249/MSS.0000000000001028

Hendrickson, R.C., Raskind, M.A., Millard, S.P., Sikkema, C., Terry, G.E., Pagulayan, K.F., Li, G., Peskind, E.R., 2018. Evidence for altered brain reactivity to norepinephrine in Veterans with a history of traumatic stress. Neurobiol. Stress 8, 103–111. https://doi.org/10.1016/j.ynstr.2018.03.001

Heng, N., Malek, N., Lawton, M.A., Nodehi, A., Pitz, V., Grosset, K.A., Ben-Shlomo, Y., Grosset, D.G., 2023. Striatal Dopamine Loss in Early Parkinson's Disease: Systematic Review and Novel Analysis of Dopamine Transporter Imaging. Mov. Disord. Clin. Pract. 10, 539–546. https://doi.org/10.1002/mdc3.13687

Henry, R.J., Meadows, V.E., Stoica, B.A., Faden, A.I., Loane, D.J., 2020a. Longitudinal Assessment of Sensorimotor Function after Controlled Cortical Impact in Mice: Comparison of Beamwalk, Rotarod, and Automated Gait Analysis Tests. J. Neurotrauma 37, 2709–2717. https://doi.org/10.1089/neu.2020.7139

Henry, R.J., Ritzel, R.M., Barrett, J.P., Doran, S.J., Jiao, Y., Leach, J.B., Szeto, G.L., Wu, J., Stoica, B.A., Faden, A.I., Loane, D.J., 2020b. Microglial Depletion with CSF1R Inhibitor During Chronic Phase of Experimental Traumatic Brain Injury Reduces Neurodegeneration and Neurological Deficits. J. Neurosci. Off. J. Soc. Neurosci. 40, 2960–2974. https://doi.org/10.1523/JNEUROSCI.2402-19.2020

Hiebert, J.B., Shen, Q., Thimmesch, A.R., Pierce, J.D., 2015. Traumatic Brain Injury and Mitochondrial Dysfunction. Am. J. Med. Sci. 350, 132–138. https://doi.org/10.1097/MAJ.0000000000000506

Higgins, J.P.T., Altman, D.G., Gotzsche, P.C., Juni, P., Moher, D., Oxman, A.D., Savovic, J., Schulz, K.F., Weeks, L., Sterne, J.A.C., Cochrane Bias Methods Group, Cochrane Statistical Methods Group, 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 343, d5928–d5928. https://doi.org/10.1136/bmj.d5928

Hilton, D., Stephens, M., Kirk, L., Edwards, P., Potter, R., Zajicek, J., Broughton, E., Hagan, H., Carroll, C., 2014. Accumulation of α-synuclein in the bowel of patients in the pre-clinical phase of Parkinson's disease. Acta Neuropathol. (Berl.) 127, 235–241. https://doi.org/10.1007/s00401-013-1214-6

Hitti, F.L., Ramayya, A.G., McShane, B.J., Yang, A.I., Vaughan, K.A., Baltuch, G.H., 2020. Long-term outcomes following deep brain stimulation for Parkinson's disease. J. Neurosurg. 132, 205–210. https://doi.org/10.3171/2018.8.JNS182081

Hoehn, M.M., Yahr, M.D., 2001. Parkinsonism: onset, progression, and mortality. 1967. Neurology 57, S11-26.

Hoffman, S.W., Virmani, S., Simkins, R.M., Stein, D.G., 2003. The Delayed Administration of Dehydroepiandrosterone Sulfate Improves Recovery of Function after Traumatic Brain Injury in Rats. J. Neurotrauma 20, 859–870. https://doi.org/10.1089/089771503322385791

Holmqvist, S., Chutna, O., Bousset, L., Aldrin-Kirk, P., Li, W., Björklund, T., Wang, Z.-Y., Roybon, L., Melki, R., Li, J.-Y., 2014. Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. Acta Neuropathol. (Berl.) 128, 805–820. https://doi.org/10.1007/s00401-014-1343-6

Homsi, S., Piaggio, T., Croci, N., Noble, F., Plotkine, M., Marchand-Leroux, C., Jafarian-Tehrani, M., 2010. Blockade of Acute Microglial Activation by Minocycline Promotes Neuroprotection and Reduces Locomotor Hyperactivity after Closed Head Injury in Mice: A Twelve-Week Follow-Up Study. J. Neurotrauma 27, 911–921. https://doi.org/10.1089/neu.2009.1223

Hooijmans, C.R., Rovers, M.M., de Vries, R.B., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14, 43. https://doi.org/10.1186/1471-2288-14-43

Horsager, J., Andersen, K.B., Knudsen, K., Skjærbæk, C., Fedorova, T.D., Okkels, N., Schaeffer, E., Bonkat, S.K., Geday, J., Otto, M., Sommerauer, M., Danielsen, E.H., Bech, E., Kraft, J., Munk, O.L., Hansen, S.D., Pavese, N., Göder, R., Brooks, D.J., Berg, D., Borghammer, P., 2020. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. Brain 143, 3077–3088. https://doi.org/10.1093/brain/awaa238

Horsager, J., Knudsen, K., Sommerauer, M., 2022. Clinical and imaging evidence of brain-first and body-first Parkinson's disease. Neurobiol. Dis. 164, 105626. https://doi.org/10.1016/j.nbd.2022.105626

Hou, J., Nelson, R., Wilkie, Z., Mustafa, G., Tsuda, S., Thompson, F.J., Bose, P., 2017. Mild and Mild-to-Moderate Traumatic Brain Injury-Induced Significant Progressive and Enduring Multiple Comorbidities. J. Neurotrauma 34, 2456–2466. https://doi.org/10.1089/neu.2016.4851

Huang, C.-H., Lin, C.-W., Lee, Y.-C., Huang, C.-Y., Huang, R.-Y., Tai, Y.-C., Wang, K.-W., Yang, S.-N., Sun, Y.-T., Wang, H., 2018. Is traumatic brain injury a risk factor for neurodegeneration? A meta-analysis of population-based studies. BMC Neurol. 18, 184. https://doi.org/10.1186/s12883-018-1187-0

Huang, H.-P., Hsu, C.F., Mao, Y.-C., Hsu, L., Chi, S., 2021. Gait Stability Measurement by Using Average Entropy. Entropy Basel Switz. 23. https://doi.org/10.3390/e23040412

Huger, F., Patrick, G., 1979. EFFECT OF CONCUSSIVE HEAD INJURY ON CENTRAL CATECHOLAMINE LEVELS AND SYNTHESIS RATES IN RAT BRAIN REGIONS. J. Neurochem. 33, 89–95. https://doi.org/10.1111/j.1471-4159.1979.tb11710.x

Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J., 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry 55, 181–184. https://doi.org/10.1136/jnnp.55.3.181

Hunter, S.K., Pereira, H.M., Keenan, K.G., 2016. The aging neuromuscular system and motor performance. J. Appl. Physiol. Bethesda Md 1985 121, 982–995. https://doi.org/10.1152/japplphysiol.00475.2016

Hutson, C.B., Lazo, C.R., Mortazavi, F., Giza, C.C., Hovda, D., Chesselet, M.-F., 2011. Traumatic Brain Injury in Adult Rats Causes Progressive Nigrostriatal Dopaminergic Cell Loss and Enhanced Vulnerability to the Pesticide Paraquat. J. Neurotrauma 28, 1783–1801. https://doi.org/10.1089/neu.2010.1723

Huynh, L.M., Burns, M.P., Taub, D.D., Blackman, M.R., Zhou, J., 2020. Chronic Neurobehavioral Impairments and Decreased Hippocampal Expression of Genes Important for Brain Glucose Utilization in a Mouse Model of Mild TBI. Front. Endocrinol. 11, 556380. https://doi.org/10.3389/fendo.2020.556380

Hyder, A.A., Wunderlich, C.A., Puvanachandra, P., Gururaj, G., Kobusingye, O.C., 2007. The impact of traumatic brain injuries: a global perspective. NeuroRehabilitation 22, 341–353.

Iacono, D., Raiciulescu, S., Olsen, C., Perl, D.P., 2021. Traumatic Brain Injury Exposure Lowers Age of Cognitive Decline in AD and Non-AD Conditions. Front. Neurol. 12, 573401. https://doi.org/10.3389/fneur.2021.573401

Ikawa, M., Okazawa, H., Yoneda, M., 2021. Molecular imaging for mitochondrial metabolism and oxidative stress in mitochondrial diseases and neurodegenerative disorders. Biochim. Biophys. Acta BBA - Gen. Subj. 1865, 129832. https://doi.org/10.1016/j.bbagen.2020.129832

Ikonomovic, M.D., Uryu, K., Abrahamson, E.E., Ciallella, J.R., Trojanowski, J.Q., Lee, V.M.-Y., Clark, R.S., Marion, D.W., Wisniewski, S.R., DeKosky, S.T., 2004. Alzheimer's pathology in human temporal cortex surgically excised after severe brain injury. Exp. Neurol. 190, 192–203. https://doi.org/10.1016/j.expneurol.2004.06.011

Illouz, T., Madar, R., Clague, C., Griffioen, K.J., Louzoun, Y., Okun, E., 2016. Unbiased classification of spatial strategies in the Barnes maze. Bioinformatics 32, 3314–3320. https://doi.org/10.1093/bioinformatics/btw376

Imamura, K., Hishikawa, N., Sawada, M., Nagatsu, T., Yoshida, M., Hashizume, Y., 2003. Distribution of major histocompatibility complex class II-positive microglia and cytokine

profile of Parkinson's disease brains. Acta Neuropathol. (Berl.) 106, 518–526. https://doi.org/10.1007/s00401-003-0766-2

Impellizzeri, D., Campolo, M., Bruschetta, G., Crupi, R., Cordaro, M., Paterniti, I., Cuzzocrea, S., Esposito, E., 2016. Traumatic Brain Injury Leads to Development of Parkinson's Disease Related Pathology in Mice. Front. Neurosci. 10. https://doi.org/10.3389/fnins.2016.00458

Incoccia, C., Formisano, R., Muscato, P., Reali, G., Zoccolotti, P., 2004. Reaction and Movement Times in Individuals with Chronic Traumatic Brain Injury with Good Motor Recovery. Cortex 40, 111–115. https://doi.org/10.1016/S0010-9452(08)70924-9

Inden, M., Kitamura, Y., Abe, M., Tamaki, A., Takata, K., Taniguchi, T., 2011. Parkinsonian Rotenone Mouse Model: Reevaluation of Long-Term Administration of Rotenone in C57BL/6 Mice. Biol. Pharm. Bull. 34, 92–96. https://doi.org/10.1248/bpb.34.92

Iraji, A., Benson, R.R., Welch, R.D., O'Neil, B.J., Woodard, J.L., Ayaz, S.I., Kulek, A., Mika, V., Medado, P., Soltanian-Zadeh, H., Liu, T., Haacke, E.M., Kou, Z., 2015. Resting State Functional Connectivity in Mild Traumatic Brain Injury at the Acute Stage: Independent Component and Seed-Based Analyses. J. Neurotrauma 32, 1031–1045. https://doi.org/10.1089/neu.2014.3610

Isaias, I.U., Marotta, G., Pezzoli, G., Sabri, O., Schwarz, J., Crenna, P., Classen, J., Cavallari, P., 2011. Enhanced catecholamine transporter binding in the locus coeruleus of patients with early Parkinson disease. BMC Neurol. 11, 88. https://doi.org/10.1186/1471-2377-11-88

Islam, M.B.A.R., Davis, B.T., Kando, M.J., Mao, Q., Procissi, D., Weiss, C., Schwulst, S.J., 2021. Differential neuropathology and functional outcome after equivalent traumatic brain injury in aged versus young adult mice. Exp. Neurol. 341. https://doi.org/10.1016/j.expneurol.2021.113714

Jafari, S., Etminan, M., Aminzadeh, F., Samii, A., 2013. Head injury and risk of Parkinson disease: A systematic review and meta-analysis: Head Injury and Risk of Parkinson Disease. Mov. Disord. 28, 1222–1229. https://doi.org/10.1002/mds.25458

Jain, S., Iverson, L.M., 2020. Glasgow Coma Scale, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Jäkälä, P., 1999. Guanfacine, But Not Clonidine, Improves Planning and Working Memory Performance in Humans. Neuropsychopharmacology 20, 460–470. https://doi.org/10.1016/S0893-133X(98)00127-4

James, S.L., Theadom, A., Ellenbogen, R.G., Bannick, M.S., Montjoy-Venning, W., Lucchesi, L.R., Abbasi, N., Abdulkader, R., Abraha, H.N., Adsuar, J.C., Afarideh, M., Agrawal, S., Ahmadi, A., Ahmed, M.B., Aichour, A.N., Aichour, I., Aichour, M.T.E., Akinyemi, R.O., Akseer, N., Alahdab, F., Alebel, A., Alghnam, S.A., Ali, B.A., Alsharif, U., Altirkawi, K.,

Andrei, C.L., Anjomshoa, M., Ansari, H., Ansha, M.G., Antonio, C.A.T., Appiah, S.C.Y., Ariani, F., Asefa, N.G., Asgedom, S.W., Atique, S., Awasthi, A., Ayala Quintanilla, B.P., Ayuk, T.B., Azzopardi, P.S., Badali, H., Badawi, A., Balalla, S., Banstola, A., Barker-Collo, S.L., Bärnighausen, T.W., Bedi, N., Behzadifar, Masoud, Behzadifar, Meysam, Bekele, B.B., Belachew, A.B., Belay, Y.A., Bennett, D.A., Bensenor, I.M., Berhane, A., Beuran, M., Bhalla, A., Bhaumik, S., Bhutta, Z.A., Biadgo, B., Biffino, M., Bijani, A., Bililign, N., Birungi, C., Boufous, S., Brazinova, A., Brown, A.W., Car, M., Cárdenas, R., Carrero, J.J., Carvalho, F., Castañeda-Orjuela, C.A., Catalá-López, F., Chaiah, Y., Champs, A.P., Chang, J.-C., Choi, J.-Y.J., Christopher, D.J., Cooper, C., Crowe, C.S., Dandona, L., Dandona, R., Daryani, A., Davitoiu, D.V., Degefa, M.G., Demoz, G.T., Deribe, K., Djalalinia, S., Do, H.P., Doku, D.T., Drake, T.M., Dubey, M., Dubljanin, E., El-Khatib, Z., Ofori-Asenso, R., Eskandarieh, S., Esteghamati, A., Esteghamati, S., Faro, A., Farzadfar, F., Farzaei, M.H., Fereshtehnejad, S.-M., Fernandes, E., Feyissa, G.T., Filip, I., Fischer, F., Fukumoto, T., Ganji, M., Gankpe, F.G., Gebre, A.K., Gebrehiwot, T.T., Gezae, K.E., Gopalkrishna, G., Goulart, A.C., Haagsma, J.A., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hamadeh, R.R., Hamidi, S., Haro, J.M., Hassankhani, H., Hassen, H.Y., Havmoeller, R., Hawley, C., Hay, S.I., Hegazy, M.I., Hendrie, D., Henok, A., Hibstu, D.T., Hoffman, H.J., Hole, M.K., Homaie Rad, E., Hosseini, S.M., Hostiuc, S., Hu, G., Hussen, M.A., Ilesanmi, O.S., Irvani, S.S.N., Jakovljevic, M., Jayaraman, S., Jha, R.P., Jonas, J.B., Jones, K.M., Jorjoran Shushtari, Z., Jozwiak, J.J., Jürisson, M., Kabir, A., Kahsay, A., Kahssay, M., Kalani, R., Karch, A., Kasaeian, A., Kassa, G.M., Kassa, T.D., Kassa, Z.Y., Kengne, A.P., Khader, Y.S., Khafaie, M.A., Khalid, N., Khalil, I., Khan, E.A., Khan, M.S., Khang, Y.-H., Khazaie, H., Khoja, A.T., Khubchandani, J., Kiadaliri, A.A., Kim, D., Kim, Y.-E., Kisa, A., Koyanagi, A., Krohn, K.J., Kuate Defo, B., Kucuk Bicer, B., Kumar, G.A., Kumar, M., Lalloo, R., Lami, F.H., Lansingh, V.C., Laryea, D.O., Latifi, A., Leshargie, C.T., Levi, M., Li, S., Liben, M.L., Lotufo, P.A., Lunevicius, R., Mahotra, N.B., Majdan, M., Majeed, A., Malekzadeh, R., Manda, A.-L., Mansournia, M.A., Massenburg, B.B., Mate, K.K.V., Mehndiratta, M.M., Mehta, V., Meles, H., Melese, A., Memiah, P.T.N., Mendoza, W., Mengistu, G., Meretoja, A., Meretoja, T.J., Mestrovic, T., Miazgowski, T., Miller, T.R., Mini, G., Mirica, A., Mirrakhimov, E.M., Moazen, B., Mohammadi, M., Molokhia, M., Monasta, L., Mondello, S., Moosazadeh, M., Moradi, G., Moradi, M., Moradi-Lakeh, M., Moradinazar, M., Morrison, S.D., Moschos, M.M., Mousavi, S.M., Murthy, S., Musa, K.I., Mustafa, G., Naghavi, M., Naik, G., Najafi, F., Nangia, V., Nascimento, B.R., Negoi, I., Nguyen, T.H., Nichols, E., Ningrum, D.N.A., Nirayo, Y.L., Nyasulu, P.S., Ogbo, F.A., Oh, I.-H., Okoro, A., Olagunju, A.T., Olagunju, T.O., Olivares, P.R., Otstavnov, S.S., Owolabi, M.O., P A, M., Pakhale, S., Pandey, A.R., Pesudovs, K., Pinilla-Monsalve, G.D., Polinder, S., Poustchi, H., Prakash, S., Qorbani, M., Radfar, A., Rafay, A., Rafiei, A., Rahimi-Movaghar, A., Rahimi-Movaghar, V., Rahman, M., Rahman, M.A., Rai, R.K., Rajati, F., Ram, U., Rawaf, D.L., Rawaf, S., Reiner, R.C., Reis, C., Renzaho, A.M.N., Resnikoff, S., Rezaei, S., Rezaeian, S., Roever, L., Ronfani, L., Roshandel, G., Roy, N., Ruhago, G.M., Saddik, B., Safari, H., Safiri, S., Sahraian, M.A., Salamati, P., Saldanha, R. de F., Samy, A.M., Sanabria, J., Santos, J.V., Santric Milicevic, M.M.M., Sartorius, B., Satpathy, M., Savuon, K., Schneider, I.J.C., Schwebel, D.C., Sepanlou, S.G., Shabaninejad, H., Shaikh, M.A.A., Shams-Beyranvand, M., Sharif, M., Sharif-Alhoseini, M., Shariful Islam, S.M., She, J., Sheikh, A., Shen, J., Sheth, K.N., Shibuya, K., Shiferaw, M.S., Shigematsu, M., Shiri, R., Shiue, I.,

Shoman, H., Siabani, S., Siddiqi, T.J., Silva, J.P., Silveira, D.G.A., Sinha, D.N., Smith, M., Soares Filho, A.M., Sobhani, S., Soofi, M., Soriano, J.B., Soyiri, I.N., Stein, D.J., Stokes, M.A., Sufiyan, M.B., Sunguya, B.F., Sunshine, J.E., Sykes, B.L., Szoeke, C.E.I., Tabarés-Seisdedos, R., Te Ao, B.J., Tehrani-Banihashemi, A., Tekle, M.G., Temsah, M.-H., Temsah, O., Topor-Madry, R., Tortajada-Girbés, M.-, Tran, B.X., Tran, K.B., Tudor Car, L., Ukwaja, K.N., Ullah, I., Usman, M.S., Uthman, O.A., Valdez, P.R., Vasankari, T.J., Venketasubramanian, N., Violante, F.S., Wagnew, F.W.S., Waheed, Y., Wang, Y.-P., Weldegwergs, K.G., Werdecker, A., Wijeratne, T., Winkler, A.S., Wyper, G.M.A., Yano, Y., Yaseri, M., Yasin, Y.J., Ye, P., Yimer, E.M., Yip, P., Yisma, E., Yonemoto, N., Yoon, S.-J., Yost, M.G., Younis, M.Z., Yousefifard, M., Yu, C., Zaidi, Z., Zaman, S.B., Zamani, M., Zenebe, Z.M., Zodpey, S., Feigin, V.L., Vos, T., Murray, C.J.L., 2019. Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 18, 56–87. https://doi.org/10.1016/S1474-4422(18)30415-0

Jan, A., Gonçalves, N.P., Vaegter, C.B., Jensen, P.H., Ferreira, N., 2021. The Prion-Like Spreading of Alpha-Synuclein in Parkinson's Disease: Update on Models and Hypotheses. Int. J. Mol. Sci. 22, 8338. https://doi.org/10.3390/ijms22158338

Jankovic, J., McDermott, M., Carter, J., Gauthier, S., Goetz, C., Golbe, L., Huber, S., Koller, W., Olanow, C., Shoulson, I., Stern, M., Tanner, C., Weiner, W., Parkinson Study Group, 1990. Variable expression of Parkinson's disease: A base-line analysis of the DAT ATOP cohort. Neurology 40, 1529–1529. https://doi.org/10.1212/WNL.40.10.1529

Jenkins, P.O., De Simoni, S., Bourke, N.J., Fleminger, J., Scott, G., Towey, D.J., Svensson, W., Khan, S., Patel, M., Greenwood, R., Cole, J.H., Sharp, D.J., 2018. Dopaminergic abnormalities following traumatic brain injury. Brain 141, 797–810. https://doi.org/10.1093/brain/awx357

Jenkins, P.O., Mehta, M.A., Sharp, D.J., 2016. Catecholamines and cognition after traumatic brain injury. Brain 139, 2345–2371. https://doi.org/10.1093/brain/aww128

Jennings, D., Siderowf, A., Stern, M., Seibyl, J., Eberly, S., Oakes, D., Marek, K., For the PARS Investigators, 2014. Imaging prodromal Parkinson disease: The Parkinson Associated Risk Syndrome Study. Neurology 83, 1739–1746. https://doi.org/10.1212/WNL.0000000000000960

Jessop, R.T., Horowicz, C., Dibble, L.E., 2006. Motor Learning and Parkinson Disease: Refinement of Movement Velocity and Endpoint Excursion in a Limits of Stability Balance Task. Neurorehabil. Neural Repair 20, 459–467. https://doi.org/10.1177/1545968306287107

Jing, L., Hou, L., Zhang, D., Li, S., Ruan, Z., Zhang, X., Hong, J.-S., Wang, Q., 2021. Microglial Activation Mediates Noradrenergic Locus Coeruleus Neurodegeneration via Complement Receptor 3 in a Rotenone-Induced Parkinson's Disease Mouse Model. J. Inflamm. Res. 14, 1341–1356. https://doi.org/10.2147/JIR.S299927

Johnson, M.E., Bobrovskaya, L., 2015. An update on the rotenone models of Parkinson's disease: Their ability to reproduce the features of clinical disease and model gene–environment interactions. NeuroToxicology 46, 101–116. https://doi.org/10.1016/j.neuro.2014.12.002

Johnson, V.E., Stewart, J.E., Begbie, F.D., Trojanowski, J.Q., Smith, D.H., Stewart, W., 2013. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain 136, 28–42. https://doi.org/10.1093/brain/aws322

Johnston, W., Heiderscheit, B., Sanfilippo, J., Brooks, M.A., Caulfield, B., 2020. Athletes with a concussion history in the last two years have impairments in dynamic balance performance. Scand. J. Med. Sci. Sports 30, 1497–1505. https://doi.org/10.1111/sms.13691

Jones, B.E., Moore, R.Y., 1977. Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. Brain Res. 127, 23–53. https://doi.org/10.1016/0006-8993(77)90378-X

Jones-Tabah, J., Martin, R.D., Chen, J.J., Tanny, J.C., Clarke, P.B.S., Hébert, T.E., 2021. Dopamine D1 receptor activation and cAMP/PKA signalling mediate Brd4 recruitment to chromatin to regulate gene expression in rat striatal neurons (preprint). Neuroscience. https://doi.org/10.1101/2021.07.01.450754

Jorge, R., Robinson, R.G., 2003. Mood disorders following traumatic brain injury. Int. Rev. Psychiatry 15, 317–327. https://doi.org/10.1080/09540260310001606700

Jourdan, C., Bayen, E., Pradat-Diehl, P., Ghout, I., Darnoux, E., Azerad, S., Vallat-Azouvi, C., Charanton, J., Aegerter, P., Ruet, A., Azouvi, P., 2016. A comprehensive picture of 4-year outcome of severe brain injuries. Results from the PariS-TBI study. Ann. Phys. Rehabil. Med. 59, 100–106. https://doi.org/10.1016/j.rehab.2015.10.009

Jovanovic, P., Wang, Y., Vit, J.-P., Novinbakht, E., Morones, N., Hogg, E., Tagliati, M., Riera, C.E., 2022. Sustained chemogenetic activation of locus coeruleus norepinephrine neurons promotes dopaminergic neuron survival in synucleinopathy. PLOS ONE 17, e0263074. https://doi.org/10.1371/journal.pone.0263074

Juengst, S.B., Kumar, R.G., Arenth, P.M., Wagner, A.K., 2014. Exploratory associations with Tumor Necrosis Factor-α, disinhibition and suicidal endorsement after traumatic brain injury. Brain. Behav. Immun. 41, 134–143. https://doi.org/10.1016/j.bbi.2014.05.020

K, P.E., J, T.N., A, K.J., 2011. Striatal Dopamine Depletion Induces Forelimb Motor Impairments and Disrupts Forelimb Movement Representations within the Motor Cortex. J. Park. Dis. 93–100. https://doi.org/10.3233/JPD-2011-11017

Kalia, L.V., Kalia, S.K., Lang, A.E., 2015. Disease-modifying strategies for Parkinson's disease. Mov. Disord. 30, 1442–1450. https://doi.org/10.1002/mds.26354

Kalia, L.V., Lang, A.E., 2015. Parkinson's disease. The Lancet 386, 896–912. https://doi.org/10.1016/S0140-6736(14)61393-3

Kalpinski, R.J., Williamson, M.L.C., Elliott, T.R., Berry, J.W., Underhill, A.T., Fine, P.R., 2013. Modeling the prospective relationships of impairment, injury severity, and participation to quality of life following traumatic brain injury. BioMed Res. Int. 2013, 102570. https://doi.org/10.1155/2013/102570

Kamath, T., Abdulraouf, A., Burris, S.J., Langlieb, J., Gazestani, V., Nadaf, N.M., Balderrama, K., Vanderburg, C., Macosko, E.Z., 2022. Single-cell genomic profiling of human dopamine neurons identifies a population that selectively degenerates in Parkinson's disease. Nat. Neurosci. 25, 588–595. https://doi.org/10.1038/s41593-022-01061-1

Karve, I.P., Taylor, J.M., Crack, P.J., 2016. The contribution of astrocytes and microglia to traumatic brain injury. Br. J. Pharmacol. 173, 692–702. https://doi.org/10.1111/bph.13125

Katayama, Y., Becker, D.P., Tamura, T., Hovda, D.A., 1990. Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J. Neurosurg. 73, 889–900. https://doi.org/10.3171/jns.1990.73.6.0889

Kędziorek, J., Błażkiewicz, M., 2020. Nonlinear Measures to Evaluate Upright Postural Stability: A Systematic Review. Entropy Basel Switz. 22. https://doi.org/10.3390/e22121357

Keil, M.F., Briassoulis, G., Stratakis, C.A., Wu, T.J., 2016. Protein Kinase A and Anxiety-Related Behaviors: A Mini-Review. Front. Endocrinol. 7, 83. https://doi.org/10.3389/fendo.2016.00083

Kennard, J., 2011. Age sensitivity of behavioral tests and brain substrates of normal aging in mice. Front. Aging Neurosci. 3. https://doi.org/10.3389/fnagi.2011.00009

Khan, F., Baguley, I.J., Cameron, I.D., 2003. 4: Rehabilitation after traumatic brain injury. Med. J. Aust. 178, 290–295. https://doi.org/10.5694/j.1326-5377.2003.tb05199.x

Kim, S., Kwon, S.-H., Kam, T.-I., Panicker, N., Karuppagounder, S.S., Lee, S., Lee, J.H., Kim, W.R., Kook, M., Foss, C.A., Shen, C., Lee, H., Kulkarni, S., Pasricha, P.J., Lee, G., Pomper, M.G., Dawson, V.L., Dawson, T.M., Ko, H.S., 2019. Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease. Neuron 103, 627-641.e7. https://doi.org/10.1016/j.neuron.2019.05.035

Kim, T.-K., Bae, E.-J., Jung, B.C., Choi, M., Shin, S.J., Park, S.J., Kim, J.T., Jung, M.K., Ulusoy, A., Song, M.-Y., Lee, J.S., Lee, H.-J., Di Monte, D.A., Lee, S.-J., 2022. Inflammation promotes synucleinopathy propagation. Exp. Mol. Med. 54, 2148–2161. https://doi.org/10.1038/s12276-022-00895-w

Kinder, H.A., Baker, E.W., West, F.D., 2019. The pig as a preclinical traumatic brain injury model: current models, functional outcome measures, and translational detection strategies. Neural Regen. Res. 14, 413–424. https://doi.org/10.4103/1673-5374.245334

King, B.R., van Ruitenbeek, P., Leunissen, I., Cuypers, K., Heise, K.-F., Santos Monteiro, T., Hermans, L., Levin, O., Albouy, G., Mantini, D., Swinnen, S.P., 2018. Age-Related Declines in Motor Performance are Associated With Decreased Segregation of Large-Scale Resting State Brain Networks. Cereb. Cortex 28, 4390–4402. https://doi.org/10.1093/cercor/bhx297

Kish, S.J., Shannak, K., Hornykiewicz, O., 1988. Uneven Pattern of Dopamine Loss in the Striatum of Patients with Idiopathic Parkinson's Disease. N. Engl. J. Med. 318, 876–880. https://doi.org/10.1056/NEJM198804073181402

Klein, C., Westenberger, A., 2012. Genetics of Parkinson's Disease. Cold Spring Harb. Perspect. Med. 2, a008888–a008888. https://doi.org/10.1101/cshperspect.a008888

Klein, M.O., Battagello, D.S., Cardoso, A.R., Hauser, D.N., Bittencourt, J.C., Correa, R.G., 2019. Dopamine: Functions, Signaling, and Association with Neurological Diseases. Cell. Mol. Neurobiol. 39, 31–59. https://doi.org/10.1007/s10571-018-0632-3

Klima, D., Morgan, L., Baylor, M., Reilly, C., Gladmon, D., Davey, A., 2019. Physical Performance and Fall Risk in Persons With Traumatic Brain Injury. Percept. Mot. Skills 126, 50–69. https://doi.org/10.1177/0031512518809203

Komoltsev, I.G., Volkova, A.A., Levshina, I.P., Novikova, M.R., Gulyaeva, N.V., 2021. Long-term sequelae of traumatic brain injury in rats: A morphological, behavioral, and electrophysiological study. Neurosci. Behav. Physiol. 51, 209–219.

Koprich, J.B., Reske-Nielsen, C., Mithal, P., Isacson, O., 2008. Neuroinflammation mediated by IL-1β increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease. J. Neuroinflammation 5, 8. https://doi.org/10.1186/1742-2094-5-8

Kordower, J.H., Olanow, C.W., Dodiya, H.B., Chu, Y., Beach, T.G., Adler, C.H., Halliday, G.M., Bartus, R.T., 2013. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. Brain 136, 2419–2431. https://doi.org/10.1093/brain/awt192

Kouli, A., Torsney, K.M., Department of Medicine for the Elderly, Cambridge University Hospitals NHS Foundation Trust, UK, Clinical Gerontology Unit, Department of Public Health and Primary Care, University of Cambridge, UK, Kuan, W.-L., John van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, UK, 2018. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis, in: John Van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, UK, Stoker, T.B., Greenland, J.C., John Van Geest Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, UK (Eds.), Parkinson's Disease: Pathogenesis and Clinical Aspects. Codon Publications, pp. 3–26. https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch1

Kraeuter, A.-K., Guest, P.C., Sarnyai, Z., 2019. The Elevated Plus Maze Test for Measuring Anxiety-Like Behavior in Rodents, in: Guest, P.C. (Ed.), Pre-Clinical Models, Methods in Molecular Biology. Springer New York, New York, NY, pp. 69–74. https://doi.org/10.1007/978-1-4939-8994-2_4

Krauss, J.K., Trankle, R., Kopp, K.-H., 1996. Post-traumatic movement disorders in survivors of severe head injury. Neurology 47, 1488–1492. https://doi.org/10.1212/WNL.47.6.1488

Krogh, K., Ostergaard, K., Sabroe, S., Laurberg, S., 2007. Clinical aspects of bowel symptoms in Parkinson?s disease. Acta Neurol. Scand. 0, 070905010742008-??? https://doi.org/10.1111/j.1600-0404.2007.00900.x

Kumar, A., Loane, D.J., 2012. Neuroinflammation after traumatic brain injury: Opportunities for therapeutic intervention. Brain. Behav. Immun. 26, 1191–1201. https://doi.org/10.1016/j.bbi.2012.06.008

Kumar, R.G., Boles, J.A., Wagner, A.K., 2015. Chronic Inflammation After Severe Traumatic Brain Injury: Characterization and Associations With Outcome at 6 and 12 Months Postinjury. J. Head Trauma Rehabil. 30, 369–381. https://doi.org/10.1097/HTR.0000000000000067

Kurowski, B.G., Backeljauw, B., Zang, H., Zhang, N., Martin, L.J., Pilipenko, V., Yeates, K., Taylor, H.G., Wade, S., 2016. Influence of Catechol-O-methyltransferase on Executive Functioning Longitudinally After Early Childhood Traumatic Brain Injury: Preliminary Findings. J. Head Trauma Rehabil. 31, E1–E9. https://doi.org/10.1097/HTR.0000000000000162

Lachman, H.M., Papolos, D.F., Saito, T., Yu, Y.-M., Szumlanski, C.L., Weinshilboum, R.M., 1996. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenet. Genomics 6, 243–250.

Lai, J.-H., Karlsson, T.E., Wu, J.C.-C., Huang, C.-Z., Chen, Y.-H., Kang, S.-J., Brodin, A.T.S., Hoffer, B.J., Olson, L., Chiang, Y.-H., Chen, K.-Y., 2019. Role of Nogo Receptor-1 for Recovery of Balance, Cognition, and Emotion after Mild Traumatic Brain Injury in Mice. J. Neurotrauma 36, 1054–1059. https://doi.org/10.1089/neu.2018.5949

Laurer, H.L., Bareyre, F.M., Lee, V.M.Y.C., Trojanowski, J.Q., Longhi, L., Hoover, R., Saatman, K.E., Raghupathi, R., Hoshino, S., Grady, M.S., McIntosh, T.K., 2001. Mild head injury increasing the brain's vulnerability to a second concussive impact. J. Neurosurg. 95, 859–870. https://doi.org/10.3171/jns.2001.95.5.0859

Lawrence, T., 2009. The Nuclear Factor NF- B Pathway in Inflammation. Cold Spring Harb. Perspect. Biol. 1, a001651–a001651. https://doi.org/10.1101/cshperspect.a001651

Leconte, C., Benedetto, C., Lentini, F., Simon, K., Ouaazizi, C., Taib, T., Cho, A., Plotkine, M., Mongeau, R., Marchand-Leroux, C., Besson, V.C., 2020. Histological and behavioral evaluation after traumatic brain injury in mice: A ten months follow-up study. J. Neurotrauma 37, 1342–1357.

Ledwidge, P.S., Patterson, J.N., Molfese, D.L., Honaker, J.A., 2019. Clinical Utility of Oculomotor and Electrophysiological Measures in Identifying Concussion History. Clin. J. Sport Med. Off. J. Can. Acad. Sport Med. 29, 292–297.

Ledwidge, P.S., Patterson, J.N., Molfese, D.L., Honaker, J.A., 2017. Clinical Utility of Oculomotor and Electrophysiological Measures in Identifying Concussion History. Clin. J. Sport Med. Publish Ahead of Print. https://doi.org/10.1097/JSM.0000000000000512

Lee Mosley, R., Benner, E.J., Kadiu, I., Thomas, M., Boska, M.D., Hasan, K., Laurie, C., Gendelman, H.E., 2006. Neuroinflammation, oxidative stress, and the pathogenesis of Parkinson's disease. Clin. Neurosci. Res. 6, 261–281. https://doi.org/10.1016/j.cnr.2006.09.006

Lee, H., Lee, S., Black, I., Salado, L., Estrada, J., Isla, K., 2020. Long-Term Impact of Mild Traumatic Brain Injuries on Multiple Functional Outcomes and Epigenetics: A Pilot Study with College Students. Appl. Sci. 10, 4131. https://doi.org/10.3390/app10124131

Lee, J.-K., Tran, T., Tansey, M.G., 2009. Neuroinflammation in Parkinson's Disease. J. Neuroimmune Pharmacol. 4, 419–429. https://doi.org/10.1007/s11481-009-9176-0

Lee, M.K., Stirling, W., Xu, Y., Xu, X., Qui, D., Mandir, A.S., Dawson, T.M., Copeland, N.G., Jenkins, N.A., Price, D.L., 2002. Human α-synuclein-harboring familial Parkinson's disease-linked Ala-53 → Thr mutation causes neurodegenerative disease with α-synuclein aggregation in transgenic mice. Proc. Natl. Acad. Sci. 99, 8968–8973. https://doi.org/10.1073/pnas.132197599

Lee, P.-C., Bordelon, Y., Bronstein, J., Ritz, B., 2012. Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease. Neurology 79, 2061–2066. https://doi.org/10.1212/WNL.0b013e3182749f28

Lehman, E.J., Hein, M.J., Baron, S.L., Gersic, C.M., 2012. Neurodegenerative causes of death among retired National Football League players. Neurology 79, 1970–1974. https://doi.org/10.1212/WNL.0b013e31826daf50

Lema Tomé, C.M., Tyson, T., Rey, N.L., Grathwohl, S., Britschgi, M., Brundin, P., 2013. Inflammation and α-Synuclein's Prion-like Behavior in Parkinson's Disease—Is There a Link? Mol. Neurobiol. 47, 561–574. https://doi.org/10.1007/s12035-012-8267-8

Levin, B.E., Brown, K.L., Pawar, G., Dunn-Meynell, A., 1995. Widespread and lateralized effects of acute traumatic brain injury on norepinephrine turnover in the rat brain. Brain Res. 674, 307–313. https://doi.org/10.1016/0006-8993(95)00032-L

Lewis, M.M., Van Scoy, L.J., De Jesus, S., Hakun, J.G., Eslinger, P.J., Fernandez-Mendoza, J., Kong, L., Yang, Y., Snyder, B.L., Loktionova, N., Duvvuri, S., Gray, D.L., Huang, X., Mailman, R.B., 2023. Dopamine D1 Agonists: First Potential Treatment for Late-Stage Parkinson's Disease. Biomolecules 13, 829. https://doi.org/10.3390/biom13050829

Li, B.-M., Mei, Z.-T., 1994. Delayed-response deficit induced by local injection of the α2-adrenergic antagonist yohimbine into the dorsolateral prefrontal cortex in young adult monkeys. Behav. Neural Biol. 62, 134–139. https://doi.org/10.1016/S0163-1047(05)80034-2

Li, C., Chen, X., Zhang, N., Song, Y., Mu, Y., 2012. Gastrodin inhibits neuroinflammation in rotenone-induced Parkinson's disease model rats. Neural Regen. Res. 7, 325–331. https://doi.org/10.3969/j.issn.1673-5374.2012.05.001

Li, N., Ragheb, K., Lawler, G., Sturgis, J., Rajwa, B., Melendez, J.A., Robinson, J.P., 2003. Mitochondrial Complex I Inhibitor Rotenone Induces Apoptosis through Enhancing Mitochondrial Reactive Oxygen Species Production. J. Biol. Chem. 278, 8516–8525. https://doi.org/10.1074/jbc.M210432200

Li, Q., Cheng, Z., Zhou, L., Darmanis, S., Neff, N.F., Okamoto, J., Gulati, G., Bennett, M.L., Sun, L.O., Clarke, L.E., Marschallinger, J., Yu, G., Quake, S.R., Wyss-Coray, T., Barres, B.A., 2019. Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. Neuron 101, 207-223.e10. https://doi.org/10.1016/j.neuron.2018.12.006

Li, X., Redus, L., Chen, C., Martinez, P.A., Strong, R., Li, S., O'Connor, J.C., 2013. Cognitive Dysfunction Precedes the Onset of Motor Symptoms in the MitoPark Mouse Model of Parkinson's Disease. PLoS ONE 8, e71341. https://doi.org/10.1371/journal.pone.0071341

Liang, Y., Zhou, T., Chen, Y., Lin, D., Jing, X., Peng, S., Zheng, D., Zeng, Z., Lei, M., Wu, X., Huang, K., Yang, L., Xiao, S., Liu, J., Tao, E., 2017. Rifampicin inhibits rotenone-induced microglial inflammation via enhancement of autophagy. NeuroToxicology 63, 137–145. https://doi.org/10.1016/j.neuro.2017.09.015

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C., Ioannidis, J.P.A., Clarke, M., Devereaux, P.J., Kleijnen, J., Moher, D., 2009. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. PLoS Med. 6, e1000100. https://doi.org/10.1371/journal.pmed.1000100

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. Clin. Interv. Aging Volume 13, 757–772. https://doi.org/10.2147/CIA.S158513

Lim, Y.H., Kim, D.H., Lee, M.Y., Joo, M.C., 2012. Bowel Dysfunction and Colon Transit Time in Brain-Injured Patients. Ann. Rehabil. Med. 36, 371. https://doi.org/10.5535/arm.2012.36.3.371

Lin, C., Zhang, M., Huang, T., Yang, L., Fu, H., Zhao, L., Zhong, L.L., Mu, H., Shi, X., Leung, C.F., Fan, B., Jiang, M., Lu, A., Zhu, L., Bian, Z., 2015. Spexin Enhances Bowel Movement through Activating L-type Voltage-dependent Calcium Channel via Galanin Receptor 2 in Mice. Sci. Rep. 5, 12095. https://doi.org/10.1038/srep12095

Lin, C.-H., Fan, J.-Y., Lin, H.-I., Chang, C.-W., Wu, Y.-R., 2018. Catechol-O-methyltransferase (COMT) genetic variants are associated with cognitive decline in patients with Parkinson's disease. Parkinsonism Relat. Disord. 50, 48–53. https://doi.org/10.1016/j.parkreldis.2018.02.015

Lin, L.-F., Liou, T.-H., Hu, C.-J., Ma, H.-P., Ou, J.-C., Chiang, Y.-H., Chiu, W.-T., Tsai, S.-H., Chu, W.-C., 2015. Balance function and sensory integration after mild traumatic brain injury. Brain Inj. 29, 41–46. https://doi.org/10.3109/02699052.2014.955881

Lin, T.-K., Cheng, C.-H., Chen, S.-D., Liou, C.-W., Huang, C.-R., Chuang, Y.-C., 2012. Mitochondrial dysfunction and oxidative stress promote apoptotic cell death in the striatum via cytochrome c/caspase-3 signaling cascade following chronic rotenone intoxication in rats. Int. J. Mol. Sci. 13, 8722–8739. https://doi.org/10.3390/ijms13078722

Lipsky, R.H., Sparling, M.B., Ryan, L.M., Xu, K., Salazar, A.M., Goldman, D., Warden, D.L., 2005. Association of COMT Val158Met genotype with executive functioning following traumatic brain injury. J. Neuropsychiatry Clin. Neurosci. 17, 465–471.

List, E.O., Berryman, D.E., Wright-Piekarski, J., Jara, A., Funk, K., Kopchick, J.J., 2013. The effects of weight cycling on lifespan in male C57BL/6J mice. Int. J. Obes. 37, 1088–1094. https://doi.org/10.1038/ijo.2012.203

Liu, A., Papale, A.E., Hengenius, J., Patel, K., Ermentrout, B., Urban, N.N., 2020. Mouse Navigation Strategies for Odor Source Localization. Front. Neurosci. 14, 218. https://doi.org/10.3389/fnins.2020.00218

Liu, G., Ou, S., Cui, H., Li, X., Yin, Z., Gu, D., Wang, Z., 2021. Head Injury and Amyotrophic Lateral Sclerosis: A Meta-Analysis. Neuroepidemiology 1–9. https://doi.org/10.1159/000510987

Liu, M., Bachstetter, A.D., Cass, W.A., Lifshitz, J., Bing, G., 2017. Pioglitazone Attenuates Neuroinflammation and Promotes Dopaminergic Neuronal Survival in the Nigrostriatal System of Rats after Diffuse Brain Injury. J. Neurotrauma 34, 414–422. https://doi.org/10.1089/neu.2015.4361

Lizhnyak, P.N., Ottens, A.K., 2015. Proteomics: in pursuit of effective traumatic brain injury therapeutics. Expert Rev. Proteomics 12, 75–82. https://doi.org/10.1586/14789450.2015.1000869

Lloyd-Donald, P., Spencer, W., Cheng, J., Romero, L., Jithoo, R., Udy, A., Fitzgerald, M.C., 2020. In adult patients with severe traumatic brain injury, does the use of norepinephrine for augmenting cerebral perfusion pressure improve neurological outcome? A systematic review. Injury 51, 2129–2134. https://doi.org/10.1016/j.injury.2020.07.054

Loane, D.J., Kumar, A., Stoica, B.A., Cabatbat, R., Faden, A.I., 2014. Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation. J. Neuropathol. Exp. Neurol. 73, 14–29. https://doi.org/10.1097/NEN.0000000000000021

López-Armada, M.J., Riveiro-Naveira, R.R., Vaamonde-García, C., Valcárcel-Ares, M.N., 2013. Mitochondrial dysfunction and the inflammatory response. Mitochondrion 13, 106–118. https://doi.org/10.1016/j.mito.2013.01.003

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I., Taskinen, J., 1995. Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 34, 4202–4210.

Luk, K.C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J.Q., Lee, V.M.-Y., 2012. Pathological α-Synuclein Transmission Initiates Parkinson-like Neurodegeneration in Nontransgenic Mice. Science 338, 949–953. https://doi.org/10.1126/science.1227157

M. Faul, Xu, Likang, Wald, Marlena M., Coronado, Victor G., 2010. Traumatic Brain Injury in the United states: Emergency Department Visits, Hospitalization and Deaths 2002-2006. Cent. Dis. Control Prev. Natl. Cent. Inj. Prev. Control.

Ma, S.Y., Röyttä, M., Rinne, J.O., Collan, Y., Rinne, U.K., 1997. Correlation between neuromorphometry in the substantia nigra and clinical features in Parkinson's disease using disector counts. J. Neurol. Sci. 151, 83–87. https://doi.org/10.1016/S0022-510X(97)00100-7

Mackay, D.F., Russell, E.R., Stewart, K., MacLean, J.A., Pell, J.P., Stewart, W., 2019. Neurodegenerative Disease Mortality among Former Professional Soccer Players. N. Engl. J. Med. 381, 1801–1808. https://doi.org/10.1056/NEJMoa1908483

Mackay, G.M., Forrest, C.M., Stoy, N., Christofides, J., Egerton, M., Stone, T.W., Darlington, L.G., 2006. Tryptophan metabolism and oxidative stress in patients with chronic brain injury. Eur. J. Neurol. 13, 30–42. https://doi.org/10.1111/j.1468-1331.2006.01220.x

Mann, A., Chesselet, M.-F., 2015. Chapter 8 - Techniques for Motor Assessment in Rodents, in: LeDoux, M.S. (Ed.), Movement Disorders (Second Edition). Academic Press, Boston, pp. 139–157. https://doi.org/10.1016/B978-0-12-405195-9.00008-1

Mannix, R., Berglass, J., Berkner, J., Moleus, P., Qiu, J., Andrews, N., Gunner, G., Berglass, L., Jantzie, L.L., Robinson, S., Meehan, W.P., 2014. Chronic gliosis and behavioral deficits in mice following repetitive mild traumatic brain injury. J. Neurosurg. 121, 1342–1350. https://doi.org/10.3171/2014.7.JNS14272

Mannix, R., Berkner, J., Mei, Z., Alcon, S., Hashim, J., Robinson, S., Jantzie, L., Meehan, W.P., Qiu, J., 2017. Adolescent Mice Demonstrate a Distinct Pattern of Injury after Repetitive Mild Traumatic Brain Injury. J. Neurotrauma 34, 495–504. https://doi.org/10.1089/neu.2016.4457

Maries, E., Dass, B., Collier, T.J., Kordower, J.H., Steece-Collier, K., 2003. The role of α-synuclein in Parkinson's disease: insights from animal models. Nat. Rev. Neurosci. 4, 727–738. https://doi.org/10.1038/nrn1199

Marinova-Mutafchieva, L., Sadeghian, M., Broom, L., Davis, J.B., Medhurst, A.D., Dexter, D.T., 2009. Relationship between microglial activation and dopaminergic neuronal loss in the substantia nigra: a time course study in a 6-hydroxydopamine model of Parkinson's disease. J. Neurochem. 110, 966–975. https://doi.org/10.1111/j.1471-4159.2009.06189.x

Marking, L.L., Bills, T.D., 1976. Toxicity of rotenone to fish in standardized laboratory tests (Report No. 72), Investigations in Fish Control.

Marmarou, A., Foda, M.A., van den Brink, W., Campbell, J., Kita, H., Demetriadou, K., 1994. A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. J. Neurosurg. 80, 291–300. https://doi.org/10.3171/jns.1994.80.2.0291

Marmarou, C., Prieto, R., Taya, K., Young, H., Marmarou, A., 2009. Marmarou Weight Drop Injury Model, in: Animal Models of Acute Neurological Injuries. pp. 393–407. https://doi.org/10.1007/978-1-60327-185-1_34

Marquez de la Plata, C.D., Hart, T., Hammond, F.M., Frol, A.B., Hudak, A., Harper, C.R., O'Neil-Pirozzi, T.M., Whyte, J., Carlile, M., Diaz-Arrastia, R., 2008. Impact of age on long-term recovery from traumatic brain injury. Arch. Phys. Med. Rehabil. 89, 896–903. https://doi.org/10.1016/j.apmr.2007.12.030

Marras, C., Hincapié, C.A., Kristman, V.L., Cancelliere, C., Soklaridis, S., Li, A., Borg, J., af Geijerstam, J.-L., Cassidy, J.D., 2014. Systematic Review of the Risk of Parkinson's Disease After Mild Traumatic Brain Injury: Results of the International Collaboration on Mild Traumatic Brain Injury Prognosis. Arch. Phys. Med. Rehabil. 95, S238–S244. https://doi.org/10.1016/j.apmr.2013.08.298

Marsden, C.D., 1994. Problems with long-term levodopa therapy for Parkinson's disease. Clin. Neuropharmacol. 17 Suppl 2, S32-44.

Marsden, C.D., 1983. Neuromelanin and Parkinson's disease. J. Neural Transm. Suppl. 19, 121–141.

Martini, Douglas N., Parrington, L., Stuart, S., Fino, P.C., King, L.A., 2021. Gait performance in people with symptomatic, chronic mild traumatic brain injury. J. Neurotrauma 38, 218–224.

Martini, D.N., Parrington, L., Stuart, S., Fino, P.C., King, L.A., 2021. Gait Performance in People with Symptomatic, Chronic Mild Traumatic Brain Injury. J. Neurotrauma 38, 218–224. https://doi.org/10.1089/neu.2020.6986

Martini, D.N., Sabin, M.J., DePesa, S.A., Leal, E.W., Negrete, T.N., Sosnoff, J.J., Broglio, S.P., 2011. The Chronic Effects of Concussion on Gait. Arch. Phys. Med. Rehabil. 92, 585–589. https://doi.org/10.1016/j.apmr.2010.11.029

Marttila, R.J., Lorentz, H., Rinne, U.K., 1988. Oxygen toxicity protecting enzymes in Parkinson's disease. J. Neurol. Sci. 86, 321–331. https://doi.org/10.1016/0022-510X(88)90108-6

Martyn, C.N., Osmond, C., 1995. Parkinson's disease and the environment in early life. J. Neurol. Sci. 132, 201–206. https://doi.org/10.1016/0022-510x(95)00148-u

Masel, B.E., DeWitt, D.S., 2010. Traumatic Brain Injury: A Disease Process, Not an Event. J. Neurotrauma 27, 1529–1540. https://doi.org/10.1089/neu.2010.1358

Mastroeni, D., Grover, A., Leonard, B., Joyce, J.N., Coleman, P.D., Kozik, B., Bellinger, D.L., Rogers, J., 2009. Microglial responses to dopamine in a cell culture model of Parkinson's disease. Neurobiol. Aging 30, 1805–1817. https://doi.org/10.1016/j.neurobiolaging.2008.01.001

Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Kubo, M., Shimozawa, A., Akiyama, H., Hasegawa, M., 2014. Pathological alpha-synuclein propagates through neural networks. Acta Neuropathol. Commun. 2, 88. https://doi.org/10.1186/s40478-014-0088-8

Mautes, A.E.M., Müller, M., Cortbus, F., Schwerdtfeger, K., Maier, B., Holanda, M., Nacimiento, A., Marzi, I., Steudel, W.-I., 2001. Alterations of Norepinephrine Levels in Plasma and CSF of Patients After Traumatic Brain Injury in Relation to Disruption of the Blood-Brain Barrier. Acta Neurochir. (Wien) 143, 51–58. https://doi.org/10.1007/s007010170138

McAllister, T.W., 2011. Neurobiological consequences of traumatic brain injury. Dialogues Clin. Neurosci. 13, 287–300. https://doi.org/10.31887/DCNS.2011.13.2/tmcallister

McAllister, T.W., McDonald, B.C., Flashman, L.A., Ferrell, R.B., Tosteson, T.D., Yanofsky, N.N., Grove, M.R., Saykin, A.J., 2011. Alpha-2 adrenergic challenge with guanfacine one month after mild traumatic brain injury: altered working memory and BOLD response. Int. J. Psychophysiol. Off. J. Int. Organ. Psychophysiol. 82, 107–114. https://doi.org/10.1016/j.ijpsycho.2011.06.022

McAteer, K.M., Corrigan, F., Thornton, E., Turner, R.J., Vink, R., 2016. Short and Long Term Behavioral and Pathological Changes in a Novel Rodent Model of Repetitive Mild Traumatic Brain Injury. PLOS ONE 11, e0160220. https://doi.org/10.1371/journal.pone.0160220

McCann, S.J., LeCouteur, D.G., Green, A.C., Brayne, C., Johnson, A.G., Chan, D., McManus, M.E., Pond, S.M., 1998. The epidemiology of Parkinson's disease in an Australian population. Neuroepidemiology 17, 310–317. https://doi.org/10.1159/000026185

McGeer, P.L., Itagaki, S., Boyes, B.E., McGeer, E.G., 1988. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38, 1285–1285. https://doi.org/10.1212/WNL.38.8.1285

McIntosh, T.K., Vink, R., Noble, L., Yamakami, I., Fernyak, S., Soares, H., Faden, A.L., 1989. Traumatic brain injury in the rat: Characterization of a lateral fluid-percussion model. Neuroscience 28, 233–244. https://doi.org/10.1016/0306-4522(89)90247-9

Mckee, A.C., Daneshvar, D.H., 2015. The neuropathology of traumatic brain injury, in: Handbook of Clinical Neurology. Elsevier, pp. 45–66. https://doi.org/10.1016/B978-0-444-52892-6.00004-0

McMillan, T.M., Teasdale, G.M., Stewart, E., 2012. Disability in young people and adults after head injury: 12–14 year follow-up of a prospective cohort. J. Neurol. Neurosurg. Psychiatry 83, 1086–1091. https://doi.org/10.1136/jnnp-2012-302746

Meeuws, S., Yue, J.K., Huijben, J.A., Nair, N., Lingsma, H.F., Bell, M.J., Manley, G.T., Maas, A.I.R., 2020. Common Data Elements: Critical Assessment of Harmonization between Current Multi-Center Traumatic Brain Injury Studies. J. Neurotrauma 37, 1283–1290. https://doi.org/10.1089/neu.2019.6867

Mendes, C.S., Bartos, I., Márka, Z., Akay, T., Márka, S., Mann, R.S., 2015. Quantification of gait parameters in freely walking rodents. BMC Biol. 13, 50. https://doi.org/10.1186/s12915-015-0154-0

Miguel, T.T., Gomes, K.S., Nunes-de-Souza, R.L., 2014. Tonic modulation of anxiety-like behavior by corticotropin-releasing factor (CRF) type 1 receptor (CRF1) within the medial prefrontal cortex (mPFC) in male mice: Role of protein kinase A (PKA). Horm. Behav. 66, 247–256. https://doi.org/10.1016/j.yhbeh.2014.05.003

Miyanishi, K., Choudhury, M.E., Watanabe, M., Kubo, M., Nomoto, M., Yano, H., Tanaka, J., 2019. Behavioral tests predicting striatal dopamine level in a rat hemi-Parkinson's disease model. Neurochem. Int. 122, 38–46. https://doi.org/10.1016/j.neuint.2018.11.005

Miyazaki, I., Isooka, N., Imafuku, F., Sun, J., Kikuoka, R., Furukawa, C., Asanuma, M., 2020. Chronic Systemic Exposure to Low-Dose Rotenone Induced Central and Peripheral Neuropathology and Motor Deficits in Mice: Reproducible Animal Model of Parkinson's Disease. Int. J. Mol. Sci. 21, 3254. https://doi.org/10.3390/ijms21093254

Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., Minami, M., Nagatsu, T., 1994a. Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients. Neurosci. Lett. 180, 147–150. https://doi.org/10.1016/0304-3940(94)90508-8

Mogi, M., Harada, M., Narabayashi, H., Inagaki, H., Minami, M., Nagatsu, T., 1996. Interleukin (IL)-1β, IL-2, IL-4, IL-6 and transforming growth factor-α levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. Neurosci. Lett. 211, 13–16. https://doi.org/10.1016/0304-3940(96)12706-3

Mogi, M., Harada, M., Riederer, P., Narabayashi, H., Fujita, K., Nagatsu, T., 1994b. Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci. Lett. 165, 208–210. https://doi.org/10.1016/0304-3940(94)90746-3

Molinoff, P.B., Axelrod, J., 1971. Biochemistry of Catecholamines. Annu. Rev. Biochem. 40, 465–500. https://doi.org/10.1146/annurev.bi.40.070171.002341

Mondello, S., Buki, A., Italiano, D., Jeromin, A., 2013. -Synuclein in CSF of patients with severe traumatic brain injury. Neurology 80, 1662–1668. https://doi.org/10.1212/WNL.0b013e3182904d43

Monje, M.H.G., Sánchez-Ferro, Á., Pineda-Pardo, J.A., Vela-Desojo, L., Alonso-Frech, F., Obeso, J.A., 2021. Motor Onset Topography and Progression in Parkinson's Disease: the Upper Limb Is First. Mov. Disord. 36, 905–915. https://doi.org/10.1002/mds.28462

Moon, D., 2022. Disorders of Movement due to Acquired and Traumatic Brain Injury. Curr. Phys. Med. Rehabil. Rep. 10, 311–323. https://doi.org/10.1007/s40141-022-00368-1

Morriss, N.J., Conley, G.M., Hodgson, N., Boucher, M., Ospina-Mora, S., Fagiolini, M., Puder, M., Mejia, L., Qiu, J., Meehan, W., Mannix, R., 2021. Visual Dysfunction after Repetitive Mild Traumatic Brain Injury in a Mouse Model and Ramifications on Behavioral Metrics. J. Neurotrauma 38, 2881–2895. https://doi.org/10.1089/neu.2021.0165

Mountney, A., Boutté, A.M., Cartagena, C.M., Flerlage, W.F., Johnson, W.D., Rho, C., Lu, X.-C., Yarnell, A., Marcsisin, S., Sousa, J., Vuong, C., Zottig, V., Leung, L.-Y., Deng-Bryant, Y., Gilsdorf, J., Tortella, F.C., Shear, D.A., 2017. Functional and Molecular Correlates after Single and Repeated Rat Closed-Head Concussion: Indices of Vulnerability after Brain Injury. J. Neurotrauma 34, 2768–2789. https://doi.org/10.1089/neu.2016.4679

Mouzon, B.C., Bachmeier, C., Ferro, A., Ojo, J.-O., Crynen, G., Acker, C.M., Davies, P., Mullan, M., Stewart, W., Crawford, F., 2014. Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model: Chronic Effects of r-mTBI. Ann. Neurol. 75, 241–254. https://doi.org/10.1002/ana.24064

Mouzon, B.C., Bachmeier, C., Ojo, J.O., Acker, C.M., Ferguson, S., Paris, D., Ait-Ghezala, G., Crynen, G., Davies, P., Mullan, M., Stewart, W., Crawford, F., 2018. Lifelong behavioral and neuropathological consequences of repetitive mild traumatic brain injury. Ann. Clin. Transl. Neurol. 5, 64–80. https://doi.org/10.1002/acn3.510

Muslimovic, D., Post, B., Speelman, J.D., Schmand, B., 2005. Cognitive profile of patients with newly diagnosed Parkinson disease. Neurology 65, 1239–1245. https://doi.org/10.1212/01.wnl.0000180516.69442.95

Myer, D.J., 2006. Essential protective roles of reactive astrocytes in traumatic brain injury. Brain 129, 2761–2772. https://doi.org/10.1093/brain/awl165

Nagamoto-Combs, K., McNeal, D.W., Morecraft, R.J., Combs, C.K., 2007. Prolonged Microgliosis in the Rhesus Monkey Central Nervous System after Traumatic Brain Injury. J. Neurotrauma 24, 1719–1742. https://doi.org/10.1089/neu.2007.0377

Namdar, I., Feldman, R., Glazer, S., Meningher, I., Shlobin, N.A., Rubovitch, V., Bikovski, L., Been, E., Pick, C.G., 2020. Motor Effects of Minimal Traumatic Brain Injury in Mice. J. Mol. Neurosci. 70, 365–377. https://doi.org/10.1007/s12031-019-01422-9

Nandhagopal, R., Kuramoto, L., Schulzer, M., Mak, E., Cragg, J., Lee, C.S., McKenzie, J., McCormick, S., Samii, A., Troiano, A., Ruth, T.J., Sossi, V., De La Fuente-Fernandez, R., Calne, D.B., Stoessl, A.J., 2009. Longitudinal progression of sporadic Parkinson's disease: a multi-tracer positron emission tomography study. Brain 132, 2970–2979. https://doi.org/10.1093/brain/awp209

Nelson, L.D., Temkin, N.R., Dikmen, S., Barber, J., Giacino, J.T., Yuh, E., Levin, H.S., McCrea, M.A., Stein, M.B., Mukherjee, P., Okonkwo, D.O., Robertson, C.S., Diaz-Arrastia, R., Manley, G.T., Adeoye, O., Badjatia, N., Boase, K., Bodien, Y., Bullock, M.R., Chesnut, R., Corrigan, J.D., Crawford, K., Duhaime, A.-C., Ellenbogen, R., Feeser, V.R., Ferguson, A., Foreman, B., Gardner, R., Gaudette, E., Gonzalez, L., Gopinath, S., Gullapalli, R., Hemphill, J.C., Hotz, G., Jain, S., Korley, F., Kramer, J., Kreitzer, N., Lindsell, C., Machamer, J., Madden, C., Martin, A., McAllister, T., Merchant, R., Noel, F., Palacios, E., Perl, D., Puccio, A., Rabinowitz, M., Rosand, J., Sander, A., Satris, G., Schnyer, D., Seabury, S., Sherer, M., Taylor, S., Toga, A., Valadka, A., Vassar, M.J., Vespa, P., Wang, K., Yue, J.K., Zafonte, R., 2019. Recovery After Mild Traumatic Brain Injury in Patients Presenting to US Level I Trauma Centers: A Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Study. JAMA Neurol. 76, 1049–1059. https://doi.org/10.1001/jamaneurol.2019.1313

Ng, K., Mikulis, D.J., Glazer, J., Kabani, N., Till, C., Greenberg, G., Thompson, A., Lazinski, D., Agid, R., Colella, B., Green, R.E., 2008. Magnetic Resonance Imaging Evidence of Progression of Subacute Brain Atrophy in Moderate to Severe Traumatic Brain Injury. Arch. Phys. Med. Rehabil. 89, S35–S44. https://doi.org/10.1016/j.apmr.2008.07.006

Nicholl, J., LaFrance, W., 2009. Neuropsychiatric Sequelae of Traumatic Brain Injury. Semin. Neurol. 29, 247–255. https://doi.org/10.1055/s-0029-1223878

Nicolson, G.L., 2014. Mitochondrial Dysfunction and Chronic Disease: Treatment With Natural Supplements. Integr. Med. Encinitas Calif 13, 35–43.

Nissinen, J., Andrade, P., Natunen, T., Hiltunen, M., Malm, T., Kanninen, K., Soares, J.I., Shatillo, O., Sallinen, J., Ndode-Ekane, X.E., Pitkänen, A., 2017. Disease-modifying effect of atipamezole in a model of post-traumatic epilepsy. Epilepsy Res. 136, 18–34. https://doi.org/10.1016/j.eplepsyres.2017.07.005

Nolan, A., Hennessy, E., Krukowski, K., Guglielmetti, C., Chaumeil, M.M., Sohal, V.S., Rosi, S., 2018. Repeated Mild Head Injury Leads to Wide-Ranging Deficits in Higher-Order Cognitive Functions Associated with the Prefrontal Cortex. J. Neurotrauma 35, 2425–2434. https://doi.org/10.1089/neu.2018.5731

Norazit, A., Meedeniya, A.C.B., Nguyen, M.N., Mackay-Sim, A., 2010. Progressive loss of dopaminergic neurons induced by unilateral rotenone infusion into the medial forebrain bundle. Brain Res. 1360, 119–129. https://doi.org/10.1016/j.brainres.2010.08.070

Norden, D.M., Godbout, J.P., 2013. Review: microglia of the aged brain: primed to be activated and resistant to regulation. Neuropathol. Appl. Neurobiol. 39, 19–34. https://doi.org/10.1111/j.1365-2990.2012.01306.x

Norris, K.L., Hao, R., Chen, L.-F., Lai, C.-H., Kapur, M., Shaughnessy, P.J., Chou, D., Yan, J., Taylor, J.P., Engelender, S., West, A.E., Lim, K.-L., Yao, T.-P., 2015. Convergence of Parkin, PINK1, and α-Synuclein on Stress-induced Mitochondrial Morphological Remodeling. J. Biol. Chem. 290, 13862–13874. https://doi.org/10.1074/jbc.M114.634063

Noyce, A.J., Bestwick, J.P., Silveira-Moriyama, L., Hawkes, C.H., Giovannoni, G., Lees, A.J., Schrag, A., 2012. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann. Neurol. 72, 893–901. https://doi.org/10.1002/ana.23687

Oertel, W.H., Henrich, M.T., Janzen, A., Geibl, F.F., 2019. The locus coeruleus: Another vulnerability target in Parkinson's disease. Mov. Disord. 34, 1423–1429. https://doi.org/10.1002/mds.27785

Ohtsuka, C., Sasaki, M., Konno, K., Koide, M., Kato, K., Takahashi, J., Takahashi, S., Kudo, K., Yamashita, F., Terayama, Y., 2013. Changes in substantia nigra and locus coeruleus in patients with early-stage Parkinson's disease using neuromelanin-sensitive MR imaging. Neurosci. Lett. 541, 93–98. https://doi.org/10.1016/j.neulet.2013.02.012

Ojha, S., Javed, H., Azimullah, S., Abul Khair, S.B., Haque, M.E., 2016. Glycyrrhizic acid Attenuates Neuroinflammation and Oxidative Stress in Rotenone Model of Parkinson's Disease. Neurotox. Res. 29, 275–287. https://doi.org/10.1007/s12640-015-9579-z

Oliveira, L.M., Tuppy, M., Moreira, T.S., Takakura, A.C., 2017. Role of the locus coeruleus catecholaminergic neurons in the chemosensory control of breathing in a Parkinson's disease model. Exp. Neurol. 293, 172–180. https://doi.org/10.1016/j.expneurol.2017.04.006

Olsson, M., Nikkhah, G., Bentlage, C., Björklund, A., 1995. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J. Neurosci. Off. J. Soc. Neurosci. 15, 3863–3875. https://doi.org/10.1523/JNEUROSCI.15-05-03863.1995

Osier, N., Dixon, C.E., 2016. The Controlled Cortical Impact Model of Experimental Brain Trauma: Overview, Research Applications, and Protocol. Methods Mol. Biol. Clifton NJ 1462, 177–192. https://doi.org/10.1007/978-1-4939-3816-2_11

Otsuka, M., Ichiya, Y., Kuwabara, Y., Hosokawa, S., Sasaki, M., Yoshida, T., Fukumura, T., Masuda, K., Kato, M., 1996. Differences in the reduced 18F-Dopa uptakes of the caudate and the putamen in Parkinson's disease: correlations with the three main symptoms. J. Neurol. Sci. 136, 169–173. https://doi.org/10.1016/0022-510X(95)00316-T

Ouchi, Y., Yoshikawa, E., Sekine, Y., Futatsubashi, M., Kanno, T., Ogusu, T., Torizuka, T., 2005. Microglial activation and dopamine terminal loss in early Parkinson's disease. Ann. Neurol. 57, 168–175. https://doi.org/10.1002/ana.20338

Özen, I., Mai, H., De Maio, A., Ruscher, K., Michalettos, G., Clausen, F., Gottschalk, M., Ansar, S., Arkan, S., Erturk, A., Marklund, N., 2022. Purkinje cell vulnerability induced by diffuse traumatic brain injury is linked to disruption of long-range neuronal circuits. Acta Neuropathol. Commun. 10, 129. https://doi.org/10.1186/s40478-022-01435-3

Padmakumar, S., Kulkarni, P., Ferris, C.F., Bleier, B.S., Amiji, M.M., 2022. Traumatic brain injury and the development of parkinsonism: Understanding pathophysiology, animal models, and therapeutic targets. Biomed. Pharmacother. 149, 112812. https://doi.org/10.1016/j.biopha.2022.112812

Pan, T., Liao, K., Roenigk, K., Daly, J.J., Walker, M.F., 2015. Static and dynamic postural stability in veterans with combat-related mild traumatic brain injury. Gait Posture 42, 550–557. https://doi.org/10.1016/j.gaitpost.2015.08.012

Pang, S.Y.-Y., Ho, P.W.-L., Liu, H.-F., Leung, C.-T., Li, L., Chang, E.E.S., Ramsden, D.B., Ho, S.-L., 2019. The interplay of aging, genetics and environmental factors in the pathogenesis of Parkinson's disease. Transl. Neurodegener. 8, 23. https://doi.org/10.1186/s40035-019-0165-9

Pan-Montojo, F., Anichtchik, O., Dening, Y., Knels, L., Pursche, S., Jung, R., Jackson, S., Gille, G., Spillantini, M.G., Reichmann, H., Funk, R.H.W., 2010. Progression of Parkinson's Disease Pathology Is Reproduced by Intragastric Administration of Rotenone in Mice. PLoS ONE 5, e8762. https://doi.org/10.1371/journal.pone.0008762

Paradiso, S., Chemerinski, E., Yazici, K.M., Tartaro, A., Robinson, R.G., 1999. Frontal lobe syndrome reassessed: comparison of patients with lateral or medial frontal brain damage. J. Neurol. Neurosurg. Psychiatry 67, 664–667. https://doi.org/10.1136/jnnp.67.5.664

Parkinson, J., 2002. An Essay on the Shaking Palsy. J. Neuropsychiatry Clin. Neurosci. 14, 223–236. https://doi.org/10.1176/jnp.14.2.223

Paterno, R., Folweiler, K.A., Cohen, A.S., 2017. Pathophysiology and Treatment of Memory Dysfunction After Traumatic Brain Injury. Curr. Neurol. Neurosci. Rep. 17, 52. https://doi.org/10.1007/s11910-017-0762-x

Patil, M., Gupta, A., Khanna, M., Taly, A.B., Soni, A., Kumar, J.K., Thennarasu, K., 2017. Cognitive and Functional Outcomes following Inpatient Rehabilitation in Patients with Acquired Brain Injury: A Prospective Follow-up Study. J. Neurosci. Rural Pract. 8, 357–363. https://doi.org/10.4103/jnrp.jnrp_53_17

Patrick, K.L., Bell, S.L., Weindel, C.G., Watson, R.O., 2019. Exploring the "Multiple-Hit Hypothesis" of Neurodegenerative Disease: Bacterial Infection Comes Up to Bat. Front. Cell. Infect. Microbiol. 9, 138. https://doi.org/10.3389/fcimb.2019.00138

Pausch, C., Schomburg, R., Wagenpfeil, S., Wollenweber, F.A., Bayer, C., Fassbender, K., Behnke, S., 2016. Neuropsychological impairment in prodromal Parkinson's disease. J. Neurol. Sci. 371, 117–120. https://doi.org/10.1016/j.jns.2016.10.007

Paxinos, G., Watson, C., 2006. The rat brain in stereotaxic coordinates: hard cover edition. Elsevier.

Pearce, A.J., Hoy, K., Rogers, M.A., Corp, D.T., Maller, J.J., Drury, H.G.K., Fitzgerald, P.B., 2014. The Long-Term Effects of Sports Concussion on Retired Australian Football Players: A Study Using Transcranial Magnetic Stimulation. J. Neurotrauma 31, 1139–1145. https://doi.org/10.1089/neu.2013.3219

Pearce, A.J., Rist, B., Fraser, C.L., Cohen, A., Maller, J.J., 2018. Neurophysiological and cognitive impairment following repeated sports concussion injuries in retired professional rugby league players. Brain Inj. 32, 498–505. https://doi.org/10.1080/02699052.2018.1430376

Pearce, R.K.B., Owen, A., Daniel, S., Jenner, P., Marsden, C.D., 1997. Alterations in the distribution of glutathione in the substantia nigra in Parkinson's disease. J. Neural Transm. 104, 661–677. https://doi.org/10.1007/BF01291884

Pierce, J.E.S., Smith, D.H., Trojanowski, J.Q., McIntosh, T.K., 1998. Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. Neuroscience 87, 359–369. https://doi.org/10.1016/S0306-4522(98)00142-0

Pitt, W., Chou, L.-S., 2020. A case-control study of gait balance control in veterans with chronic symptoms following mTBI. Gait Posture 76, 188–192. https://doi.org/10.1016/j.gaitpost.2019.12.010

Ponsen, M.M., Stoffers, D., Booij, J., Van Eck-Smit, B.L.F., Wolters, E.Ch., Berendse, H.W., 2004. Idiopathic hyposmia as a preclinical sign of Parkinson's disease. Ann. Neurol. 56, 173–181. https://doi.org/10.1002/ana.20160

Ponsford, J.L., Olver, J.H., Curran, C., 1995. A profile of outcome: 2 years after traumatic brain injury. Brain Inj. 9, 1–10. https://doi.org/10.3109/02699059509004565

Postuma, R.B., 2014. Prodromal Parkinson's disease – Using REM sleep behavior disorder as a window. Parkinsonism Relat. Disord. 20, S1–S4. https://doi.org/10.1016/S1353-8020(13)00400-8

Postuma, R.B., Berg, D., 2019. Prodromal Parkinson's Disease: The Decade Past, the Decade to Come. Mov. Disord. 34, 665–675. https://doi.org/10.1002/mds.27670

Postuma, R.B., Lang, A.E., Gagnon, J.F., Pelletier, A., Montplaisir, J.Y., 2012. How does parkinsonism start? Prodromal parkinsonism motor changes in idiopathic REM sleep behaviour disorder. Brain 135, 1860–1870. https://doi.org/10.1093/brain/aws093

Pouchieu, C., Piel, C., Carles, C., Gruber, A., Helmer, C., Tual, S., Marcotullio, E., Lebailly, P., Baldi, I., 2018. Pesticide use in agriculture and Parkinson's disease in the AGRICAN cohort study. Int. J. Epidemiol. 47, 299–310. https://doi.org/10.1093/ije/dyx225

Poudel, G.R., Dominguez D, J.F., Verhelst, H., Vander Linden, C., Deblaere, K., Jones, D.K., Cerin, E., Vingerhoets, G., Caeyenberghs, K., 2020. Network diffusion modeling predicts neurodegeneration in traumatic brain injury. Ann. Clin. Transl. Neurol. 7, 270–279. https://doi.org/10.1002/acn3.50984

Prins, M., Greco, T., Alexander, D., Giza, C.C., 2013. The pathophysiology of traumatic brain injury at a glance. Dis. Model. Mech. 6, 1307–1315. https://doi.org/10.1242/dmm.011585

Pruitt, D., Hays, S., Schmid, A., Choua, C., Kim, L., Trieu, J., Kilgard, M.P., Rennaker, R.L., 2014. Controlled-cortical impact reduces volitional forelimb strength in rats. Brain Res. 1582, 91–98. https://doi.org/10.1016/j.brainres.2014.07.039

Pruitt, D.T., Danaphongse, T.T., Schmid, A.N., Morrison, R.A., Kilgard, M.P., Rennaker, R.L., Hays, S.A., 2017. Traumatic Brain Injury Occludes Training-Dependent Cortical Reorganization in the Contralesional Hemisphere. J. Neurotrauma 34, 2495–2503. https://doi.org/10.1089/neu.2016.4796

Pupillo, E., Bianchi, E., Vanacore, N., Montalto, C., Ricca, G., Robustelli Della Cuna, F.S., Fumagalli, F., Castellani, M., Poli, F., Romeo, F., Tommasi, D., Lazzaro, P., Beghi, E., 2020. Increased risk and early onset of ALS in professional players from Italian Soccer Teams.

Amyotroph. Lateral Scler. Front. Degener. 21, 403–409. https://doi.org/10.1080/21678421.2020.1752250

Qiu, J., Peng, G., Tang, Y., Li, S., Liu, Z., Zheng, J., Wang, Y., Liu, H., Wei, L., Su, Y., Lin, Y., Dai, W., Zhang, Z., Chen, X., Ding, L., Guo, W., Zhu, X., Xu, P., Mo, M., 2023. Lipid profiles in the cerebrospinal fluid of rats with 6-hydroxydopamine-induced lesions as a model of Parkinson's disease. Front. Aging Neurosci. 14, 1077738. https://doi.org/10.3389/fnagi.2022.1077738

Radad, K., Al-Shraim, M., Al-Emam, A., Wang, F., Kranner, B., Rausch, W.-D., Moldzio, R., 2019. Rotenone: from modelling to implication in Parkinson's disease. Folia Neuropathol. 57, 317–326. https://doi.org/10.5114/fn.2019.89857

Radad, K., Rausch, W.-D., Gille, G., 2006. Rotenone induces cell death in primary dopaminergic culture by increasing ROS production and inhibiting mitochondrial respiration. Neurochem. Int. 49, 379–386. https://doi.org/10.1016/j.neuint.2006.02.003

Rämä, P., Linnankoski, I., Tanila, H., Pertovaara, A., Carlson, S., 1996. Medetomidine, atipamezole, and guanfacine in delayed response performance of aged monkeys. Pharmacol. Biochem. Behav. 55, 415–422. https://doi.org/10.1016/s0091-3057(96)00111-6

Ramlackhansingh, A.F., Brooks, D.J., Greenwood, R.J., Bose, S.K., Turkheimer, F.E., Kinnunen, K.M., Gentleman, S., Heckemann, R.A., Gunanayagam, K., Gelosa, G., Sharp, D.J., 2011. Inflammation after trauma: Microglial activation and traumatic brain injury. Ann. Neurol. 70, 374–383. https://doi.org/10.1002/ana.22455

Rana, A., Singh, S., Deshmukh, R., Kumar, A., 2020. Pharmacological potential of tocopherol and doxycycline against traumatic brain injury-induced cognitive/motor impairment in rats. Brain Inj. 34, 1039–1050. https://doi.org/10.1080/02699052.2020.1772508

Rasman, B.G., Forbes, P.A., Tisserand, R., Blouin, J.-S., 2018. Sensorimotor Manipulations of the Balance Control Loop-Beyond Imposed External Perturbations. Front. Neurol. 9, 899. https://doi.org/10.3389/fneur.2018.00899

Rastoldo, G., Marouane, E., El Mahmoudi, N., Péricat, D., Bourdet, A., Timon-David, E., Dumas, O., Chabbert, C., Tighilet, B., 2020. Quantitative Evaluation of a New Posturo-Locomotor Phenotype in a Rodent Model of Acute Unilateral Vestibulopathy. Front. Neurol. 11, 505. https://doi.org/10.3389/fneur.2020.00505

Raymont, V., Salazar, A.M., Lipsky, R., Goldman, D., Tasick, G., Grafman, J., 2010. Correlates of posttraumatic epilepsy 35 years following combat brain injury. Neurology 75, 224–229. https://doi.org/10.1212/WNL.0b013e3181e8e6d0

Recasens, A., Dehay, B., Bové, J., Carballo-Carbajal, I., Dovero, S., Pérez-Villalba, A., Fernagut, P.-O., Blesa, J., Parent, A., Perier, C., Fariñas, I., Obeso, J.A., Bezard, E., Vila, M., 2014. Lewy body extracts from Parkinson disease brains trigger α-synuclein pathology and

neurodegeneration in mice and monkeys. Ann. Neurol. 75, 351–362. https://doi.org/10.1002/ana.24066

Redell, J.B., Dash, P.K., 2007. Traumatic brain injury stimulates hippocampal catechol-O-methyl transferase expression in microglia. Neurosci. Lett. 413, 36–41. https://doi.org/10.1016/j.neulet.2006.11.060

Reilly, N., Prebor, J., Moxey, J., Schussler, E., 2020. Chronic impairments of static postural stability associated with history of concussion. Exp. Brain Res. 238, 2783–2793. https://doi.org/10.1007/s00221-020-05934-4

Ren, Y., Feng, J., 2007. Rotenone selectively kills serotonergic neurons through a microtubule-dependent mechanism. J. Neurochem. 103, 303–311. https://doi.org/10.1111/j.1471-4159.2007.04741.x

Reynolds, A.D., Glanzer, J.G., Kadiu, I., Ricardo-Dukelow, M., Chaudhuri, A., Ciborowski, P., Cerny, R., Gelman, B., Thomas, M.P., Mosley, R.L., Gendelman, H.E., 2008. Nitrated alpha-synuclein-activated microglial profiling for Parkinson's disease. J. Neurochem. 104, 1504–1525. https://doi.org/10.1111/j.1471-4159.2007.05087.x

Richardson, J.R., Quan, Y., Sherer, T.B., Greenamyre, J.T., Miller, G.W., 2005. Paraquat Neurotoxicity is Distinct from that of MPTP and Rotenone. Toxicol. Sci. 88, 193–201. https://doi.org/10.1093/toxsci/kfi304

Riederer, P., Sofic, E., Rausch, W., Schmidt, B., Reynolds, G.P., Jellinger, K., Youdim, M.B.H., 1989. Transition Metals, Ferritin, Glutathione, and Ascorbic Acid in Parkinsonian Brains. J. Neurochem. 52, 515–520. https://doi.org/10.1111/j.1471-4159.1989.tb09150.x

Rinne, J.O., Ruottinen, H., Bergman, J., Haaparanta, M., Sonninen, P., Solin, O., 1999. Usefulness of a dopamine transporter PET ligand [18F]beta -CFT in assessing disability in Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 67, 737–741. https://doi.org/10.1136/jnnp.67.6.737

Ritzel, R.M., Doran, S.J., Glaser, E.P., Meadows, V.E., Faden, A.I., Stoica, B.A., Loane, D.J., 2019. Old age increases microglial senescence, exacerbates secondary neuroinflammation, and worsens neurological outcomes after acute traumatic brain injury in mice. Neurobiol. Aging 77, 194–206. https://doi.org/10.1016/j.neurobiolaging.2019.02.010

Ritzel, R.M., Li, Y., He, J., Khan, N., Doran, S.J., Faden, A.I., Wu, J., 2020. Sustained neuronal and microglial alterations are associated with diverse neurobehavioral dysfunction long after experimental brain injury. Neurobiol. Dis. 136, 104713. https://doi.org/10.1016/j.nbd.2019.104713

Ritzel, R.M., Li, Y., Lei, Z., Carter, J., He, J., Choi, H.M.C., Khan, N., Li, H., Allen, S., Lipinski, M.M., Faden, A.I., Wu, J., 2022. Functional and transcriptional profiling of microglial activation during the chronic phase of TBI identifies an age-related driver of poor

outcome in old mice. GeroScience 44, 1407–1440. https://doi.org/10.1007/s11357-022-00562-y

Robertson, D., Goldberg, M.R., Onrot, J., Hollister, A.S., Wiley, R., Thompson, J.G., Robertson, R.M., 1986. Isolated Failure of Autonomic Noradrenergic Neurotransmission. N. Engl. J. Med. 314, 1494–1497. https://doi.org/10.1056/NEJM198606053142307

Rocha, S.M., Bantle, C.M., Aboellail, T., Chatterjee, D., Smeyne, R.J., Tjalkens, R.B., 2022. Rotenone induces regionally distinct α-synuclein protein aggregation and activation of glia prior to loss of dopaminergic neurons in C57Bl/6 mice. Neurobiol. Dis. 167, 105685. https://doi.org/10.1016/j.nbd.2022.105685

Rojo, A.I., Cavada, C., De Sagarra, M.R., Cuadrado, A., 2007. Chronic inhalation of rotenone or paraquat does not induce Parkinson's disease symptoms in mice or rats. Exp. Neurol. 208, 120–126. https://doi.org/10.1016/j.expneurol.2007.07.022

Rosenblum, D.J., Walton, S.R., Erdman, N.K., Broshek, D.K., Hart, J.M., Resch, J.E., 2020. If not now, when? An absence of neurocognitive and postural stability deficits in collegiate athletes with one or more concussions. J. Neurotrauma 37, 1211–1220.

Rosenfeld, C.S., Ferguson, S.A., 2014. Barnes Maze Testing Strategies with Small and Large Rodent Models. J. Vis. Exp. 51194. https://doi.org/10.3791/51194

Rowe, R.K., Ziebell, J.M., Harrison, J.L., Law, L.M., Adelson, P.D., Lifshitz, J., 2016. Aging with Traumatic Brain Injury: Effects of Age at Injury on Behavioral Outcome following Diffuse Brain Injury in Rats. Dev. Neurosci. 38, 195–205. https://doi.org/10.1159/000446773

Ruan, Z., Zhang, D., Huang, R., Sun, W., Hou, L., Zhao, J., Wang, Q., 2022. Microglial Activation Damages Dopaminergic Neurons through MMP-2/-9-Mediated Increase of Blood-Brain Barrier Permeability in a Parkinson's Disease Mouse Model. Int. J. Mol. Sci. 23, 2793. https://doi.org/10.3390/ijms23052793

Ruet, A., Bayen, E., Jourdan, C., Ghout, I., Meaude, L., Lalanne, A., Pradat-Diehl, P., Nelson, G., Charanton, J., Aegerter, P., Vallat-Azouvi, C., Azouvi, P., 2019. A Detailed Overview of Long-Term Outcomes in Severe Traumatic Brain Injury Eight Years Post-injury. Front. Neurol. 10, 120. https://doi.org/10.3389/fneur.2019.00120

Rusiecki, J., Levin, L.I., Wang, L., Byrne, C., Krishnamurthy, J., Chen, L., Galdzicki, Z., French, L.M., 2020. Blast traumatic brain injury and serum inflammatory cytokines: a repeated measures case-control study among U.S. military service members. J. Neuroinflammation 17, 20. https://doi.org/10.1186/s12974-019-1624-z

Russell, A., Drozdova, A., Wang, W., Thomas, M., 2014. The Impact of Dementia Development Concurrent with Parkinson's Disease: A New Perspective. CNS Neurol. Disord. - Drug Targets 13, 1160–1168. https://doi.org/10.2174/1871527313666140917122739

Russell, E.R., Mackay, D.F., Lyall, D., Stewart, K., MacLean, J.A., Robson, J., Pell, J.P., Stewart, W., 2022. Neurodegenerative disease risk among former international rugby union players. J. Neurol. Neurosurg. Psychiatry 93, 1262–1268. https://doi.org/10.1136/jnnp-2022-329675

Sabbagh, J.J., Fontaine, S.N., Shelton, L.B., Blair, L.J., Hunt, J.B., Zhang, B., Gutmann, J.M., Lee, D.C., Lloyd, J.D., Dickey, C.A., 2016. Noncontact Rotational Head Injury Produces Transient Cognitive Deficits but Lasting Neuropathological Changes. J. Neurotrauma 33, 1751–1760. https://doi.org/10.1089/neu.2015.4288

Saggu, H., Cooksey, J., Dexter, D., Wells, F.R., Lees, A., Jenner, P., Marsden, C.D., 1989. A Selective Increase in Particulate Superoxide Dismutase Activity in Parkinsonian Substantia Nigra. J. Neurochem. 53, 692–697. https://doi.org/10.1111/j.1471-4159.1989.tb11759.x

Samet, J.M., Wages, P.A., 2018. Oxidative Stress from Environmental Exposures. Curr. Opin. Toxicol. 7, 60–66.

Sampaio, T.B., Marques, N.F., Binder, L.B., Tasca, C.I., Prediger, R.D., 2020. Role of Prefrontal Cortex on Recognition Memory Deficits in Rats following 6-OHDA-Induced Locus Coeruleus Lesion. Oxid. Med. Cell. Longev. 2020, 8324565. https://doi.org/10.1155/2020/8324565

Sandhaug, M., Andelic, N., Langhammer, B., Mygland, A., 2015. Functional level during the first 2 years after moderate and severe traumatic brain injury. Brain Inj. 29, 1431–1438. https://doi.org/10.3109/02699052.2015.1063692

Sanyal, J., Bandyopadhyay, S.K., Banerjee, T.K., Mukherjee, S.C., Chakraborty, D.P., Ray, B.C., Rao, V.R., 2009. Plasma levels of lipid peroxides in patients with Parkinson's disease. Eur. Rev. Med. Pharmacol. Sci. 13, 129–132.

Sarajuuri, J., Pasanen, M., Rinne, M., Vartiainen, M., Lehto, T., Alaranta, H., 2013. Relationship between cognitive and motor performance in physically well-recovered men with traumatic brain injury. J. Rehabil. Med. 45, 38–46. https://doi.org/10.2340/16501977-1060

Scalzo, P., Kümmer, A., Cardoso, F., Teixeira, A.L., 2009. Increased serum levels of soluble tumor necrosis factor-α receptor-1 in patients with Parkinson's disease. J. Neuroimmunol. 216, 122–125. https://doi.org/10.1016/j.jneuroim.2009.08.001

Scherman, D., Desnos, C., Darchen, F., Pollak, P., Javoy-Agid, F., Agid, Y., 1989. Striatal dopamine deficiency in parkinson's disease: Role of aging. Ann. Neurol. 26, 551–557. https://doi.org/10.1002/ana.410260409

Schönfeld, L.-M., Jahanshahi, A., Lemmens, E., Schipper, S., Dooley, D., Joosten, E., Temel, Y., Hendrix, S., 2017. Long-Term Motor Deficits after Controlled Cortical Impact in Rats

Can Be Detected by Fine Motor Skill Tests but Not by Automated Gait Analysis. J. Neurotrauma 34, 505–516. https://doi.org/10.1089/neu.2016.4440

Schwartz, M., Groshar, D., Inzelberg, R., Hocherman, S., 2004. Dopamine-transporter imaging and visuo-motor testing in essential tremor, practical possibilities for detection of early stage Parkinson's disease. Parkinsonism Relat. Disord. 10, 385–389. https://doi.org/10.1016/j.parkreldis.2004.03.004

Schwerin, S.C., Hutchinson, E.B., Radomski, K.L., Ngalula, K.P., Pierpaoli, C.M., Juliano, S.L., 2017. Establishing the ferret as a gyrencephalic animal model of traumatic brain injury: Optimization of controlled cortical impact procedures. J. Neurosci. Methods 285, 82–96. https://doi.org/10.1016/j.jneumeth.2017.05.010

Scudamore, O., Ciossek, T., 2018. Increased Oxidative Stress Exacerbates α-Synuclein Aggregation In Vivo. J. Neuropathol. Exp. Neurol. 77, 443–453. https://doi.org/10.1093/jnen/nly024

Segovia-Oropeza, M., Santiago-Castañeda, C., Orozco-Suárez, S.A., Concha, L., Rocha, L., n.d. Sodium Cromoglycate Decreases Sensorimotor Impairment and Hippocampal Alterations Induced by Severe Traumatic Brain Injury in Rats 9.

Seidler, A., Hellenbrand, W., Robra, B.P., Vieregge, P., Nischan, P., Joerg, J., Oertel, W.H., Ulm, G., Schneider, E., 1996. Possible environmental, occupational, and other etiologic factors for Parkinson's disease: a case-control study in Germany. Neurology 46, 1275–1284. https://doi.org/10.1212/wnl.46.5.1275

Seidler, R.D., Bernard, J.A., Burutolu, T.B., Fling, B.W., Gordon, M.T., Gwin, J.T., Kwak, Y., Lipps, D.B., 2010. Motor control and aging: links to age-related brain structural, functional, and biochemical effects. Neurosci. Biobehav. Rev. 34, 721–733. https://doi.org/10.1016/j.neubiorev.2009.10.005

Sell, S.L., Johnson, K., DeWitt, D.S., Prough, D.S., 2017. Persistent Behavioral Deficits in Rats after Parasagittal Fluid Percussion Injury. J. Neurotrauma 34, 1086–1096. https://doi.org/10.1089/neu.2016.4616

S.f, C., M, S., S.r, S., R, R., 2020. Inhibitory neuronal changes following a mixed diffuse-focal model of traumatic brain injury. J. Comp. Neurol. 528.

Shahnawaz, M., Mukherjee, A., Pritzkow, S., Mendez, N., Rabadia, P., Liu, X., Hu, B., Schmeichel, A., Singer, W., Wu, G., Tsai, A.-L., Shirani, H., Nilsson, K.P.R., Low, P.A., Soto, C., 2020. Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy. Nature 578, 273–277. https://doi.org/10.1038/s41586-020-1984-7

Shakya, S., Prevett, J., Hu, X., Xiao, R., 2022. Characterization of Parkinson's Disease Subtypes and Related Attributes. Front. Neurol. 13, 810038. https://doi.org/10.3389/fneur.2022.810038

Shao, W., Zhang, S., Tang, M., Zhang, X., Zhou, Z., Yin, Y., Zhou, Q., Huang, Y., Liu, Y., Wawrousek, E., Chen, T., Li, S., Xu, M., Zhou, Jiang-ning, Hu, G., Zhou, Jia-wei, 2013. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin. Nature 494, 90–94. https://doi.org/10.1038/nature11748

Sharma, M., Kaur, J., Rakshe, S., Sharma, N., Khunt, D., Khairnar, A., 2022. Intranasal Exposure to Low-Dose Rotenone Induced Alpha-Synuclein Accumulation and Parkinson's Like Symptoms Without Loss of Dopaminergic Neurons. Neurotox. Res. 40, 215–229. https://doi.org/10.1007/s12640-021-00436-9

Sharma, M., Sharma, N., Khairnar, A., 2023. Intranasal Rotenone Induces Alpha-Synuclein Accumulation, Neuroinflammation and Dopaminergic Neurodegeneration in Middle-Aged Mice. Neurochem. Res. 48, 1543–1560. https://doi.org/10.1007/s11064-022-03847-y

Shear, D.A., Lu, X.-C.M., Bombard, M.C., Pedersen, R., Chen, Z., Davis, A., Tortella, F.C., 2010. Longitudinal characterization of motor and cognitive deficits in a model of penetrating ballistic-like brain injury. J. Neurotrauma 27, 1911–1923. https://doi.org/10.1089/neu.2010.1399

Sherer, M., Struchen, M.A., Yablon, S.A., Wang, Y., Nick, T.G., 2007. Comparison of indices of traumatic brain injury severity: Glasgow Coma Scale, length of coma and post-traumatic amnesia. J. Neurol. Neurosurg. Psychiatry 79, 678–685. https://doi.org/10.1136/jnnp.2006.111187

Sherer, T.B., Betarbet, R., Testa, C.M., Seo, B.B., Richardson, J.R., Kim, J.H., Miller, G.W., Yagi, T., Matsuno-Yagi, A., Greenamyre, J.T., 2003a. Mechanism of toxicity in rotenone models of Parkinson's disease. J. Neurosci. Off. J. Soc. Neurosci. 23, 10756–10764. https://doi.org/10.1523/JNEUROSCI.23-34-10756.2003

Sherer, T.B., Kim, J.-H., Betarbet, R., Greenamyre, J.T., 2003b. Subcutaneous Rotenone Exposure Causes Highly Selective Dopaminergic Degeneration and α-Synuclein Aggregation. Exp. Neurol. 179, 9–16. https://doi.org/10.1006/exnr.2002.8072

Shibuya N., Tachibana H., Kawabata K., Sugita M., 2001. Memory Function in Patients with Parkinson's Disease: In Relation to Neuropsychological Tests and Cerebral Blood Flow. Nippon Ronen Igakkai Zasshi 38, 193–200. https://doi.org/10.3143/geriatrics.38.193

Shin, L.M., 2006. Amygdala, Medial Prefrontal Cortex, and Hippocampal Function in PTSD. Ann. N. Y. Acad. Sci. 1071, 67–79. https://doi.org/10.1196/annals.1364.007

Shin, L.M., Liberzon, I., 2010. The Neurocircuitry of Fear, Stress, and Anxiety Disorders. Neuropsychopharmacology 35, 169–191. https://doi.org/10.1038/npp.2009.83

Shultz, S.R., MacFabe, D.F., Foley, K.A., Taylor, R., Cain, D.P., 2011. A single mild fluid percussion injury induces short-term behavioral and neuropathological changes in the Long–

Evans rat: Support for an animal model of concussion. Behav. Brain Res. 224, 326–335. https://doi.org/10.1016/j.bbr.2011.06.012

Shultz, S.R., McDonald, S.J., Corrigan, F., Semple, B.D., Salberg, S., Zamani, A., Jones, N.C., Mychasiuk, R., 2020. Clinical Relevance of Behavior Testing in Animal Models of Traumatic Brain Injury. J. Neurotrauma 37, 2381–2400. https://doi.org/10.1089/neu.2018.6149

Shultz, S.R., McDonald, S.J., Corrigan, F., Semple, B.D., Salberg, S., Zamani, A., Jones, N.C., Mychasiuk, R., 2020. Clinical Relevance of Behavior Testing in Animal Models of Traumatic Brain Injury. J. Neurotrauma 37, 2381–2400. https://doi.org/10.1089/neu.2018.6149

Sian, J., Dexter, D.T., Lees, A.J., Daniel, S., Agid, Y., Javoy-Agid, F., Jenner, P., Marsden, C.D., 1994. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann. Neurol. 36, 348–355. https://doi.org/10.1002/ana.410360305

Sidaros, A., Skimminge, A., Liptrot, M., Sidaros, K., Engberg, A., Herning, M., Paulson, O., Jernigan, T., Rostrup, E., 2009. Long-term global and regional brain volume changes following severe traumatic brain injury: A longitudinal study with clinical correlates. NeuroImage 44, 1–8. https://doi.org/10.1016/j.neuroimage.2008.08.030

Sies, H., Jones, D.P., 2020. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat. Rev. Mol. Cell Biol. 21, 363–383. https://doi.org/10.1038/s41580-020-0230-3

Simola, N., Morelli, M., Carta, A.R., 2007. The 6-Hydroxydopamine model of parkinson's disease. Neurotox. Res. 11, 151–167. https://doi.org/10.1007/BF03033565

Singh, S., Chauhan, K., 2022. Pharmacological approach using doxycycline and tocopherol in rotenone induced oxidative stress, neuroinflammation and Parkinson's like symptoms. Int. J. Neurosci. 1–16. https://doi.org/10.1080/00207454.2022.2154670

Siquier, A., Andrés, P., 2021. Cognitive and Behavioral Inhibition Deficits in Parkinson's Disease: The Hayling Test as a Reliable Marker. Front. Aging Neurosci. 12, 621603. https://doi.org/10.3389/fnagi.2020.621603

Sitte, H.H., Pifl, C., Rajput, A.H., Hörtnagl, H., Tong, J., Lloyd, G.K., Kish, S.J., Hornykiewicz, O., 2017. Dopamine and noradrenaline, but not serotonin, in the human claustrum are greatly reduced in patients with Parkinson's disease: possible functional implications. Eur. J. Neurosci. 45, 192–197. https://doi.org/10.1111/ejn.13435

Smargiassi, A., Mutti, A., De Rosa, A., De Palma, G., Negrotti, A., Calzetti, S., 1998. A case-control study of occupational and environmental risk factors for Parkinson's disease in the Emilia-Romagna region of Italy. Neurotoxicology 19, 709–712.

Smith, C., Gentleman, S.M., Leclercq, P.D., Murray, L.S., Griffin, W.S.T., Graham, D.I., Nicoll, J.A.R., 2013. The neuroinflammatory response in humans after traumatic brain injury. Neuropathol. Appl. Neurobiol. 39, 654–666. https://doi.org/10.1111/nan.12008

Smith, Y., Kieval, J.Z., 2000. Anatomy of the dopamine system in the basal ganglia. Trends Neurosci. 23, S28–S33. https://doi.org/10.1016/S1471-1931(00)00023-9

Smits, R.P., Steinbusch, H.W., Mulder, A.H., 1990. Distribution of dopamine-immunoreactive cell bodies in the guinea-pig brain. J. Chem. Neuroanat. 3, 101–123.

So, R.W.L., Watts, J.C., 2023. α-Synuclein Conformational Strains as Drivers of Phenotypic Heterogeneity in Neurodegenerative Diseases. J. Mol. Biol. 435, 168011. https://doi.org/10.1016/j.jmb.2023.168011

Soblosky, J.S., Colgin, L.L., Chorney-Lane, D., Davidson, J.F., Carey, M.E., 1997. Some functional recovery and behavioral sparing occurs independent of task-specific practice after injury to the rat's sensorimotor cortex. Behav. Brain Res. 89, 51–59. https://doi.org/10.1016/S0166-4328(97)00049-1

Sofic, E., Lange, K.W., Jellinger, K., Riederer, P., 1992. Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci. Lett. 142, 128–130. https://doi.org/10.1016/0304-3940(92)90355-B

Soman, K., Nelson, C.A., Cerono, G., Goldman, S.M., Baranzini, S.E., Brown, E.G., 2023. Early detection of Parkinson's disease through enriching the electronic health record using a biomedical knowledge graph. Front. Med. 10, 1081087. https://doi.org/10.3389/fmed.2023.1081087

Song, L., Li, Y., Qian, X., Xu, N., Yu, Z., Dai, J., Xu, B., Hu, X., 2023. Parkinson's disease constipation effect of electroacupuncture at ST25 through colonic motility and enteric neuropathology. Front. Neurol. 13, 1092127. https://doi.org/10.3389/fneur.2022.1092127

Song, Y.-M., Qian, Y., Su, W.-Q., Liu, X.-H., Huang, J.-H., Gong, Z.-T., Luo, H.-L., Gao, C., Jiang, R.-C., 2019. Differences in pathological changes between two rat models of severe traumatic brain injury. Neural Regen. Res. 14, 1796–1804. https://doi.org/10.4103/1673-5374.257534

Sonntag, K.C., Brenhouse, H.C., Freund, N., Thompson, B.S., Puhl, M., Andersen, S.L., 2014. Viral over-expression of D1 dopamine receptors in the prefrontal cortex increase high-risk behaviors in adults: comparison with adolescents. Psychopharmacology (Berl.) 231, 1615–1626. https://doi.org/10.1007/s00213-013-3399-8

Sordillo, P.P., Sordillo, L.A., Helson, L., 2016. Bifunctional role of pro-inflammatory cytokines after traumatic brain injury. Brain Inj. 30, 1043–1053. https://doi.org/10.3109/02699052.2016.1163618

Sosnoff, J.J., Broglio, S.P., Ferrara, M.S., 2008. Cognitive and motor function are associated following mild traumatic brain injury. Exp. Brain Res. 187, 563–571. https://doi.org/10.1007/s00221-008-1324-x

Sosnoff, J.J., Broglio, S.P., Shin, S., Ferrara, M.S., 2011. Previous Mild Traumatic Brain Injury and Postural-Control Dynamics. J. Athl. Train. 46, 85–91. https://doi.org/10.4085/1062-6050-46.1.85

Spangenberg, S., Hannerz, H., Tüchsen, F., Mikkelsen, K.L., 2009. A nationwide population study of severe head injury and Parkinson's disease. Parkinsonism Relat. Disord. 15, 12–14. https://doi.org/10.1016/j.parkreldis.2008.02.004

Speelberg, D.H.B., Janssen Daalen, J.M., Bloem, B.R., Gagnon, J.-F., Post, B., Darweesh, S.K.L., 2022. Prodromal Cognitive Deficits and the Risk of Subsequent Parkinson's Disease. Brain Sci. 12, 199. https://doi.org/10.3390/brainsci12020199

Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R., Goedert, M., 1997. α-Synuclein in Lewy bodies. Nature 388, 839–840. https://doi.org/10.1038/42166

Stålnacke, B.-M., Saveman, B.-I., Stenberg, M., 2019. Long-Term Follow-Up of Disability, Cognitive, and Emotional Impairments after Severe Traumatic Brain Injury. Behav. Neurol. 2019, 1–7. https://doi.org/10.1155/2019/9216931

Stefanis, L., 2012. -Synuclein in Parkinson's Disease. Cold Spring Harb. Perspect. Med. 2, a009399–a009399. https://doi.org/10.1101/cshperspect.a009399

Stern, M.B., 1991. Head trauma as a risk factor for Parkinson's disease. Mov. Disord. Off. J. Mov. Disord. Soc. 6, 95–97. https://doi.org/10.1002/mds.870060202

Stocchetti, N., Zanier, E.R., 2016. Chronic impact of traumatic brain injury on outcome and quality of life: a narrative review. Crit. Care 20, 148. https://doi.org/10.1186/s13054-016-1318-1

Stokholm, M.G., Danielsen, E.H., Hamilton-Dutoit, S.J., Borghammer, P., 2016. Pathological α-synuclein in gastrointestinal tissues from prodromal P arkinson disease patients. Ann. Neurol. 79, 940–949. https://doi.org/10.1002/ana.24648

Stuart, S., Parrington, L., Morris, R., Martini, D.N., Fino, P.C., King, L.A., 2020. Gait measurement in chronic mild traumatic brain injury: A model approach. Hum. Mov. Sci.

Sugama, S., Takenouchi, T., Hashimoto, M., Ohata, H., Takenaka, Y., Kakinuma, Y., 2019. Stress-induced microglial activation occurs through β-adrenergic receptor: noradrenaline as a key neurotransmitter in microglial activation. J. Neuroinflammation 16, 266. https://doi.org/10.1186/s12974-019-1632-z

Sulzer, D., 2007. Multiple hit hypotheses for dopamine neuron loss in Parkinson's disease. Trends Neurosci. 30, 244–250. https://doi.org/10.1016/j.tins.2007.03.009

Sun, M., McDonald, S.J., Brady, R.D., Collins-Praino, L., Yamakawa, G.R., Monif, M., O'Brien, T.J., Cloud, G.C., Sobey, C.G., Mychasiuk, R., Loane, D.J., Shultz, S.R., 2020. The need to incorporate aged animals into the preclinical modeling of neurological conditions. Neurosci. Biobehav. Rev. 109, 114–128. https://doi.org/10.1016/j.neubiorev.2019.12.027

Surgent, O.J., Dadalko, O.I., Pickett, K.A., Travers, B.G., 2019. Balance and the brain: A review of structural brain correlates of postural balance and balance training in humans. Gait Posture 71, 245–252. https://doi.org/10.1016/j.gaitpost.2019.05.011

Swaine, B.R., Sullivan, S.J., 1996. Longitudinal profile of early motor recovery following severe traumatic brain injury. Brain Inj. 10, 347–366. https://doi.org/10.1080/026990596124368

Sysoev, Y.I., Prikhodko, V.A., Chernyakov, R.T., Idiyatullin, R.D., Musienko, P.E., Okovityi, S.V., 2021. Effects of Alpha-2 Adrenergic Agonist Mafedine on Brain Electrical Activity in Rats after Traumatic Brain Injury. Brain Sci. 11, 981. https://doi.org/10.3390/brainsci11080981

Szot, P., Franklin, A., Sikkema, C., Wilkinson, C.W., Raskind, M.A., 2012. Sequential Loss of LC Noradrenergic and Dopaminergic Neurons Results in a Correlation of Dopaminergic Neuronal Number to Striatal Dopamine Concentration. Front. Pharmacol. 3. https://doi.org/10.3389/fphar.2012.00184

Tabet, M., El-Kurdi, M., Haidar, M.A., Nasrallah, L., Reslan, M.A., Shear, D., Pandya, J.D., El-Yazbi, A.F., Sabra, M., Mondello, S., Mechref, Y., Shaito, A., Wang, K.K., El-Khoury, R., Kobeissy, F., 2022. Mitoquinone supplementation alleviates oxidative stress and pathologic outcomes following repetitive mild traumatic brain injury at a chronic time point. Exp. Neurol. 351. https://doi.org/10.1016/j.expneurol.2022.113987

Taguchi, T., Ikuno, M., Yamakado, H., Takahashi, R., 2020. Animal Model for Prodromal Parkinson's Disease. Int. J. Mol. Sci. 21, 1961. https://doi.org/10.3390/ijms21061961

Taipa, R., Pereira, C., Reis, I., Alonso, I., Bastos-Lima, A., Melo-Pires, M., Magalhães, M., 2016. DJ-1 linked parkinsonism (PARK7) is associated with Lewy body pathology. Brain 139, 1680–1687. https://doi.org/10.1093/brain/aww080

Tajiri, N., Hernandez, D., Acosta, S., Shinozuka, K., Ishikawa, H., Ehrhart, J., Diamandis, T., Gonzales-Portillo, C., Borlongan, M.C., Tan, J., Kaneko, Y., Borlongan, C.V., 2014. Suppressed cytokine expression immediatey following traumatic brain injury in neonatal rats indicates an expeditious endogenous anti-inflammatory response. Brain Res. 1559, 65–71. https://doi.org/10.1016/j.brainres.2014.02.041

Takahashi, H., Watanabe, Y., Tanaka, H., Mochizuki, H., Kato, H., Hatazawa, J., Tomiyama, N., 2019. Quantifying the Severity of Parkinson Disease by Use of Dopaminergic Neuroimaging. Am. J. Roentgenol. 213, 163–168. https://doi.org/10.2214/AJR.18.20655

Tan, X.L., Zheng, P., Wright, D.K., Sun, M., Brady, R.D., Liu, S., McDonald, S.J., Mychasiuk, R., Cenap, S., Jones, N.C., O'Brien, T.J., Shultz, S.R., 2020. The genetic ablation of tau improves long-term, but not short-term, functional outcomes after experimental traumatic brain injury in mice. Brain Inj. 34, 131–139.

Tang, C., Wang, W., Shi, M., Zhang, N., Zhou, X., Li, X., Ma, C., Chen, G., Xiang, J., Gao, D., 2019. Meta-Analysis of the Effects of the Catechol-O-Methyltransferase Val158/108Met Polymorphism on Parkinson's Disease Susceptibility and Cognitive Dysfunction. Front. Genet. 10, 644. https://doi.org/10.3389/fgene.2019.00644

Tanila, H., Rämä, P., Carlson, S., 1996. The effects of prefrontal intracortical microinjections of an alpha-2 agonist, alpha-2 antagonist and lidocaine on the delayed alternation performance of aged rats. Brain Res. Bull. 40, 117–119. https://doi.org/10.1016/0361-9230(96)00026-3

Tanner, C.M., Goldman, S.M., 1996. Epidemiology of Parkinson's disease. Neurol. Clin. 14, 317–335.

Tanner, C.M., Kamel, F., Ross, G.W., Hoppin, J.A., Goldman, S.M., Korell, M., Marras, C., Bhudhikanok, G.S., Kasten, M., Chade, A.R., Comyns, K., Richards, M.B., Meng, C., Priestley, B., Fernandez, H.H., Cambi, F., Umbach, D.M., Blair, A., Sandler, D.P., Langston, J.W., 2011. Rotenone, Paraquat, and Parkinson's Disease. Environ. Health Perspect. 119, 866–872. https://doi.org/10.1289/ehp.1002839

Tansey, M.G., Goldberg, M.S., 2010. Neuroinflammation in Parkinson's disease: Its role in neuronal death and implications for therapeutic intervention. Neurobiol. Dis. 37, 510–518. https://doi.org/10.1016/j.nbd.2009.11.004

Taylor, C.A., Bell, J.M., Breiding, M.J., Xu, L., 2017. Traumatic Brain Injury–Related Emergency Department Visits, Hospitalizations, and Deaths — United States, 2007 and 2013. MMWR Surveill. Summ. 66, 1–16. https://doi.org/10.15585/mmwr.ss6609a1

Taylor, C.A., Saint-Hilaire, M.H., Cupples, L.A., Thomas, C.A., Burchard, A.E., Feldman, R.G., Myers, R.H., 1999. Environmental, medical, and family history risk factors for Parkinson's disease: a New England-based case control study. Am. J. Med. Genet. 88, 742–749.

Taylor, F.B., Russo, J., 2001. Comparing Guanfacine and Dextroamphetamine for the Treatment of Adult Attention-Deficit/Hyperactivity Disorder. J. Clin. Psychopharmacol. 21.

Taylor, J.M., Main, B.S., Crack, P.J., 2013. Neuroinflammation and oxidative stress: Co-conspirators in the pathology of Parkinson's disease. Neurochem. Int. 62, 803–819. https://doi.org/10.1016/j.neuint.2012.12.016

Teasdale, G., Jennett, B., 1974. ASSESSMENT OF COMA AND IMPAIRED CONSCIOUSNESS. The Lancet 304, 81–84. https://doi.org/10.1016/S0140-6736(74)91639-0

Teleanu, D.M., Niculescu, A.-G., Lungu, I.I., Radu, C.I., Vladâcenco, O., Roza, E., Costăchescu, B., Grumezescu, A.M., Teleanu, R.I., 2022. An Overview of Oxidative Stress, Neuroinflammation, and Neurodegenerative Diseases. Int. J. Mol. Sci. 23, 5938. https://doi.org/10.3390/ijms23115938

Temmel, A.F.P., Quint, C., Schickinger-Fischer, B., Klimek, L., Stoller, E., Hummel, T., 2002. Characteristics of Olfactory Disorders in Relation to Major Causes of Olfactory Loss. Arch. Otolaryngol. Neck Surg. 128, 635. https://doi.org/10.1001/archotol.128.6.635

Testa, C.M., Sherer, T.B., Greenamyre, J.T., 2005. Rotenone induces oxidative stress and dopaminergic neuron damage in organotypic substantia nigra cultures. Mol. Brain Res. 134, 109–118. https://doi.org/10.1016/j.molbrainres.2004.11.007

Thakur, P., Nehru, B., 2015. Inhibition of Neuroinflammation and Mitochondrial Dysfunctions by Carbenoxolone in the Rotenone Model of Parkinson's Disease. Mol. Neurobiol. 51, 209–219. https://doi.org/10.1007/s12035-014-8769-7

Thau-Zuchman, O., Svendsen, L., Dyall, S.C., Paredes-Esquivel, U., Rhodes, M., Priestley, J.V., Feichtinger, R.G., Kofler, B., Lotstra, S., Verkuyl, J.M., Hageman, R.J., Broersen, L.M., van Wijk, N., Silva, J.P., Tremoleda, J.L., Michael-Titus, A.T., 2021. A new ketogenic formulation improves functional outcome and reduces tissue loss following traumatic brain injury in adult mice. Theranostics 11, 346–360. https://doi.org/10.7150/thno.48995

Theadom, A., Parag, V., Dowell, T., McPherson, K., Starkey, N., Barker-Collo, S., Jones, K., Ameratunga, S., Feigin, V.L., 2016. Persistent problems 1 year after mild traumatic brain injury: a longitudinal population study in New Zealand. Br. J. Gen. Pract. J. R. Coll. Gen. Pract. 66, e16-23. https://doi.org/10.3399/bjgp16X683161

Thomas, B., Beal, M.F., 2007. Parkinson's disease. Hum. Mol. Genet. 16, R183–R194. https://doi.org/10.1093/hmg/ddm159

Thompson, H.J., McCormick, W.C., Kagan, S.H., 2006. Traumatic brain injury in older adults: epidemiology, outcomes, and future implications. J. Am. Geriatr. Soc. 54, 1590–1595. https://doi.org/10.1111/j.1532-5415.2006.00894.x

Till, C., Colella, B., Verwegen, J., Green, R.E., 2008. Postrecovery Cognitive Decline in Adults With Traumatic Brain Injury. Arch. Phys. Med. Rehabil. 89, S25–S34. https://doi.org/10.1016/j.apmr.2008.07.004

Tong, J., Hornykiewicz, O., Kish, S.J., 2006. Inverse Relationship Between Brain Noradrenaline Level and Dopamine Loss in Parkinson Disease: A Possible Neuroprotective Role for Noradrenaline. Arch. Neurol. 63, 1724. https://doi.org/10.1001/archneur.63.12.1724

Toshkezi, G., Kyle, M., Longo, S.L., Chin, L.S., Zhao, L.-R., 2018. Brain repair by hematopoietic growth factors in the subacute phase of traumatic brain injury. J. Neurosurg. 129, 1286–1294. https://doi.org/10.3171/2017.7.JNS17878

Troncoso-Escudero, P., Parra, A., Nassif, M., Vidal, R.L., 2018. Outside in: Unraveling the Role of Neuroinflammation in the Progression of Parkinson's Disease. Front. Neurol. 9, 860. https://doi.org/10.3389/fneur.2018.00860

Troshev, D., Voronkov, D., Pavlova, A., Abaimov, D., Latanov, A., Fedorova, T., Berezhnoy, D., 2022. Time Course of Neurobehavioral Disruptions and Regional Brain Metabolism Changes in the Rotenone Mice Model of Parkinson's Disease. Biomedicines 10, 466. https://doi.org/10.3390/biomedicines10020466

Tsai, C.H., Lo, S.K., See, L.C., Chen, H.Z., Chen, R.S., Weng, Y.H., Chang, F.C., Lu, C.S., 2002. Environmental risk factors of young onset Parkinson's disease: a case-control study. Clin. Neurol. Neurosurg. 104, 328–333. https://doi.org/10.1016/s0303-8467(02)00027-6

Tschuor, C., Asmis, L.M., Lenzlinger, P.M., Tanner, M., Härter, L., Keel, M., Stocker, R., Stover, J.F., 2008. In vitro norepinephrine significantly activates isolated platelets from healthy volunteers and critically ill patients following severe traumatic brain injury. Crit. Care 12, R80. https://doi.org/10.1186/cc6931

Tsunemi, T., Ishiguro, Y., Yoroisaka, A., Valdez, C., Miyamoto, K., Ishikawa, K., Saiki, S., Akamatsu, W., Hattori, N., Krainc, D., 2020. Astrocytes Protect Human Dopaminergic Neurons from α-Synuclein Accumulation and Propagation. J. Neurosci. 40, 8618–8628. https://doi.org/10.1523/JNEUROSCI.0954-20.2020

Tucker, L.B., Fu, A.H., McCabe, J.T., 2016. Performance of Male and Female C57BL/6J Mice on Motor and Cognitive Tasks Commonly Used in Pre-Clinical Traumatic Brain Injury Research. J. Neurotrauma 33, 880–894. https://doi.org/10.1089/neu.2015.3977

Tucker, L.B., Velosky, A.G., Fu, A.H., McCabe, J.T., 2019. Chronic Neurobehavioral Sex Differences in a Murine Model of Repetitive Concussive Brain Injury. Front. Neurol. 10, 509. https://doi.org/10.3389/fneur.2019.00509

Turner, R.C., VanGilder, R.L., Naser, Z.J., Lucke-Wold, B.P., Bailes, J.E., Matsumoto, R.R., Huber, J.D., Rosen, C.L., 2014. Elucidating the severity of preclinical traumatic brain injury models: a role for functional assessment? Neurosurgery 74, 382–394; discussion 394. https://doi.org/10.1227/NEU.0000000000000292

Tyurin, V.A., Tyurina, Y.Y., Borisenko, G.G., Sokolova, T.V., Ritov, V.B., Quinn, P.J., Rose, M., Kochanek, P., Graham, S.H., Kagan, V.E., 2000. Oxidative Stress Following Traumatic Brain Injury in Rats: Quantitation of Biomarkers and Detection of Free Radical Intermediates. J. Neurochem. 75, 2178–2189. https://doi.org/10.1046/j.1471-4159.2000.0752178.x

Ujvári, B., Pytel, B., Márton, Z., Bognár, M., Kovács, L.Á., Farkas, J., Gaszner, T., Berta, G., Kecskés, A., Kormos, V., Farkas, B., Füredi, N., Gaszner, B., 2022. Neurodegeneration in the centrally-projecting Edinger–Westphal nucleus contributes to the non-motor symptoms of Parkinson's disease in the rat. J. Neuroinflammation 19, 31. https://doi.org/10.1186/s12974-022-02399-w

Ungerstedt, U., 1976. 6-Hydroxydopamine-induced degeneration of the nigrostriatal dopamine pathway: The turning syndrome. Pharmacol. Ther. [B] 2, 37–40. https://doi.org/10.1016/0306-039X(76)90016-7

Uryu, K., Chen, X.-H., Martinez, D., Browne, K.D., Johnson, V.E., Graham, D.I., Lee, V.M.-Y., Trojanowski, J.Q., Smith, D.H., 2007. Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. Exp. Neurol. 208, 185–192. https://doi.org/10.1016/j.expneurol.2007.06.018

Uryu, K., Giasson, B.I., Longhi, L., Martinez, D., Murray, I., Conte, V., Nakamura, M., Saatman, K., Talbot, K., Horiguchi, T., McIntosh, T., Lee, V.M.-Y., Trojanowski, J.Q., 2003. Age-dependent synuclein pathology following traumatic brain injury in mice. Exp. Neurol. 184, 214–224. https://doi.org/10.1016/S0014-4886(03)00245-0

USEPA, (U.S. Environmental Protection Agency), 2006. ROTENONE: Final HED Chapter of the Reregistration Eligibility Decision Document (RED)PC Code: 071003. DP Barcode: D328478. Memorandum from Charles Smith, et al., HED/OPP and R. David Jones, EFED/OPP to Katie Hall, SRRD/OPP. 71 p. Docket # EPA-HQ-OPP-2005-0494-0034.

Useros Olmo, A.I., Periañez, J.A., Martínez-Pernía, D., Miangolarra Page, J.C., 2020a. Effects of spatial working memory in balance during dual tasking in traumatic brain injury and healthy controls. Brain Inj. 34, 1159–1167. https://doi.org/10.1080/02699052.2020.1792984

Useros Olmo, A.I., Perianez, J.A., Martinez-Pernia, D., Miangolarra Page, J.C., 2020b. Effects of spatial working memory in balance during dual tasking in traumatic brain injury and healthy controls. Brain Inj. 34, 1159–1167.

Ustinova, K.I., 2017. Decomposition of postural movements in individuals with mild TBI while reaching to intercept a moving virtual target. Physiother. Theory Pract. 33, 527–534. https://doi.org/10.1080/09593985.2017.1325958

Valles, S.L., Singh, S.K., Campos-Campos, J., Colmena, C., Campo-Palacio, I., Alvarez-Gamez, K., Caballero, O., Jorda, A., 2023. Functions of Astrocytes under Normal Conditions and after a Brain Disease. Int. J. Mol. Sci. 24, 8434. https://doi.org/10.3390/ijms24098434

van Bregt, D.R., Thomas, T.C., Hinzman, J.M., Cao, T., Liu, M., Bing, G., Gerhardt, G.A., Pauly, J.R., Lifshitz, J., 2012. Substantia nigra vulnerability after a single moderate diffuse brain injury in the rat. Exp. Neurol. 234, 8–19. https://doi.org/10.1016/j.expneurol.2011.12.003

Van Laar, A.D., Webb, K.R., Keeney, M.T., Van Laar, V.S., Zharikov, A., Burton, E.A., Hastings, T.G., Glajch, K.E., Hirst, W.D., Greenamyre, J.T., Rocha, E.M., 2023. Transient exposure to rotenone causes degeneration and progressive parkinsonian motor deficits, neuroinflammation, and synucleinopathy. Npj Park. Dis. 9, 121. https://doi.org/10.1038/s41531-023-00561-6

Vanderploeg, R.D., Curtiss, G., Luis, C.A., Salazar, A.M., 2007. Long-term morbidities following self-reported mild traumatic brain injury. J. Clin. Exp. Neuropsychol. 29, 585–598. https://doi.org/10.1080/13803390600826587

Vasudevan, E.V.L., Glass, R.N., Packel, A.T., 2014. Effects of Traumatic Brain Injury on Locomotor Adaptation: J. Neurol. Phys. Ther. 38, 172–182. https://doi.org/10.1097/NPT.0000000000000049

Verbaan, D., Marinus, J., Visser, M., Van Rooden, S.M., Stiggelbout, A.M., Van Hilten, J.J., 2007. Patient-reported autonomic symptoms in Parkinson disease. Neurology 69, 333–341. https://doi.org/10.1212/01.wnl.0000266593.50534.e8

Vidal-Itriago, A., Radford, R.A.W., Aramideh, J.A., Maurel, C., Scherer, N.M., Don, E.K., Lee, A., Chung, R.S., Graeber, M.B., Morsch, M., 2022. Microglia morphophysiological diversity and its implications for the CNS. Front. Immunol. 13, 997786. https://doi.org/10.3389/fimmu.2022.997786

Vijiaratnam, N., Simuni, T., Bandmann, O., Morris, H.R., Foltynie, T., 2021. Progress towards therapies for disease modification in Parkinson's disease. Lancet Neurol. 20, 559–572. https://doi.org/10.1016/S1474-4422(21)00061-2

Vink, R., 2018. Large animal models of traumatic brain injury. J. Neurosci. Res. 96, 527–535. https://doi.org/10.1002/jnr.24079

Vogel, A., Wilken-Schmitz, A., Hummel, R., Lang, M., Gurke, R., Schreiber, Y., Schäfer, M.K.E., Tegeder, I., 2020. Low brain endocannabinoids associated with persistent non-goal directed nighttime hyperactivity after traumatic brain injury in mice. Sci. Rep. 10, 14929. https://doi.org/10.1038/s41598-020-71879-x

Wagner, Amy K., Chen, X., Kline, A.E., Li, Y., Zafonte, R.D., Dixon, C.E., 2005. Gender and environmental enrichment impact dopamine transporter expression after experimental traumatic brain injury. Exp. Neurol. 195, 475–483. https://doi.org/10.1016/j.expneurol.2005.06.009

Wagner, A.K., Scanion, J.M., Becker, C.R., Ritter, A.C., Niyonkuru, C., Dixon, C.E., Conley, Y.P., Price, J.C., 2014. The Influence of Genetic Variants on Striatal Dopamine Transporter and D2 Receptor Binding after TB. J. Cereb. Blood Flow Metab. 34, 1328–1339. https://doi.org/10.1038/jcbfm.2014.87

Wagner, A. K., Sokoloski, J.E., Ren, D., Chen, X., Khan, A.S., Zafonte, R.D., Michael, A.C., Dixon, C.E., 2005. Controlled cortical impact injury affects dopaminergic transmission in the rat striatum. J. Neurochem. 95, 457–465. https://doi.org/10.1111/j.1471-4159.2005.03382.x

Wake, H., Moorhouse, A.J., Jinno, S., Kohsaka, S., Nabekura, J., 2009. Resting Microglia Directly Monitor the Functional State of Synapses In Vivo and Determine the Fate of Ischemic Terminals. J. Neurosci. 29, 3974–3980. https://doi.org/10.1523/JNEUROSCI.4363-08.2009

Walf, A.A., Frye, C.A., 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat. Protoc. 2, 322–328. https://doi.org/10.1038/nprot.2007.44

Walker, W.C., 2007. Motor impairment after severe traumatic brain injury: A longitudinal multicenter study. J. Rehabil. Res. Dev. 44, 975–982. https://doi.org/10.1682/JRRD.2006.12.0158

Walker, W.C., Hirsch, S., Carne, W., Nolen, T., Cifu, D.X., Wilde, E.A., Levin, H.S., Brearly, T.W., Eapen, B.C., Williams, R., 2018a. Chronic Effects of Neurotrauma Consortium (CENC) multicentre study interim analysis: Differences between participants with positive versus negative mild TBI histories. Brain Inj. 32, 1079–1089. https://doi.org/10.1080/02699052.2018.1479041

Walker, W.C., Nowak, K.J., Kenney, K., Franke, L.M., Eapen, B.C., Skop, K., Levin, H., Agyemang, A.A., Tate, D.F., Wilde, E.A., Hinds, S., Nolen, T.L., 2018b. Is balance performance reduced after mild traumatic brain injury?: Interim analysis from chronic effects of neurotrauma consortium (CENC) multi-centre study. Brain Inj. 32, 1156–1168. https://doi.org/10.1080/02699052.2018.1483529

Walker, W.C., Pickett, T.C., 2007. Motor impairment after severe traumatic brain injury: A longitudinal multicenter study. J. Rehabil. Res. Dev. 44, 975–982. https://doi.org/10.1682/jrrd.2006.12.0158

Wang, D., Xu, X., Wu, Y.-G., Lyu, L., Zhou, Z.-W., Zhang, J.-N., 2018. Dexmedetomidine attenuates traumatic brain injury: action pathway and mechanisms. Neural Regen. Res. 13, 819. https://doi.org/10.4103/1673-5374.232529

Wang, F., Franco, R., Skotak, M., Hu, G., Chandra, N., 2014. Mechanical stretch exacerbates the cell death in SH-SY5Y cells exposed to paraquat: mitochondrial dysfunction and oxidative stress. NeuroToxicology 41, 54–63. https://doi.org/10.1016/j.neuro.2014.01.002

Wang, Q., Oyarzabal, E.A., Song, S., Wilson, B., Santos, J.H., Hong, J.-S., 2020. Locus coeruleus neurons are most sensitive to chronic neuroinflammation-induced neurodegeneration. Brain. Behav. Immun. 87, 359–368. https://doi.org/10.1016/j.bbi.2020.01.003

Wang, T., Nowrangi, D., Yu, L., Lu, T., Tang, J., Han, B., Ding, Y., Fu, F., Zhang, J.H., 2018. Activation of dopamine D1 receptor decreased NLRP3-mediated inflammation in intracerebral hemorrhage mice. J. Neuroinflammation 15, 2. https://doi.org/10.1186/s12974-017-1039-7

Wang, T.-F., Wu, S.-Y., Pan, B.-S., Tsai, S.-F., Kuo, Y.-M., 2022. Inhibition of Nigral Microglial Activation Reduces Age-Related Loss of Dopaminergic Neurons and Motor Deficits. Cells 11, 481. https://doi.org/10.3390/cells11030481

Wang, Z., Wang, S., Xu, T., Hong, C., Cheng, M., Zhu, P., Lin, J., Su, D., Miao, C., 2020a. Cerebral organoids transplantation improves neurological motor function in rat brain injury. CNS Neurosci. Ther. 26, 682–697. https://doi.org/10.1111/cns.13286

Wanneveich, M., Moisan, F., Jacqmin-Gadda, H., Elbaz, A., Joly, P., 2018. Projections of prevalence, lifetime risk, and life expectancy of Parkinson's disease (2010-2030) in France. Mov. Disord. 33, 1449–1455. https://doi.org/10.1002/mds.27447

Wassall, R.D., Teramoto, N., Cunnane, T.C., 2009. Noradrenaline, in: Encyclopedia of Neuroscience. Elsevier, pp. 1221–1230. https://doi.org/10.1016/B978-008045046-9.00681-1

Watanabe, Y., Watanabe, T., 2017. Meta-analytic evaluation of the association between head injury and risk of amyotrophic lateral sclerosis. Eur. J. Epidemiol. 32, 867–879. https://doi.org/10.1007/s10654-017-0327-y

Webster, K.M., Wright, D.K., Sun, M., Semple, B.D., Ozturk, E., Stein, D.G., O'Brien, T.J., Shultz, S.R., 2015. Progesterone treatment reduces neuroinflammation, oxidative stress and brain damage and improves long-term outcomes in a rat model of repeated mild traumatic brain injury. J. Neuroinflammation 12, 238. https://doi.org/10.1186/s12974-015-0457-7

Weinshilboum, R., Axelrod, J., 1971. Serum Dopamine-Beta-Hydroxylase Activity. Circ. Res. 28, 307–315. https://doi.org/10.1161/01.RES.28.3.307

Weintraub, D., Chahine, L.M., Hawkins, K.A., Siderowf, A., Eberly, S., Oakes, D., Seibyl, J., Stern, M.B., Marek, K., Jennings, D., the PARS Investigators, 2017. Cognition and the course of prodromal Parkinson's disease. Mov. Disord. 32, 1640–1645. https://doi.org/10.1002/mds.27189

Wilkerson, G.B., Nabhan, D.C., Perry, T.S., 2021. A Novel Approach to Assessment of Perceptual-Motor Efficiency and Training-Induced Improvement in the Performance Capabilities of Elite Athletes. Front. Sports Act. Living 3. https://doi.org/10.3389/fspor.2021.729729

Williams-Gray, C.H., Wijeyekoon, R., Yarnall, A.J., Lawson, R.A., Breen, D.P., Evans, J.R., Cummins, G.A., Duncan, G.W., Khoo, T.K., Burn, D.J., Barker, R.A., on behalf of the ICICLE-PD study group, 2016. Serum immune markers and disease progression in an

incident Parkinson's disease cohort (ICICLE-PD). Mov. Disord. 31, 995–1003. https://doi.org/10.1002/mds.26563

Williams, D.B., Annegers, J.F., Kokmen, E., O'Brien, P.C., Kurland, L.T., 1991. Brain injury and neurologic sequelae: A cohort study of dementia, parkinsonism, and amyotrophic lateral sclerosis. Neurology 41, 1554–1554. https://doi.org/10.1212/WNL.41.10.1554

Williams, G., Morris, M.E., Schache, A., McCrory, P.R., 2010. People Preferentially Increase Hip Joint Power Generation to Walk Faster Following Traumatic Brain Injury. Neurorehabil. Neural Repair 24, 550–558. https://doi.org/10.1177/1545968309357925

Williams, G., Morris, M.E., Schache, A., McCrory, P.R., 2009. Incidence of Gait Abnormalities After Traumatic Brain Injury. Arch. Phys. Med. Rehabil. 90, 587–593. https://doi.org/10.1016/j.apmr.2008.10.013

Williams, G., Robertson, V., Greenwood, K., 2004. Measuring High-Level Mobility After Traumatic Brain Injury: Am. J. Phys. Med. Rehabil. 83, 910–920. https://doi.org/10.1097/01.PHM.0000146503.25221.1D

Williams, G., Schache, A., Morris, M.E., 2013. Running abnormalities after traumatic brain injury. Brain Inj. 27, 434–443. https://doi.org/10.3109/02699052.2012.750754

Williams, G., Schache, A.G., 2016. The distribution of positive work and power generation amongst the lower-limb joints during walking normalises following recovery from traumatic brain injury. Gait Posture 43, 265–269. https://doi.org/10.1016/j.gaitpost.2015.10.009

Williamson, M.L.C., Elliott, T.R., Bogner, J., Dreer, L.E., Arango-Lasprilla, J.C., Kolakowsky-Hayner, S.A., Pretz, C.R., Lequerica, A., Perrin, P.B., 2016. Trajectories of Life Satisfaction Over the First 10 Years After Traumatic Brain Injury: Race, Gender, and Functional Ability. J. Head Trauma Rehabil. 31, 167–179. https://doi.org/10.1097/HTR.0000000000000111

Wood, D.M., Alsahaf, H., Streete, P., Dargan, P.I., Jones, A.L., 2005. Fatality after deliberate ingestion of the pesticide rotenone: a case report. Crit. Care Lond. Engl. 9, R280-284. https://doi.org/10.1186/cc3528

Woollacott, M., Shumway-Cook, A., 2002. Attention and the control of posture and gait: a review of an emerging area of research. Gait Posture 16, 1–14. https://doi.org/10.1016/S0966-6362(01)00156-4

Wright, D.K., Liu, S., van der Poel, C., McDonald, S.J., Brady, R.D., Taylor, L., Yang, L., Gardner, A.J., Ordidge, R., O'Brien, T.J., Johnston, L.A., Shultz, S.R., 2017. Traumatic Brain Injury Results in Cellular, Structural and Functional Changes Resembling Motor Neuron Disease. Cereb. Cortex N. Y. N 1991 27, 4503–4515. https://doi.org/10.1093/cercor/bhw254

Wright, W.G., Handy, J.D., Avcu, P., Ortiz, A., Haran, F.J., Doria, M., Servatius, R.J., 2018. Healthy Active Duty Military with Lifetime Experience of Mild Traumatic Brain Injury Exhibits Subtle Deficits in Sensory Reactivity and Sensory Integration During Static Balance. Mil. Med. 183, 313–320. https://doi.org/10.1093/milmed/usx182

Wright, W.G., McDevitt, J., Tierney, R., Haran, F.J., Appiah-Kubi, K.O., Dumont, A., 2017. Assessing subacute mild traumatic brain injury with a portable virtual reality balance device. Disabil. Rehabil. 39, 1564–1572. https://doi.org/10.1080/09638288.2016.1226432

Wu, J., Vogel, T., Gao, X., Lin, B., Kulwin, C., Chen, J., 2018. Neuroprotective effect of dexmedetomidine in a murine model of traumatic brain injury. Sci. Rep. 8, 4935. https://doi.org/10.1038/s41598-018-23003-3

Wüllner, U., Schmitz-Hübsch, T., Antony, G., Fimmers, R., Spottke, A., Oertel, W.H., Deuschl, G., Klockgether, T., Eggert, K., on behalf of the KNP e.V., 2007. Autonomic dysfunction in 3414 Parkinson's disease patients enrolled in the German Network on Parkinson's disease (KNP e.V.): the effect of ageing: Autonomic dysfunction in Parkinson's disease. Eur. J. Neurol. 14, 1405–1408. https://doi.org/10.1111/j.1468-1331.2007.01982.x

Xie, B.-S., Wang, Y.-Q., Lin, Y., Mao, Q., Feng, J.-F., Gao, G.-Y., Jiang, J.-Y., 2019. Inhibition of ferroptosis attenuates tissue damage and improves long-term outcomes after traumatic brain injury in mice. CNS Neurosci. Ther. 25, 465–475.

Xiong, N., Huang, J., Zhang, Zhentao, Zhang, Zhaowen, Xiong, J., Liu, X., Jia, M., Wang, F., Chen, C., Cao, X., Liang, Z., Sun, S., Lin, Z., Wang, T., 2009. Stereotaxical Infusion of Rotenone: A Reliable Rodent Model for Parkinson's Disease. PLoS ONE 4, e7878. https://doi.org/10.1371/journal.pone.0007878

Xiong, Y., Gu, Q., Peterson, P.L., Muizelaar, J.P., Lee, C.P., 1997. Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. J. Neurotrauma 14, 23–34. https://doi.org/10.1089/neu.1997.14.23

Xiong, Y., Mahmood, A., Chopp, M., 2018. Current understanding of neuroinflammation after traumatic brain injury and cell-based therapeutic opportunities. Chin. J. Traumatol. 21, 137–151. https://doi.org/10.1016/j.cjtee.2018.02.003

Xiong, Y., Mahmood, A., Chopp, M., 2013. Animal models of traumatic brain injury. Nat. Rev. Neurosci. 14, 128–142. https://doi.org/10.1038/nrn3407

Xu, S.-Y., Liu, M., Gao, Y., Cao, Y., Bao, J.-G., Lin, Y.-Y., Wang, Y., Luo, Q.-Z., Jiang, J.-Y., Zhong, C.-L., 2019. Acute histopathological responses and long-term behavioral outcomes in mice with graded controlled cortical impact injury. Neural Regen. Res. 14, 997–1003. https://doi.org/10.4103/1673-5374.250579

Yadav, D., Kumar, P., 2022. Restoration and targeting of aberrant neurotransmitters in Parkinson's disease therapeutics. Neurochem. Int. 156, 105327. https://doi.org/10.1016/j.neuint.2022.105327

Yan, D., Zhang, Y., Liu, L., Shi, N., Yan, H., 2018. Pesticide exposure and risk of Parkinson's disease: Dose-response meta-analysis of observational studies. Regul. Toxicol. Pharmacol. 96, 57–63. https://doi.org/10.1016/j.yrtph.2018.05.005

Yang, W., Hamilton, J.L., Kopil, C., Beck, J.C., Tanner, C.M., Albin, R.L., Ray Dorsey, E., Dahodwala, N., Cintina, I., Hogan, P., Thompson, T., 2020. Current and projected future economic burden of Parkinson's disease in the U.S. Npj Park. Dis. 6, 15. https://doi.org/10.1038/s41531-020-0117-1

Yogev-Seligmann, G., Hausdorff, J.M., Giladi, N., 2008. The role of executive function and attention in gait. Mov. Disord. Off. J. Mov. Disord. Soc. 23, 329–342; quiz 472. https://doi.org/10.1002/mds.21720

You, W.-C., Wang, C., Pan, Y., Zhang, Xin, Zhou, X., Zhang, Xiang-sheng, Shi, J., Zhou, M., 2013. Activation of Nuclear Factor-κB in the Brain after Experimental Subarachnoid Hemorrhage and Its Potential Role in Delayed Brain Injury. PLoS ONE 8, e60290. https://doi.org/10.1371/journal.pone.0060290

Zapparoli, L., Mariano, M., Paulesu, E., 2022. How the motor system copes with aging: a quantitative meta-analysis of the effect of aging on motor function control. Commun. Biol. 5, 79. https://doi.org/10.1038/s42003-022-03027-2

Zarow, C., Lyness, S.A., Mortimer, J.A., Chui, H.C., 2003b. Neuronal Loss Is Greater in the Locus Coeruleus Than Nucleus Basalis and Substantia Nigra in Alzheimer and Parkinson Diseases. Arch. Neurol. 60, 337. https://doi.org/10.1001/archneur.60.3.337

Zeevalk, G.D., Razmpour, R., Bernard, L.P., 2008. Glutathione and Parkinson's disease: Is this the elephant in the room? Biomed. Pharmacother. 62, 236–249. https://doi.org/10.1016/j.biopha.2008.01.017

Zehr, E.P., Barss, T.S., Dragert, K., Frigon, A., Vasudevan, E.V., Haridas, C., Hundza, S., Kaupp, C., Klarner, T., Klimstra, M., Komiyama, T., Loadman, P.M., Mezzarane, R.A., Nakajima, T., Pearcey, G.E.P., Sun, Y., 2016. Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. Exp. Brain Res. 234, 3059–3081. https://doi.org/10.1007/s00221-016-4715-4

Zhang, C., Saatman, K.E., Royo, N.C., Soltesz, K.M., Millard, M., Schouten, J.W., Motta, M., Hoover, R.C., McMillan, A., Watson, D.J., Lee, V.M.-Y., Trojanowski, J.Q., McIntosh, T.K., 2005a. Delayed Transplantation of Human Neurons following Brain Injury in Rats: A Long-Term Graft Survival and Behavior Study. J. Neurotrauma 22, 1456–1474. https://doi.org/10.1089/neu.2005.22.1456

Zhang, J., Wang, R.-J., Chen, M., Liu, X.-Y., Ma, K., Xu, H.-Y., Deng, W.-S., Ye, Y.-C., Li, W.-X., Chen, X.-Y., Sun, H.-T., 2021. Collagen/heparan sulfate porous scaffolds loaded with neural stem cells improve neurological function in a rat model of traumatic brain injury. Neural Regen. Res. 16, 1068–1077. https://doi.org/10.4103/1673-5374.300458

Zhang, L., Abreu, B.C., Gonzales, V., Huddleston, N., Ottenbacher, K.J., 2002. The effect of predictable and unpredictable motor tasks on postural control after traumatic brain injury. NeuroRehabilitation 17, 225–230. https://doi.org/10.3233/NRE-2002-17308

Zhang, N., Dou, D., Ran, X., Kang, T., 2018. Neuroprotective effect of arctigenin against neuroinflammation and oxidative stress induced by rotenone. RSC Adv. 8, 2280–2292. https://doi.org/10.1039/C7RA10906G

Zhang, W., Wang, T., Pei, Z., Miller, D.S., Wu, X., Block, M.L., Wilson, B., Zhang, Wanqin, Zhou, Y., Hong, J.-S., Zhang, J., 2005. Aggregated α-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB J. 19, 533–542. https://doi.org/10.1096/fj.04-2751com

Zhang, Y., Chen, Y., Wu, J., Manaenko, A., Yang, P., Tang, J., Fu, W., Zhang, J.H., 2015. Activation of Dopamine D2 Receptor Suppresses Neuroinflammation Through αB-Crystalline by Inhibition of NF-κB Nuclear Translocation in Experimental ICH Mice Model. Stroke 46, 2637–2646. https://doi.org/10.1161/STROKEAHA.115.009792

Zhang, Z.-N., Zhang, J.-S., Xiang, J., Yu, Z.-H., Zhang, W., Cai, M., Li, X.-T., Wu, T., Li, W.-W., Cai, D.-F., 2017. Subcutaneous rotenone rat model of Parkinson's disease: Dose exploration study. Brain Res. 1655, 104–113. https://doi.org/10.1016/j.brainres.2016.11.020

Zhou, C., Guo, T., Bai, X., Wu, J., Gao, T., Guan, X., Liu, X., Gu, L., Huang, P., Xuan, M., Gu, Q., Xu, X., Zhang, B., Zhang, M., 2021. Locus coeruleus degeneration is associated with disorganized functional topology in Parkinson's disease. NeuroImage Clin. 32, 102873. https://doi.org/10.1016/j.nicl.2021.102873

Zhou, J., Peng, C., Li, Q., Yan, X., Yang, L., Li, M., Cao, X., Xie, X., Chen, D., Rao, C., Huang, S., Peng, F., Pan, X., 2022. Dopamine Homeostasis Imbalance and Dopamine Receptors-Mediated AC/cAMP/PKA Pathway Activation are Involved in Aconitine-Induced Neurological Impairment in Zebrafish and SH-SY5Y Cells. Front. Pharmacol. 13, 837810. https://doi.org/10.3389/fphar.2022.837810

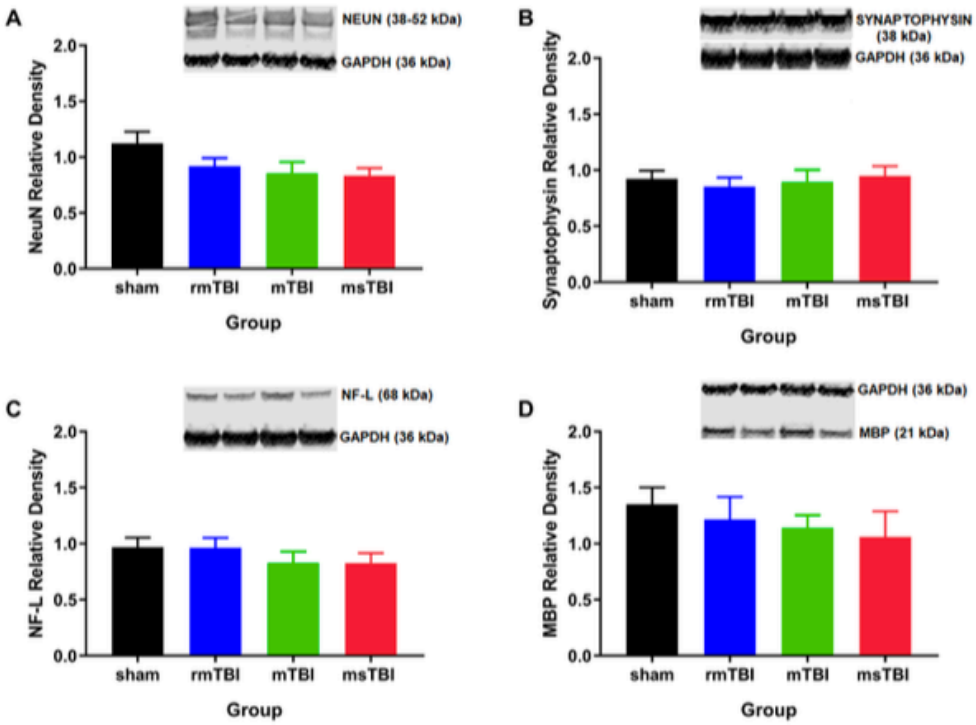
Zhou, Z.D., Yi, L.X., Wang, D.Q., Lim, T.M., Tan, E.K., 2023. Role of dopamine in the pathophysiology of Parkinson's disease. Transl. Neurodegener. 12, 44. https://doi.org/10.1186/s40035-023-00378-6

Ziebell, J.M., Morganti-Kossmann, M.C., 2010. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. Neurotherapeutics 7, 22–30. https://doi.org/10.1016/j.nurt.2009.10.016

Ziebell, M., Andersen, B.B., Thomsen, G., Pinborg, L.H., Karlsborg, M., Hasselbalch, S.G., Knudsen, G.M., 2012. Predictive value of dopamine transporter SPECT imaging with [123I]PE2I in patients with subtle parkinsonian symptoms. Eur. J. Nucl. Med. Mol. Imaging 39, 242–250. https://doi.org/10.1007/s00259-011-1976-9

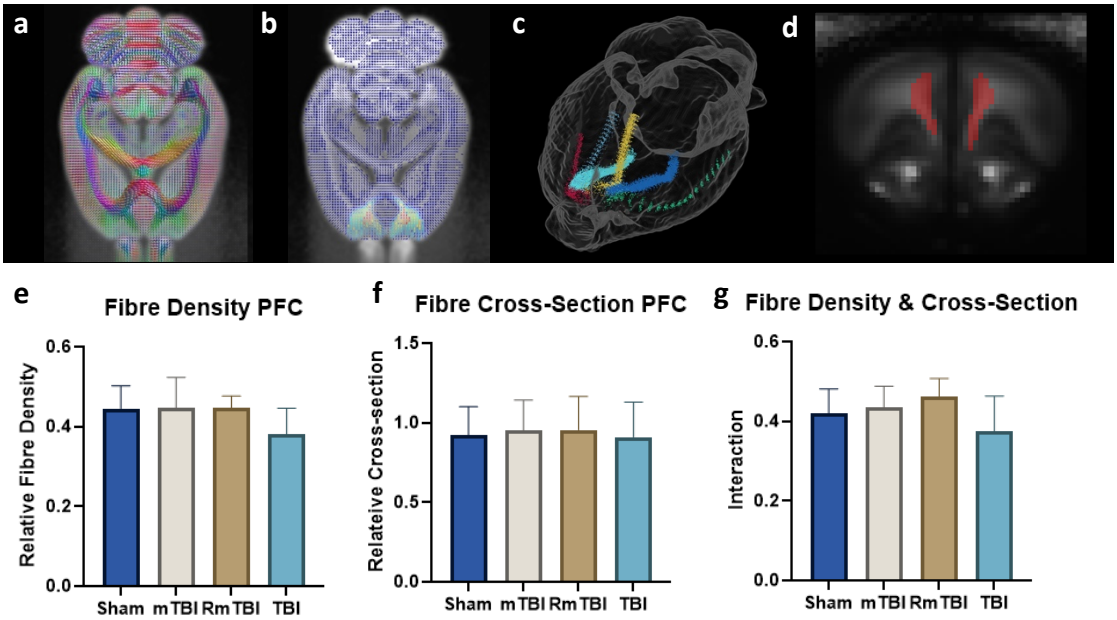
APPENDIX

Supplementary Table 1. Effect values from two-way ANOVA analysis			
	TBI effect	**Rotenone effect**	**Interaction**
Gross motor function			
GT-Stride length	$F_{1, 42}$= 0.054, p=0.82	$F_{1, 42}$= 0.352, p=0.56	$F_{1, 42}$= 1.03, p=0.32
GT-Stance length	$F_{1, 42}$= 2.185, p=0.15	$F_{1, 42}$= 1.102, p=0.3	$F_{1, 42}$= 0.017, p=0.9
GT-Stance width	$F_{1, 43}$= 2.8, p=0.102	$F_{1, 43}$= 0.324, p=0.57	$F_{1, 43}$= 0.628, p=0.432
GT-Hindlimb BOS	$F_{1, 43}$= 1.18, p=0.284	$F_{1, 43}$= 2.64, p=0.11	$F_{1, 43}$= 3.13, p=1.043
Sensorimotor function			
PMT-Time consumed/pasta	$F_{1, 33}$= 0.34, p=0.56	$F_{1, 33}$= 2.62, p=0.115	$F_{1, 33}$= 0.74, p=0.4
PMT-Atypical pattern/pasta	$F_{1, 33}$= 0.07, p=0.79	$F_{1, 33}$= 0.03, p=0.87	$F_{1, 33}$= 0.143, p=0.71
Anxiety			
OF-time in centre	$F_{1, 41}$= 0.43, p=0.51	$F_{1, 41}$= 0.0002, p=0.99	$F_{1, 41}$= 1.359, p=0.25
EPM-time in open arms	$F_{1, 43}$= 2.97, p=0.09	$F_{1, 43}$= 0.33, p=0.57	$F_{1, 43}$= 1.47, p=0.23
EPM-number of arm entries	$F_{1, 43}$= 0.05, p=0.82	$F_{1, 43}$= 0.002, p=0.96	$F_{1, 43}$= 0.46, p=0.5
Cognition			
BM-Frequency of revisit to old box location	$F_{1, 43}$= 0.27, p=0.61	$F_{1, 43}$= 1.91, p=0.17	$F_{1, 43}$= 0.12, p=0.73
BM-reference memory error	$F_{1, 42}$= 1.68, p=0.2	$F_{1, 42}$= 0.69, p=0.41	$F_{1, 42}$= 0.34, p=0.57
BM-working memory error	$F_{1, 38}$= 0.46, p=0.5	$F_{1, 38}$= 0.0003, p=0.99	$F_{1, 38}$= 0.0003, p=0.99



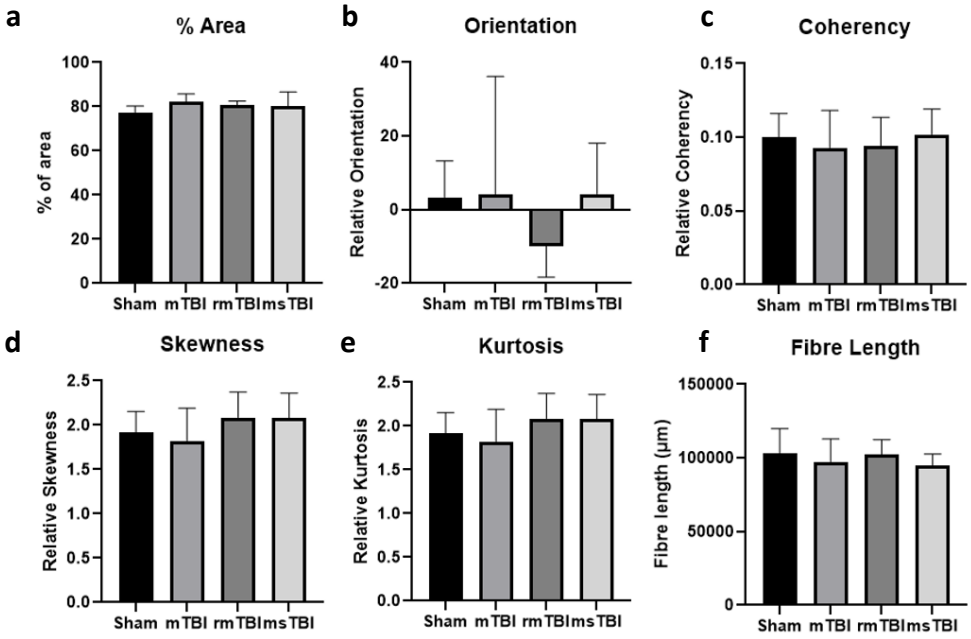
Supplementary Figure 1 (Arulsamy 2019)

Molecular analysis on the prefrontal cortex at 12 months was measured using semi-quantitative western blotting to analyse A) neuronal survival (total neurons, NeuN marker), B) integrity (synaptophysin marker) and C-D) structure damage (NF-L and MBP markers). GAPDH was used as a housekeeper protein for all analysis. Graphs represent the mean± SEM. Representative images of the western blots were extracted from Image Studio Lite.



Supplementary Figure 2

MRI analysis in PFC at 12-months post different severities of injury. (a) Template image-Fibre orientation distribution; (b) Fixels originate from the PFC, with fixels assessed in colours other than navy blue. (c) Fixels assessed in the analysis (d) Area determined as PFC by ITKS-SNAP, (e) Fibre density, (f) Fibre Cross-section, (g)Interaction between Fibre density and cross section in PFC. Data are presented as mean±SEM, One-way ANOVA was performed.



Supplementary Figure 3

Immunohistochemistry analysis of MBP in PFC at 12-months post different severities of injury. (a) Percentage of area, (b) orientation, (c) coherency, (d) skewness, (e) kurtosis, and (f) fibre length. Data are presented as mean±SEM, One-way ANOVA was performed.

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EDITED BY
John K. Yue,
University of California, San Francisco,
United States

REVIEWED BY
Brandon Peter Lucke-Wold,
University of Florida, United States
Catherine Suen,
University of Utah Hospital, United States
J. Russell Huie,
University of California, San Francisco,
United States

*CORRESPONDENCE
Lyndsey E. Collins-Praino
✉ lyndsey.collins-praino@adelaide.edu.au

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Chronic motor performance following different traumatic brain injury severity—A systematic review

Frances Corrigan[1], Ing Chee Wee[2] and Lyndsey E. Collins-Praino[2]*

[1]Head Injury Lab, School of Biomedicine, The University of Adelaide, Adelaide, SA, Australia, [2]Cognition, Ageing and Neurodegenerative Disease Laboratory, School of Biomedicine, The University of Adelaide, Adelaide, SA, Australia

Introduction: Traumatic brain injury (TBI) is now known to be a chronic disease, causing ongoing neurodegeneration and linked to increased risk of neurodegenerative motor diseases, such as Parkinson's disease and amyotrophic lateral sclerosis. While the presentation of motor deficits acutely following traumatic brain injury is well-documented, however, less is known about how these evolve in the long-term post-injury, or how the initial severity of injury affects these outcomes. The purpose of this review, therefore, was to examine objective assessment of chronic motor impairment across the spectrum of TBI in both preclinical and clinical models.

Methods: PubMed, Embase, Scopus, and PsycINFO databases were searched with a search strategy containing key search terms for TBI and motor function. Original research articles reporting chronic motor outcomes with a clearly defined TBI severity (mild, repeated mild, moderate, moderate–severe, and severe) in an adult population were included.

Results: A total of 97 studies met the inclusion criteria, incorporating 62 preclinical and 35 clinical studies. Motor domains examined included neuroscore, gait, fine-motor, balance, and locomotion for preclinical studies and neuroscore, fine-motor, posture, and gait for clinical studies. There was little consensus among the articles presented, with extensive differences both in assessment methodology of the tests and parameters reported. In general, an effect of severity was seen, with more severe injury leading to persistent motor deficits, although subtle fine motor deficits were also seen clinically following repeated injury. Only six clinical studies investigated motor outcomes beyond 10 years post-injury and two preclinical studies to 18–24 months post-injury, and, as such, the interaction between a previous TBI and aging on motor performance is yet to be comprehensively examined.

Conclusion: Further research is required to establish standardized motor assessment procedures to fully characterize chronic motor impairment across the spectrum of TBI with comprehensive outcomes and consistent protocols. Longitudinal studies investigating the same cohort over time are also a key for understanding the interaction between TBI and aging. This is particularly critical, given the risk of neurodegenerative motor disease development following TBI.

KEYWORDS

traumatic brain injury, neurodegenerative movement disorder, motor performance, long-term outcomes, systematic review

1. Introduction

Although once thought of as an acute event, it is now well recognized that traumatic brain injury (TBI) leads to long-lasting disability in a subset of individuals (1–3), including persistent impairments in memory, decision-making, and motor function. Following even mild TBI, 53% of individuals still report functional limitations at 12 months post-injury (4). Such impairments significantly impact an individual's quality of life, affecting social relationships and ability to return to work (5). Mobility, in particular, has been shown to be an important mediator of the relationship between TBI and quality of life following injury (6) with more functional impairment associated with decreases in life satisfaction (7).

Acutely, TBI leads to several neuromotor deficits which are injury severity dependent. Mild TBI most commonly presents with balance disturbance and poor coordination, (8, 9) while severe TBI can lead to spastic paralysis, impaired motor coordination with postural instability and gait abnormalities, and reduced fine motor control (10). Motor impairment has been particularly well-characterized to occur following moderate–severe TBI, with nearly 78% of individuals reporting some level of impairment on gross neuromotor examination during acute rehabilitation (10). Studies focused on the 1st year post-injury in moderate–severe TBI have shown that most motor recovery is reached within 6 months post-injury (11–13), with patients not showing significant functional improvement over the latter part of the year (12, 14, 15). In line with this, 30% of individuals reported difficulty in walking unaided up to 2 years following moderate–severe injury (16), with 25% of individuals still reporting upper- or lower-limb motor difficulty and 43% reporting balance difficulties, even 4 years after a severe brain injury (17). Conversely, following a mild TBI, impairments generally resolve within days to weeks post-injury, although some level of motor dysfunction may persist in at least a subset of individuals [see Chmieliewski et al. for review (18)]. In support of this, slowed motor execution speed and impaired postural control have been reported up to 9 months following concussion in university football players, compared to healthy, non-concussed controls (19).

Despite evidence that motor impairment may persist chronically following TBI, however, examination of the evolution of specific motor deficits long-term following TBI has received comparatively little attention in the literature. Indeed, particularly in clinical research, published TBI outcome studies are skewed toward global measures and/or measures within the behavioral and cognitive, rather than physical, domains. In addition, of studies that do report physical outcomes, most utilize gross functional or disability instruments, rather than dissecting specific types of motor impairment. For example, utilizing the Rivermead Concussion scale, Theadom found 28.5% of participants reporting dizziness at 12 months following mild TBI, (20) which is in line with an earlier Ponsford et al. study, where, on structured interview 2 years post-TBI, 36% of patients reported dizziness (21). Studies where specific motor impairments are reported typically examine only one motor domain; for example, Williams et al. examined chronic gait dysfunction following severe TBI (22–24) and Pearce et al. the effects of prior concussion on fine motor performance (25, 26). Even in preclinical studies, the behavioral batteries employed typically only consist of 1–2 motor specific tasks (27–36) and, thus, cannot provide a comprehensive overview on how TBI influences motor performance as a whole.

Understanding the persistent nature of motor impairment following TBI is critical, as impaired motor control following concussion has been shown to increase risk for subsequent musculoskeletal injury (18) and falling (37). TBI is also linked to an elevated risk of developing neurodegenerative diseases associated with motor symptoms, including motor neuron disease (38) and Parkinson's disease (PD) (39). For example, multiple studies have established a link between TBI and the later development of PD, with Gardner et al. (40) recently reporting that mild TBI increases risk of PD by 56%, while moderate/severe TBI increases PD risk by 83%. More recently, Russell and colleagues reported in a retrospective cohort study that Scottish former rugby players had a higher incident rate of neurodegenerative diseases, including both PD [HR/OR (95% CI) = 3.04 (1.51–6.10)] and motor neuron disease [HR/OR (95% CI) = 15.17 (2.10–178.96)] compared to a matched comparison group from the general population over a 32-year median follow-up period from study entry (11.4 vs. 5.4%) (41). This is consistent with growing neuroimaging evidence that TBI leads to ongoing neurodegeneration. In the months to years following injury, progressive lesion expansion occurs concomitant with white and gray matter atrophy and loss of white matter integrity (42–45). Importantly, structures affected include those critical for motor function, such as the striatum (46), thalamus (47), and cerebellum (47).

Considering the high prevalence of TBI, a fuller description of neuromotor deficits, stratified by motor domain, in the gross or fine motor will provide insight into the global recovery process and rehabilitation needs of persons with TBI. In addition, given that motor function may play a crucial role in linking TBI to the later emergence of neurodegenerative movement disorders, examining specific motor changes that occur long-term following injury could serve as a novel method for identifying the risk of these diseases. As such, the aim of this systematic review was to review all original research reports that assessed chronic motor outcomes following TBI, stratified by injury severity in both preclinical models and patient populations.

2. Methods

2.1. Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (48) were used. A comprehensive literature search was performed in May 2019, with an updated search undertaken in March 2022, using the electronic databases PubMed, Embase, Scopus, and PsycINFO to identify relevant publications. The search strategy was developed based on an initial scoping search and in consultation with a health and medical sciences librarian. The search terms used were "traumatic brain injury", "Parkinson's disease", "motor neuron disease" and "motor performance," or variations thereof that were combined using "AND" and "OR" search operators. The developed search

strategy is depicted in Supplementary Table 1. Further searches were performed in the reference lists from included studies.

2.2. Study selection: inclusion and exclusion criteria

Following the search, identified articles were imported into EndNote X9.3.3 and duplicates were removed either by the EndNote "delete duplicates" function or deleted manually. Titles and abstracts were then screened, with clinical studies reporting motor outcomes >1 year post-injury and preclinical studies reporting motor outcomes >30 days post-injury retained. For articles that passed this preliminary assessment, the full-text article was retrieved and screened for eligibility against the inclusion and exclusion criteria. The eligibility of articles was assessed by two independent reviewers. Any conflicts were resolved via discussion, and if a consensus could not be reached, a third reviewer was consulted. A flowchart with reasons for the exclusion of studies is displayed in Figure 1.

2.3. Inclusion criteria

The following inclusion criteria were utilized:

(i) An original research article published in English.
(ii) Investigated an adult population (preclinical: 8 weeks or older; clinical: 18 years or older) with a prior history of TBI.
(iii) Assessed long-term motor performance (preclinical: >30 days post-injury; clinical: mean time since TBI > 1 year).
(iv) Clear classification of TBI severity [Preclinical: required a comprehensive description of the TBI model and parameters used to induce injury; clinical: Provided Glasgow Coma Scale (GCS), Westmead Post-Traumatic Amnesia scale (PTA), and/or loss of consciousness (LOC) duration].
(v) Compared motor performance with a control group.

The search had no restrictions on the year of publication; however, only English language publications were included. Databases were searched from inception.

2.4. Exclusion criteria

Studies were excluded from further consideration as follows:

(i) Reported outcome measurements that were not purely motor (e.g., cognition, visuomotor integration/coordination, social preference, or quality of life).
(ii) Did not specifically state month/time post-TBI, injury severity, or motor outcomes assessed.
(iii) Pilot studies that had a sample size of a single group of less than 5.
(iv) Studies were single case reports/expert options.
(v) Studies were review articles or conference abstracts.
(vi) No specific statistical comparison was reported for injured compared to sham/naïve animals in treatment studies, with treatment effects not the focus of the current review.

2.5. Data extraction and synthesis

Data were extracted from the included studies by two independent reviewers. Data extracted included study characteristics (author, year of publication, study design, motor function measurement, injury method in preclinical studies, description of TBI severity for clinical studies); participant/TBI preclinical model characteristics (sample size, age, sex, history of TBI/frequency of injury, time point assessed post-injury, mechanism of injury, and TBI severity); primary methods/functional tests used to measure motor performance, and primary or secondary outcome(s) of motor performance. A copy of the data extraction template is found in Supplementary Table 2.

Due to the large diversity of motor outcome measures used across the study, the measurements were categorized into different motor functions and analyzed separately. The categorization of the motor outcome measures is outlined in Supplementary Table 3. In order to assess the effect of TBI severity on motor performance, outcomes were further stratified by injury severity. The evaluation of TBI severity was classified as described in Supplementary Table 4. Injury severity in preclinical studies was separated into five groups: (i) single mild; (ii) repetitive mild; (iii) moderate; (iv) moderate–severe; and (v) severe. A similar classification system was used for clinical TBI, with minor modifications. As some individuals had experienced more than one injury, the following groups were used: (i) single mild, (ii) the combination of single and repetitive mild (prior TBI history ranged from 1 to more), (iii) repetitive mild (>1 prior TBI), (iv) moderate–severe, and (v) severe.

2.6. Assessment of methodological quality

Articles included in the study were assessed for methodological quality by one reviewer (IW), with confirmation provided by a second reviewer (LCP or FC), by using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool (preclinical) (49) and the Cochrane Risk of Bias Tool (clinical) (50). Studies were judged as having a low, unclear, or high risk of bias in the following domains: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. The overall risk of bias for each included study was categorized as "strong quality" if the risk of bias was low in 70% or more of the criteria, "low quality" if the risk of bias was high in at least 30% of the criteria, and "moderate quality" if the risk of bias fell between these two parameters. Disagreements were resolved by consensus. Summary graphs were created in Review Manager (RevMan) ([Computer program], Version 5.4.1 Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014).

3. Results

3.1. Search outcomes

The initial search yielded 28,172 articles. From these, 9,324 duplicates were removed with the Endnote function, and another 18,002 were excluded after reviewing the title and abstracts

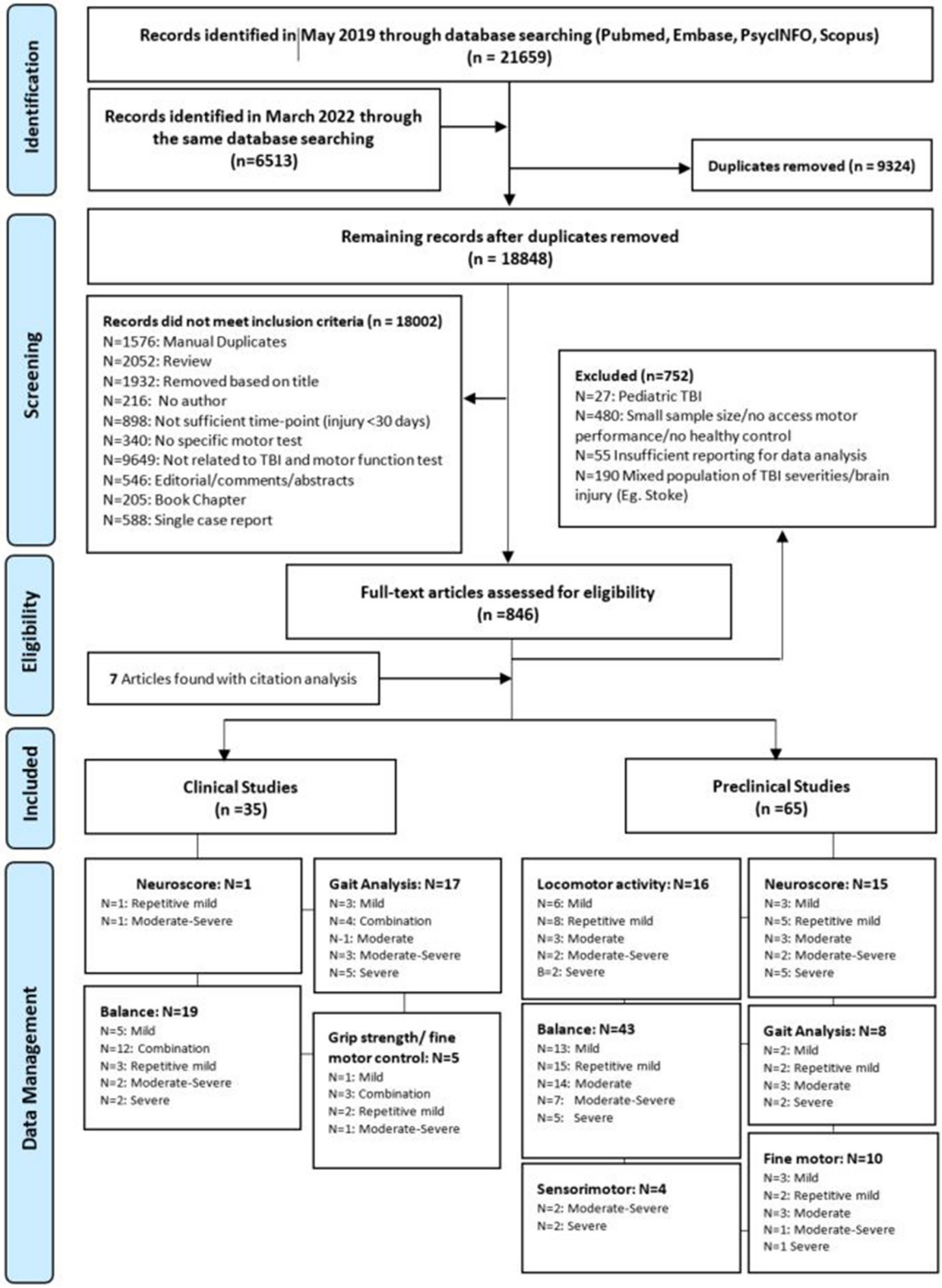

FIGURE 1
PRIMSA flow diagram outlining the article selection and screening process and subsequent data management. The sum of each domain indicates the total number of publications investigated that motor function. One study could report more than one injury severity or more than one motor functional test.

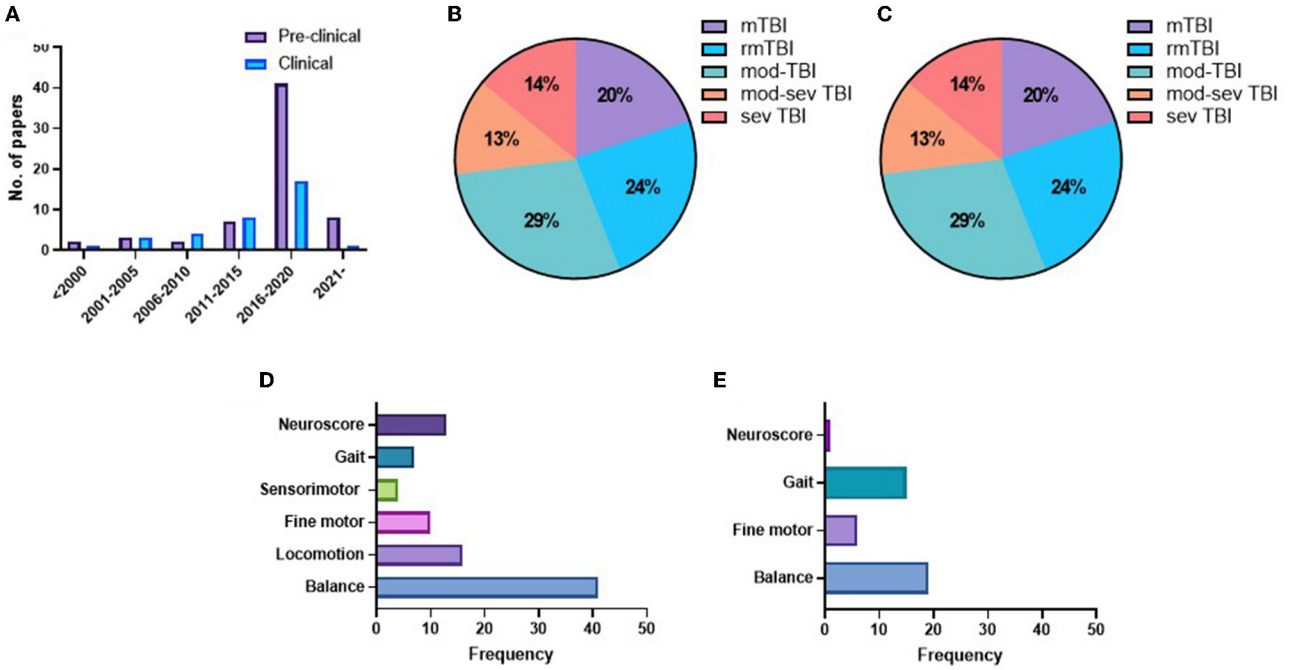

FIGURE 2
Number of publications per year **(A)**. Pie charts indicating the representation of different injury severities in preclinical **(B)** and clinical studies **(C)**. Frequency charts indicating the number of studies that reported on each motor domain in preclinical **(D)** and clinical studies **(E)**.

(Figure 1). Full-text analysis was then performed on the remaining 846 articles, of which only 93 met inclusion criteria, with seven additional articles identified from a search of the citation lists of included studies. All articles ($n = 100$) were then separated into preclinical ($n = 65$) and clinical ($n = 35$) subgroups for further analysis. Stratification based on motor outcomes and injury severity was performed as described above. Details of this process are described in the PRISMA diagram (Figure 1).

3.2. Study characteristics

The earliest included study was published in 1997, and the rate of publications/year was low until 2016, when the rate of publications increased markedly, particularly for preclinical studies (Figure 2A). In the preclinical studies, there was an even split between the use of mice (50%) and rats (50%). The vast majority of preclinical studies used male animals (82%), with only 11.3% reporting the use of female animals, a further 1.7% using both sexes and four studies (5%) not mentioning the sex of the animals used. In clinical studies, the majority of studies (23/35) had more than 60% male TBI participants (19, 22–26, 40, 51–66), including five studies with only male participants (19, 25, 26, 55, 64), nine studies had a TBI cohort with 70–45% female participants (67–75), and three studies did not state the sex of TBI participants (76–78).

Sample sizes varied across the studies ranging from 6 to 49 animals in a group in preclinical studies. The sample size for the included clinical studies was consistently low, with the majority ranging from 16 to 66 participants, with only a few moderately sized (111–453 individuals)[32–395–125–125–125–12] and one large study (4,007 subjects) (55). The source populations of clinical studies varied widely including rehabilitation institutes (22, 23, 59, 67), professional athletes (25, 26), college students (54, 71, 78), college athletes (19, 60, 61, 63, 68, 73, 76), military veterans (40, 52–56, 65, 69), and the community (51, 54, 58, 64, 70, 72, 75, 77).

In preclinical studies, there was a relatively even split across the injury severities, with 20% mTBI, 24% rmTBI, 29% moderate TBI, 13% moderate–severe TBI, and 14% severe TBI (Figure 2B). In comparison, the majority of clinical studies examined a combination of single and repetitive mild TBI (42.5%), with a further 17% reporting single mTBI and 12.5% rmTBI. Moderate–severe TBI was included in only 12.5% of studies and severe TBI in just 15% of studies (Figure 2C). The motor domain most commonly examined in preclinical studies was balanced (43/63 studies), with a much smaller number examining locomotion (16), neuroscore (15), fine motor (10), and gait (8) (Figure 2D). In contrast, in clinical studies, balance (19) and gait (15) were most commonly tested, with fewer studies investigating fine motor control (6) or a neuroscore (1) (Figure 2E).

3.3. Risk of bias

Examination of risk of bias found that of the preclinical studies, only seven were of strong quality, 24 were of moderate quality, and 31 studies were of low quality (Supplementary Figure 1 for complete evaluation and Figure 3 for summary data). Of the clinical studies, only one was of strong quality, one of moderate quality, and the remaining 32 were of low quality (Figure 4). Key sources of bias for preclinical studies included selection bias and detection bias,

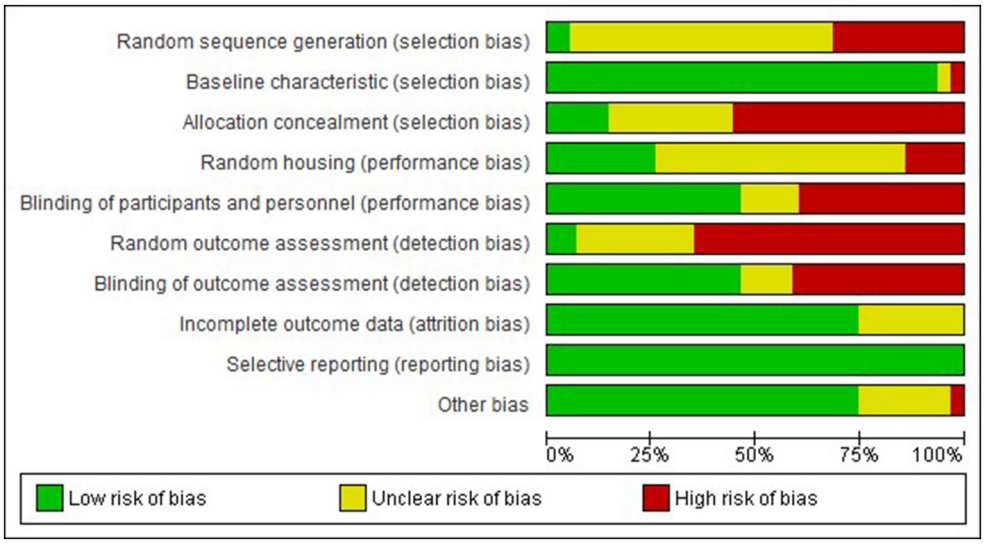

FIGURE 3
Risk of bias graph-Preclinical. Review authors' judgement about each risk of bias item presented as percentages across all included studies.

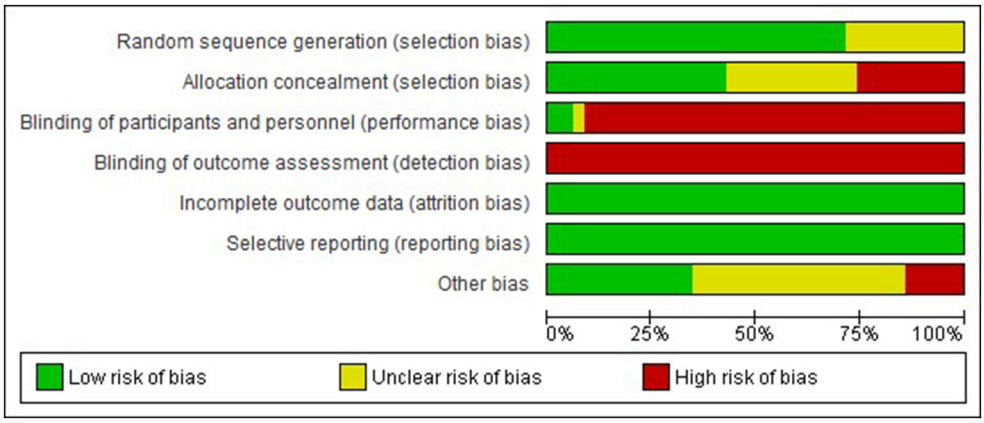

FIGURE 4
Risk of bias graph-Clinical. Review authors' judgement about each risk of bias item presented as percentages across all included studies.

whereas for clinical studies, they included selection, performance, and detection bias.

3.4. Preclinical motor outcomes

3.4.1. Neuroscore

Overall, a focal injury was required to lead to persistent decreases in neuroscore, with minimal changes seen with diffuse injury. It should be noted the neurological severity scoring system varied widely among the 15 included studies, with the standard neurological severity score (79–85), modified neurological severity score (86–88), and revised neurological severity score (89) all represented. Even articles that used the same names for their scoring systems incorporated different tasks within their behavioral battery (Supplementary Table 4). The most commonly included items were those from the standard neurological severity score, including forelimb and hindlimb flexion on tail suspension (10 of 15 studies) (79, 81–85, 87, 89–91), forelimb/hindlimb placement on a flat surface (5/15) (79, 83, 84, 87, 90), and resistance to lateral pulsion (6/15) (79, 82, 84, 85, 90, 91). Other tests that were included were simple reflexes (limb, tail, corneal, and startle), with different combinations used in seven studies (30, 80, 81, 87–89, 92). Measures of hemiparesis, either via direct assessment of hemiplegia (30, 80, 86) or circling behavior (80, 86, 87, 91), were included in six studies. Balance was examined as ability to stay on a round beam (80, 86, 87), a flat surface (80, 86), or an inclined plane (79, 82, 83), as well as the ability to walk across beams of different widths (1, 2, and 3 cm) (80, 86, 88, 89, 92), with a measure of balance incorporated into nine of the 15 studies. General activity was assessed either directly (90) or via ability to exit a circle (86) or seeking behavior (86, 92) in three studies. Direct assessment of gait

as performance on the treadmill was included in one study (30). Given the different combinations of tests which reflect different types of motor behaviour were included in the neurological severity score across the studies, direct comparisons between studies is difficult. Nonetheless, in studies utilizing focal or mixed moderate (30, 81, 87), moderate–severe (90, 91), and severe TBI models (82–85, 92), a consistent impairment was found in neuroscore, regardless of the scoring system implemented, out to 8 months post-injury, with no further deficits noted in the one study that included a 12-month time point (Table 1) (82). In general, no chronic deficits in neuroscore were noted in preclinical models of smTBI (79, 80) or rmTBI (79, 80, 86, 89), with 7 months as the latest time point examined. Only one study utilizing a projectile concussive impact where a steel ball is projected at the rat head which is protected by a steel helmet found chronic neuroscore deficits at 3 months post-injury in both single and repeated (4 x mTBI 1 h apart) animals (88).

3.4.2. Gait analysis

Gait was analyzed post-TBI using automated systems such as the CatWalk, requiring animals to actively walk across a platform (28, 29, 31, 88, 93, 94), or the DigiGait apparatus, which utilizes a treadmill at a steady speed (27, 30). In general, the studies were selective in reporting gait parameters, not providing a comprehensive overview of the different measures in the methods and often only reporting selective results, with some exceptions (88) (Table 2). No single impact study reported chronic changes in speed or cadence following TBI, with this either directly reported (29, 31, 94), or not included within the results (27, 28, 30, 93), with an acute decrease in speed only noted at 24 h following severe focal injury (31). Indeed persistent reductions in cadence were only noted when four impacts were delivered 1 h apart, with this deficit persisting to 3 months post-injury (88).

However, following even single mTBI, subtle gait alterations were noted with free ambulation of the CatWalk including a decreased front base of support seen acutely and persisting up to 3 months post-injury (1, 2). Namdar et al. (93), with a decrease in hind base of support also developing at the 3-month time point (88). Repeated injury led to more prominent gait abnormalities, with the 4 × 1 h apart injury model leading to persistent deficits to 3 months post-injury in single-stance time, stride length, stand time, and front base of support, with the deficit in hind base support again only developing at 3 months post-injury (88). In contrast, on a treadmill, repeat injury (5× mTBI 24 or 48 h apart) led to no deficits in gait either acutely or at 1 month, although different gait parameters were reported across studies, including gait symmetry, paw contact area, and hindlimb shared stance (27).

Mixed results were also found for gait following more severe injury. Following focal moderate CCI injury, no deficits were detected from 24 h to 1 month post-injury on the CatWalk, with reporting of measures like paw contract area, stance, swing speed, base of support, and interlimb coordination (28). In contrast, Ritzel et al. only examined gait at 26 weeks post-moderate CCI injury and reported a number of impairments, including reduced stride length of the contralateral right hindlimb and reduced swing speed in the right hind and forelimb, but no overall change in average speed (29). Similarly, Daglas et al. utilizing the DigiGait treadmill apparatus found significant reductions in contralateral swing duration of the right hindlimb with compensatory right forelimb propulsion duration from 1–32 weeks post-moderate CCI injury (30). Surprisingly, minimal chronic deficits were reported following severe CCI injury, with Cline et al. only detecting deficits at 24 h, but not 1 month, post-injury, with the exception of swing duration of the contralateral hindlimb (31). Similarly, Schönfeld et al. also found no alterations in stride length, base of support, or three limb support at 1 month following injury (94).

3.4.3. Sensorimotor control

The sensorimotor function was primarily evaluated with the adhesive removal test (1–4), with severe injury required to produce chronic deficits. The number of trials evaluated varied between studies ranging from 1 to 6, as did the presentation of results, which included % sham (95), latency to remove the adhesive (83, 96), and difference in performance between preferred and non-preferred paw (94) (Table 3). Severe injury, either focal (94) or mixed (83), led to persistent deficits in adhesive removal out to 16 weeks post-injury, the latest time point assessed. In contrast, following moderate–severe injury, neither focal (95) nor diffuse (96) injury led to chronic deficits in adhesive removal, with deficits noted up to 6 days following diffuse injury and 3 weeks following focal injury, with no further impairment noted to 41 days post-injury, the latest time point assessed. Interestingly, the whisker-evoked forepaw placement task did detect chronic deficits in the diffuse injury moderate–severe model with impaired forepaw placement out to 41 days post-injury (96), indicating potential task-dependent effects. To date, no studies have evaluated sensorimotor function chronically following either a single mTBI or repeated mTBI.

3.4.4. Grip strength and fine motor

Grip strength and fine motor ability were assessed via the grip strength meter (30, 93, 97–100), isometric pull task (33, 101), pellet reaching tasking (34), and Montoya staircase test (94) (Table 4). The grip strength meter requires minimal pre-training, whereas the other tasks involve more extensive training with food restriction, with the test relying on the animal's desire to obtain sugar pellets as a reward for completing the task successfully. Overall, with single diffuse injury, moderate (32, 33, 101), but not mild, injury (93, 97, 98) led to chronic grip strength deficits. However, the protocol for assessing grip strength varied between studies, with the number of trials examined ranging from 1 to 10. Following diffuse single mTBI, no deficits were detected on grip strength up to 12 weeks post-injury (93, 97, 98), with only one study finding an acute deficit at 1 week post-injury (98). In contrast, diffuse moderate TBI did produce deficits in grip strength at 28 days post-injury, the latest time point assessed (32, 33, 101). For repeated mild injury, bilateral mTBI weekly for 5 weeks led to impaired grip strength at 40–45 weeks post-injury (100), whereas 3 impacts 24 h apart led to no deficits at 30 days post-injury, (99) with these the only time points assessed in these studies. Notably, with the negative result, the average of three trials relative to body weight was recorded (99), whereas the positive result was only one trial reported as maximum force achieved, introducing a potential confound (100). To date, no

TABLE 1 Preclinical neurological severity score evaluation.

References	Years	Method	Severity	Type injury	Sample	Pre	<72h	1–3wks	1–2mo	3–5mo	6–11mo	12–24mo
Laurer et al. (79)	2001	CCI-CS	Mild	Diffuse	T:16–24; C:14–23		+	-	-			
Fehily et al. (80)	2019	WD	Mild	Diffuse	T:15; C:15					-		
Mountney et al. (88)	2017	PCS	Mild	Diffuse	T/C: 8–10					+		
Mountney et al. (88)	2017	PCS	Rep-Mild	Diffuse 4 × TBI/1hr	T/C: 8–10					+++		
Feng et al. (89)	2021	CHIMERA	Rep-Mild	Diffuse 3xTBI/day × 2 48 hrs	T:9–13; C:12–14		-	-	-			
Laurer et al. (79)	2001	CCI-CS	Rep-Mild	Diffuse 2xTBI 24 hrs	T:16–24; C:14–23		+++	+++	-			
Fehily et al. (80)	2019	WD	Rep-Mild	Diffuse 2xTBI 24 hr	T:15; C:15					-		
			Rep-Mild	Diffuse 3xTBI 24 hr	T:15; C:15					-		
Huynh et al. (86)	2020	CCI-CS	Rep-Mild	Left sided 5xTBI, 48 hr	T:15; C:15					-	-	
Zhang et al. (87)	2021	CCI	Moderate	Focal	T:10; C:10		++	++	++			
Daglas et al. (30)	2019	CCI	Moderate	Focal	T:12; C:12		++++	++++	++++	++++	++++	
Sell et al. (81)	2017	LFP	Moderate	Mixed	T:11–14; C:12–14		+++	/	/	+++	-	-
Shear et al. (91)	2010	PBBI	Mod-Sev	Focal	T/C=10		+	+	+			
Wang et al. (90)	2019	Punch	Mod-Sev	Focal	T:8; C:8		++	++	++			
Segovia et al. (85)	2020	LFP	Severe	Mixed	T:7; C:7	-	+++		+++			
Nissinen et al. (84)	2017	LFP	Severe	Mixed	T:35; C:16	-	+++	+++	+++			
Zhau	2021	CCI	Severe	Focal	T:10; C:10		+++	+++	+++			
Zhang et al. (83)	2005	LFP	Severe	Mixed	T:14; C:24			++	++	++		
Pierce et al. (82)	1998	LFP	Severe	Mixed	T:12–16; C:11–15		+	+	+		+	-

$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; $^{++++}p < 0.0001$; -, not significant; Gray, not evaluated. CCI-CS, controlled cortical impact-closed skull; WD, weight drop; CHIMERA, Closed-Head Impact Model of Engineered Rotational Acceleration; LFP, lateral fluid percussion; CCI, controlled cortical impact; PBBI, penetrating ballistic like impact.

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TABLE 2 Preclinical gait evaluation.

References	Years	Method	Severity	Type injury	Apparatus	Parameters examined	Sample	Pre	<72h	1–3wk	1–2mo	3–5mo	6–11mo	12–24mo
Namdar et al. (93)	2020	WD	Mild	Diffuse	CatWalk	Front base of support	T:15; C:13			+	+++			
						Standing on diagonal two				+	-			
						Standing on three				++	+			
						Hind base of support				-	-			
Mountney et al. (88)	2017	PCI	Mild	Diffuse	CatWalk	Stand (sec)	T/C: 8–10		+	-	-			
						Stand index			+	-	-			
						Swing (sec)			-	-	-			
						Step cycle (sec)			-	-	-			
						Single stance			-	-	-			
						Stride length			-	-	+			
						Front base of support			++	+	+++			
						Hind base of support			-	-	+++			
						Three limb support			-	-	-			
						Cadence			-	-	-			
Bolton	2016	CCI-CS	Rep-Mild	5 × TBI 24 hrs	Digigait, treadmill 15 cm/s	Gait symmetry	T/C: 10		-		-			
						Hindlimb shared stance			-		-			
						Paw contact area			-		-			
Bolton et al. (27)	2016	CCI-CS	Rep-Mild	5 × TBI 48 hrs	Digigait, treadmill 15 cm/s	Gait symmetry			-		-			
						Hindlimb shared stance			-		-			
						Paw contact area								
Mountney et al. (88)	2017	PCI	Mild	Diffuse 4 × TBI/1hr	CatWalk	Stand (sec)	T/C: 8–10		+++	+	-			
						Stand index			++	++	++			
						Swing (sec)			+++	+	+			
						Step cycle (sec)			+++	+++	+			

(Continued)

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TABLE 2 (Continued)

References	Years	Method	Severity	Type injury	Apparatus	Parameters examined	Sample	Pre	<72h	1–3wk	1–2mo	3–5mo	6–11mo	12–24mo
						Single stance			+++	+	+			
						Stride length			-	+	-			
						Front base of support			++	+	+++			
						Hind base of support			-	-	+++			
						Three limb support			++	+	-			
						Cadence			+++	+++	++			
Henry et al. (28)	2020	CCI	Moderate	Focal	CatWalk	Paw contact area	T:12; C:12	-	-	-	-			
						Stance, swing								
						Speed								
						Interlimb coordination								
						Base of support								
						%Support time								
Ritzel et al. (29)	2020	CCI	Moderate	Focal	CatWalk	Step Sequence	T/C: 16–23						+	
						Stride length (RH)							+	
						Swing speed (RF,RH)							+	
						Print position							+	
						Average speed, number of steps							-	
Daglas et al. (30)	2019	CCI	Moderate	Focal	DigiGait 15 cm/s	Swing duration (RH)	T:12; C:12	-		+	+	+	++	
						Propulsion duration (RF)		-		-	++	+	+++	
Cline et al. (31)	2017	CCI	Severe	Focal	CatWalk	Cadence	T:15; C:14		++		-			
						Average Speed			++		-			
						Swing duration (RH)			++		+			
						Average Swing speed (LF, RF, RH)			+		-			
Schönfeld et al. (94)	2017	CCI	Severe	Focal	CatWalk	Stride length Base of support Three limb support; Speed Cadence	T:10; C:7	-		-	-			

$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; -, not significant; Gray, not evaluated. T, TBI; C, control; RH, right hindlimb; RF, right forelimb; LF, left forelimb; CCI-CS, controlled cortical impact-closed skull; WD, weight drop; PCI, projectile concussive impact.

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TABLE 3 Preclinical sensorimotor evaluation.

References	Years	Method	Severity	Type Injury	Test	Parameters examined	Sample	Pre	<72h	1–3wks	1–2mo	3–5 mo	6–11 mo	12–24mo
Hoffman et al. (95)	2003	CCI	Mod-Sev	Focal	Adhesive Test	3 × 2 min trials %sham	T:8; C:8			+	-			
Alwis et al. (96)	2012	WD	Mod-Sev	Diffuse	Adhesive Test	1 trial Latency	T:19; C: 12	-	+	-	-			
					Whisker-evoked forepaw placement	10 trials No. correct	T:4; C:4		+	+	+			
Schönfeld et al. (94)	2017	CCI	Severe	Focal	Adhesive Test	3 trials Paw difference	T:10; C: 6	-		+	+			
Zhang et al. (83)	2005	LFP	Severe	Mixed	Adhesive Test	6 trials Latency	T:14; C:13			‡‡	‡‡	‡‡		

$^{+}p < 0.05$; $^{++}p < 0.01$; -, not significant; Gray, not evaluated. T, TBI; C, control/; CCI, control cortical impact; WD, weight drop injury; LFP, lateral fluid percussion injury.

study has assessed grip strength alterations chronically following a severe TBI. Another test of strength, the isometric pull task, was used in two studies of focal moderate TBI, in which the lesion was specifically located over the motor cortex (33, 101). In this task, rats were trained prior to injury to reach a force threshold of at least 120 g within 2 s on a pull lever in order to receive a food reward. Following injury, rats had a decrease in maximal force produced, a decrease in the % of successful trials and decrease in the speed of force generation from weeks 1–6 post-injury (33, 101). However, in successful trials, the time taken to reach the 120 g threshold was only significantly increased in Weeks 1–2, returning to sham level from Week 3. This mirrored results for total trials, which were significantly decreased from Weeks 1–2 before returning to sham level, potentially indicating reduced motivation as well (33). However, given the focal nature of the injury in these studies, it is difficult to know whether these findings would transfer to a diffuse injury model.

Fine motor skills were assessed by Adkins et al. (34) and Schönfeld et al. (94) using variations of a pellet reaching task following moderate–severe and severe focal injury, respectively, with significant deficits found out to 6 weeks post-injury. In the Adkins et al. study, the pellets were located on a flat surface in front of the animals (34), while Schönfeld et al. (94) used a staircase with pellets placed on increasing higher steps to enhance difficulty. In the pellet reaching task, injured animals had a decrease in the % successful reaching attempts to 42 days post-injury (34). In the Montoya staircase task, injured rats obtained significantly fewer pellets across all steps, made less reaching attempts, and misplaced more pellets on the upper steps (94).

3.4.5. Locomotor activity

The open-field test, the most commonly used task to measure general locomotor activity levels by examining the total distance traveled over a test period (5–60 min), was used across all included studies (Table 5). Following diffuse mTBI, either single or repeated, locomotor results varied depending on the species (rat vs. mice), strain, protocol, and apparatus employed (Table 5). The utilization of a smaller apparatus (19 × 11 cm) following weight drop TBI in Swiss mice over a 60-min period found persistent hyperactivity from 48 h to 12 weeks following injury (102). In contrast, closed skull CCI injury in C57BL/6J mice led to no changes in locomotion over 30 min in a larger 49 × 36 cm arena either acutely or chronically up to 90 days post-injury (103). Indeed, repeated impacts over a short interval (Morriss et al.: 5 × 24 h; Tucker et al.: 3 × 24h) were required to replicate this hyperactivity in mice in a larger arena (40 × 40 cm), with this behavior developing at 3 months post-injury (104, 105) and persisting to 12 months post-injury (105), the latest time point examined. With 2 CCI-CS impacts over 3 days, changes in locomotion were no longer observed in mice in a similar size arena over 30 min on day 1, day 7, or 12 weeks post-injury (103).

Varied results have been reported in rat studies. Mild weight drop TBI in Sprague-Dawley rats found no difference in locomotion in a 60 × 60 cm arena over 10 min at either 1 or 4 weeks post-injury (93), although a decrease in locomotion has been reported at 6 weeks, resolving by 12 weeks post-injury in a larger

TABLE 4 Preclinical grip strength and fine motor evaluation.

References	Years	Method	Severity	Type Injury	Test	Parameters examined	Sample	Pre	<72h	1–3 wks	1–2mo	3–5mo	6–11mo	12–24mo
Evans et al. (98)	2014	CCI-CS	Mild	Diffuse	Grip strength meter	Average 10 trials	T9; C8		-	+	-	-		
Namdar et al. (93)	2020	WD	Mild	Diffuse	Grip strength meter	5 trials, average best 3	T:10; C:8			-	-			
Evans et al. (97)	2015	CCI-CS	Mild	Diffuse	Grip strength meter	Average 10 trials	T:12; C:11		-	-	-			
Tabet et al. (99)	2022	CCI-CS	Rep-Mild	3× TBI 24 hrs	Grip strength meter	Average 3 trials relative to weight	T:11; C:11				-			
Dhillon et al. (100)	2020	CCI-CS	Rep-Mild	2xTBI ($L^{+}R$) 5 × weekly	Grip strength meter	1 trial	T:10; C:8						+	
Rana et al. (32)	2020	WD	Moderate	Diffuse	Grip strength meter	1 trial	T:7; C:5			+	+			
Pruitt et al. (33)	2014	CCI	Moderate	Focal (motor cortex)	Isometric pull task	Maximal Force	T:15; C:11	-		+	+			
						% Successful Trials		-		+	+			
						Time to 120 g threshold		-		+	-			
						Speed force generation		-		+	+			
						Total Trials		-		+	-			
Pruitt et al. (97)	2017	CCI	Moderate	Focal (motor cortex)	Isometric pull task	Maximal Force	T:6; C: 6	-		+	+			
						% Successful Trials				+	+			
Adkins et la. (34)	2015	CCI	Mod-Sev	Focal	Pellet reaching Test	% successful	T:41; C:31	-	+++	+++	+++			
Schönfeld et al. (94)	2017	CCI	Severe	Focal	Montoya staircase test	Pellets eaten	T:8; C:7	-		+++	++			

$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; -, not significant; Gray, not evaluated. T, TBI; C, control/Sham; CCI, control cortical impact; CCI-CS, control cortical impact-closed skull; WD, weight drop injury.

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TABLE 5 Preclinical locomotor activity evaluation.

References	Year	Method	Severity	Type injury	Test	Time	Size	Sample	Pre	<72 hr	1–3 wks	1–2 mo	3–5 mo	6–11 mo	12–24 mo
Namdar et al. (93)	2020	WD	Mild	Diffuse	Open Field	10 mins	60 × 60 cm	T:15; C:13			-	-			
Homsi et al. (102)	2010	WD	Mild	Diffuse	Open Field	60 mins	19 × 11 cm	T/C: 10–12		+++	++	++	+		
Bajwa et al. (103)	2016	CCI-CS	Mild	Diffuse	Open Field	30 mins	49 × 36 cm	T:10; C:10		-	-		-		
McAteer et la. (106)	2016	WD	Mild	Diffuse	Open Field	5 mins	1 × 1 m	T:9; C: 9				+	-		
Arulsamy et al. (108)	2019	WD	Mild	Diffuse	Open Field	5 mins	1 × 1 m	T:14; T:14							-
Arun et al. (114)	2020	Blast	Mild	Blast	Open Field	60 mins	40 × 40 cm	T/C: 10–31		-	-	-	-	+	-
Feng et al. (89)	2021	Chimera	Rep-Mild	Diffuse 2 × 3 d	Open Field	Unknown	40 × 40 cm	T^{+}C:81		-	-	-			
Bajwa et al. (103)	2016	CCI-CS	Rep-Mild	Diffuse 2 × 3 d	Open Field	30 mins	49 × 36 cm	T:10; C:10		-	-		-		
Corrigan et al. (107)	2017	WD	Rep-Mild	Diffuse 3 × 5 d	Open Field	5 mins	1 × 1 m	T/C: 8–10					+		
McAteer et al. (106)	2016	WD	Rep-Mild	Diffuse 3 × 5 d	Open Field	5 mins	1 × 1 m	T:7; C: 9				+	+		
Morriss et al. (104)	2021	WD	Rep-Mild	Diffuse 5x 24 hrs	Open Field	Unknown	Unknown	T:11; C:10				-	+	+	
Arulsamy et al. (108)	2019	WD	Rep-Mild	Diffuse 3 × 5 d	Open Field	5 mins	1 × 1 m	T:14; C:14							-
Arun et al. (114)	2020	Blast	Rep-Mild	Blast 2 × 2 mins	Open Field	60 mins	40 × 40 cm	T/C: 10–31		++	-	-	+	++	++
Tucker et al. (105)	2019	CCI-CS	Rep-Mild	Diffuse 3x 24 hrs	Open Field	20 mins	40 × 40 cm	T/C: 17–21				-	+++	+++	+++
Bajwa et al. (103)	2016	CCI	Moderate	Focal	Open Field	30 mins	49 × 36 cm	T:10; C:10		-	+++		-		
Leconte et al. (109)	2020	CCI	Moderate	Focal	Open Field	9 mins	1 m × 1 m	T:15; C:13					+		

(Continued)

TABLE 5 (Continued)

References	Year	Method	Severity	Type injury	Test	Time	Size	Sample	Pre	<72 hr	1–3 wks	1–2 mo	3–5 mo	6–11 mo	12–24 mo
Rowe et al. (111)	2016	LFP	Moderate	Mixed	Open Field	5 mins	70 × 70 cm	T/C:11–12: 2M				-	-	-	
								T/C:11–12: 4M				-	-		
								T/C:11–12: 6M				-			
Arulsamy et al. (113)	2018	WD	Mod-Sev	Diffuse	Open Field	5 mins	1 x1 m	T:14; C:13				-	+		
Arulsamy et al. (108)	2019	WD	Mod-Sev	Diffuse	Open Field	5 mins	1 x1 m	T:12; C: 14							-
Islam et al. (110)	2021	CCI	Severe	Focal	Open Field	5 mins	54.5 × 54.5 cm	T,C: 9–13					+		
Komoltsev et al. (112)	2021	LFP	Severe	Mixed	Open Field	5 mins	1 × 1 m	T:13; C:7						-	

$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; -, not significant; Gray, not evaluated. T, TBI; C, control; CCI, control cortical impact; WD, weight drop injury; LFP, lateral fluid percussion injury.

1 × 1 m enclosure over 5 min (106). In contrast, with repeated injury (3 × 5 days), both a decrease in locomotor activity at 6 and 12 weeks (107) and an increase in locomotor activity at 12 weeks (107) have been reported utilizing the same testing parameters. Notably, the decrease was recorded with manual counting of squares crossed (106), whereas the increase was detected using automated software of distance traveled (107). Nonetheless, no differences in locomotion were noted at 12 months post-injury in the same injury model (108), nor in a repeated CHIMERA model (2 × 3 days) in a smaller open field (40 cm × 40 cm) from day 1 to 12 weeks post-injury (89).

Increasing injury severity had little effect on chronic locomotor activity. Moderate focal CCI injury in C57/BL6 mice found a transient decrease in distance traveled at 7 days, but this resolved by 12 weeks (103). Leconte et al. similarly showed no difference compared to naïve animals at 5 months following CCI injury in rats (109). Even with more severe injury, locomotor performance was unchanged at 10 weeks following injury in young mice, with a significant difference only seen in mice injured at 18 months of age (110). Similarly, mixed focal/diffuse injury via LFP had no effect on locomotor activity, as measured via distance traveled over 5 min out to 6 months post-injury (111, 112). The pattern of deficits differed slightly with moderate–severe diffuse injury, with no differences noted at 4 weeks, a decrease in distance traveled at 12 weeks (113), with recovery by 12 months, the latest time point examined (108). A similar pattern was seen following a single blast injury (114). No changes were seen from day 1 to 3 months post-injury, but this was followed by a subsequent significant decrease in total distance traveled over 60 min in a 40 × 40 cm arena at 6 and 9 months, with recovery by 12 months post-injury (114). Indeed, two 19 PSI injuries delivered within 2 min were required to lead to a persistent decrease in locomotor activity at 12 months, with deficits seen within the first 3 days post-injury, resolving at 4 weeks post-injury, prior to re-emerging at 3 months, and then persisting to the 12 month time point (114).

3.4.6. Balance and coordination

Balance and coordination were the most common motor domains evaluated in preclinical studies via tasks encompassing the balance beam (28, 81, 83, 84, 90, 96, 100, 103, 111, 115–122), ladder, (80, 93, 99, 123) rotating pole (79, 83, 114), grid walk, (31, 103), string suspension (124), pole climbing (99, 109), and rotarod tasks (28, 35, 36, 83, 88–90, 93, 96–98, 100, 103–106, 113–115, 119, 124–132) (Table 6). The tests conducted varied between studies including variation in the size of the beam and speed of the rotarod and rotating pole. Furthermore, the parameters examined varied between studies. For example, beam performance was analyzed via time to traverse beam (81, 111, 117, 119, 121), number of foot faults (28, 31, 79, 103, 111, 118, 119, 122), or a ranking scale for performance (81, 83, 84, 90, 96, 124). For the rotarod, performance was evaluated on one trial (93, 96, 97, 106, 108) or an average across up to eight trials (35, 36, 89, 93, 105, 124–126, 131) which may influence results.

Following diffuse TBI, moderate-to-severe injury was more likely to lead to acute balance deficits as seen as impaired rotarod performance (96, 113), time to traverse a beam (96), or a score

evaluating performance encompassing foot faults or falls (124). In contrast, acute deficits were not seen in most models of mTBI, (93, 106, 131) with only one diffuse mTBI study reporting acute deficits (<72 h) on the rotarod (97), with these deficits persisting to 1 month following injury and resolving by 3 months (98). Nonetheless following diffuse injury, the overall consensus was that no chronic deficits were seen on the rotarod, regardless of injury severity, from 3 to 24 months post-injury (35, 36, 88, 98, 103, 106, 113). In fact, only a single study found long-term impairment on the rotarod following either mild or moderate diffuse TBI (131). In the mild diffuse TBI group, deficits emerged at 8 weeks following injury and persisted to 18 weeks, whereas with a moderate injury deficits emerged at 4 weeks and similarly persisted to 18 weeks, the latest time point examined (131). The use of other balance tests did detect balance deficits up to 2 months post-injury following mTBI on both time to traverse a 0.5 cm beam (117) and increased missteps on the Erasmus ladder (93). Furthermore, following mod-sev diffuse TBI, worse performance was noted both on an 0.8 cm beam, with performance scored from 0 to 3 depending on how mice were able to traverse the beam and number of falls and foot faults, and on a string suspension assay (124). However, this has not been consistently reported, with other studies investigating mild (80, 103) and moderate-to-severe diffuse TBI (96) showing no deficits when traversing larger beams (0.65–2 cm) (96, 103) or on forelimb placement in the ladder task (80) up to 3 months post-injury, indicating task-dependent effects and that more difficult tasks are required to detect subtle motor deficits.

Compared to diffuse injury, the focal injury was more likely to cause chronic balance deficits. Following moderate focal injury, deficits on the grid walk, balance beam, and rotarod tasks were noted to be 3 months post-injury (28, 103, 120–122, 128, 133), the latest time point assessed. Differing results were seen on the pole test, with an increase in time to turn only emerging at 4.5 months post-injury, with recovery by 6.5 months, which persisted to 9 months post-injury (109). These results were supported by studies in moderate–severe focal TBI, where Hanscom et al. found significantly increased foot faults on the ledged beam from 1 day to 2 months post-injury (116), and a focal punch injury similarly resulted in impaired beam performance up to 6 weeks post-injury (90). With a severe focal injury, balance deficits were consistently noted up to 10 weeks post-injury, the latest time point assessed on the balance beam, rotarod, cylinder test, and grid walk tasks (31, 94, 121, 129). In contrast, two studies did not report chronic balance deficits following moderate–severe focal TBI, although these used a larger beam (118) and altered rotarod parameters (mice were placed on the rotarod already spinning at 36 RPM, rather than gradually increasing speed from 3 RPM) (132). The larger beam would have reduced the complexity of the task, whereas the increased rotarod starting speed may have made the task too difficult for the shams, masking any injury effect.

With a mixed focal and diffuse injury via LFP, mixed results for balance and coordination were seen with a moderate injury. Wright et al. noted increased foot faults and decreased time to cross the beam (119), and Tan et al. found impaired rotarod performance at 3 months post-injury (130). Rowe et al. found a similar pattern in animals injured at 2 months of age, with increased foot faults and time to cross the beam at both 1 and 3 months post-injury but interestingly not in animals injured at 4 or 6 months (111). In contrast, Carron et al. noted acute deficits in performance on the rotarod and tapered ledged beam, which had recovered by 1 week following injury, with no further deficits seen to 2 months post-injury (115). However, with more severe injury, LFP resulted in impaired performance on both the balance beam and rotating pole tasks out to 4 months post-injury, the latest time point examined (83, 84).

In models of repeated mTBI, a higher number of injuries or a shorter interval between injuries were generally associated with more persistent balance deficits. Following 5–7 injuries, chronic deficits on the balance beam, string suspension, and rotarod were noted up to 12 months post-injury (100, 104, 124, 125), although no deficits were seen by 24 months post-injury (36). Only the Mannix et al. (125, 126) and the Mouzon et al. (35) studies failed to find chronic balance deficits following 4–7 impacts. Following three injuries with a 24-h interval between injuries, increased latency to fall on the rotarod was seen up to 6 months post-injury (99, 105), with recovery by 12 months (105). Extending the interval between injuries to 5 days meant that three injuries no longer led to deficits on the rotarod up to 3 months post-injury (106). Interestingly, unlike the rotarod, no deficits were noted on forelimb placement in the ladder walk (80) nor in pole climbing time (99) with 3 injuries, 24 h apart. With two injuries spaced 24 h apart, the number of foot faults on a rotating pole was increased at 3 days post-injury, before returning to sham levels from 7–28 days, before a deficit re-emerged at 2 months following injury (79). By increasing the interval between the two injuries to 3 days, deficits were no longer noted on the balance beam, rotarod, or grid walk tasks either acutely or chronically up to 2 months post-injury (103).

Finally, neither single nor repeated blast injury was sufficient to produce persistent motor deficits at 6 months post-injury, regardless of initial injury severity. With a mild blast injury at 19psi, a single injury led to no balance deficits on the rotating pole or rotarod task to 6 months post-injury (114). In contrast, when two 19 PSI injuries were delivered within 2 min, balance deficits emerged at 6 days, persisted to 4 weeks, with recovery and no further impairment noted following this time point up to 6 months post-injury (114). Similarly, following a single moderate blast impact (50 PSI), acute deficits in latency to fall on the rotarod were noted, which had recovered by 6.5 weeks post-injury (65).

3.5. Clinical studies

3.5.1. Motor function test

Overall long-term motor function following TBI was assessed in a single study using the Unified Parkinson's Disease Rating Scale (UPDRS) Motor Examination, which was used to calculate a modified UPDRS (mUPDRS) global motor score, as well as four domain scores: tremor, rigidity, bradykinesia, and posture/gait (40). In retired military veterans (M: 76.4 ± 10.0 years of age) who self-reported TBI [median TBIs = 2 (1.2), 53.2 ± 18.1 years since first TBI, 37.0 ± 22.5 years ago since last TBI], those with a history of moderate–severe, but not mTBI, had a significantly worse mUPDRS global motor score, as well as a worsened score for

posture/gait, but not for tremor, rigidity, or bradykinesia, compared to those without a history of TBI (M: 79.4 ± 8.2 years of age) (40) (Table 7).

3.5.2. Grip strength and fine motor control

Six studies identified herein (19, 25, 26, 51, 53, 76) evaluated chronic alterations in grip strength or fine motor control (Table 8). Grip strength was assessed only in one study comparing individuals with a history of TBI 1–26 years earlier to healthy controls. No differences were seen following either mild or moderate–severe TBI, in either the dominant or non-dominant hand, although the mTBI group was significantly more variable than healthy controls across 10 trials (51). This study also investigated finger dexterity as time taken to touch each of their fingers to their thumb three times. The moderate–severe TBI group at 12.2 years post-injury (range 1–25), but not the mild TBI group at 7.1 years post-injury (range 1–27), had slower finger dexterity in both the non-dominant and dominant hands over 10 trials compared to healthy controls (51). This was not related to age, given that both groups had a similar mean age (35.4 vs. 37.6 years). Similarly, no effects of at least one mTBI (range 1–12, median = 2) in military veterans at a median of 8 years post the most recent TBI were noted in the grooved pegboard task, where pins must be manipulated and rotated to fit a hole (53). With a higher number of repeated concussions and a longer time-period post-injury, however, deficits in fine motor control were seen chronically. Retired rugby league players (mean 8.5 concussions) at almost 20 years post-injury took longer in the O'Connor Finger Dexterity Test, where the time taken to place pegs in holes is recorded compared to controls (26), with similar findings in amateur Australian football players (mean 3.2 concussion) at 22.12 ± 6.73 years following their last injury on the same task (25). Similarly, chronic, but not acute, deficits were seen in a RAM task consisting of rapid wrist supination–pronation movements. Significantly lower movement velocity was found in athletes who sustained their last concussion 30 years earlier (range 27–41 years) (76) but not in those who had sustained their last concussion only 9–34 months earlier (19). Importantly, both groups had the same range of 1–5 concussions, suggesting that these effects may be due to time elapsed since injury, rather than number of injuries.

3.5.3. Gait

Several characteristics can be used to assess gait, including spatiotemporal factors, such as cadence, stride length and single and double support time, kinematics in regard to the motion of joints, and kinetics to describe the measurement of the forces required to make a movement (22). Clinical studies varied widely in regards to the gait parameters examined, the tests employed, and equipment used (Table 9).

No difference in gait speed over a distance of 3–4 m was seen either in a group of military veterans (53) or in a cohort recruited from the general population (51) on average a decade following their last injury. Conversely, more sophisticated analysis employing an 8-m electronic walkway found that students with a history of concussion with a mean time since injury of 6.32 years had greater time in double-leg stance support and less time in single-leg stance support, throughout the gait cycle (66). The more difficult task of heel to toe walking was also found to be affected by previous mTBI, with veterans with a history of mTBI approximately 16 years ago being three times as likely as normal controls to have their performance ranked as abnormal by a neurologist (55). Other studies investigating mTBI specifically recruited patient populations with persistent symptoms. Stuart et al. aimed to develop a model to describe differences in gait seen in individuals with a history of mTBI sustained approximately 18 months ago (median 551 days) who had self-reported balance instability. Participants walked over a 13-m distance for 2 min with inertial sensors detecting gait. Differences in the mTBI cohort compared to healthy controls related to increased variability, decreased rhythm, and reduced pace in parameters such as stride length and time, alongside increased turn duration and velocity (75). These results were only partially supported by another study, which recruited individuals with a history of mTBI with symptoms persisting >3 months but did not require these symptoms to be specifically balance related. Participants were on average a year from injury, with statistically significant differences only detected in pace and turning, but not in rhythm and variability, over a ∼200-m walk with multiple 180° turns (124). It should be noted that the Stuart et al. study did not report *p*-values but rather investigated effect size only, which could also account for the differences between these studies. Conversely, a much smaller study ($n = 16$) of symptomatic veterans with a history of mTBI 3.5 ± 1.7 years previously found no difference in gait speed or stride length over 10 m (33). Given that the Stuart et al. study included 111 participants (75) and the Martini et al. study 68 (124), any differences may relate to the small sample size.

Indeed, in comparison with symptomatic mTBI, moderate–severe TBI at least 18 months earlier (mean: 35.5 ± 20.2 months) found no difference in cadence on a treadmill at 3 km/h for 2 min (77). Similar findings were found more chronically, with a moderate TBI (range 1–26 years prior) not producing deficits in walking or turning speed on a walkway (51) or treadmill or elliptical trainer task (77). With a more difficult task, participants were placed on split-belt treadmill, such that the speed required for each leg could be varied (58). Those with a history of moderate–severe injury an average of 2.9 ± 1.7 years prior took longer to adapt to the belts being at different speeds, seen as an decrease in step symmetry, but were no different in the baseline task or post-adaptation when the two belts were at the same speed (58).

Self-selected walking (22) and running (23) speeds were slower in individuals with a previous history of severe TBI an average of 5–6 years earlier, with participants chosen for their ability to walk and run independently, respectively, while still attending physiotherapy for mobility limitations. When healthy controls matched these speeds, no difference in either cadence or stance time were seen with walking (22), whereas, with running, a previous history of TBI led to increased cadence and shorter stride length to produce the same speed (23). In the only study which did not report a difference in walking speed, 19 of 52 participants were unable to walk at the faster speed, negating the measurement (22). Nonetheless, numerous kinetic and kinematic alterations were associated with both running and walking following severe TBI, including alterations in ankle power generation (22, 24) and knee

TABLE 6 Preclinical balance and coordination evaluation.

References	Years	Method	Severity	Injury	Test	Parameters	Sample	Pre	<72h	1–3wk	1–2mo	3–5mo	6–11 mo	7–11mo	12–24mo
Evans et al. (97)	2015	CCI-CS	Mild	Diffuse	Rotarod (4–40 prm)	Latency to fall	T:12; C: 11		+	+	-				
Namdar et al. (93)	2020	WD	Mild	Diffuse	Rotarod (4–40 rpm)	Latency to fall	T:15; C:13			-	-				
					Eramus Ladder	Correct steps				-	+				
						Missteps				-	+				
						Time				-	-				
Lai et al. (117)	2019	WD	Mild	Diffuse	0.5 cm Beam	Traverse time	T:7; C:7	-		+++	+++				
Laurer et al. (79)	2001	CCI-CS	Mild	Diffuse	3 cm Rotating pole 1,3,5 rpm	Foot-faults	T:16–24; C:14–23		-	-	-				
Bajwa et al. (103)	2016	CCI-CS	Mild	Diffuse	0.65 cm Beam 2.5 cm Grid Walk Rotarod	Traverse Time Foot faults Latency to fall	T:10; C:10		-	-		-			
Evans et al. (98)	2014	CCI-CS	Mild	Diffuse	Rotarod (4–40 prm)	Latency to fall	T:9; C: 8			+	+	-			
Fehily et al. (80)	2019	WD	Mild	Diffuse	Ladder walk	% Stepping errors	T:15; C:15					-			
McAteer et la. (106)	2016	WD	Mild	Diffuse	Rotarod (3–30 rpm)	Latency to fall	T:9; C:9		-	-	-	-			
Hou et al. (131)	2017	WD	Mild	Diffuse	Rotarod (3–30 rpm)	Average 3 trials	T:8; C: 8	-		-	+	++			
Mouzon et al. (35)	2014	CCI-CS	Mild	Diffuse	Rotarod (5–50 rpm)	Average 3 trials	T:12; C:12						-		
Mouzon et al. (36)	2018	CCI-CS	Mild	Diffuse	Rotarod (5–50 rpm)	Average 3 trials	T:7; C:8								-
Xu et al. (121)	2019	CCI	Mild	Diffuse	2 cm Beam	Traverse Time	T:10; C10		-	-	-				
Mountney et al. (88)	2017	PCS	Mild	Diffuse	Rotarod (0.1 rpm/sec) Three sets × 5 with 2 min intertrial interval	Latency to fall	T/C: 8–10					-			

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TABLE 6 (Continued)

References	Years	Method	Severity	Injury	Test	Parameters	Sample	Pre	<72h	1–3wk	1–2mo	3–5mo	6–11 mo	7–11mo	12–24mo
Mountney et al. (88)	2017	PCS	Rep-Mild	Diffuse 4xTBI 1 hr apart	Rotarod (0.1 rpm/sec) Three sets × 5 with 2 min intertrial interval	Latency to fall	T/C: 8–10					+			
Albayram et al. (124)	2017	WD	Rep-Mild	Diffuse 7 in 9D	0.8 cm beam	Score	T/C: 9–10						++		
					String Suspension (3 trials)	Score							++		
					Rotarod (4–40 opm) 5 mins; 4x day for 2 days	Latency to fall Average 8 trials							+		
Feng et al. (89)	2021	Chimera	Rep-Mild	Diffuse 3xTBI/day × 2 48 hrs apart	Rotarod (5–40 rpm)	Latency to fall-average 3 trials	T:9; C:9				-				
Tabet et al. (99)	2022	CCI-CS	Rep-Mild	Diffuse 3x TBI 24hr	Ladder rung	% Foot faults to baseline	T:10; C:10				+				
					Pole climbing	Time (3 trials)	T:10; C:10				-				
Laurer et al. (79)	2001	CCI-CS	Rep-Mild	Diffuse 2xTBI 24 hrs	3 cm Rotating pole	(1, 3, 5 rpm)	T:49; C:36		++	-	++				
Bajwa et al. (103)	2016	CCI-CS	Rep-Mild	Diffuse 2x 3d	0.65 cm Beam 2.5 cm Grid Walk Rotarod (2x 5 rpm, 2x 3 rpm/5s, 2x 3 rpm/3s	All measures Foot faults Average of trials	T:10; C:10		-	-		-			
Mannix et al. (125)	2014	WD	Rep-Mild	7inj/9D	Rotarod (0.1 rpm/sec)	Average 4 trials	T:32; C:21		+			+			
Mannix et al. (126)	2017	WD	Rep-Mild	7inj/9D	Rotarod (0.1 rpm/sec)	Average 4 trials	T:12; C:11		+			-			

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TABLE 6 (Continued)

References	Years	Method	Severity	Injury	Test	Parameters	Sample	Pre	<72h	1–3wk	1–2mo	3–5mo	6–11 mo	7–11mo	12–24mo
McAteer et la. (106)	2016	WD	Rep-Mild	Diffuse 3 × 5d	Rotarod (3–30 rpm)	Latency to fall	T:7; C:9		-	-	-	-			
Fehily et al. (80)	2019	WD	Rep-Mild	Diffuse 2xTBI 24 hr	Ladder walk	% Stepping errors	T:15; C:15					-			
				Diffuse 3xTBI 24 hr	Ladder walk	% Stepping errors						-			
Mouzon et al. (35)	2014	CCI-CS	Rep-Mild	Diffuse 5xTBI 24 hr	Rotarod (5–50 rpm)	Latency to fall-average 3 trials	T:12; C:12						-		
Morriss et al. (104)	2021	WD	Rep-Mild	Diffuse 5xTBI 24 hr	Rotarod	Latency to fall 4 trials	T:11; C:10							++	
Dhillon et al. (100)	2020	CCI-CS	Rep-Mild	Bilateral 5x TBI/5 weeks	Rotarod (3–30 rpm)	Fall- 3 trials	T:10; C:8	-	-	+	+	+	+	+	+
					2.5 cm beam	Hindlimb rating								+	
Tucker et al. (105)	2019	CCI-CS	Rep-Mild	Diffuse 3xTBI 24hr	Rotarod (4–60 rpm)	Latency to fall-average 3 trials	T:19–21; C17–19		+++		-	+	+		-
Mouzon et al. (36)	2018	CCI-CS	Rep-Mild	Diffuse 5xTBI 24hr	Rotarod (5–50 rpm)	Latency to fall-average 3 trials	T:7; C:7								-
Hou et al. (131)	2017	WD	Moderate	Diffuse	Rotarod (3–30 rpm)	Average 3 trials	T:8; C: 8	-		-	+	+++			
Toshkezi et al. (128)	2018	CCI	Moderate	Focal	Rotarod (2–20 rpm)	Latency to fall	T:9; C: 5				+++				
Barrett et al. (122)	2020	CCI	Moderate	Focal	0.5 cm Beam	Foot Faults	T/C: 8–13	-	+++	+++	+++				
Henry et al. (28)	2020	CCI	Moderate	Focal	0.5 cm Beam	Foot Faults	T:11; C:12	-	+++	+++	+++				
					Rotarod (1–30 rpm)	%Baseline		-	+++	-	+++				
Xie et al. (120)	2019	CCI	Moderate	Focal	0.6 cm Beam	Foot Faults	T:10; C:10	-	+++	+++	+++				
Xu et al. (121)	2019	CCI	Moderate	Focal	2 cm Beam	Traverse Time	T/C: 38		-	+	+				
Chen et al. (123)	2016	NY	Moderate	Focal	Ladder test	Errors	T:20; C:10	-	++	-	-				
Bajwa et al. (103)	2016	CCI	Moderate	Focal	0.65 cm Beam	Time Active	T:10; C:10		+	-		-			

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TABLE 6 (Continued)

References	Years	Method	Severity	Injury	Test	Parameters	Sample	Pre	<72h	1–3wk	1–2mo	3–5mo	6–11 mo	7–11mo	12–24mo
						Falls			++	-		-			
					2.5 cm Grid walk	Foot faults			+++	++		++			
					Rotarod (2x 5 RPM, 2x 3 rpm/5s, 2x 3 rpm/3s	Average trials			+++	-		++			
Carron et al. (115)	2019	LFP	Moderate	Mixed	Rotarod (1.5 rpm/3s)	% Baseline	T:10; C:10	-	+++	-	-				
					Tapered Beam	Ranking		-	+++	-	-				
					Tapered Beam	Foot faults		-	+++	-	-				
Tan et al. (130)	2020	LFP	Moderate	Mixed	Rotarod (4–40 rpm)	Average 3 trials	T:18; C: 10			-		+			
Wright et al. (119)	2017	LFP	Moderate	Mixed	2 cm Beam	Foot faults	T:10; C:10			+		+			
					2 cm Beam	Traverse time	T:10; C:10			+		+			
Rowe et al. (111)	2016	LFP	Moderate	Mixed	3 cm Beam	Foot faults	T/C:11–12 2M				+	-	-		
							T/C:11–12 4M				-	-			
							T/C:11–12 6M				-				
					3 cm Beam	Traverse Time	T/C:11–12 2M				-	-	-		
							T/C:11–12 4M				-	-			
							T/C:11–12 6M				-				
Sell et al. (81)	2017	LFP	Moderate	Mixed	2.5 cm Beam	Traverse Time	T:37; C:39	-	+			-	-		-
					1.75 cm Beam	Ranking		-	+			+	-		-

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TABLE 6 (Continued)

References	Years	Method	Severity	Injury	Test	Parameters	Sample	Pre	<72h	1–3wk	1–2mo	3–5mo	6–11 mo	7–11mo	12–24mo
Alwis et al. (96)	2012	WD	Mod-Sev	Diffuse	Rotarod (3–30 rpm)	% Baseline	T:19; C: 12	-	+	+	+				
					2 cm Beam	Ranking	T:19; C: 12	-	+	+	+				
Arulsamy et al. (113)	2018	WD	Mod-Sev	Diffuse	Rotarod (3–30 rpm)	Latency to fall	T:6; C: 6			+++	-	-			
Albayram et al. (124)	2017	WD	Mod-Sev	Diffuse	0.8 cm beam	Score	T/C = 9–10			+			+		
					String Suspension (3 trials)	Score				+			+		
Soblosky et al. (120)	1997	CCI	Mod-Sev	Focal	2.5 cm Beam	Ranking	T:10; C: 10	-	+	+	+	-			
					Pegged 2.5 cm Beam	Foot faults	T:10–13; C:10–14	-		+	+	-			
Wang et al. (90)	2019	Punch	Mod-Sev	Focal	2 cm Beam	Ranking	T:8; C:8		++	++	++				
Hanscom et al. (116)	2021	CCI	Mod-Sev	Focal	5 mm beam	Foot faults	T/C: 14–21	-	+++	+++	+++				
Vogel et al. (132)	2020	CCI	Mod-Sev	Focal	Rotarod 36 rpm	Latency to fall	T:16; C:16			-			-		
					Rotarod:accelerating		T:20 C:10			-		-			
Cline et al. (31)	2017	CCI	Severe	Focal	2.5 cm Gridwalk	Foot faults	T:15; C:14	-	+		+				
Xu et al. (121)	2019	CCI	Severe	Focal	2 cm Beam	Traverse Time	T/C: 38		-	+	+				
He et al. (129)	2020	CCI	Severe	Focal	Rotarod (4–40 rpm)	Average 3 trials	T:11; C: 7				+	+			
Nissinen et al. (84)	2017	LFP	Severe	Mixed	2 cm Beam	Ranking	T:23; C:10	-	+++	+++	++	-			
Zhang et al. (83)	2005	LFP	Severe	Mixed	Rotating pole (5 rpm)	Ranking	T:14; C: 24				++	++			
					2 cm Beam	Ranking					++	++			

$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; -, not significant; Gray, not evaluated. T, TBI; C, control; CCI, controlled cortical impact; CCI-CS, control cortical impact, closed skull; WD, weight drop injury; LFP, lateral fluid percussion.

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TABLE 7 Clinical neuroscore evaluation.

References	Year	Severity	Population	Sample size	Age (mean)	Sex %male	Motor test	<5 yrs	6–10 yrs	11–25 yrs	>25yrs
Gardner et al. (40)	2017	Rep-Mild	Military veterans	T:31–34 C:65–68	T: 79.4 C: 76.4	T:82.4% C:94.9%	mUPDRS Global Score				-
							mUPDRS Tremor Score				-
							mUPDRS Rigidity Score				-
							mUPDRS Bradykinesia Score				-
							mUPDRS Posture/Gait Score				-
		Moderate-Severe		T:20 C:65–68			**mUPDRS Global Score**				+
							mUPDRS Tremor Score				-
							mUPDRS Rigidity Score				-
							mUPDRS Bradykinesia Score				-
							mUPDRS Posture/Gait Score				+

$^{+}p < 0.05$; -, not significant; gray, not evaluated.

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TABLE 8 Clinical fine motor evaluation.

References	Year	Severity	Population	Sample size	Age	Sex %male	Motor test	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
Burton et al. (51)	2002	Mild	Community	T:19; C:26	T:35.36 C: 32.77	T:78.9 C:46.15	Fine motor	Touch fingers to thumb		-		
							Grip strength	Dynamometer		-		
Walker et al. (52)	2018	Combination	Military veterans	T:380; C 73	T:36 C:40.5	T:88% C:79.5%	Fine motor	Grooved Pegboard		-		
De Beaumont et al. (19)	2011	Combination	College Athletes	T:21; C:15	T/C: 22.3	T/C: 100%	Rapid alternating movement Wrist supination-pronation	Velocity	+ (↑)			
								Sharpness	-			
								Bimanual co-ordination	-			
De Beaumont et al. (76)	2009	Combination	College Athletes	T:19; C:21	T:61; C:59	Not stated	Rapid alternating movement Wrist supination-pronation	Duration				-
								Range				-
								Sharpness				-
								Velocity				+
Pearce et al. (25)	2014	Rep-Mild	Professional Athletes	T:40; C:20	T:49.3 C:47.6	T/C: 100%	Fine motor	O'Connor Finger Dexterity Test			+	
Pearce et al. (26)	2018	Rep-Mild	Professional Athletes	T:25; C:25	T:48.4 C:48.8	T/C: 100%	Fine motor	O'Connor Finger Dexterity Test			+	
Burton et al. (51)	2002	Mod-Sev	Community	T:9; C:26	T:37.56 C: 32.77	T:66.67% C:46.15	Fine motor	Touch fingers to thumb			+	
							Grip strength	Dynamometer			–	

$^{+}p < 0.05$; -, not significant; gray, not evaluated; ↑, increased.

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TABLE 9 Clinical gait studies.

References	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	<5 yrs	6–10 yrs	11–25 yrs	>25 yrs
Burton et al. (51)	2002	Mild	Community	T:19; C:26	T:35.36 C:32.77	T:78.9 C:46.15	Turn 360°	None	Speed		-		
							Timed 4 m walk	None	Speed		-		
Vanderploeg et al. (55)	2007	Mild	Military veterans	T:254; C:3214 MVA:539	T:37.8 C:38.5 MVA:37.8	100%	Heel toe walk	None	Abnormal/normal			3x	
Stuart et al. (75)	2020	Mild	Community Self-reported balance instability > 3 months post-injury	T:52; C:59	T:39.6 C:37.0	T:30.8% C:42.4%	13 m walkway, 2 mins	Five sensors strapped to feet, L5, sternum and head	**Stride length**	**			
									Speed	***			
									Foot stride angle	**			
									Toe off angle	-			
									Single support time	**			
									Double support time	**			
									Stride time	**			
									Turn duration	**			
									Turn step number	*			
									Turn velocity	***			
Martini et al. (54)	2021	Combination	Community Symptoms persisting > 3 months following TBI	T:65; C:57	T:39.6 C:36.9	T:64% C:55%	208 m walk	Five sensors strapped to feet, L5, sternum and head	**Speed**	+			-
									Variability	-			
									Rhythm	-			
									Turning	+			
							Dual task Above + auditory Stroop		**Speed**	+			

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TABLE 9 (Continued)

References	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	<5 yrs	6–10 yrs	11–25 yrs	>25 yrs
									Variability	-			
									Rhythm	+			
									Turning	+			
Walker et al. (52)	2018	Combination	Military veterans	T:258;C:47	T:36 C:40.5	T:88% C:79.5%	4 m walk	None	Speed			-	
Martini et al. (66)	2011	Combination	College students	T:25; C:25	T:21 C:20.7	T:60.7% C:50%	4 m walk	GAITr walkway	**Speed**		+		
									Double Stance support		+		
							4 m walk + obstacle		Speed		-		
									Double Stance support		–		
							Brooks Spatial Memory task + 4 m walk		Speed		-		
									Double Stance support		-		
							Brooks Spatial Memory task + 4 m walk + obstacle		Speed		-		
									Double Stance support		+		
Pitt et al. (56)	2020	Combination	Military veterans with chronic symptoms	T:8; C:8	T:32.5 C:33.3	T:87.5% C:87.5%	10 m walk, 8–10 trials	Whole body retroreflective marker set with 12 motion analysis system	Speed	-			
									Step length	-			
									Step width	-			
									Medial-lateral COM displacement	+			
									Peak medial-lateral COM velocity	-			

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TABLE 9 (Continued)

References	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	<5 yrs	6–10 yrs	11–25 yrs	>25 yrs
							Above + auditory Stroop task		Speed	-			
									Step length	-			
									Step width	-			
									Medial-lateral COM displacement	+			
									Peak medial-lateral COM velocity	-			
Burton et al. (51)	2002	Mod-Sev	Community	T:9; C:26	T:37.56 C:32.77	T:66.67% C:46.15	Turn 360°	None	Speed		-		
							Timed 4 m walk	None	Speed		-		
Useros Olmo et al. (57)	2020	Mod-Sev	Hospital	T:20; C:19	T:36.1 C:38.2	T:85% C:89.5%	Treadmill 3 kms/hr,	Gait Trainer2	Cadence	-			
							Treadmill 3 kms/hr + cognitive task			-			
Vasudevan et al. (58)	2014	Mod-Sev	Community	T14; C:11	T:29.7 C:31.1	T:71.4% C:81.8%	Split treadmill: same speed	Markers on toe, ankle, knee, hip, pelvis, shoulder	Stride length	-			
									Stance time	-			
									Step symmetry	-			
									Center of oscillation	-			
									Temporal coordination	-			
							Split treadmill: different speed		Stride length	-			
									Stance time	-			
									Step symmetry	+			
									Center of oscillation	-			

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TABLE 9 (Continued)

References	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	<5 yrs	6–10 yrs	11–25 yrs	>25 yrs
									Temporal coordination	-			
							Split treadmill: same speed: Post-adaption		Stride length	-			
									Stance time	-			
									Symmetry	-			
									Center of oscillation	-			
									Temporal coordination	-			
Buster et al. (77)	2013	Severe	Community	T:10; C:10	T:36: C:34	Not stated	Elliptical trainer, comfortable stride length, 3 mins	Reflective markers on the pelvis, hip, knee, ankle and foot	Speed		-		
									Cadence		-		
									Stride length		-		
									Motion profile		-		
									Joint angles		+		
							Treadmill, comfortable speed 3 mins		Speed		-		
									Cadence		-		
									Stride length		-		
									Motion profile		-		
									Joint angles		-		
Williams et al. (59)	2009	Severe	Hospital Able to walk independently 20 m	T:41; C:25	T:29.1 C:27.8	T:75.6% C:64%	12 m walk: Self-selected speed	25 reflective markers on pelvis and lower limbs	**Double support**		+		
									Speed		+		
									Cadence		+		
									Stride length		+		

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TABLE 9 (Continued)

References	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	<5 yrs	6–10 yrs	11–25 yrs	>25 yrs
									Stance duration		+		
									Base of support		+		
				T:41; C:15			12 m walk: Matched speed		**Trunk angle**		+		
									Pelvic angle		-		
									Hip angle		-		
									Knee angle		+		
									Ankle angle		-		
									Lateral COM displacement		+		
Williams et al. (22)	2010	Severe	Hospital Able to walk independently 20 m	T:55; C:10	T:28.5 C:27.3	T:72.7% C:50%	12 m walk: Matched speed	25 reflective markers on pelvis and lower limbs	Speed		-		
									Cadence		-		
									Stride length		+		
									Stance time		-		
									Double support time		-		
									Lateral COM displacement		+		
									Peak ankle power		-		
									Peak hip power (initial)		+		
									Peak hip power (prewswing)		+		
				T:36;C:10	T:27.3 C:27.3	T:77.8% C:50%	12 m walk: Fastest speed		Speed		-		
									Cadence		-		
									Stride length		-		
									Stance time		-		
									Double support time		-		

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TABLE 9 (Continued)

References	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	<5 yrs	6–10 yrs	11–25 yrs	>25 yrs
									Lateral COM displacement		+		
									Peak ankle power		+		
									Peak hip power (initial)		+		
									Peak hip power (prewswing)		-		
Williams et al. (23)	2013	Severe	Hospital Able to run independently 20 m	T:44; C:15	T:27.9 C:28.1	T:81.8% C:73.3%	15 m run: Matched speed	25 reflective markers on pelvis and lower limbs	Speed	-			
									Cadence	+			
									Stride length	+			
									Stance time	+			
									Flight phase	+			
									Base of support	-			
									Trunk flexion	-			
									Pelvic rotation	+			
									Hip extension/adduction	-			
									Knee flexion	+			
									Ankle flexion	-			
									Lateral COM displacement	+			
									Ankle power	+			
									Knee power	+			
									Hip power	+			
							15 m run: Fastest speed		**Speed**	+			
									Cadence	+			
									Stride length	+			

(Continued)

TABLE 9 (Continued)

References	Year	Severity	Population	Sample size	Age (mean)	Sex (%male)	Procedure	Equipment	Measure	<5 yrs	6–10 yrs	11–25 yrs	>25 yrs
									Stance time	-			
									Flight phase	+			
									Base of support	+			
Williams et al. (24)	2016	Severe	Hospital Able to walk independently 20m	T:35; C:25	T:28.4 C:27.8	T:74.3% C:64%	12 m walkway: matched speed	25 reflective markers on pelvis and lower limbs	Hip work	-			
									Knee work	-			
									Ankle work	+			
									Hip power	-			
									Knee power	-			
									Ankle power	+			

*$p < 0.05$, significant; ***strong power; ** medium power; *low power; -, non-significant; gray, not assessed.

stability (23), which likely drive these alterations in gait. In a follow-up study, these authors followed patients for 6 months following severe TBI an average of 2 years earlier with access to a rehabilitation program (83). At baseline, previous TBI was again associated with significantly slower self-selected walking speed than healthy controls (83). However, at the 6-month follow-up, walking speed had significantly improved, such that there was no longer any difference compared to healthy controls, with an associated improvement in ankle power generation, indicating that these deficits can improve (83).

In addition to assessing gait alone, several studies investigated the effect of increasing difficulty via the inclusion of obstacles (54) and/or cognitive tasks (54, 56, 66) on gait following mild TBI (combined single and multiple). In symptomatic individuals <5 years following the last injury, adding an auditory Stroop task had little effect on gait (54, 56). In a community cohort, changes in rhythm were seen in the dual vs. single task in those with a history of mTBI compared to controls (54), whereas in a small cohort of military veterans no additive effect was seen with alterations in center of mass displacement seen in both walking alone and the dual task (56). Adding obstacles or a spatial memory task actually reduced the performance in healthy controls, such that differences seen in speed and double stance support time with walking alone compared to those with a history of TBI were no longer present (66). Indeed, only combining the memory task with obstacles while walking re-introduced the increase in double stance support time in those with a history of mTBI 6 years earlier (66). Hence, abnormalities in gait following mTBI may be detected by single task alone, without requiring the more difficult combined tasks.

3.5.4. Posture and balance

The effects of a prior TBI on chronic alterations in static balance were assessed under several conditions, with the majority of studies only investigating 1–5 years post-injury (52, 53, 57, 60, 63, 64, 67–69, 74, 78, 134) and no studies looking at greater than 10 years post-injury (Table 10). In healthy populations, maintenance of postural stability does not require large amount of conscious effort and is regulated by subconscious reflexive actions of the CNS to interpret and act in accordance to perceived sensory feedback information from the visual, somatosensory, and vestibular systems (135). Manipulating the type and/or amount of information being processed by these three sensory feedback systems increases the difficulty of balance tasks and can reveal injury effects (136–138). Across the included studies, postural control was examined primarily by evaluating alterations in center of mass while standing, with concomitant manipulation of the sensory information available via altering the standing surface, closing eyes, or altering the visual surroundings. Studies also incorporated the effects on balance in functional reaching involving either the leg (60) or arm (67, 119) as well as the effects of bimanual lifting of weights (64).

A history of asymptomatic mTBI <5 years earlier had no effects on postural control while standing, regardless of alterations in the support surface or visual feedback in a variety of cohorts including military veterans (65, 119), college athletes (68), and university students (78). This included measures of the center of pressure sway

area (69), sway path length (65), trunk pitch and roll angle (65), and the degree of anterior–posterior sway (68). On the one hand, in an arm functional reach task, mTBI, an average of 5.8 years earlier, was associated with reduced postural angular velocity, although no changes in angular displacement were noted (73). On the other hand, symptomatic mTBI was associated with greater alterations in balance, with increased postural sway both during quiet standing (65, 74) and when suddenly perturbed (65).

Repeat mild injury, either investigated as a separate cohort (68, 69) or via combining those with a history of single and repeated injuries (60, 63, 68–72, 76, 134), had more mixed effects on balance. In general, more difficult tasks were required to detect differences between those with a history of mTBI (1 or more) and healthy controls (52, 63, 69–71, 78). For example, in college students, no changes in amplitude, velocity, frequency, or regularity were seen in quiet standing in those with a mean time since the last injury of 7.1 years (mean 2.5 injuries) (71). However, two studies were able to detect postural changes with quiet standing alone, with collegiate athletes 19 months since their last injury (range 1–5 injuries) exhibiting an increase in center of mass oscillation irregularity (76) and a cohort recruited from the community with one or more mTBIs also at 19 months post-injury having a larger body sway area, a larger displacement amplitude in the medio-lateral direction, a slower body oscillation in both directions, and a more irregular pattern of body oscillation (72). In contrast, Wright et al. only noted a difference compared to healthy controls in individuals with more than one mTBI with the last injury at least a year ago in the most difficult condition, where participants were required to stand on a foam surface with a dynamic visual surrounding, leading to the increased center of pressure sway area (69). Similarly, Helmich et al. only saw an increase in effort of balance in symptomatic individuals within a mean of 2 years post-injury during balance on an unstable surface, with eyes closed or a combination of both, but not on a stable surface or with eyes open (78). At a year post-injury, Reilly et al. also found no effect of previous mTBI (combined single and multiple) on bipedal or unipedal stance alone but did see an increase in sway and decreased regularity when combined with a cognitive task (70). Conversely, Rosenblum et al. found no differences on the Sensory Organization Test at 2–3 years following last injury in a population of collegiate athletes (68). The Sensory Organization Test evaluates quiet standing under six different conditions (either fixed/sway surface, eyes open/closed, or surrounding normal/sway-referenced), thus involving increasing task difficulty. This was regardless of whether analysis looked at single vs. multiple injuries compared to healthy controls, or when those with a history of single or rmTBI were combined (68). In contrast, the same test in military veterans at an average of 10 years post-injury did find task-specific effects, with a decrease in equilibrium score in the eyes closed/fixed surface and eyes open, fixed surface/sway surroundings conditions only when looking at combined single and rmTBI and in these conditions, alongside the sway surface with eyes open or closed conditions, when analysis investigated the rmTBI cohort separate from single injuries (52, 53). Only one study utilized a different task, the Y Balance Test, where participants stand on one leg and reach the other in an anterior, posteromedial, or posterolateral direction (60). Acute deficits, with an increased amplitude of center of mass in the posteromedial and posterolateral direction in those with a history of TBI a median of 294 days ago, had resolved in those with their last injury a median of 3.5 years ago, with no deficits noted compared to healthy controls (60).

With a more severe injury, the same level of analysis examining multiple parameters such as regularity, amplitude, frequency, and velocity changes in the center of pressure has yet to occur, but consistent alterations in balance have been reported out to 10 years post-injury. A prior moderate–severe TBI a mean of 3 years earlier was associated with the increased center of pressure displacement across three tasks: standing only, standing with a numerical task, and standing with a spatial memory task, with no significant alterations in performance between the different tasks (57). Similarly, a prior severe TBI 10 years earlier led to a decrease in dynamic post-urography scores, where postural changes in response to a tilt platform were examined, alongside a higher Berg balance score (77). A functional reach task where participants were able to sit in a wheelchair while moving their arm to touch a target that appeared in either a predictable or unpredictable fashion found a medium–large effect size of prior moderate–severe TBI on stability ratio during the task (67). In a bimanual lifting task, although a history of previous severe TBI 2–10 years earlier was associated with greater instability in the quiet stance phase, the postural adjustments that occurred to lift 4 or 8 kg weights were not different from that of healthy subjects (64). Thus, balance changes appear to be similarly evident, particularly with quiet standing following more severe injury.

4. Discussion

This systematic review investigated chronic motor outcomes following TBI and the effect of injury severity. The results of this study provide a comprehensive overview of the current state of understanding of motor changes following TBI, highlighting limitations and gaps of existing research that are critical to filling in order to suggest guidelines for rehabilitation programs following TBI. There was little consensus across the articles presented, with a wide variety of motor domains examined, as well as significant differences in the methodology of the tests utilized and parameters reported. Indeed, the lack of consensus in the approaches used in assessing and reporting chronic motor outcomes in both preclinical and clinical models of TBI limits the generalizability of the findings. In the future, more standardized testing parameters and protocols for motor tasks would assist in comparing findings. For example, the development of common data elements for both preclinical and clinical studies would be of benefit, given that standardization and harmonization of data collection are of paramount importance (139).

Overall, there was a paucity of clinical studies investigating motor outcomes beyond 10 years post-injury, with only six identified within this review. The majority of these studies investigated fine motor control (25, 26, 51, 76), meaning that the long-term effects of TBI on gross motor functions, such as gait and postural control, have not been extensively studied. Furthermore, there was a lack of longitudinal clinical studies investigating how motor performance changes over time in the same cohort in the

TABLE 10 Clinical postural stability studies.

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test	Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
Geurts et al. (74)	1999	Mild	Hospital Persistent symptoms	T:15;C:20	T:35.9 C:35.4	T:53.3% C:60%	Postural control: Eyes open, closed or with simple cognitive task	Balance board 2x 30 secs per variable	**Anteroposterior sway**	+			
									Lateral sway	+			
									Weight shifting	+			
Wright et al. (69)	2018	Mild	Military veterans	T:9;C:19	T:25.95 C:33.57	T/C: 45%	Postural control Firm or foam, eyes open or closed, static or rolling scene	Virtual environment TBI screening 3 × 30secs per variable	Sway area	-			
Pan et al. (65)	2015	Mild	Military veterans Non-symptomatic	T:6, C:10	T:26.5 C: Not stated	T:100% C:Not stated	Postural control: On floor or foam; Eyes open or closed	Markers on sacrum, pelvis, C7 and shoulder.	Pelvic postural sway	-			
									Pelvic sway path length	-			
									Pitch trunk angle	-			
									Roll Trunk angle	-			
							Postural control^{+} perturbation: On floor or foam; Eyes open or closed		Upper trunk sway path length	-			
									Pelvic sway path length	-			
									Oscillations	-			
			Military veterans Symptomatic	T:8; C:10		T:87.5% C:Not stated	Postural control: On floor or foam; Eyes open or closed		**Pelvic postural sway**	+			
									Pelvic sway path length	+			
									Pitch trunk angle	+			

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test	Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
									Roll trunk angle	+			
							Postural control^{+} perturbation: On floor or foam; Eyes open or closed		**Upper trunk sway path length**	+			
									Pelvic sway path length	-			
									Oscillations	+			
Rosenblum et al. (68)	2020	Mild	College Athletes	T:91;C:129	T:18.9; C:19.1	T:56.6% C:56.6%	Sensory organization test Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding,	Smart Balance Master System 3 × 20secs per variable	Anterior-posterior sway (equilibrium score)	-			
									Somatosensory sensory ratio	-			
									Visual sensory ratio	-			
									Vestibular sensory ratio	-			
Ustinova (73)	2017	Mild	College Athletes	T:13; C:13	T:34.9 C:33.8	T:30.8% C:38.4%	Functional reach Interception of targets via arm at 5 fixed location	3D video game 30 reflective body markers 10 × 90sec games	Postural angular displacement		-		
									Postural angular velocity		+		
									Arm angular displacement		-		
									Arm angular velocity		+		
Wright et al. (69)	2018	Combination	Military veterans	T:14; C:19	T:25.95 C:33.57	T/C: 45%	Postural control Firm or foam, eyes open or closed, static or rolling scene	Virtual environment TBI screening 3 × 30secs per variable	Sway area	+			

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test	Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
Helmich et al. (78)	2016	Combination	University students w/o symptoms	T:13; C:10	T:29 C:27	T:Not stated C:40%	Postural control Stable vs. unstable surface, eyes open, closed or blurred	Force platform 10 × 10sec trials per variable	COP path	-			
									COP area	-			
									Effort of balance	-			
							Balance Error Scoring System		Score	-			
			University students with symptoms	T:7; C:10	T:26 C:26	T:Not stated C:40%	Postural control Stable vs. unstable surface, eyes open, closed or blurred		COP path	-			
									COP area	-			
									Effort of balance	+			
							Balance Error Scoring System		**Score**	+			
Degani et al. (72)	2017	Combination	Community	T:11; C:11	T: 29.4; C: 26.8	T:45.5% C:36%	Postural control Natural stance vs crossed arms	Force platform 10 mins	**Body sway area**	+			
									Amplitude of COP displacement	+			
									Mean velocity of COP displacement	+			
									Frequency of COP displacement	+			
									Regularity of COP displacement	+			
De Beaumont et al. (76)	2011	Combination	Collegiate athletes	T:21; C:15	T/C: 22.3	T/C: 100%	Postural control	Force platform 2 × 30 sec trials	**Regularity of COP displacement**	+			

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test	Method	Parameters		< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
									Amplitude of COP displacement		-			
Johnston et al. (60)	2020	Combination	Collegiate athletes	T:30; C:90	T:20.3 C:20.2	T/C: 83.5%	Y Balance test (Stand on one leg, reach other in anterior, posteromedial and posterolateral direction)	Lumbar inertial sensor 3 trials	Anterior	Reach distance	-			
										Regularity	-			
										Amplitude	-			
									Posteromedial	Reach distance	-			
										Regularity	-			
										Amplitude	-			
									Posterolateral	Reach distance	-			
										Regularity	-			
										Amplitude	-			
Ledwidge et al. (134)	2020	Combination	Collegiate athletes	T:21; C:24	T:20.17 C:20.03	T:90% C:79%	BESS balance test Feet together, non-dominant only, tandem on firm or foam surface	-	Score		-			
Lee et al. (71)	2020	Combination	College students	T:11; C:14	T:28.7 C:22	T:52% C:35.7	Postural stability	Force platform 120 sec	Body sway area			-		
									Amplitude			-		
									Mean velocity			-		
									Frequency			-		
									Regularity			-		

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test		Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
										Asynchrony AP and ML		-		
Reilly et al. (70)	2020	Combination	Community	T:27; C:27	T:26.1 C:28.6	T:44.4% C:77.8%	Postural stability	Bipedal only	Force platform	Mean velocity		-		
										Path length		-		
										AP sway		-		
										ML sway		-		
										Body sway area		-		
										Regularity (AP)		-		
										Regularity (ML)		-		
								Bipedal + cog task		Mean velocity		-		
										Path length		-		
										AP sway		+		
										ML sway		-		
										Body sway area		+		
										Regularity (AP)		+		
										Regularity (ML)		-		
								Unipedal only		Mean velocity		-		
										Path length		-		
										AP sway		-		
										ML sway		-		
										Body sway area		-		
										Regularity (AP)		+		
										Regularity (ML)		+		
								Unipedal + cog task		Mean velocity		-		
										Path length		-		

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test		Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
										AP sway		-		
										ML sway		+		
										Body sway area		+		
										Regularity (AP)		+		
										Regularity (ML)		+		
Rosenblum et al. (68)	2020	Combination	College Athletes	T177;C:129	T:19.1 C:19.1	T:57.6% C:56.6%	Sensory organization test Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding,		Smart Balance Master System 3 × 20secs per variable	Anterior-posterior sway (equilibrium score)	-			
										Somatosensory sensory ratio	-			
										Visual sensory ratio	-			
										Vestibular sensory ratio	-			
Sosnoff et al. (63)	2011	Combination	College athletes	T:62; C:162	T/C: 20.04	T/C: 67.8%	Sensory organization test Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding		NeuroCom Smart Balance Master 3 × each test	Composite balance score	-			
										Somatosensory sensory ratio	-			
										Visual sensory ratio	-			
										Vestibular sensory ratio	-			
										Regularity (AP)	+			
										Regularity (ML)	+			
Walker et al. (52)	2018	Combination	Military veterans	T:248; C:47	T:36 C:40.5	T:88% C:79.5%	Sensory organization test	Eyes open/fixed surface	NeuroCom Smart Balance Master 3 × each test	Equilibrium score		-		
								Eyes closed/fixed surface		**Equilibrium score**		+		

(Continued)

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test		Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
								Eyes open/fixed surface/sway surroundings		**Equilibrium score**		+		
								Eyes open, sway surface, fixed surroundings		Equilibrium score		-		
								Eyes closed, sway surrounding		Equilibrium score		-		
								Eyes open, sway surface, sway surrounding		Equilibrium score		-		
								Composite score		**Equilibrium score**		+		
Walker et al. (52)	2018	Combination	Military veterans	T:414; C:78	T:36 C:40.5	T:88.2% C:79.5%	Sensory organization test	Eyes open/fixed surface		Equilibrium score		-		
								Eyes closed/fixed surface		**Equilibrium score**		+		
								Eyes open/fixed surface/sway surroundings		**Equilibrium score**		+		
								Eyes open, sway surface, fixed surroundings		Equilibrium score				
								Eyes closed, sway surrounding		Equilibrium score				

(Continued)

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test		Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
								Eyes open, sway surface, sway surrounding		Equilibrium score				
Wright et al. (69)	2018	rmTBI	Military veterans	T:5;C:19	T:25.95 C:33.57	T/C: 45%	Postural control Firm or foam, eyes open or closed, static or rolling scene		Virtual environment TBI screening 3 × 30secs per variable	**Sway area**	+			
Rosenblum et al. (68)	2020	rmTBI	College Athletes	2*TBI:52 3*TBI:34; C: 129	2*T:19.1 3*T:19.8 C:18.1	2*T:62% 3*T:52% C:56.6%	Sensory organization test Fixed/ sway surface, eyes open/closed, surrounding/sway-referenced surrounding,		Smart Balance Master System 3 × 20secs per variable	Anterior-posterior sway (equilibrium score)	-			
										Somatosensory sensory ratio	-			
										Visual sensory ratio	-			
										Vestibular sensory ratio	-			
Walker et al. (52)	2018	rmTBI	Military veterans	T:248; C:47	T:41 C:46	T:88.3% C:78.7%	Sensory organization test	Eyes open/fixed surface	NeuroCom Smart Balance Master 3 × each test	Equilibrium score		-		
								Eyes closed/fixed surface		**Equilibrium score**		+		
								Eyes open/fixed surface/sway surroundings		**Equilibrium score**		+		
								Eyes open, sway surface, fixed surroundings		**Equilibrium score**		+		
								Eyes closed, sway surface		**Equilibrium score**		+		

(Continued)

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TABLE 10 (Continued)

References	Year	Severity	Population	Sample size	Age	Sex (%male)	Motor test		Method	Parameters	< 5 yrs	6–10 yrs	11–25 yrs	>25 yrs
								Eyes open, sway surface, sway surrounding		Equilibrium score		-		
								Composite score		**Composite Equilibrium Score**		+		
Useros Olmo et al. (57)	2020	Mod-sev	Hospital	T:20; C:19	T:36.1 C:38.2	T:85% C:89.5%	Postural control	Standing only	Force platform 2 mins recording	Displacement of COP	+			
								Standing + numerical cog task			+			
								Standing + spatial memory cog task			+			
Zhang et al. (83)	2002	Mod-sev	Rehabilitation	T:10; C:10	T:30.6 C:31.8	T:50% C:50%	Functional reach	Unpredictable	Force platform Board with two lights	**Stability ratio**	**			
								L predictable			**			
								R predictable			***			
Arce	2004	Severe	Community	T:7; C:10	T:26.4 C: 24.9	T:100% C:100%	Postural control: Bimanual lifting	No load × 3	Tetrax posturography system- two force platforms	**Stability score: standing**	+			
								4kg × 6		Forward weight shift	-			
								8kg × 6		% change vertical ground reaction force	-			
Buster et al. (77)	2013	Severe	Community	T;10: C:10	T:36; C:24	Not stated	Berg Balance test		None	**Score**		+		
							Dynamic posturography		Tilt platform 3 × 120 secs	**Dynamic movement analysis score**		+		

$^{+}p < 0.05$, -, not significant, ***strong power, **medium power.

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chronic phase post-injury. Similarly, in preclinical work, only nine studies investigated to 12 months post-injury (35, 36, 81, 82, 100, 105, 108, 114) and, of these, only two studies to 18–24 months post-injury (35, 36). More chronic studies are, therefore, needed to understand how a history of TBI interacts with normal aging to affect motor performance. Imaging studies have suggested that a history of TBI accelerates the rate of brain atrophy (25, 26, 40, 52, 55, 76) and studies investigating cognition have suggested TBI is associated with an earlier age of cognitive decline, not necessarily associated with a specific neurodegenerative disorder (140). Whether TBI similarly leads to earlier physical decline needs further investigation. This is particularly relevant given the growing literature linking a history of TBI to the later risk of neurodegenerative motor disorder development, particularly motor neuron disease (MND) and PD. For example, Wright et al. reported ALS-like pathological changes, accompanied by persistent motor deficits, at 12 weeks, but not 1 week, following a moderate experimental TBI in rats (141). This suggests that TBI may begin an insidious neurodegenerative process that predisposes an individual to the later development of motor neuron disease. This is in line with several previous studies conducted with professional athletes, including National Football League (NFL), (142, 143) soccer (144–147), and rugby union players (41). Overall, meta-analyses have suggested a 1.3- to 1.7-fold increase in motor neuron disease risk due to a prior history of TBI (38, 148, 149); however, not all literature has been consistent (150). Similar findings have also been reported for PD, with a doubling of deaths due to PD in former professional soccer players compared to a matched control group drawn from the general population (146). Even a mild TBI has been shown to increase risk of PD by 56% in US military veterans, after adjusting for demographics and comorbidities (40). Several potential biological mechanisms have been proposed to explain this link, including chronic neuroinflammation, metabolic dysregulation, and pathological upregulation of several key PD-linked proteins, including alpha-synuclein, hyperphosphorylated tau, amyloid precursor protein, TDP-43 and, more recently, leucine-rich repeat kinase 2 (LRRK2) and its Rab protein substrates [see Delic et al. for review (151)]. Motor dysfunction may also play a role in other neurodegenerative diseases linked to TBI, including chronic traumatic encephalopathy (CTE), which is characterized by the accumulation of hyperphosphorylated tau aggregates (148). Clinical data from 298 donors diagnosed with CTE identified motor symptoms in a large portion of cases, with gait and balance disturbance noted in 51% and signs of parkinsonism in up to 28% of cases (149). Thus, tracking alterations in motor function longitudinally in those with a prior history of TBI may allow for earlier identification, and subsequently treatment, of those at risk for the development of MND, PD, or CTE, currently a major area of clinical need.

Despite this significant gap, key findings from clinical studies conducted to date of chronic motor alterations following TBI suggest that measures of balance, including postural control and gait, could differentiate between levels of injury severity in those who had suffered there injury in the last 10 years and, importantly, could discriminate between symptomatic and asymptomatic mTBI sufferers. Balance requires multiple input and integration centers spanning the entire brain, with damage to any of these structures or their associated white matter networks resulting in balance impairment (136). A key feature of post-concussion syndrome may be disruption of these networks, subtlety impairing balance control. Given that stressing the sensorimotor integration centers of the brain elicited the greatest degree of impairment, it suggests that, following injury, there may be limited access to neural resources capable of compensating for reductions in sensory feedback information (either visual, vestibular, or somatosensory), as opposed to gross decreases in musculoskeletal or aerobic functional capacities (138). Entropy measures of postural sway parameters were particularly shown to be affected by symptomatic mTBI, with these measuring the regularity of center of pressure oscillations (137). From a motor control perspective, more regular values are interpreted as indicating a less stable system, as damage to neural tissue results in a reduced capacity for the complex oscillatory networks within the brain to produce and maintain upright posture under a wider variety of movement patterns (152). Decreased entropy values have been reported acutely following mTBI (153, 154) and are shown here to persist in a subpopulation of symptomatic sufferers. The specific mechanisms driving these balance disruptions, however, require further investigation.

Mechanistic investigations may be limited to date, due to significant differences in the examination of balance in preclinical models compared to measures employed clinically. Relatively few preclinical studies incorporated gait analysis, which could be due to the technology required to perform detailed analysis. Surprisingly, the one consistent finding seen in more severe clinical TBI, a reduction in speed, was not replicated in preclinical studies. Indeed, minimal gait deficits, in general, were found in preclinical studies, with only a reduction in swing speed and stride length at 6 months following moderate focal injury (29, 30) but no deficits at 1 month following a more severe injury (94). This may reflect the differing mechanisms of injury and severity of preclinical compared to clinical models. Preclinical models are limited in their abilities to model more severe TBI, which are associated with prolonged stays within the intensive care unit and long periods of rehabilitation, which may impact upon motor function (155). Furthermore, the location of contusional injuries differs in preclinical compared to clinical models, typically found in the pre-frontal and temporal lobes clinically (156) and the parietal lobes in preclinical models (157). Key differences in gait are also obviously evident in biped vs. quadrupeds, with center of mass higher in bipeds than quadrupeds (158) and increased frequency of gait patterns at higher speeds, such as trotting and galloping, in rodents, which are generally not seen in bipedal human (159). However, there were some preclinical findings that were supported clinically, with a model of mTBI finding alterations in base of support to 3 months post-injury (88), with clinical studies similarly showing an alteration in the equivalent double vs. single-stance support at 6 years post-injury (66). Thus, incorporation of longitudinal gait analysis out to more chronic time points in preclinical models of TBI would be useful.

Furthermore, there are no static tests of balance utilized in preclinical studies. Instead, balance assessment pre-clinically incorporates transitional movements utilizing tasks such as the rotarod, balance beam, grid walk, and ladder walk, which are all scored with gross parameters, such as number of foot faults, latency to cross, and speed achieved on the rotarod. These measures

may not be sensitive enough to detect subtle deficits, particularly in models of mTBI, especially given that the read out measures are relatively crude, a limitation that has previously been noted elsewhere (160). Indeed, even in more severe models of diffuse injury, chronic (>6 months) impairments in motor performance were not seen, unlike focal or mixed injury models (30, 100, 122), where more widespread disruption of motor pathways may occur. It has previously been noted that the lack of functional deficits in preclinical models is surprising given the amount of histological damage (161). Refinement of motor tests is needed to discern whether this is because the damage is not sufficient to drive functional changes, or whether the motor tests used are not sensitive enough. For example, utilizing center of pressure measurements may be an option for future studies, with this successfully employed previously in models of vestibular injury in rodents (162), given the sensitivity of the task in clinical work.

Another discrepancy between clinical and preclinical studies is the incorporation of fine-motor specific tasks. Although some of the balance tasks outlined above, such as the grid walk and rotarod, incorporate aspects of fine motor performance, the effects of injury specifically on this domain cannot be discerned. Furthermore, tasks like the adhesive removal test may be complicated by the presence of sensory deficits (163). Notably, given that a history of repeated injury clinically appeared to be associated with poorer performance on dexterity tasks (25, 26), the need for greater inclusion of these within preclinical work is supported. Only two studies incorporated a fine-motor specific task in the pellet reaching (34) and Montoya staircase tasks (94). These were only utilized following moderate–severe and focal injury, noting deficits to 6 weeks post-injury, making comparisons with the clinical work, where moderate–severe injury led to deficits at 10 years post-injury (51), but repeated mild TBI at >20 years, (25, 26) difficult. Investigation of other forepaw dexterity-based tasks, such as the vermicelli or cappelini handling tests (163), would also be useful to add to motor behavioral batteries post-TBI.

Alongside the need to utilize a wider variety of preclinical motor tasks, the field would also benefit from a broadening of the animals used. Preclinical rodent models have some limitations in the ability to fully model the types of white matter damage encountered in the diffuse injury of any severity due to the relative lack of myelinated tracks (164). Indeed, single diffuse or mixed injury models did not produce long-term motor deficits (>6 months) (36, 81, 108, 111, 112) compared to purely focal injury (29, 30), although there were obviously few studies that investigated these chronic time points. Promising gyrencephalic models with more extensive white matter tracts may provide a key bridge between preclinical and clinical work with development of porcine (165), ferret (166), and sheep (164) TBI models that could be utilized for future investigation of longitudinal motor deficits. A number of motor tasks have already been developed for these models including gait analysis (160) which provide useful information about the longitudinal trajectory of motor impairment.

In addition, studies to date have failed to take into consideration what effect age at injury may have on long-term motor outcomes following TBI. Motor function is well known to decline substantially with advancing age, with changes at the level of the motor unit (167), as well as at the neural level [for review, see Seidler et al. (168)]. For example, King et al. have demonstrated that age-related declines in motor performance are associated with stronger internetwork resting-state connectivity, suggesting breakdown of organization of large-scale brain networks (169). Similar changes in resting-state functional connectivity have been noted following TBI (170). It is, therefore, reasonable to hypothesize that advanced age may exacerbate alterations in motor performance following injury. In line with this, older age is known to be associated with poorer outcome following TBI, with older adults having the highest rate of hospitalization and death following TBI (171). Older adults have also been shown to experience greater decline in Disability Rating Scale scores over the first 5 years following injury (172). Despite this, however, to date, no clinical studies have investigated the effect of age at injury on chronic motor outcomes. Pre-clinically, only one study incorporated rats injured at different time points in adulthood, with no effect on overall long-term motor performance noted (111). Future studies should, therefore, be designed to include assessment of how age at time of injury may influence motor response. Indeed, in the preclinical literature, there is a growing call to include aged animals in the modeling of neurological disease more generally (173).

Although the current study was limited to chronic motor outcomes, it should be noted that these do not occur independent of effects on other functional domains, such as cognition. For example, following mTBI, it was found that there was a significant association between cognitive and motor function following injury but not prior (174). A potential explanation is that the effects of injury on attentional networks, which are a driver of cognitive dysfunction (175), also impair performance on numerous motor tasks, including postural control (176), gait (177), and the ability to perform fine motor tasks (178). Executive function more broadly is also key for motor performance, with patients with normal measures of executive function following moderate–severe TBI demonstrating better balance and agility and increased speed of walking and running than those with executive function deficits (179). Further study should, therefore, examine chronic motor outcomes in the context of the effects on broader functional domains, including cognition, in order to better explore the bidirectional nature of these relationships. This is particularly important given that effects on cognitive function can affect ability to participate in physical rehabilitation programs, which can have detrimental consequences for functional motor recovery.

Finally, it should also be noted that a high risk of bias was noted for the majority of preclinical and clinical studies included here, which may have influenced the results. For the preclinical studies, key sources of bias included random outcome assessment and blinding of outcome assessment, as well as allocation concealment. For clinical studies, a key source of bias was blinding of outcome assessment, as well and blinding of participants and personnel. If researchers are not blinded, this will have implicit biases on the data recording process and potentially the randomization of the study and random outcome detection, making it difficult to truly interpret results. Given the high risk of bias and high degree of heterogeneity between studies, it was not feasible to conduct a meta-analysis in the current study; however, in the future, it may be of interest to consider meta-analysis on specific data subsets.

In conclusion, despite the known relationship between mobility and quality of life following TBI (6), chronic motor performance following injury is less well studied than either cognitive or affective outcomes. Additionally, across the few studies conducted, significant differences in experimental paradigms employed in both clinical and preclinical studies make it difficult to discern the pattern of motor deficits in the subacute and chronic phase following injury, with more standardized protocols required. Furthermore, a broadening of the motor batteries utilized within preclinical studies is warranted to more closely mirror the types of balance and fine motor deficits identified clinically. This review highlights the need for more consistent investigation and reporting of long-term motor deficits to allow an understanding of their evolution over time. An understanding is a key to allow full insight into the recovery process, and rehabilitation needs of those with TBI and how chronic motor changes post-TBI could provide a novel method for identifying risk of neurodegenerative movement disorders.

Author contributions

FC, IC, and LC-P contributed to conception and design of the study, reviewed articles for inclusion, contributed to the results, and wrote sections of the manuscript. IC performed the search and organized the database. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.1180353/full#supplementary-material

References

1. Masel BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. *J Neurotrauma.* (2010) 27:1529–40. doi: 10.1089/neu.2010.1358

2. Brett BL, Gardner RC, Godbout J, Dams-O'Connor K, Keene CD. Traumatic brain injury and risk of neurodegenerative disorder trauma brain. *Inj Ment Health.* (2022) 91:498–507. doi: 10.1016/j.biopsych.2021.05.025

3. Corrigan JD, Hammond FM. Traumatic brain injury as a chronic health condition. *Arch Phys Med Rehabil.* (2013) 94:1199–201. doi: 10.1016/j.apmr.2013.01.023

4. Nelson LD, Temkin NR, Dikmen S, Barber J, Giacino JT, Yuh E, et al. Recovery after mild traumatic brain injury in patients presenting to US Level I trauma centers: a transforming research and clinical knowledge in traumatic brain injury (TRACK-TBI) Study. *JAMA Neurol.* (2019) 76:1049–59. doi: 10.1001/jamaneurol.2019.1313

5. Stocchetti N, Zanier ER. Chronic impact of traumatic brain injury on outcome and quality of life: a narrative review. *Crit Care Lond Engl.* (2016) 20:148. doi: 10.1186/s13054-016-1318-1

6. Kalpinski RJ, Williamson MLC, Elliott TR, Berry JW, Underhill AT, Fine PR. Modeling the prospective relationships of impairment, injury severity, and participation to quality of life following traumatic brain injury. *BioMed Res Int.* (2013) 2013:102570. doi: 10.1155/2013/102570

7. Williamson MLC, Elliott TR, Bogner J, Dreer LE, Arango-Lasprilla JC, Kolakowsky-Hayner SA, et al. Trajectories of Life Satisfaction Over the First 10 Years After Traumatic Brain Injury: Race, Gender, and Functional Ability. *J Head Trauma Rehabil.* (2016) 31:167–79. doi: 10.1097/HTR.0000000000000111

8. Gera G, Chesnutt J, Mancini M, Horak FB, King LA. Inertial sensor-based assessment of central sensory integration for balance after mild traumatic brain injury. *Mil Med.* (2018) 183:327–32. doi: 10.1093/milmed/usx162

9. Lin L-F, Liou T-H, Hu C-J, Ma H-P, Ou J-C, Chiang Y-H, et al. Balance function and sensory integration after mild traumatic brain injury. *Brain Inj.* (2015) 29:41–6. doi: 10.3109/02699052.2014.955881

10. Walker WC, Pickett TC. Motor impairment after severe traumatic brain injury: A longitudinal multicenter study. *J Rehabil Res Dev.* (2007) 44:975–82. doi: 10.1682/JRRD.2006.12.0158

11. Swaine BR, Sullivan SJ. Longitudinal profile of early motor recovery following severe traumatic brain injury. *Brain INJ.* (1996) 10:347–66. doi: 10.1080/026990596124368

12. Agrawal M, Joshi M. Impact of rehabilitation on functional outcome during the first year of moderate and severe traumatic brain injury. *Brain Inj.* (2014) 28:292–7. doi: 10.3109/02699052.2013.865266

13. Patil M, Gupta A, Khanna M, Taly AB, Soni A, Kumar JK, et al. Cognitive and functional outcomes following inpatient rehabilitation in patients with acquired brain injury: a prospective follow-up study. *J Neurosci Rural Pract.* (2017) 8:357–63. doi: 10.4103/jnrp.jnrp_53_17

14. Sandhaug M, Andelic N, Langhammer B, Mygland A. Functional level during the first 2 years after moderate and severe traumatic brain injury. *Brain Inj.* (2015) 29:1431–8. doi: 10.3109/02699052.2015.1063692

15. Hart T, Kozlowski AJ, Whyte J, Poulsen I, Kristensen K, Nordenbo A, et al. Functional recovery after severe traumatic brain injury: an individual growth curve approach. *Arch Phys Med Rehabil.* (2014) 95:2103–10. doi: 10.1016/j.apmr.2014.07.001

16. Dikmen S, Machamer J, Temkin N. Psychosocial outcome in patients with moderate to severe head injury: 2-year follow-up. *Brain Inj.* (1993) 7:113–24. doi: 10.3109/02699059309008165

17. Jourdan C, Bayen E, Pradat-Diehl P, Ghout I, Darnoux E, Azerad S, et al. A comprehensive picture of 4-year outcome of severe brain injuries. Results from the PariS-TBI study. *Ann Phys Rehabil Med.* (2016) 59:100–6. doi: 10.1016/j.rehab.2015.10.009

18. Chmielewski TL, Tatman J, Suzuki S, Horodyski M, Reisman DS, Bauer RM, et al. Impaired motor control after sport-related concussion could increase risk for musculoskeletal injury: Implications for clinical management and rehabilitation. *J Sport Health Sci.* (2021) 10:154–61. doi: 10.1016/j.jshs.2020.11.005

19. De Beaumont L, Mongeon D, Tremblay S, Messier J, Prince F, Leclerc S, et al. Persistent motor system abnormalities in formerly concussed athletes. *J Athl Train.* (2011) 46:234–40. doi: 10.4085/1062-6050-46.3.234

20. Theadom A, Parag V, Dowell T, McPherson K, Starkey N, Barker-Collo S, et al. Persistent problems 1 year after mild traumatic brain injury: a longitudinal population study in New Zealand. *Br J Gen Pract J R Coll Gen Pract.* (2016) 66:e16–23. doi: 10.3399/bjgp16X683161

21. Ponsford JL, Olver JH, Curran C, A. profile of outcome: 2 years after traumatic brain injury. *Brain Inj.* (1995) 9:1–10. doi: 10.3109/02699059509004565

22. Williams G, Morris ME, Schache A, McCrory PR. People Preferentially Increase Hip Joint Power Generation to Walk Faster Following Traumatic Brain Injury. *Neurorehabil Neural Repair.* (2010) 24:550–8. doi: 10.1177/1545968309357925

23. Williams G, Schache A, Morris ME. Running abnormalities after traumatic brain injury. *Brain Inj.* (2013) 27:434–43. doi: 10.3109/02699052.2012.750754

24. Williams G, Schache AG. The distribution of positive work and power generation amongst the lower-limb joints during walking normalises following recovery from traumatic brain injury. *Gait Posture.* (2016) 43:265–9. doi: 10.1016/j.gaitpost.2015.10.009

25. Pearce AJ, Hoy K, Rogers MA, Corp DT, Maller JJ, Drury HGK, et al. The long-term effects of sports concussion on retired Australian football players: a study using transcranial magnetic stimulation. *J Neurotrauma.* (2014) 31:1139–45. doi: 10.1089/neu.2013.3219

26. Pearce AJ, Rist B, Fraser CL, Cohen A, Maller JJ. Neurophysiological and cognitive impairment following repeated sports concussion injuries in retired professional rugby league players. *Brain Inj.* (2018) 32:498–505. doi: 10.1080/02699052.2018.1430376

27. Hall ANB, Joseph B, Brelsfoard JM, Saatman KE. Repeated closed head injury in mice results in sustained motor and memory deficits and chronic cellular changes. *Borlongan CV ed PLOS ONE.* (2016) 11:e0159442. doi: 10.1371/journal.pone.0159442

28. Henry RJ, Meadows VE, Stoica BA, Faden AI, Loane DJ. Longitudinal assessment of sensorimotor function after controlled cortical impact in mice: comparison of beamwalk, rotarod, and automated gait analysis tests. *J Neurotrauma.* (2020) 37:2709–17. doi: 10.1089/neu.2020.7139

29. Ritzel RM Li Y, He J, Khan N, Doran SJ, Faden AI, et al. Sustained neuronal and microglial alterations are associated with diverse neurobehavioral dysfunction long after experimental brain injury. *Neurobiol Dis.* (2020) 136:104713. doi: 10.1016/j.nbd.2019.104713

30. Daglas M, Draxler DF, Ho H, McCutcheon F, Galle A, Au AE, et al. Activated CD8(+) T cells cause long-term neurological impairment after traumatic brain injury in mice. *Cell Rep.* (2019). 29:1178–1191.e6. doi: 10.1016/j.celrep.2019.09.046

31. Cline MM, Yumul JC, Hysa L, Murra D, Garwin GG, Cook DG, et al. Novel application of a Radial Water Tread maze can distinguish cognitive deficits in mice with traumatic brain injury. *Brain Res.* (2017) 1657:140–7. doi: 10.1016/j.brainres.2016.11.027

32. Rana A, Singh S, Deshmukh R, Kumar A. Pharmacological potential of tocopherol and doxycycline against traumatic brain injury-induced cognitive/motor impairment in rats. *Brain Inj.* (2020) 34:1039–50. doi: 10.1080/02699052.2020.1772508

33. Pruitt D, Hays S, Schmid A, Choua C, Kim L, Trieu J, et al. Controlled-cortical impact reduces volitional forelimb strength in rats. *Brain Res.* (2014) 1582:91–8. doi: 10.1016/j.brainres.2014.07.039

34. Adkins DL, Ferguson L, Lance S, Pevtsov A, McDonough K, Stamschror J, et al. Combining multiple types of motor rehabilitation enhances skilled forelimb use following experimental traumatic brain injury in rats. *Neurorehabil Neural Repair.* (2015) 29:989–1000. doi: 10.1177/1545968315576577

35. Mouzon BC, Bachmeier C, Ferro A, Ojo J-O, Crynen G, Acker CM, et al. Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model: Chronic Effects of r-mTBI. *Ann Neurol.* (2014) 75:241–54. doi: 10.1002/ana.24064

36. Mouzon BC, Bachmeier C, Ojo JO, Acker CM, Ferguson S, Paris D, et al. Lifelong behavioral and neuropathological consequences of repetitive mild traumatic brain injury. *Ann Clin Transl Neurol.* (2018) 5:64–80. doi: 10.1002/acn3.510

37. Klima D, Morgan L, Baylor M, Reilly C, Gladmon D, Davey A. Physical performance and fall risk in persons with traumatic brain injury. *Percept Mot Skills.* (2019) 126:50–69. doi: 10.1177/0031512518809203

38. Chen H, Richard M, Sandler DP, Umbach DM, Kamel F. Head injury and amyotrophic lateral sclerosis. *Am J Epidemiol.* (2007) 166:810–6. doi: 10.1093/aje/kwm153

39. Crane PK, Gibbons LE, Dams-O'Connor K, Trittschuh E, Leverenz JB, Keene CD, et al. Association of Traumatic Brain Injury With Late-Life Neurodegenerative Conditions and Neuropathologic Findings. *JAMA Neurol.* (2016) 73:1062–9. doi: 10.1001/jamaneurol.2016.1948

40. Gardner RC, Peltz CB, Kenney K, Covinsky KE, Diaz-Arrastia R, Yaffe K. Remote traumatic brain injury is associated with motor dysfunction in older military veterans. *J Gerontol Ser A.* (2017) 72:1233–8. doi: 10.1093/gerona/glw341

41. Russell ER, Mackay DF, Lyall D, Stewart K, MacLean JA, Robson J, et al. Neurodegenerative disease risk among former international rugby union players. *J Neurol Neurosurg Psychiatry.* (2022) 93:1262–8. doi: 10.1136/jnnp-2022-329675

42. Adnan A, Crawley A, Mikulis D, Moscovitch M, Colella B, Green R. Moderate–severe traumatic brain injury causes delayed loss of white matter integrity: Evidence of fornix deterioration in the chronic stage of injury. *Brain Inj.* (2013) 27:1415–22. doi: 10.3109/02699052.2013.823659

43. Green R, Collella B, Maller J, Bayley M, Glazer J, Mikulis D, et al. Scale and pattern of atrophy in the chronic stages of moderate-severe TBI. *Front-Hum Neurosci.* (2014) 8. doi: 10.3389/fnhum.2014.00067

44. Greenberg G, Mikulis DJ, Ng K, DeSouza D, Green RE. Use of diffusion tensor imaging to examine subacute white matter injury progression in moderate to severe traumatic brain injury. *Arch Phys Med Rehabil.* (2008) 89:S45–50. doi: 10.1016/j.apmr.2008.08.211

45. Bendlin BB, Ries ML, Lazar M, Alexander AL, Dempsey RJ, Rowley HA, et al. Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. *Neuroimage.* (2008) 42:503–14. doi: 10.1016/j.neuroimage.2008.04.254

46. Poudel GR JFD D, Verhelst H, Vander Linden C, Deblaere K, Jones DK, et al. Network diffusion modeling predicts neurodegeneration in traumatic brain injury. *Ann Clin Transl Neurol.* (2020) 7:270–9. doi: 10.1002/acn3.50984

47. Harris TC, de Rooij R, Kuhl E. The Shrinking Brain: Cerebral Atrophy Following Traumatic Brain Injury. *Ann Biomed Eng.* (2019) 47:1941–59. doi: 10.1007/s10439-018-02148-2

48. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* (2009) 6:e1000100. doi: 10.1371/journal.pmed.1000100

49. Hooijmans CR, Rovers MM, De Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol.* (2014) 14:43. doi: 10.1186/1471-2288-14-43

50. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* (2011) 343:d5928–d5928. doi: 10.1136/bmj.d5928

51. Burton CL, Hultsch DF, Strauss E, Hunter MA. Intraindividual Variability in Physical and Emotional Functioning: Comparison of Adults With Traumatic Brain Injuries and Healthy Adults. *Clin Neuropsychol.* (2002) 16:264–79. doi: 10.1076/clin.16.3.264.13854

52. Walker WC, Nowak KJ, Kenney K, Franke LM, Eapen BC, Skop K, et al. Is balance performance reduced after mild traumatic brain injury? Interim analysis from chronic effects of neurotrauma consortium (CENC) multi-centre study. *Brain Inj.* (2018) 32:1156–68. doi: 10.1080/02699052.2018.1483529

53. Walker WC, Hirsch S, Carne W, Nolen T, Cifu DX, Wilde EA, et al. Chronic Effects of Neurotrauma Consortium (CENC) multicentre study interim analysis: Differences between participants with positive versus negative mild TBI histories. *Brain Inj.* (2018) 32:1079–89. doi: 10.1080/02699052.2018.1479041

54. Martini DN, Parrington L, Stuart S, Fino PC, King LA. Gait performance in people with symptomatic, chronic mild traumatic brain injury. *J Neurotrauma.* (2021) 38:218–24. doi: 10.1089/neu.2020.6986

55. Vanderploeg RD, Curtiss G, Luis CA, Salazar AM. Long-term morbidities following self-reported mild traumatic brain injury. *J Clin Exp Neuropsychol.* (2007) 29:585–98. doi: 10.1080/13803390600826587

56. Pitt W, Chou L-S, A. case-control study of gait balance control in veterans with chronic symptoms following mTBI. *Gait Posture.* (2020) 76:188–92. doi: 10.1016/j.gaitpost.2019.12.010

57. Olmo AIU, Periañez JA, Martínez-Pernía D, Page JCM. Effects of spatial working memory in balance during dual tasking in traumatic brain injury and healthy controls. *Brain Inj.* (2020) 34:1159–67. doi: 10.1080/02699052.2020.1792984

58. Vasudevan EVL, Glass RN, Packel AT. Effects of traumatic brain injury on locomotor adaptation. *J Neurol Phys Ther.* (2014) 38:172–82. doi: 10.1097/NPT.0000000000000049

59. Williams G, Morris ME, Schache A, McCrory PR. Incidence of Gait Abnormalities After Traumatic Brain Injury. *Arch Phys Med Rehabil.* (2009) 90:587–93. doi: 10.1016/j.apmr.2008.10.013

60. Johnston W, Heiderscheit B, Sanfilippo J, Brooks MA, Caulfield B. Athletes with a concussion history in the last two years have impairments in dynamic balance performance. *Scand J Med Sci Sports.* (2020) 30:1497–505. doi: 10.1111/sms.13691

61. Ledwidge PS, Patterson JN, Molfese DL, Honaker JA. Clinical Utility of Oculomotor and Electrophysiological Measures in Identifying Concussion History. *Clin J Sport Med.* (2017). 29:292. doi: 10.1097/JSM.0000000000000512

62. Williams G, Robertson V, Greenwood K. Measuring High-Level Mobility After Traumatic Brain Injury. *Am J Phys Med Rehabil.* (2004) 83:910–20. doi: 10.1097/01.PHM.0000146503.25221.1D

63. Sosnoff JJ, Broglio SP, Shin S, Ferrara MS. Previous mild traumatic brain injury and postural-control dynamics. *J Athl Train.* (2011) 46:85–91. doi: 10.4085/1062-6050-46.1.85

64. Arce FI, Katz N, Sugarman H. The scaling of postural adjustments during bimanual load-lifting in traumatic brain-injured adults. *Hum Mov Sci.* (2004) 22:749–68. doi: 10.1016/j.humov.2003.12.002

65. Pan T, Liao K, Roenigk K, Daly JJ, Walker MF. Static and dynamic postural stability in veterans with combat-related mild traumatic brain injury. *Gait Posture.* (2015) 42:550–7. doi: 10.1016/j.gaitpost.2015.08.012

66. Martini DN, Sabin MJ, DePesa SA, Leal EW, Negrete TN, Sosnoff JJ, et al. The chronic effects of concussion on gait. *Arch Phys Med Rehabil.* (2011) 92:585–9. doi: 10.1016/j.apmr.2010.11.029

67. Zhang L, Abreu BC, Gonzales V, Huddleston N, Ottenbacher KJ. The effect of predictable and unpredictable motor tasks on postural control after traumatic brain injury. *NeuroRehabilitation.* (2002) 17:225–30. doi: 10.3233/NRE-2002-17308

68. Rosenblum DJ, Walton SR, Erdman NK, Broshek DK, Hart JM, Resch JE. If not now, when? An absence of neurocognitive and postural stability deficits in collegiate athletes with one or more concussions. *J Neurotrauma.* (2020) 37:1211–20. doi: 10.1089/neu.2019.6813

69. Wright WG, Handy JD, Avcu P, Ortiz A, Haran FJ, Doria M, et al. Healthy active duty military with lifetime experience of mild traumatic brain injury exhibits subtle deficits in sensory reactivity and sensory integration during static balance. *Mil Med.* (2018) 183:313–20. doi: 10.1093/milmed/usx182

70. Reilly N, Prebor J, Moxey J, Schussler E. Chronic impairments of static postural stability associated with history of concussion. *Exp Brain Res.* (2020) 238:2783–93. doi: 10.1007/s00221-020-05934-4

71. Lee H, Lee S, Black I, Salado L, Estrada J, Isla K. Long-term impact of mild traumatic brain injuries on multiple functional outcomes and epigenetics: a pilot study with college students. *Appl Sci.* (2020) 10:4131. doi: 10.3390/app10124131

72. Degani AM, Santos MM, Leonard CT, Rau TF, Patel SA, Mohapatra S, et al. The effects of mild traumatic brain injury on postural control. *Brain Inj.* (2017) 31:49–56. doi: 10.1080/02699052.2016.1225982

73. Ustinova KI. Decomposition of postural movements in individuals with mild TBI while reaching to intercept a moving virtual target. *Physiother Theory Pract.* (2017) 33:527–34. doi: 10.1080/09593985.2017.1325958

74. Geurts ACH, Knoop JA, van Limbeek J. Is postural control associated with mental functioning in the persistent postconcussion syndrome? *Arch Phys Med Rehabil.* (1999) 80:144–9. doi: 10.1016/S0003-9993(99)90111-9

75. Stuart S, Parrington L, Morris R, Martini DN, Fino PC, King LA. Gait measurement in chronic mild traumatic brain injury: A model approach. *Hum Mov Sci.* (2020) 69:102557. doi: 10.1016/j.humov.2019.102557

76. De Beaumont L, Théoret H, Mongeon D, Messier J, Leclerc S, Tremblay S, et al. Brain function decline in healthy retired athletes who sustained their last sports concussion in early adulthood. *Brain.* (2009) 132:695–708. doi: 10.1093/brain/awn347

77. Buster T, Burnfield J, Taylor AP, Stergiou N. Lower extremity kinematics during walking and elliptical training in individuals with and without traumatic brain injury. *J Neurol Phys Ther.* (2013) 37:176–86. doi: 10.1097/NPT.0000000000000022

78. Helmich I, Berger A, Lausberg H. Neural control of posture in individuals with persisting postconcussion symptoms. *Med Sci Sports Exerc.* (2016) 48:2362–9. doi: 10.1249/MSS.0000000000001028

79. Laurer HL, Bareyre FM, Lee VM, Trojanowski JQ, Longhi L, Hoover R, et al. Mild head injury increasing the brain's vulnerability to a second concussive impact. *J Neurosurg.* (2001) 95:859–70. doi: 10.3171/jns.2001.95.5.0859

80. Fehily B, Bartlett CA, Lydiard S, Archer M, Milbourn H, Majimbi M, et al. Differential responses to increasing numbers of mild traumatic brain injury in a rodent closed-head injury model. *J Neurochem.* (2019) 149:660–78. doi: 10.1111/jnc.14673

81. Sell SL, Johnson K, DeWitt DS, Prough DS. Persistent behavioral deficits in rats after parasagittal fluid percussion injury. *J Neurotrauma.* (2017) 34:1086–96. doi: 10.1089/neu.2016.4616

82. Pierce JE, Smith DH, Trojanowski JQ, McIntosh TK. Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience.* (1998) 87:359–69. doi: 10.1016/S0306-4522(98)00142-0

83. Zhang C, Saatman KE, Royo NC, Soltesz KM, Millard M, Schouten JW, et al. Delayed transplantation of human neurons following brain injury in rats: a long-term graft survival and behavior study. *J Neurotrauma.* (2005) 22:1456–74. doi: 10.1089/neu.2005.22.1456

84. Nissinen J, Andrade P, Natunen T, Hiltunen M, Malm T, Kanninen K, et al. Disease-modifying effect of atipamezole in a model of post-traumatic epilepsy. *Epilepsy Res.* (2017) 136:18–34. doi: 10.1016/j.eplepsyres.2017.07.005

85. Segovia-Oropeza M, Santiago-Castaneda C, Orozco-Suárez SA, Concha L, Rocha L. Sodium cromoglycate decreases sensorimotor impairment and hippocampal alterations induced by severe traumatic brain injury in rats. *J Neurotrauma.* (2020) 37:2595–603. doi: 10.1089/neu.2019.6975

86. Huynh LM, Burns MP, Taub DD, Blackman MR, Zhou J. Chronic neurobehavioral impairments and decreased hippocampal expression of genes important for brain glucose utilization in a mouse model of mild TBI. *Front Endocrinol.* (2020) 11:556380. doi: 10.3389/fendo.2020.556380

87. Zhang J, Wang R-J, Chen M, Liu X-Y, Ma K, Xu H-Y, et al. Collagen/heparan sulfate porous scaffolds loaded with neural stem cells improve neurological function in a rat model of traumatic brain injury. *Neural Regen Res.* (2021) 16:1068–77. doi: 10.4103/1673-5374.300458

88. Mountney A, Boutté AM, Cartagena CM, Flerlage WF, Johnson WD, Rho C, et al. Functional and molecular correlates after single and repeated rat closed-head concussion: indices of vulnerability after brain injury. *J Neurotrauma.* (2017) 34:2768–89. doi: 10.1089/neu.2016.4679

89. Feng Y, Li K, Roth E, Chao D, Mecca CM, Hogan QH, et al. Repetitive mild traumatic brain injury in rats impairs cognition, enhances prefrontal cortex neuronal activity, and reduces pre-synaptic mitochondrial function. *Front Cell Neurosci.* (2021) 15:689334. doi: 10.3389/fncel.2021.689334

90. Wang Z, Wang S-N, Xu T-Y, Hong C, Cheng M-H, Zhu P-X, et al. Cerebral organoids transplantation improves neurological motor function in rat brain injury. *CNS Neurosci Ther.* (2020) 26:682–97. doi: 10.1111/cns.13286

91. Shear DA, Lu X-CM, Bombard MC, Pedersen R, Chen Z, Davis A, et al. Longitudinal characterization of motor and cognitive deficits in a model of penetrating ballistic-like brain injury. *J Neurotrauma.* (2010) 27:1911–23. doi: 10.1089/neu.2010.1399

92. Thau-Zuchman O, Svendsen L, Dyall SC, Paredes-Esquivel U, Rhodes M, Priestley JV, et al. A new ketogenic formulation improves functional outcome and reduces tissue loss following traumatic brain injury in adult mice. *Theranostics.* (2021) 11:346–60. doi: 10.7150/thno.48995

93. Namdar I, Feldman R, Glazer S, Meningher I, Shlobin NA, Rubovitch V, et al. Motor effects of minimal traumatic brain injury in mice. *J Mol Neurosci.* (2020) 70:365–77. doi: 10.1007/s12031-019-01422-9

94. Schönfeld L-M, Jahanshahi A, Lemmens E, Schipper S, Dooley D, Joosten E, et al. Long-term motor deficits after controlled cortical impact in rats can be detected by fine motor skill tests but not by automated gait analysis. *J Neurotrauma.* (2017) 34:505–16. doi: 10.1089/neu.2016.4440

95. Hoffman SW, Virmani S, Simkins RM, Stein DG. The delayed administration of dehydroepiandrosterone sulfate improves recovery of function after traumatic brain injury in rats. *J Neurotrauma.* (2003) 20:859–70. doi: 10.1089/089771503322385791

96. Alwis DS, Yan EB, Morganti-Kossmann M-C, Rajan R. Sensory Cortex Underpinnings of Traumatic Brain Injury Deficits. *PLoS ONE.* (2012) 7:e52169. doi: 10.1371/journal.pone.0052169

97. Evans TM, Jaramillo CA, Sataranatarajan K, Watts L, Sabia M, Qi W, et al. The effect of mild traumatic brain injury on peripheral nervous system pathology in wild-type mice and the G93A mutant mouse model of motor neuron disease. *Neuroscience.* (2015) 298:410–23. doi: 10.1016/j.neuroscience.2015.04.041

98. Evans TM, Van Remmen H, Purkar A, Mahesula S, Gelfond JA, Sabia M, et al. Microwave and magnetic (M2) proteomics of a mouse model of mild traumatic brain injury. *Transl Proteomics.* (2014) 3:10–21. doi: 10.1016/j.trprot.2014.03.002

99. Tabet M, El-Kurdi M, Haidar MA, Nasrallah L, Reslan MA, Shear D, et al. Mitoquinone supplementation alleviates oxidative stress and pathologic outcomes following repetitive mild traumatic brain injury at a chronic time point. *Exp Neurol.* (2022) 351:113987. doi: 10.1016/j.expneurol.2022.113987

100. Dhillon NK, Linaval NT, O'Rourke J, Barmparas G, Yang A, Cho N, et al. How repetitive traumatic injury alters long-term brain function. *J Trauma Acute Care Surg.* (2020) 89:955–61. doi: 10.1097/TA.0000000000002811

101. Pruitt DT, Danaphongse TT, Schmid AN, Morrison RA, Kilgard MP, Rennaker RL, et al. Traumatic brain injury occludes training-dependent cortical reorganization in the contralesional hemisphere. *J Neurotrauma.* (2017) 34:2495–503. doi: 10.1089/neu.2016.4796

102. Homsi S, Piaggio T, Croci N, Noble F, Plotkine M, Marchand-Leroux C, et al. Blockade of acute microglial activation by minocycline promotes neuroprotection and reduces locomotor hyperactivity after closed head injury in mice: a twelve-week follow-up study. *J Neurotrauma.* (2010) 27:911–21. doi: 10.1089/neu.2009.1223

103. Bajwa NM, Halavi S, Hamer M, Semple BD, Noble-Haeusslein LJ, Baghchechi M, et al. Mild concussion, but not moderate traumatic brain injury, is associated with long-term depression-like phenotype in mice. *PLoS ONE.* (2016) 11:e0146886. doi: 10.1371/journal.pone.0146886

104. Morriss NJ, Conley GM, Hodgson N, Boucher M, Ospina-Mora S, Fagiolini M, et al. Visual dysfunction after repetitive mild traumatic brain injury in a mouse model and ramifications on behavioral metrics. *J Neurotrauma.* (2021) 38:2881–95. doi: 10.1089/neu.2021.0165

105. Tucker LB, Velosky AG, Fu AH, McCabe JT. Chronic Neurobehavioral Sex Differences in a Murine Model of Repetitive Concussive Brain Injury. *Front Neurol.* (2019) 10:509. doi: 10.3389/fneur.2019.00509

106. McAteer KM, Corrigan F, Thornton E, Turner RJ, Vink R. Short and long term behavioral and pathological changes in a novel rodent

model of repetitive mild traumatic brain injury. *PLoS ONE.* (2016) 11:e0160220. doi: 10.1371/journal.pone.0160220

107. Corrigan F, Arulsamy A, Collins-Praino LE, Holmes JL, Vink R. Toll like receptor 4 activation can be either detrimental or beneficial following mild repetitive traumatic brain injury depending on timing of activation. *Brain Behav Immun.* (2017) 64:124–39. doi: 10.1016/j.bbi.2017.04.006

108. Arulsamy A, Corrigan F, Collins-Praino LE. Cognitive and neuropsychiatric impairments vary as a function of injury severity at 12 months post-experimental diffuse traumatic brain injury: Implications for dementia development. *Behav Brain Res.* (2019) 365:66–76. doi: 10.1016/j.bbr.2019.02.045

109. Leconte C, Benedetto C, Lentini F, Simon K, Ouaazizi C, Taib T, et al. Histological and behavioral evaluation after traumatic brain injury in mice: A ten months follow-up study. *J Neurotrauma.* (2020) 37:1342–57. doi: 10.1089/neu.2019.6679

110. Islam MBAR, 4th BTD, Kando MJ, Mao Q, Procissi D, Weiss C, et al. Differential neuropathology and functional outcome after equivalent traumatic brain injury in aged versus young adult mice. *Exp Neurol.* (2021) 341:113714. doi: 10.1016/j.expneurol.2021.113714

111. Rowe RK, Ziebell JM, Harrison JL, Law LM, Adelson PD, Lifshitz J. Aging with traumatic brain injury: effects of age at injury on behavioral outcome following diffuse brain injury in rats. *Dev Neurosci.* (2016) 38:195–205. doi: 10.1159/000446773

112. Ajao DO, Pop V, Kamper JE, Adami A, Rudobeck E, Huang L, et al. Long-term sequelae of traumatic brain injury in rats: A morphological, behavioral, and electrophysiological study. *Neurosci Behav Physiol.* (2021) 51:209–19. doi: 10.1007/s11055-021-01059-5

113. Arulsamy A, Teng J, Colton H, Corrigan F, Collins-Praino L. Evaluation of early chronic functional outcomes and their relationship to pre-frontal cortex and hippocampal pathology following moderate-severe traumatic brain injury. *Behav Brain Res.* (2018) 348:127–38. doi: 10.1016/j.bbr.2018.04.009

114. Arun P, Wilder DM, Eken O, Urioste R, Batuure A, Sajja S, et al. Long-Term Effects of Blast Exposure: A Functional Study in Rats Using an Advanced Blast Simulator. *J Neurotrauma.* (2020) 37:647–55. doi: 10.1089/neu.2019.6591

115. Carron SF, Sun M, Shultz SR. Rajan R.Inhibitory neuronal changes following a mixed diffuse-focal model of traumatic brain injury. *J Comp Neurol.* (2020) 528:175–98. doi: 10.1002/cne.24746

116. Hanscom M, Loane DJ, Aubretch T, Leser J, Molesworth K, Hedgekar N, et al. Acute colitis during chronic experimental traumatic brain injury in mice induces dysautonomia and persistent extraintestinal, systemic, and CNS inflammation with exacerbated neurological deficits. *J Neuroinflammation.* (2021) 18:24. doi: 10.1186/s12974-020-02067-x

117. Lai J-H, Karlsson TE, Wu JC-C, Huang C-Z, Chen Y-H, Kang S-J, et al. Role of nogo receptor-1 for recovery of balance, cognition, and emotion after mild traumatic brain injury in mice. *J Neurotrauma.* (2019) 36:1054–9. doi: 10.1089/neu.2018.5949

118. Soblosky JS, Colgin LL, Chorney-Lane D, Davidson JF, Carey ME. Some functional recovery and behavioral sparing occurs independent of task-specific practice after injury to the rat's sensorimotor cortex. *Behav Brain Res.* (1997) 89:51–9. doi: 10.1016/S0166-4328(97)00049-1

119. Wright WG, McDevitt J, Tierney R, Haran FJ, Appiah-Kubi KO, Dumont A. Assessing subacute mild traumatic brain injury with a portable virtual reality balance device. *Disabil Rehabil.* (2017) 39:1564–72. doi: 10.1080/09638288.2016.1226432

120. Xie B-S, Wang Y-Q, Lin Y, Mao Q, Feng J-F, Gao G-Y, et al. Inhibition of ferroptosis attenuates tissue damage and improves long-term outcomes after traumatic brain injury in mice. *CNS Neurosci Ther.* (2019) 25:465–75. doi: 10.1111/cns.13069

121. Xu S-Y, Liu M, Gao Y, Cao Y, Bao J-G, Lin Y-Y, et al. Acute histopathological responses and long-term behavioral outcomes in mice with graded controlled cortical impact injury. *Neural Regen Res.* (2019) 14:997–1003. doi: 10.4103/1673-5374.250579

122. Barrett JP, Henry RJ, Shirey KA, Doran SJ, Makarevich OD, Ritzel RM, et al. Interferon-β plays a detrimental role in experimental traumatic brain injury by enhancing neuroinflammation that drives chronic neurodegeneration. *J Neurosci.* (2020) 40:2357–70. doi: 10.1523/JNEUROSCI.2516-19.2020

123. Chen H, Chan YL, Nguyen LT, Mao Y, de Rosa A, Beh IT, et al. Moderate traumatic brain injury is linked to acute behaviour deficits and long term mitochondrial alterations. *Clin Exp Pharmacol Physiol.* (2016) 43:1107–14. doi: 10.1111/1440-1681.12650

124. Albayram O, Kondo A, Mannix R, Smith C, Tsai C-Y, Li C, et al. Cis P-tau is induced in clinical and preclinical brain injury and contributes to post-injury sequelae. *Nat Commun.* (2017) 8:1000. doi: 10.1038/s41467-017-01068-4

125. Mannix R, Berglass J, Berkner J, Moleus P, Qiu J, Andrews N, et al. Chronic gliosis and behavioral deficits in mice following repetitive mild traumatic brain injury. *J Neurosurg.* (2014) 121:1342–50. doi: 10.3171/2014.7.JNS14272

126. Mannix R, Berkner J, Mei Z, Alcon S, Hashim J, Robinson S, et al. Adolescent Mice Demonstrate a Distinct Pattern of Injury after Repetitive Mild Traumatic Brain Injury. *J Neurotrauma.* (2017) 34:495–504. doi: 10.1089/neu.2016.4457

127. Sabbagh JJ, Fontaine SN, Shelton LB, Blair LJ, Jr JBH, Zhang B, et al. Noncontact Rotational Head Injury Produces Transient Cognitive Deficits but Lasting Neuropathological Changes. *J Neurotrauma.* (2016) 33:1751–60. doi: 10.1089/neu.2015.4288

128. Toshkezi G, Kyle M, Longo SL, Chin LS, Zhao L-R. Brain repair by hematopoietic growth factors in the subacute phase of traumatic brain injury. *J Neurosurg.* (2018) 129:1286–94. doi: 10.3171/2017.7.JNS17878

129. He J, Russell T, Qiu X, Hao F, Kyle M, Chin L, et al. The contribution of stem cell factor and granulocyte colony-stimulating factor in reducing neurodegeneration and promoting neurostructure network reorganization after traumatic brain injury. *Brain Res.* (2020) 1746:147000. doi: 10.1016/j.brainres.2020.147000

130. Tan XL, Zheng P, Wright DK, Sun M, Brady RD, Liu S, et al. The genetic ablation of tau improves long-term, but not short-term, functional outcomes after experimental traumatic brain injury in mice. *Brain Inj.* (2020) 34:131–9. doi: 10.1080/02699052.2019.1667539

131. Hou J, Nelson R, Wilkie Z, Mustafa G, Tsuda S, Thompson FJ, et al. Mild and Mild-to-Moderate Traumatic Brain Injury-Induced Significant Progressive and Enduring Multiple Comorbidities. *J Neurotrauma.* (2017) 34:2456–66. doi: 10.1089/neu.2016.4851

132. Vogel A, Wilken-Schmitz A, Hummel R, Lang M, Gurke R, Schreiber Y, et al. Low brain endocannabinoids associated with persistent non-goal directed nighttime hyperactivity after traumatic brain injury in mice. *Sci Rep.* (2020) 10:14929. doi: 10.1038/s41598-020-71879-x

133. Henry RJ, Ritzel RM, Barrett JP, Doran SJ, Jiao Y, Leach JB, et al. Microglial Depletion with CSF1R Inhibitor During Chronic Phase of Experimental Traumatic Brain Injury Reduces Neurodegeneration and Neurological Deficits. *J Neurosci Off J Soc Neurosci.* (2020) 40:2960–74. doi: 10.1523/JNEUROSCI.2402-19.2020

134. Ledwidge PS, Patterson JN, Molfese DL, Honaker JA. Clinical utility of oculomotor and electrophysiological measures in identifying concussion history. *Clin J Sport Med.* (2019) 29:292–7.

135. Rasman BG, Forbes PA, Tisserand R, Blouin J-S. Sensorimotor manipulations of the balance control loop-beyond imposed external perturbations. *Front Neurol.* (2018) 9:899. doi: 10.3389/fneur.2018.00899

136. Surgent OJ, Dadalko OI, Pickett KA, Travers BG. Balance and the brain: A review of structural brain correlates of postural balance and balance training in humans. *Gait Posture.* (2019) 71:245–52. doi: 10.1016/j.gaitpost.2019.05.011

137. Kedziorek J, Błazkiewicz M. Nonlinear measures to evaluate upright postural stability: a systematic review. *Entropy Basel Switz.* (2020) 22:1357. doi: 10.3390/e22121357

138. Caccese JB, Santos FV, Yamaguchi FK, Buckley TA, Jeka JJ. Persistent visual and vestibular impairments for postural control following concussion: a cross-sectional study in university students. *Sports Med Auckl NZ.* (2021) 51:2209–20. doi: 10.1007/s40279-021-01472-3

139. Meeuws S, Yue JK, Huijben JA, Nair N, Lingsma HF, Bell MJ, et al. Common data elements: critical assessment of harmonization between current multi-center traumatic brain injury studies. *J Neurotrauma.* (2020) 37:1283–90. doi: 10.1089/neu.2019.6867

140. Iacono D, Raiciulescu S, Olsen C, Perl DP. Traumatic brain injury exposure lowers age of cognitive decline in AD and Non-AD Conditions. *Front Neurol.* (2021) 12:573401. doi: 10.3389/fneur.2021.573401

141. Wright DK, Liu S, van der Poel C, McDonald SJ, Brady RD, Taylor L, et al. Traumatic Brain Injury Results in Cellular, Structural and Functional Changes Resembling Motor Neuron Disease. *Cereb Cortex N Y N.* (2017) 27:4503–4515. doi: 10.1093/cercor/bhw254

142. Vanacore N. Neurodegenerative causes of death among retired National Football League players. *Neurology.* (2012) 79:1970–4. doi: 10.1212/WNL.0b013e31826daf50

143. Daneshvar DH, Mez J, Alosco ML, Baucom ZH, Mahar I, Baugh CM, et al. Incidence of and Mortality From Amyotrophic Lateral Sclerosis in National Football League Athletes. *JAMA Netw Open.* (2021) 4:e2138801. doi: 10.1001/jamanetworkopen.2021.38801

144. Belli S, Vanacore N. Proportionate mortality of Italian soccer players: is amyotrophic lateral sclerosis an occupational disease? *Eur J Epidemiol.* (2005) 20:237–42. doi: 10.1007/s10654-004-6879-7

145. Chiò A, Benzi G, Dossena M, Mutani R, Mora G. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. *Brain J Neurol.* (2005) 128:472–476. doi: 10.1093/brain/awh373

146. Mackay DF, Russell ER, Stewart K, MacLean JA, Pell JP, Stewart W. Neurodegenerative Disease Mortality among Former Professional Soccer Players. *N Engl J Med.* (2019) 381:1801–8. doi: 10.1056/NEJMoa1908483

147. Pupillo E, Bianchi E, Vanacore N, Montalto C, Ricca G, Cuna FSRD, et al. Increased risk and early onset of ALS in professional players from Italian Soccer Teams. *Amyotroph Lateral Scler Front Degener.* (2020) 21:403–9. doi: 10.1080/21678421.2020.1752250

148. Gu D, Ou S, Tang M, Yin Z, Wang Z, Liu G. Trauma and amyotrophic lateral sclerosis: a systematic review and meta-analysis. *Amyotroph Lateral Scler Front Degener.* (2021) 22:170–85. doi: 10.1080/21678421.2020.1861024

149. Liu G, Ou S, Cui H, Li X, Yin Z, Gu D, et al. Head injury and amyotrophic lateral sclerosis: a meta-analysis. *Neuroepidemiology.* (2021) 55:11–19. doi: 10.1159/000510987

150. Watanabe Y, Watanabe T. Meta-analytic evaluation of the association between head injury and risk of amyotrophic lateral sclerosis. *Eur J Epidemiol.* (2017) 32:867–79. doi: 10.1007/s10654-017-0327-y

151. Delic V, Beck KD, Pang KC, Citron BA. Biological links between traumatic brain injury and Parkinson's disease. *Acta Neuropathol Commun.* (2020) 8:45. doi: 10.1186/s40478-020-00924-7

152. Huang H-P, Hsu CF, Mao Y-C, Hsu L, Chi S. Gait Stability Measurement by Using Average Entropy. *Entropy Basel Switz.* (2021) 23:412. doi: 10.3390/e23040412

153. Gao J, Hu J, Buckley T, White K, Hass C. Shannon and Renyi entropies to classify effects of Mild Traumatic Brain Injury on postural sway. *PLoS ONE.* (2011) 6:e24446. doi: 10.1371/journal.pone.0024446

154. Cavanaugh JT, Guskiewicz KM, Giuliani C, Marshall S, Mercer VS, Stergiou N. Recovery of postural control after cerebral concussion: new insights using approximate entropy. *J Athl Train.* (2006) 41:305–13.

155. Dos Santos C, Hussain SNA, Mathur S, Picard M, Herridge M, Correa J, et al. Mechanisms of Chronic Muscle Wasting and Dysfunction after an Intensive Care Unit Stay. A Pilot Study. *Am J Respir Crit Care Med.* (2016) 194:821–30. doi: 10.1164/rccm.201512-2344OC

156. Adatia K, Newcombe VFJ, Menon DK. Contusion progression following traumatic brain injury: a review of clinical and radiological predictors, and influence on outcome. *Neurocrit Care.* (2021) 34:312–24. doi: 10.1007/s12028-020-00994-4

157. Osier N, Dixon CE. The Controlled cortical impact model of experimental brain trauma: overview, research applications, and protocol. *Methods Mol Biol Clifton NJ.* (2016) 1462:177–92. doi: 10.1007/978-1-4939-3816-2_11

158. Zehr EP, Barss TS, Dragert K, Frigon A, Vasudevan EV, Haridas C, et al. Neuromechanical interactions between the limbs during human locomotion: an evolutionary perspective with translation to rehabilitation. *Exp Brain Res.* (2016) 234:3059–81. doi: 10.1007/s00221-016-4715-4

159. Mendes CS, Bartos I, Márka Z, Akay T, Márka S, Mann RS. Quantification of gait parameters in freely walking rodents. *BMC Biol.* (2015) 13:50. doi: 10.1186/s12915-015-0154-0

160. Shultz SR, McDonald SJ, Corrigan F, Semple BD, Salberg S, Zamani A, et al. Clinical Relevance of Behavior Testing in Animal Models of Traumatic Brain Injury. *J Neurotrauma.* (2020) 37:2381–400. doi: 10.1089/neu.2018.6149

161. Turner RC, VanGilder RL, Naser ZJ, Lucke-Wold BP, Bailes JE, Matsumoto RR, et al. Elucidating the severity of preclinical traumatic brain injury models: a role for functional assessment? *Neurosurgery.* (2014) 74:382–394. doi: 10.1227/NEU.0000000000000292

162. Rastoldo G, Marouane E, El Mahmoudi N, Péricat D, Bourdet A, Timon-David E, et al. Quantitative evaluation of a new posturo-locomotor phenotype in a rodent model of acute unilateral vestibulopathy. *Front Neurol.* (2020) 11:505. doi: 10.3389/fneur.2020.00505

163. Mann A, Chesselet M-F. Chapter 8 - Techniques for Motor Assessment in Rodents. In: *Movement Disorders (Second Edition)*. LeDoux MS. Boston: Academic Press. (2015). p. 139–157. doi: 10.1016/B978-0-12-405195-9.00008-1

164. Vink R. Large animal models of traumatic brain injury. *J Neurosci Res.* (2018) 96:527–35. doi: 10.1002/jnr.24079

165. Kinder HA, Baker EW, West FD. The pig as a preclinical traumatic brain injury model: current models, functional outcome measures, and translational detection strategies. *Neural Regen Res.* (2019) 14:413–24. doi: 10.4103/1673-5374.245334

166. Schwerin SC, Hutchinson EB, Radomski KL, Ngalula KP, Pierpaoli CM, Juliano SL. Establishing the ferret as a gyrencephalic animal model of traumatic brain injury: Optimization of controlled cortical impact procedures. *J Neurosci Methods.* (2017) 285:82–96. doi: 10.1016/j.jneumeth.2017.05.010

167. Hunter SK, Pereira HM, Keenan KG. The aging neuromuscular system and motor performance. *J Appl Physiol Bethesda.* (2016) 121:982–995. doi: 10.1152/japplphysiol.00475.2016

168. Seidler RD, Bernard JA, Burutolu TB, Fling BW, Gordon MT, Gwin JT, et al. Motor control and aging: links to age-related brain structural, functional, and biochemical effects. *Neurosci Biobehav Rev.* (2010) 34:721–33. doi: 10.1016/j.neubiorev.2009.10.005

169. King BR, van Ruitenbeek P, Leunissen I, Cuypers K, Heise K-F, Monteiro TS, et al. Age-related declines in motor performance are associated with decreased segregation of large-scale resting state brain networks. *Cereb Cortex.* (2018) 28:4390–402. doi: 10.1093/cercor/bhx297

170. Iraji A, Benson RR, Welch RD, O'Neil BJ, Woodard JL, Ayaz SI, et al. Resting state functional connectivity in mild traumatic brain injury at the acute stage: independent component and seed-based analyses. *J Neurotrauma.* (2015) 32:1031–45. doi: 10.1089/neu.2014.3610

171. Thompson HJ, McCormick WC, Kagan SH. Traumatic brain injury in older adults: epidemiology, outcomes, and future implications. *J Am Geriatr Soc.* (2006) 54:1590–5. doi: 10.1111/j.1532-5415.2006.00894.x

172. Plata CDMd, Hart T, Hammond FM, Frol AB, Hudak A, Harper CR, et al. Impact of age on long-term recovery from traumatic brain injury. *Arch Phys Med Rehabil.* (2008). 89:896–903. doi: 10.1016/j.apmr.2007.12.030

173. Sun M, McDonald SJ, Brady RD, Collins-Praino L, Yamakawa GR, Monif M, et al. The need to incorporate aged animals into the preclinical modeling of neurological conditions. *Neurosci Biobehav Rev.* (2020) 109:114–28. doi: 10.1016/j.neubiorev.2019.12.027

174. Sosnoff JJ, Broglio SP, Ferrara MS. Cognitive and motor function are associated following mild traumatic brain injury. *Exp Brain Res.* (2008) 187:563–71. doi: 10.1007/s00221-008-1324-x

175. Halterman CI, Langan J, Drew A, Rodriguez E, Osternig LR, Chou L-S, et al. Tracking the recovery of visuospatial attention deficits in mild traumatic brain injury. *Brain J Neurol.* (2006) 129:747–753. doi: 10.1093/brain/awh705

176. Woollacott M, Shumway-Cook A. Attention and the control of posture and gait: a review of an emerging area of research. *Gait Posture.* (2002) 16:1–14. doi: 10.1016/S0966-6362(01)00156-4

177. Yogev-Seligmann G, Hausdorff JM, Giladi N. The role of executive function and attention in gait. *Mov Disord.* (2008). 23(3):329–342. doi: 10.1002/mds.21720

178. Dias da Silva MR, Postma M. Straying Off Course: The Negative Impact of Mind Wandering on Fine Motor Movements. *J Mot Behav.* (2022) 54:186–202. doi: 10.1080/00222895.2021.1937032

179. Sarajuuri J, Pasanen M, Rinne M, Vartiainen M, Lehto T, Alaranta H. Relationship between cognitive and motor performance in physically well-recovered men with traumatic brain injury. *J Rehabil Med.* (2013) 45:38–46. doi: 10.2340/16501977-1060

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Long-Term Impact of Diffuse Traumatic Brain Injury on Neuroinflammation and Catecholaminergic Signaling: Potential Relevance for Parkinson's Disease Risk

Ing Chee Wee [1], Alina Arulsamy [2], Frances Corrigan [3] and Lyndsey Collins-Praino [1,*]

[1] Cognition, Ageing and Neurodegenerative Disease Laboratory, School of Biomedicine, The University of Adelaide, Adelaide, SA 5005, Australia; ingchee.wee@adelaide.edu.au
[2] Neuropharmacology Research Laboratory, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway 47500, Selangor Darul Ehsan, Malaysia; alina.arulsamy@monash.edu
[3] Head Injury Lab, School of Biomedicine, The University of Adelaide, Adelaide, SA 5005, Australia; frances.corrigan@adelaide.edu.au
* Correspondence: lyndsey.collins-praino@adelaide.edu.au

Abstract: Traumatic brain injury (TBI) is associated with an increased risk of developing Parkinson's disease (PD), though the exact mechanisms remain unclear. TBI triggers acute neuroinflammation and catecholamine dysfunction post-injury, both implicated in PD pathophysiology. The long-term impact on these pathways following TBI, however, remains uncertain. In this study, male Sprague-Dawley rats underwent sham surgery or Marmarou's impact acceleration model to induce varying TBI severities: single mild TBI (mTBI), repetitive mild TBI (rmTBI), or moderate–severe TBI (msTBI). At 12 months post-injury, astrocyte reactivity (GFAP) and microglial levels (IBA1) were assessed in the striatum (STR), substantia nigra (SN), and prefrontal cortex (PFC) using immunohistochemistry. Key enzymes and receptors involved in catecholaminergic transmission were measured via Western blot within the same regions. Minimal changes in these markers were observed, regardless of initial injury severity. Following mTBI, elevated protein levels of dopamine D1 receptors (DRD1) were noted in the PFC, while msTBI resulted in increased alpha-2A adrenoceptors (ADRA2A) in the STR and decreased dopamine beta-hydroxylase (DβH) in the SN. Neuroinflammatory changes were subtle, with a reduced number of GFAP+ cells in the SN following msTBI. However, considering the potential for neurodegenerative outcomes to manifest decades after injury, longer post-injury intervals may be necessary to observe PD-relevant alterations within these systems.

Keywords: traumatic brain injury; Parkinson's disease; neuroinflammation; dopamine; noradrenaline

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1. Introduction

Traumatic brain injury (TBI) is a significant global cause of both mortality and disability [1]. In 2013, the United States alone recorded approximately 2.8 million TBI-related ED visits, hospitalizations, and deaths [2,3]. Worldwide, using the Global Burden of Disease Study 2016 data, 27.08 million (24.30–30.30 million) new cases of TBI were reported in 2016 compared to 2015 [4], although some estimates have suggested that this number may be as high as 50 million new cases globally each year [5]. While conventional belief once held that the effects of TBI could resolve over time, it is now well-recognized that TBI is, in fact, an ongoing disease process that may increase the risk for the later development of various neurodegenerative disorders, including Parkinson's disease (PD), in a dose-dependent manner (for review, see Brett et al. 2022) [6]. In line with this, following a moderate–severe TBI, the likelihood of developing PD was higher compared to mild TBI (hazard ratio 1.50 vs. 1.24), with the risk of PD development increasing further with the cumulative effect of multiple TBIs in comparison to a single TBI (hazard ratio 1.87 vs. 1.45) [7].

Despite this, the precise neural mechanisms that underlie the relationship between TBI and the risk of PD development remain a subject of ongoing exploration. One such mechanism is the disruption in catecholamines, including dopamine (DA) and noradrenaline (NA), following injury. The loss of dopaminergic (DA) neurons within the substantia nigra (SN) is the pathological hallmark of PD [8–12]. This loss results in a disruption in the intricate balance of dopamine within the dorsal striatum (STR)—a region that receives DA projections directly from the SN [13–15]—subsequently leading to the manifestation of the cardinal motor symptoms observed in PD [8,16–19]. However, PD also involves the loss of noradrenergic (NA) neurons, with a 20–90% loss of NA neurons within the locus coeruleus (LC), a small brainstem structure that is the principal source of noradrenaline for the brain [20–23]. In fact, the LC is among the first brain regions to be affected in PD [24], with the loss of neurons in the LC beginning prior to, and to a greater extent, than that observed within the SN [20,24]. This depletion of LC neurons disrupts the supply of NA to regions such as the pre-frontal cortex (PFC), which is thought to contribute to various non-motor symptoms experienced by PD patients, including cognitive impairment, depression, and anxiety [8,25–27].

The depletion of dopamine and noradrenaline observed in PD can trigger a cascade of events involving the immune system, driving microglial and astrocyte activation and the concomitant release of pro-inflammatory molecules [28–34]. This neuroinflammatory process persists over time, in turn exacerbating the degeneration of DA and NA neurons and creating a self-perpetuating cycle of inflammation and neurodegeneration [35,36]. Evidence from a multitude of both in vitro and toxin-based animal model studies collectively underscores the vulnerability of SN-DA and LC-NA neurons in a chronic neuroinflammatory environment, with these neurons being among the most sensitive neurons in the brain to the effects of neuroinflammation [28,31,37,38]. There is also a growing body of neuropathological and biochemical evidence showing elevated levels of activated microglia and pro-inflammatory cytokines within the brains of individuals with PD, hinting at the pivotal involvement of neuroinflammation in disease pathology [39–42]. Further, the degeneration of LC-NA neurons leads to a loss of immune cell modulation by NA, further exacerbating SN-DA neuronal loss and disease pathogenesis (for review, see Butkovich et al. 2018) [43].

Critically, the disruption in catecholamines seen in PD is also present following TBI [44–48]. Studies in rodents have shown that, at 4 weeks following a moderate TBI induced via either controlled cortical impact or lateral fluid percussion, there are noticeable changes in dopamine metabolism, coupled with significant reductions in protein expression of the dopamine transporter (DAT), within both the STR and SN [48–50]. Similarly, disruptions in NA turnover rates were found in male Sprague-Dawley rats that sustained a moderate unilateral contusion induced by an air piston following the initial 30 min after injury [51]. Notably, a significant increase in NA turnover of 72% compared to uninjured controls was reported in the LC [51], with this upregulation found to decrease from 1 to 8 weeks post moderate diffuse injury in the same model [52]. Clinically, among 10 patients who experienced severe TBI several months prior, Single Photon Emission Computed Tomography (SPECT) imaging demonstrated significant disturbances in nigrostriatal function, as evidenced by reduced striatal DAT and D2-like receptor binding [53]. While there has been limited clinical research to date exploring changes in noradrenaline levels following TBI, the utilization of NA-targeting medications in TBI patients has shown promise, with improved recovery and reduced mortality [54–56]. Particularly noteworthy, a meta-analysis consisting of 17 randomized controlled trials investigating methylphenidate, a medication that enhances NA activity, suggested that methylphenidate administration led to improved processing speed in those with a history of TBI, particularly with prolonged drug duration, implying that NA level may be compromised following TBI, and that promoting NA activity could potentially restore this disrupted NA balance and lead to improvements in cognitive function [57].

Similarly, upregulation and prolonged neuroinflammation across different brain regions (including those relevant to PD pathology are some of the key consequential responses

that the brain employs in response to the initial injury [58–60]. For instance, in mice with a diffuse axonal injury induced by the weight drop acceleration model, significant increases in proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, were observed in the medial PFC up to 9 days following a diffuse mild injury [61]. Furthermore, an increase in the transcription factor NF-κB and the proinflammatory enzymes Cox-2 and iNOS, as well as an upregulation in total microglial population, was demonstrated within the SN in mice at 4 weeks following a focal moderate TBI induced by controlled cortical impact [50]. Similarly, following a moderate midline fluid percussion injury, Sprague-Dawley rats demonstrated an elevation of proinflammatory cytokines, including IL-1β, IL-6, TNF-α, and CXCL1, in the cortex, STR, and SN, as early as 3–6 h post-injury [62]. This pro-inflammatory response appears to persist, with male Sprague-Dawley rats showing upregulated TSPO and CD45 gene expression in the SN, indicative of microglial activation, up to 28 days post-injury in a moderate diffuse injury induced by midline fluid percussion [48]. Similarly, within the STR, higher numbers of activated microglia have been reported up to 8 weeks following moderate injury induced by controlled cortical impact compared to sham uninjured animals [63]. Clinically, in a study of 10 patients with moderate–severe TBI, microglial activation has been shown to persist in several relevant brain regions, including the putamen, thalamus, and posterior limb of the internal capsule, even 17 years after the initial injury [64].

Taken together, this raises the possibility that persistent disruptions in catecholamine signaling and concomitant increases in neuroinflammation could, at least in part, help to set the stage for the later emergence of PD. However, despite this evident pathophysiological overlap, key questions remain. To date, only a limited number of preclinical studies have investigated the disruption of catecholamines following TBI, and the duration of observation often concludes prior to 6 months post-injury. In line with this, the longest follow-up investigated time point that we could identify in the literature was 28 weeks post-injury, where a 30% reduction in dopaminergic neurons was observed in animals subjected to moderate fluid percussion injury [65]. How catecholamine signaling may change at more chronic time points post-injury is not yet known. In contrast, there has been some long-term investigation of changes in neuroinflammation, with persistent microglial activity reported up to 24 months following TBI [59,66–68]. Noteworthy among these is the work of Mouzon and colleagues, demonstrating subtle, yet elevated, neuroinflammation in the corpus callosum from 12 to 24 months following both single and repetitive mild closed-head diffuse injuries [67,68]. Nevertheless, whether this post-TBI neuroinflammatory response is severity-dependent and how it may correlate with alterations in catecholamines remains to be explored.

As such, this study aimed to explore alterations in markers related to both catecholaminergic pathways and neuroinflammation within key PD-related regions (i.e., the STR, SN, and PFC at 12-months post-injury in a preclinical model of diffuse axonal injury of varying severities, including single mild, repetitive mild, and moderate–severe TBI. We hypothesized that there would be decreases in the integrity of both catecholaminergic pathways, with concomitant increases in markers of inflammation, at this chronic time point post-injury. This is significant because if markers of catecholaminergic signaling and neuroinflammation are chronically altered following TBI, monitoring such changes could potentially provide a means of identifying those at risk for later PD development, allowing for earlier identification, prior to the clinical onset of motor symptoms.

2. Results

2.1. Subtle Alterations in Neuroinflammation Observed following Moderate–Severe TBI at 12-Months Post-Injury

Analysis of the total number of GFAP+ cells at 12 months post-injury found no significant difference in the PFC ($F(3,21) = 0.57$, $p = 0.64$) or the STR ($F(3,20) = 1.292$, $p = 0.3$) (Figure 1a,c), but an overall effect was found in the SN ($F(3,18) = 4.293$, $p = 0.02$), with the msTBI group showing a significantly lower number of GFAP+ cells/mm^2 compared to the sham group (326.41 ± 22.17 cells/mm^2 vs. 448.32 ± 35.27 cells/mm^2, $p = 0.013$) (Figure 1e).

No significant injury effect was found in any region for the percentage of reactive astrocytes (Figure 1b,d,f).

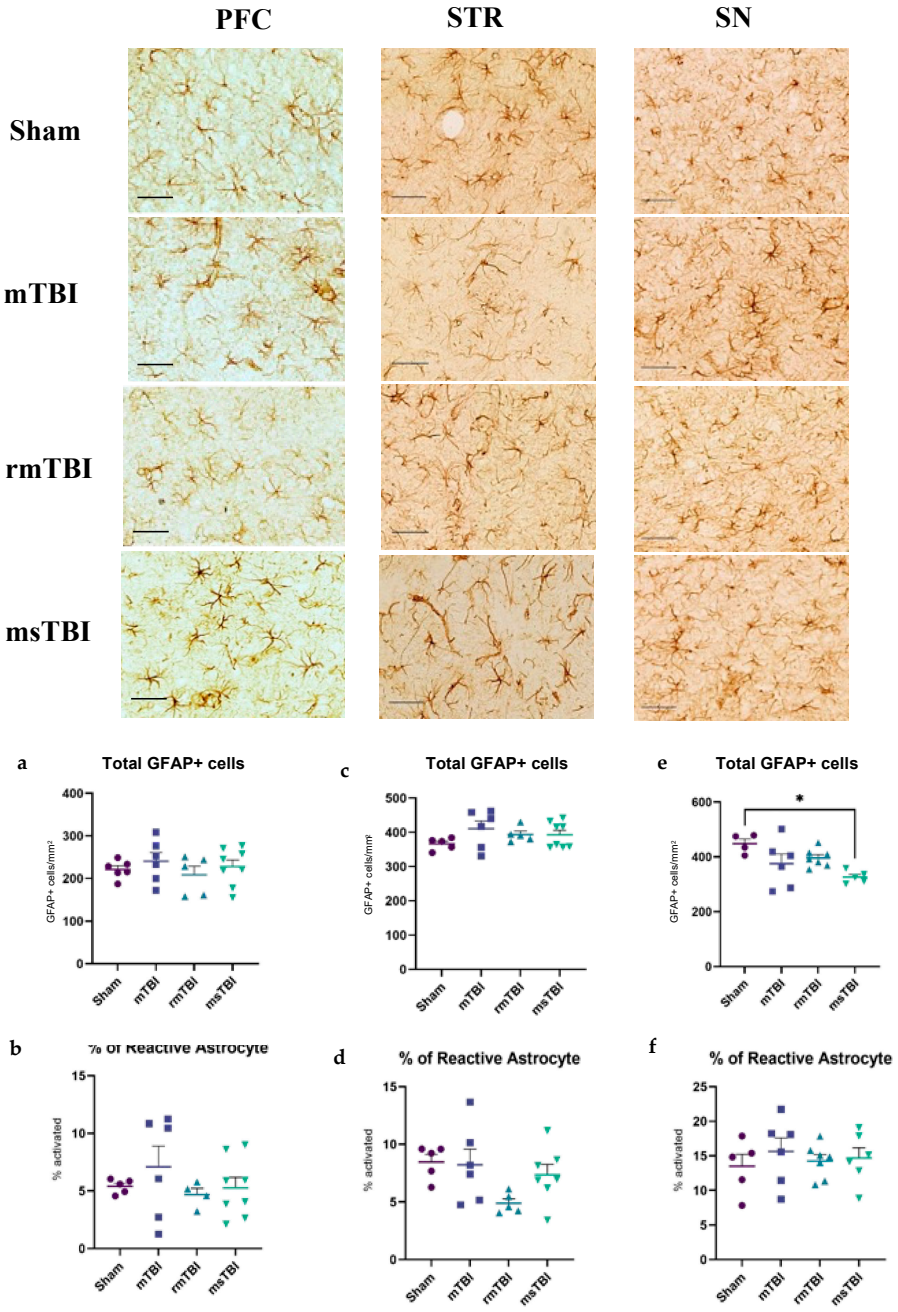

Figure 1. Neuroinflammation was assessed by glial fibrillary acidic protein (GFAP) at 12 months following different severities of injury. Representative images and the corresponding analysis of GFAP are shown in the left column for the prefrontal cortex (PFC), in the middle column for the striatum (STR), and in the right column for the substantia nigra (SN). (**a**,**b**) Total GFAP+ cells per mm^2 and percentage of reactive astrocyte in PFC. (**c**,**d**) Total GFAP+ cells per mm^2 and percentage of reactive astrocyte in STR. (**e**,**f**) Total GFAP+ cells per mm^2 and percentage of reactive astrocyte in SN. Outliers were removed (n = 0–2 per group), and one-way ANOVA was performed. n = 4–8 per group. Data are presented as mean ± SEM, * $p < 0.05$. Scale bar = 50 µm.

For microglia, no significant difference in the number of IBA1+ cells was noted in the PFC ($F(3,21) = 0.99$, $p = 0.42$) or the SN ($F(3,22) = 0.61$, $p = 0.62$) at 12 months post-injury (Figure 2a,e). Notably, a significant effect of injury was identified in the STR ($F(3,20) = 0.41$,

$p = 0.02$) (Figure 3c). Sham, mTBI, and rmTBI animals had similar numbers of IBA1+ cells (142.9 ± 15.13 cells/mm^2, 134.61 ± 6.17 cells/mm^2, 130.5 ± 8.37 cells/mm^2, respectively), while an increase was observed in msTBI animals (166.8 ± 34.6 IBA1+ cells/mm^2). However, post hoc analysis indicated that this elevation was not significantly different from shams ($p > 0.05$; Figure 2c).

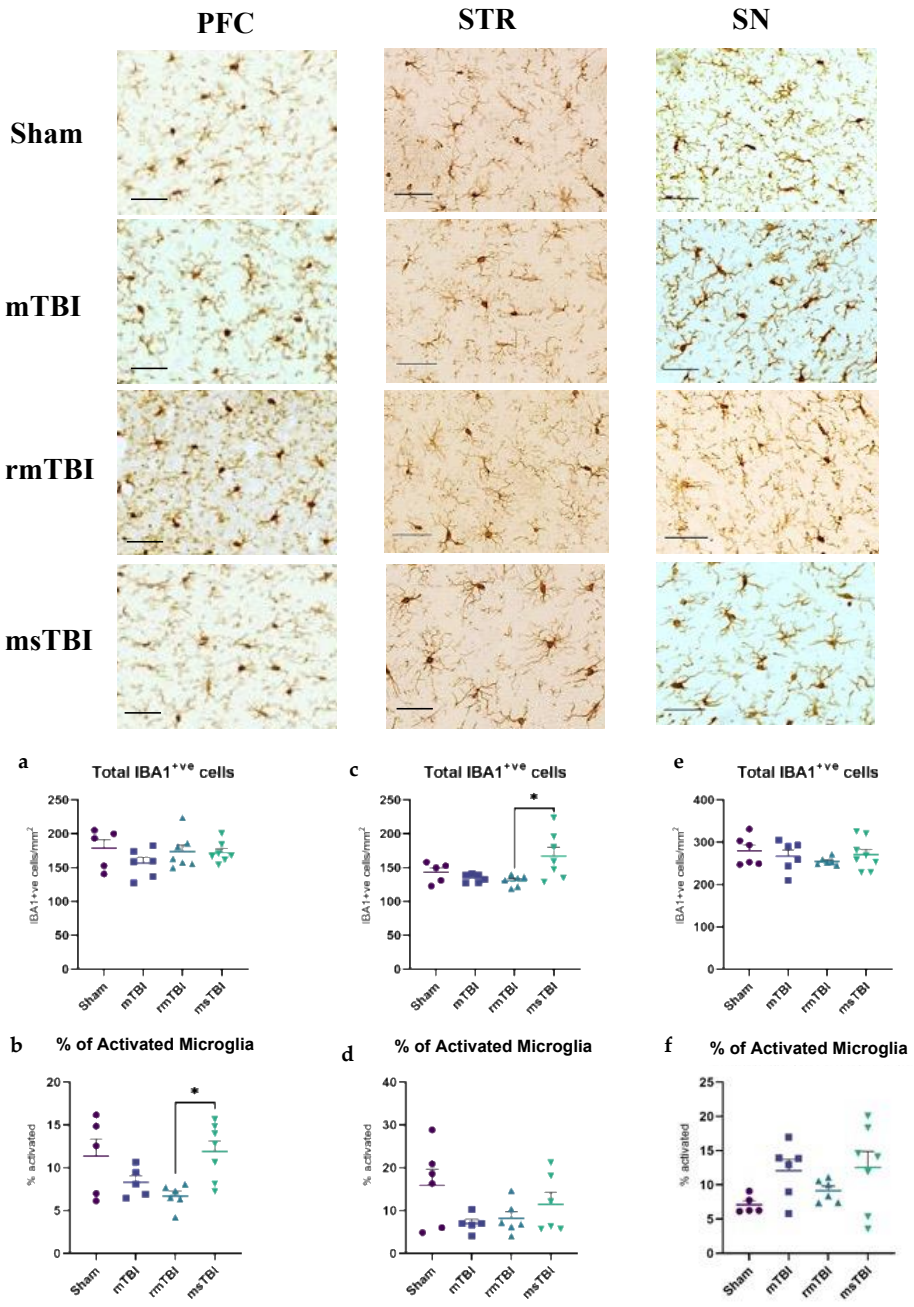

Figure 2. Neuroinflammation was assessed by ionized calcium-binding adaptor molecule 1 (Iba1) at 12 months following different severities of injury. Representative images and the corresponding analysis of IBA1 are shown in the left column for the prefrontal cortex (PFC), in the middle column for the striatum (STR), and in the right column for the substantia nigra (SN). (**a**,**b**) Total IBA1+ cells per mm^2 and percentage of activated microglia in PFC. (**c**,**d**) Total IBA1+ cells per mm^2 and percentage of activated microglia in STR. (**e**,**f**) Total IBA1+ cells per mm^2 and percentage of activated microglia in SN. Outliers were removed (n = 0–1 per group) and one-way ANOVA was performed. n = 5–8 per group. Data are presented as mean ± SEM, * $p < 0.05$. Scale bar = 50 µm.

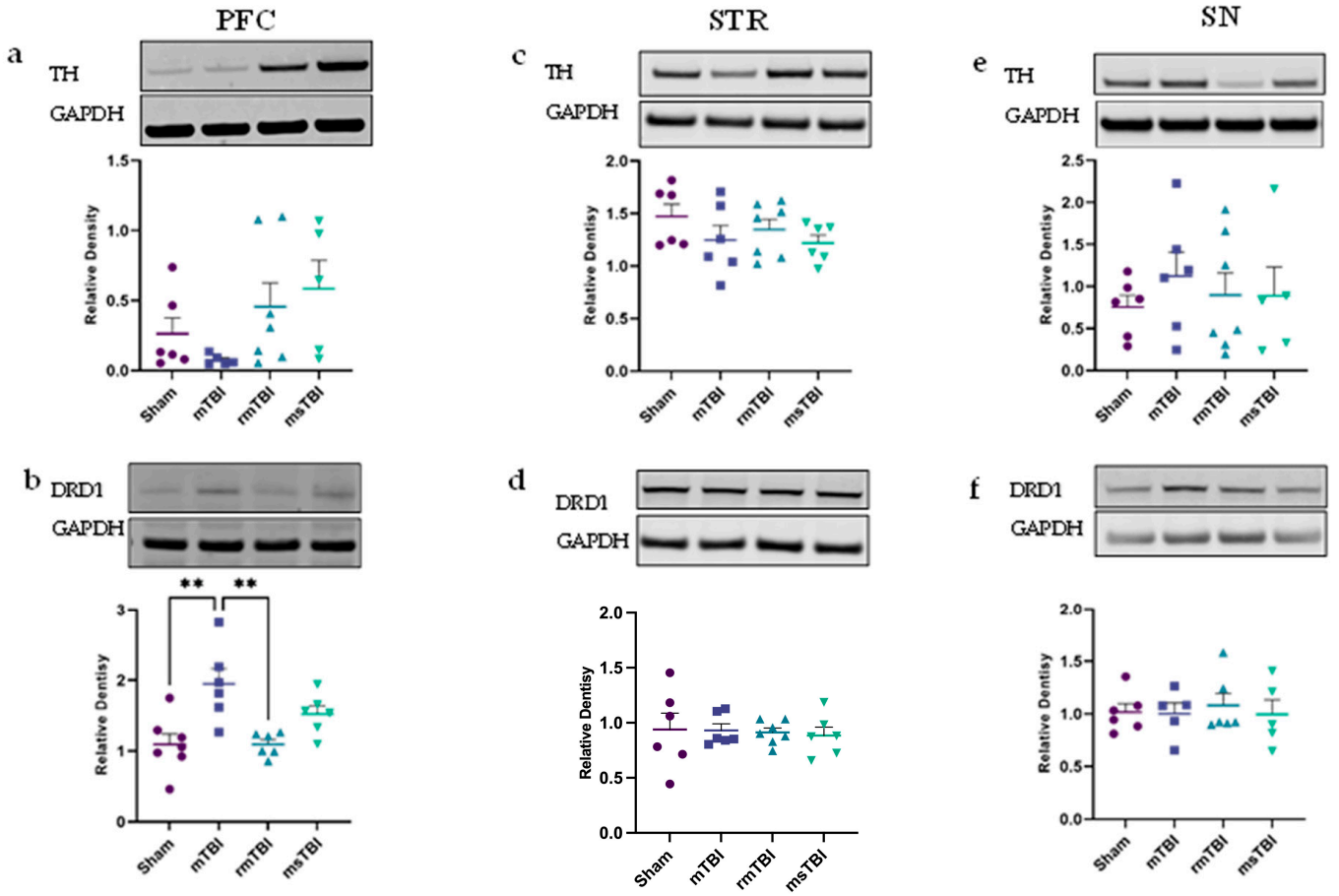

Figure 3. Dopaminergic pathway was assessed by TH and DRD1 at 12 months post different severities of injury within the PFC (**Left**), STR (**Middle**) and SN (**Right**). (**a**,**c**,**e**) Relative density of TH. (**b**,**d**,**f**) Relative density of Drd1. GAPDH was used as a housekeeper protein for analysis. Outliers were removed (n = 0–2 per group), and one-way ANOVA was performed. Data are presented as mean ± SEM, ** $p < 0.01$. n = 5–7 per group. Representative images of the Western blots were extracted from Image Studio Lite version 5.2.

No significant differences in the % activated microglia were noted in the STR ($F(3,19) = 2.32$, $p = 0.11$) or the SN ($F(3,20) = 2.29$, $p = 0.11$) at this time point following injury (Figure 2d,f); however, a significant effect was found in the PFC ($F(3,19) = 4.11$, $p = 0.02$) (Figure 2b). Sham and msTBI animals had a similar number of % activated microglia (11.34 ± 0.46% and 11.88 ± 3.30%), with lower numbers in the mTBI (8.30 ± 1.75%) and rmTBI animals (6.70 ± 1.38%). Post hoc analysis, however, found no significant differences relative to shams ($p > 0.05$; Figure 2b). A summary of the changes observed for all inflammatory markers is presented in Table 1.

2.2. DRD1 Elevation Observed in PFC, but Not STR or SN, following Single Mild TBI at 12 Months Post-Injury

To assess potential alterations in the dopaminergic pathway at 12 months following TBI, we conducted Western blot analysis of relevant proteins involved in this pathway. These included TH (tyrosine hydroxylase), responsible for dopamine production [69,70], and one of the dopamine D1-like receptors, DRD1. No alteration in the relative expression of TH was found in any of the regions examined, including PFC ($F(3,19) = 2.01$, $p = 0.15$), STR ($F(3,21) = 0.08$, $p = 0.97$), and SN ($F(3,20) = 0.34$, $p = 0.8$) (Figure 3a,c,e, Supplementary Figure S2). However, a significant change was observed for the relative expression of DRD1 in the PFC only ($F(3,21) = 7.56$, $p < 0.01$) (Figure 3b), primarily driven by an increase in mTBI animals (1.95 ± 0.53), such that they were significantly different from shams

(1.09 ± 0.39, $p = 0.002$) and rmTBI animals (1.09 ± 0.17, $p = 0.003$), but not msTBI rats (1.52 ± 0.29, $p = 0.22$) (Figure 3b, Table 2, Supplementary Figure S3). To note, other players in the dopaminergic pathway, such as another member of the D1-like receptor family, DRD5, and D2-like receptors, including DRD2 and DRD4, as well as the dopamine transporter (DAT), were included in the original experimental plan. However, due to the poor antibody quality, further analysis of their expression levels and functional roles could not be reliably conducted for the purposes of this study.

Table 1. Summary of neuroinflammatory marker results; changes following different TBI severities compared to shams at 12 months post-injury in the prefrontal cortex, striatum, and substantia nigra regions.

Marker Analysis	PFC		STR		SN	
	TBI Effect	Post hoc	TBI Effect	Post hoc	TBI Effect	Post hoc
Total GFAP+ cells	ns; $p = 0.64$	$\Delta^{mTBI} = -19.22$ $\Delta^{rmTBI} = 12.85$ $\Delta^{msTBI} = -6.70$	ns; $p = 0.3$	$\Delta^{mTBI} = -44.45$ $\Delta^{rmTBI} = -27.20$ $\Delta^{msTBI} = -26.55$	* $p = 0.02$	$\Delta^{mTBI} = -73.03$ $\Delta^{rmTBI} = 52.48$ $\Delta^{msTBI} = 121.9$ *
% of Reactive Astrocyte	ns; $p = 0.53$	$\Delta^{mTBI} = -1.7$ $\Delta^{rmTBI} = 0.71$ $\Delta^{msTBI} = 0.13$	ns; $p = 0.08$	$\Delta^{mTBI} = 0.25$ $\Delta^{rmTBI} = 3.58$ $\Delta^{msTBI} = 1.11$	ns; $p = 0.81$	$\Delta^{mTBI} = -2.15$ $\Delta^{rmTBI} = -0.75$ $\Delta^{msTBI} = -1.18$
Total IBA1+ cells	ns; $p = 0.42$	$\Delta^{mTBI} = 21.90$ $\Delta^{rmTBI} = 4.67$ $\Delta^{msTBI} = 6.38$	* $p = 0.02$	$\Delta^{mTBI} = 8.32$ $\Delta^{rmTBI} = 12.38$ $\Delta^{msTBI} = -23.91$ $\Delta^{rmTBI\text{-}msTBI} = -36.29$ *	ns; $p = 0.62$	$\Delta^{mTBI} = 12.77$ $\Delta^{rmTBI} = 24.57$ $\Delta^{msTBI} = 9.06$
% of Activated Microglial	* $p = 0.02$	$\Delta^{mTBI} = 3.04$ $\Delta^{rmTBI} = 4.64$ $\Delta^{msTBI} = -0.54$ $\Delta^{rmTBI\text{-}msTBI} = -5.2$ *	ns; $p = 0.11$	$\Delta^{mTBI} = 8.94$ $\Delta^{rmTBI} = 7.70$ $\Delta^{msTBI} = 4.43$	ns; $p = 0.11$	$\Delta^{mTBI} = -4.98$ $\Delta^{rmTBI} = -2.05$ $\Delta^{msTBI} = -5.46$

Note: Δ = mean of sham-mean TBI (s), negative value = increase value in mean 2 when compared to mean 1, positive value = decrease value in mean 2 when compared to mean 1, ns = not significant, * = $p < 0.05$. Bold text indicates statistical significance between treatment groups.

2.3. Moderate–Severe TBI Leads to Chronic Changes in STR ADRA2A and SN DβH Levels at 12 Months Post-Injury

The analysis of markers within the noradrenergic pathway included the examination of DβH (dopamine beta-hydroxylase), an enzyme responsible for noradrenaline production [71], as well as the noradrenergic receptors ADRA1a, ADRA2a, and ADRB1. Evaluation of noradrenaline synthesis using DβH showed no significant differences in the PFC ($F(3,21) = 1.89$, $p = 0.16$) (Figure 4a) or the STR ($F(3,19) = 1.35$, $p = 0.29$) (Figure 4e). However, an injury effect was observed in the SN ($F(3,19) = 4.95$, $p = 0.01$) (Figure 4i). Further post hoc analysis revealed that the msTBI group exhibited significantly lower relative expression of DβH (0.57 ± 0.39), compared to both the sham (1.46 ± 0.51, $p = 0.009$) and rmTBI (1.32 ± 0.45, $p = 0.026$) groups, while no significant difference was observed compared to the mTBI group (1.22 ± 0.19, $p = 0.089$). As the DβH bands are not visibly apparent on the grey-scale blot (Supplementary Figure S4), their position and intensity were cross-verified through the fluorescent intensity analysis tab in the Licor image studio and are presented here (Figure 4a,e,i).

Conversely, no changes were detected in ADRA1a levels in the PFC ($F(3,21) = 0.59$, $p = 0.63$), STR ($F(3,18) = 1.07$, $p = 0.39$) or SN ($F(3,21) = 2.44$, $p = 0.09$) (Figure 4b,f,j; Supplementary Figure S5). Similarly, for ADRB1, no changes were noted within any of these regions (PFC: $F(3,20) = 0.42$, $p = 0.74$; STR: $F(3,19) = 2.48$, $p = 0.09$; $F(3,20) = 1.88$, $p = 0.17$) (Figure 4d,h,l; Supplementary Figure S7). While there did appear to be a trend towards reduction in this receptor in the STR following both rmTBI and msTBI, this failed to reach statistical significance. However, a significant effect of injury was observed in ADRA2a, specifically in the STR region ($F(3,19) = 4.07$, $p = 0.02$) (Figure 4g), with msTBI animals expressing significantly higher levels of ADRA2a (1.24 ± 0.47) compared to the

sham (0.65 ± 0.28, $p = 0.04$) or rmTBI (0.66 ± 0.31, $p = 0.03$) groups. In contrast, no such effect was observed in either the PFC ($F(3,18) = 2.21$, $p = 0.13$) or SN ($F(3,20) = 0.52$, $p = 0.68$) (Figure 4c,k) (Supplementary Figure S6).

Table 2. Summary of catecholamine pathway marker results; changes following different TBI severities compared to shams at 12 months post-injury in the prefrontal cortex, striatum, and substantia nigra regions.

Marker Analysis	PFC		STR		SN	
	TBI Effect	**Post hoc**	**TBI Effect**	**Post hoc**	**TBI Effect**	**Post hoc**
TH	ns; $p = 0.15$	$\Delta^{S\text{-}mTBI} = 0.19$ $\Delta^{S\text{-}rmTBI} = -0.19$ $\Delta^{S\text{-}msTBI} = -0.32$	ns; $p = 0.38$	$\Delta^{S\text{-}mTBI} = 0.23$ $\Delta^{S\text{-}rmTBI} = 0.13$ $\Delta^{S\text{-}msTBI} = 0.25$	ns; $p = 0.8$	$\Delta^{S\text{-}mTBI} = -0.37$ $\Delta^{S\text{-}rmTBI} = -0.14$ $\Delta^{S\text{-}msTBI} = -0.13$
DRD1	** $p = 0.0013$	$\Delta^{S\text{-}mTBI} = -0.86$ ** $\Delta^{S\text{-}rmTBI} = -0.002$ $\Delta^{S\text{-}msTBI} = -0.43$ $\Delta^{mTBI\text{-}rmTBI} = 0.85$ **	ns; $p = 0.97$	$\Delta^{S\text{-}mTBI} = 0.008$ $\Delta^{S\text{-}rmTBI} = 0.03$ $\Delta^{S\text{-}msTBI} = 0.06$	ns; $p = 0.94$	$\Delta^{S\text{-}mTBI} = 0.015$ $\Delta^{S\text{-}rmTBI} = -0.06$ $\Delta^{S\text{-}msTBI} = 0.02$
DβH	ns; $p = 0.16$	$\Delta^{S\text{-}mTBI} = -0.4$ $\Delta^{S\text{-}rmTBI} = 0.26$ $\Delta^{S\text{-}msTBI} = 0.29$	ns; $p = 0.29$	$\Delta^{S\text{-}mTBI} = -0.27$ $\Delta^{S\text{-}rmTBI} = -0.42$ $\Delta^{S\text{-}msTBI} = -0.20$	* $p = 0.01$	$\Delta^{S\text{-}mTBI} = 0.24$ $\Delta^{S\text{-}rmTBI} = 0.14$ $\Delta^{S\text{-}msTBI} = 0.89$ ** $\Delta^{rmTBI\text{-}msTBI} = 0.74$ *
ADRA1a	ns; $p = 0.63$	$\Delta^{S\text{-}mTBI} = -0.12$ $\Delta^{S\text{-}rmTBI} = -0.11$ $\Delta^{S\text{-}msTBI} = -0.05$	ns; $p = 0.86$	$\Delta^{S\text{-}mTBI} = -0.03$ $\Delta^{S\text{-}rmTBI} = 0.08$ $\Delta^{S\text{-}msTBI} = 0.03$	ns; $p = 0.09$	$\Delta^{S\text{-}mTBI} = 0.16$ $\Delta^{S\text{-}rmTBI} = 0.20$ $\Delta^{S\text{-}msTBI} = 0.12$
ADRA2a	ns; $p = 0.12$	$\Delta^{S\text{-}mTBI} = -0.10$ $\Delta^{S\text{-}rmTBI} = 0.21$ $\Delta^{S\text{-}msTBI} = -0.44$	* $p = 0.02$	$\Delta^{S\text{-}mTBI} = -0.006$ $\Delta^{S\text{-}rmTBI} = -0.01$ $\Delta^{S\text{-}msTBI} = -0.60$ * $\Delta^{rmTBI\text{-}msTBI} = -0.59$ *	ns; $p = 0.68$	$\Delta^{S\text{-}mTBI} = 0.33$ $\Delta^{S\text{-}rmTBI} = 0.21$ $\Delta^{S\text{-}msTBI} = 0.23$
ADRB1	ns; $p = 0.74$	$\Delta^{S\text{-}mTBI} = 0.01$ $\Delta^{S\text{-}rmTBI} = 0.01$ $\Delta^{S\text{-}msTBI} = -0.04$	ns; $p = 0.09$	$\Delta^{S\text{-}mTBI} = -0.1$ $\Delta^{S\text{-}rmTBI} = 0.27$ $\Delta^{S\text{-}msTBI} = 0.23$	ns; $p = 0.17$	$\Delta^{S\text{-}mTBI} = 0.12$ $\Delta^{S\text{-}rmTBI} = 0.03$ $\Delta^{S\text{-}msTBI} = 0.06$
mbCOMT	ns; $p = 0.5$	$\Delta^{S\text{-}mTBI} = 0.32$ $\Delta^{S\text{-}rmTBI} = 0.30$ $\Delta^{S\text{-}msTBI} = 0.80$	ns; $p = 0.72$	$\Delta^{S\text{-}mTBI} = 0.27$ $\Delta^{S\text{-}rmTBI} = -0.26$ $\Delta^{S\text{-}msTBI} = 0.09$	ns; $p = 0.83$	$\Delta^{S\text{-}mTBI} = -0.15$ $\Delta^{S\text{-}rmTBI} = -0.09$ $\Delta^{S\text{-}msTBI} = -0.13$
sCOMT	ns; $p = 0.92$	$\Delta^{S\text{-}mTBI} = 0.12$ $\Delta^{S\text{-}rmTBI} = 0.02$ $\Delta^{S\text{-}msTBI} = 0.13$	ns; $p = 0.44$	$\Delta^{S\text{-}mTBI} = -0.11$ $\Delta^{S\text{-}rmTBI} = -0.34$ $\Delta^{S\text{-}msTBI} = -0.18$	ns; $p = 0.35$	$\Delta^{S\text{-}mTBI} = -0.25$ $\Delta^{S\text{-}rmTBI} = -0.15$ $\Delta^{S\text{-}msTBI} = -0.41$

Note: Δ = mean 1 − mean 2, negative value = increase value in mean 2 when compared to mean 1, positive value = decrease value in mean 2 when compared to mean 1, S = Sham, mTBI = single mild TBI, rmTBI = repetitive mild TBI, msTBI = moderate–severe TBI, ns = not significant, * = $p < 0.05$, ** $p < 0.01$. Bold text indicates statistical significance between treatment groups.

2.4. Expression of COMT Was Not Altered by Chronic TBI

At 12 months post-injury, there were no changes in COMT, the enzyme that degrades dopamine and noradrenaline [72], in any region, regardless of whether soluble (sCOMT) or membrane-bound (mbCOMT) was examined in the PFC (sCOMT: $F(3,22) = 0.17$, $p = 0.92$; mbCOMT $F(3,22) = 0.81$, $p = 0.5$); STR (sCOMT $F(3,21) = 0.9323$, $p = 0.44$; mbCOMT: $F(3,20) = 0.45$, $p = 0.72$); and SN (sCOMT $F(3,21) = 1.15$, $p = 0.35$; mbCOMT $F(3,21) = 0.3$, $p = 0.83$) (Figure 5; Supplementary Figure S8). A summary of the changes observed for all catecholaminergic markers is presented in Table 2.

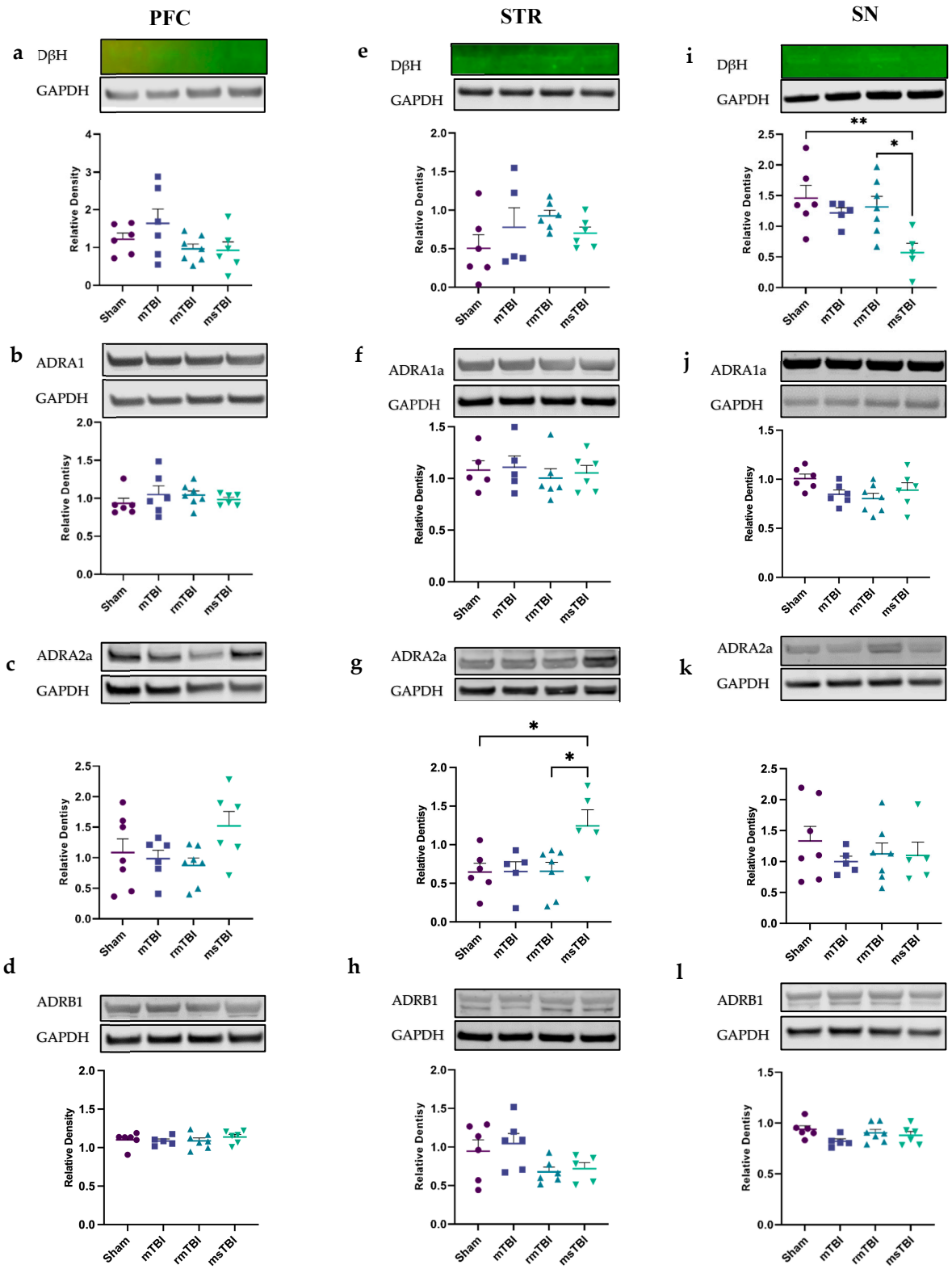

Figure 4. Noradrenergic pathway was assessed by DβH, ADRA1a, ADRA2a, and ADRB1 at 12 months post different severities of injury within PFC (left), STR (middle) and SN (right). (**a**,**e**,**i**) Relative density of DβH. (**b**,**f**,**j**) Relative density of ADRA1a. (**c**,**g**,**k**) Relative density of ADRA2a. (**d**,**h**,**l**) Relative density of ADRB1. GAPDH was used as a housekeeper protein for analysis. Outliers were removed (n = 0–2 per group) and one-way ANOVA was performed. Data are presented as mean ± SEM, * $p < 0.05$, ** $p < 0.01$. n = 5–7 per group. Representative images of the Western blots were extracted from Image Studio Lite. For clearer DBH images, please refer to Supplementary Figure S4.

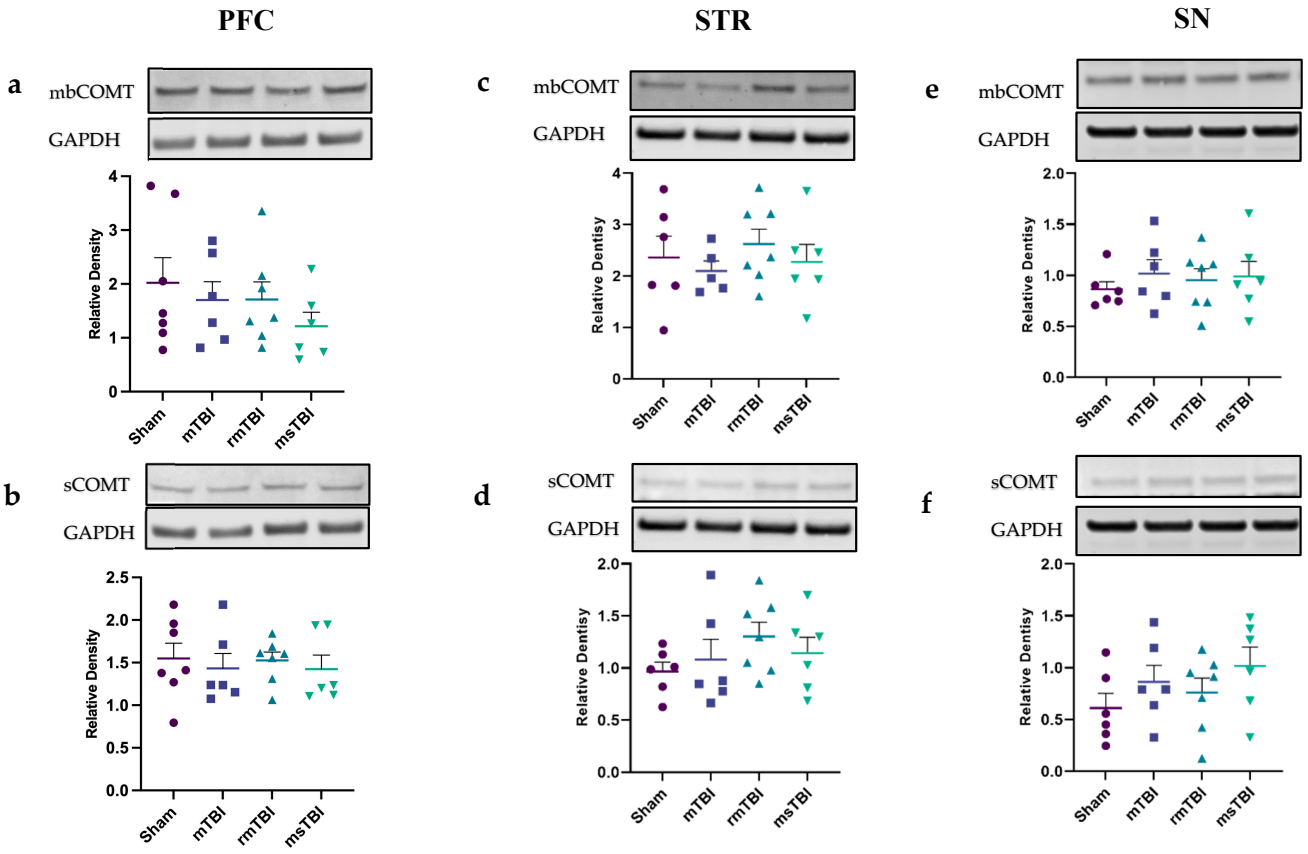

Figure 5. Catecholamine degradation rate was assessed by COMT at 12 months post different severities of injury within PFC (**left**), STR (**middle**) and SN (**right**). (**a**,**c**,**e**) Relative density of mbCOMT. (**b**,**d**,**f**) Relative density of sCOMT. GAPDH was used as a housekeeper protein for analysis. Data are presented as mean ± SEM. Outliers were removed (n = 0–2) and one-way ANOVA was performed. n = 5–7 per group. Representative images of the Western blots were extracted from Image Studio Lite.

3. Materials and Methods

3.1. Animals

This study utilized previously generated tissue from fifty-four adult male Sprague-Dawley rats (10–12 weeks, 420–480 g), originally obtained from Laboratory Animal Services (The University of Adelaide, AU). Animals were housed under conventional laboratory conditions (2 animals per cage), with a 12 h light–dark cycle and access to food and water ad libitum. All animals were housed in standard wireframe open-top cages on corncob bedding with a variety of enrichment provided within the cage, including wooden blocks, toilet paper roll tubes, and empty boxes. This study was performed under the approval of the University of Adelaide Animal Ethics Committee (M-2015-187).

3.2. Experimental Groups and Study Design

Animals were randomly allocated to receive either sham surgery (n = 14), a single mild TBI (smTBI) (n = 12), repetitive mild TBI (rmTBI) (n = 14), or moderate–severe TBI (msTBI) (n = 14). To control for anesthesia and analgesia exposure, animals in the sham group received identical procedures excluding injury, with repetitive sham animals receiving the incision three times, with 5-day intervals between each incision. At 12 months following the last TBI or sham procedure, each animal underwent a comprehensive functional battery assessing motor, neuropsychiatric, and cognitive function as previously reported [73,74]. This 12-month time point was selected for investigation as no study to date has investigated changes in catecholaminergic signaling beyond 28 weeks post-injury. Further, this repre-

sents a life stage comparable to early middle age in humans [75], which is an important period for investigating underlying pathophysiological alterations that may be indicative of later risk of PD development. This is particularly relevant given that pathological changes can occur in the brain for decades prior to the onset of the motor symptoms of PD [76]. At the end of the behavioral assessment, animals were randomly assigned for either (1) molecular analysis or (2) immunohistochemistry using simple randomization procedures (computerized random numbers) (Figure 6).

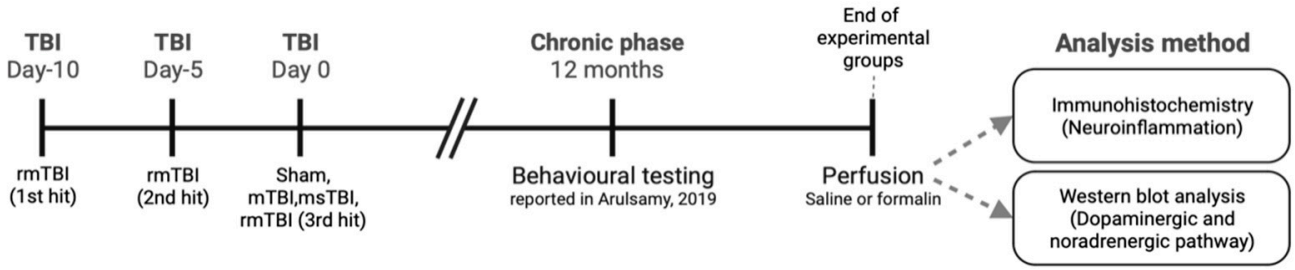

Figure 6. Experimental design of the study. During the chronic phase (12 months post-injury), animals underwent neurological and behavioral testing [73]. At the end of behavioral testing, the animals underwent perfusion using either saline or formalin fixative. This perfusion process was carried out for subsequent analyses: Western blot and immunohistochemistry, respectively.

3.3. Injury Protocol and Postoperative Care

Briefly, animals were injured with the Marmarou impact-acceleration injury method [77], an extensively validated diffuse injury model [78]. The msTBI group was intubated and mechanically ventilated, while all other groups were maintained on anesthesia via a nose cone. A 450 g weight was released from either 2 m (msTBI) or 0.75 m (mTBI, rmTBI) onto a metal disc affixed centrally to the rat's skull, with the msTBI animals exposed to 10 min of hypoxic conditions (2 L/min nitrogen; 0.2 L/min oxygen) to imitate the clinical effects of more severe head trauma [73,74,78]. The rmTBI animals received 3 hits in 10 days (5-day intervals between each injury). After the TBI surgery, animals underwent regular weight checks and multiple daily welfare assessments for up to 2 weeks post-injury to monitor animal health. As confirmation of the success of the injury, animals subjected to msTBI displayed balance and motor coordination deficits on the rotarod test compared to sham animals, persisting up to 4 days post-injury, with no significant difference for the subsequent 3 months (refer to Arulsamy et al. 2018 for further details) [79]. We maintained ongoing attention to the well-being of the animals throughout the 12-month study duration. Unfortunately, four animals were lost during this time due to age-related health complications. Additional details can be found in Arulsamy et al. [73]. Previous studies have reported on impairments in cognitive flexibility following msTBI [73], as well as decreases in locomotion (following both rmTBI and msTBI) and anxiety (following mTBI [80]), when compared to age-matched sham animals within this cohort.

3.4. Immunohistochemistry

A subset of animals (6 sham, 6 mTBI, 7 rmTBI, and 8 msTBI) were transcardially perfused with formalin, and the brain was removed. Brains were exposed to 30% sucrose solution for cryoprotection and then segmented into 2–3 mm blocks. Blocks were embedded in Optimal Cutting Temperature compound (Tissue-Tek O.C.T. compound, Proscitech, Kirwan, Australia) and snap-frozen using freezing isopentane (2-methylbutane, Sigma Aldrich, St. Louis, MO, USA, M32631). Coronal sections of 20 µm were obtained from the desired locations for each animal using a cryostat and a rat brain atlas [81] (Table 3). The frozen sections were mounted on slides and stored in a −20 °C freezer.

Table 3. Regions of interest.

Region	Coronal Coordinates (Bregma) [81]		Region of Interest (Both Left and Right)
Prefrontal cortex	3.7 mm to 3.2 mm		Prelimbic Area Anterior cingulate area Infralimbic Area
Striatum	Early	1.0 mm to 0.48 mm	Caudoputamen
	Middle I	0.20 mm to −0.40 mm	
	Middle II	−0.80 mm to −1.30 mm	
	Late	−1.5 mm to −2.10 mm	
Substantia Nigra	Early	−4.5 mm to −5.2 mm	-Substantia nigra, compact part -Substantia nigra, reticular part
	Middle	−5.2 mm to −5.8 mm	
	Late	−5.8 mm to −6.3 mm	

For immunohistochemistry, slides were air-dried overnight at room temperature and rehydrated using ethanol. Endogenous peroxidases were blocked with 0.5% hydrogen peroxide in methanol (Thermo Fisher, Waltham, MA, USA), and antigen retrieval was performed using citrate buffer. Two phosphate-buffered saline (PBS) washes of 3 min each were performed, followed by a 30 min incubation in normal horse serum (1:30, Thermo Gibco, Waltham, MA, USA) and an overnight incubation with the primary antibody (Table 4).

Table 4. Antibodies investigated using immunohistochemistry.

Primary Antibody	Species	Conc.	Catalogue#	Analysis Target	Analysis Platform and Parameters
Ionized calcium-binding adaptor molecule 1 (IBA1)	Rabbit	1:20,000	Wako-019-19741	Microglial reactivity	Halo microglial activation module: Min cell body diameter—3.4 µm ·Contrast threshold—0.3 pixel ·Min process OD—0.25 pixel ·Max process Radius—12 µm ·Max fragmentation length—2.5 µm ·Activation process thickness—2.12 µm
Glial fibrillary acidic protein (GFAP)	Rabbit	1:40,000	Dako-Z0334	Astrocyte reactivity	Image J [version 1.53b]: ·Manual identification of astrocyte morphology

On the following day, the slides were washed with 0.1% triton-X-100 in PBS, followed by the application of the appropriate biotinylated secondary antibody (1:250, Vector Laboratories, Newark, California, United States) for 30 min. Three PBS washes of 3 min each were performed between the application of the secondary and tertiary antibodies. Subsequently, the tertiary antibody streptavidin peroxidase conjugate (1:1000, Vector) was applied and incubated for one hour, and the bound antibody was detected using 3,3′-Diaminobenzidinetetrahydrochloride (1:50, Sigma Aldrich, St. Louis, MO, USA) for 7 min. After staining, the sections were mounted with coverslips using DPX mountant (Sigma Aldrich). The slides were air-dried in a fume hood for at least 2 days before being scanned with a Nanozoomer (Hamamatsu, Shizuoka, Japan) at 7 layers, with a separation of 1 µm between layers. The scanned images were viewed using the associated NDP view software (version 2).

3.5. Image Analysis

For analysis, 3 × 20× images were taken from the clearest layer in the region of interest (Table 1) and were exported and stacked with Image J to allow visualization of cell structure. GFAP immunoreactivity was assessed quantitatively by counting the reactive and immuno-positive cells per mm^2 within the same area. For IBA1 analysis, all the stacked images were exported and analyzed by using the HALO image analysis platform (Indica Labs, Albuquerque, NM, USA). Analysis settings were based on the microglial activation module (v1.2) for automated counting. In brief, the images were set according to the scanning resolution, 20× mode, 0.46µm/pixel. After that, the total microglia population and activation state were determined based on morphological parameters of IBA1+ cells (Table 4, Supplementary Figure S1). The experimenter was blinded to the experimental group during the analysis.

3.6. Western Blot

Another subset of animals (8 sham, 6 mTBI, 7 rmTBI, and 6 msTBI) were transcardially perfused with 9% cold saline. The brains were dissected and immediately frozen in liquid nitrogen and stored at −80 °C. For further investigation, a 2–3 mm region of interest was cut from each brain tissue sample (Table 1). These samples were sonicated in freshly prepared RIPA buffer (20 mM Tris-HCl pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 140 mM 2-mercaptoethanol) supplemented with a protease inhibitor (cOmplete Mini, EDTA free, Sigma, St. Louis, MO, USA). Each sample underwent three bursts of a 10 s duration (at least 1 min gap) using a sonicator probe. After sonication, the homogenized samples were centrifuged at 14,000 rpm and 4 °C for 30 min, and the supernatant was collected. The protein concentration of each sample was determined using the Pierce BCA Protein Assay (Thermo Scientific, Waltham, MA, USA) by measuring the absorbance at 650 nm.

For Western blot analysis, samples were prepared by adding sample buffer (Bolt™, Tallinn, Estonia, 4× LDS Sample buffer) and reducing agent (Bolt™, 10× sample reducing agent) to achieve a concentration of 1 mg/µL. A total of 20 mg of protein was loaded in each well. To maintain consistency across different blots, a sham sample was utilized as a standard, resulting in the inclusion of the sham number in the analysis (n = 7). Gel electrophoresis was performed using Bolt 4–12% Bis–Tris Plus gels (Invitrogen, Carlsbad, CA, USA) to separate the protein samples, followed by transfer onto a PVDF membrane using the iBlot 2 Dry Blotting System (Invitrogen). The membranes were then incubated with 5% milk-Tris buffer saline with 0.1% Tween 20 for 2 h and then with the appropriate primary antibody (Table 5) diluted in 2% bovine serum albumin (BSA, Sigma Aldrich) overnight at 4 °C. Afterward, the membranes were incubated with the corresponding secondary antibodies (donkey anti-rabbit, 1:10,000 and donkey anti-chicken, 1:10,000) for 2 h at room temperature. The western blots were imaged using an Odyssey Infrared Imaging System (model 9120; software version 3.0.21) (LI-COR, Inc., Lincoln, NE, USA) at a resolution of 169 µm. Quantitative analysis of the band signals was performed using Image Studio Lite version 5.2.

3.7. Statistics

Data analysis was performed using Prism software (GraphPad v.9.0). Statistical outliers were identified and removed based on the interquartile range in a box plot in SPSS. An ordinary one-way ANOVA (Analysis of Variance) with Tukey's multiple comparison post hoc test was conducted to determine statistical significance. All values are presented as mean ± SEM, and a significance level of $p < 0.05$ was considered statistically significant.

Table 5. Primary antibodies investigated via Western blot.

Primary Antibody	Species	Conc.	Catalogue#	Analysis Target
Tyrosine Hydroxylase (TH)	Rabbit	1:1000	Abcam-ab112	Catalytic enzyme for conversion of tyrosine to DA
Dopamine Beta Hydroxylase (DβH)	Rabbit	1:500	Abcam-ab209487	Enzyme converts dopamine to norepinephrine
Dopamine receptor D1 (DrD1)	Rabbit	1:1000	Abcam-ab20066	Receptor from $D1_R$ family
Rabbit anti-Dopamine receptor D4 (DrD4)	Rabbit	1:1000	Abcam-ab20424	Receptor from $D2_R$ family
Rabbit anti-Catechol-O-methyltransferase (COMT)	Rabbit	1:1000	Abcam-ab226938	Enzyme that degrades catecholamines
Rabbit anti-alpha 1a Adrenergic receptor (ADRA1A)	Rabbit	1:1000	Abcam-ab137123	Alpha-1 adrenergic receptor subtypes
Rabbit anti-alpha 2a Adrenergic receptor (ADRA2A)	Rabbit	1:1000	Abcam-ab85570	Alpha-1 adrenergic receptor subtypes
Rabbit anti-beta 1 Adrenergic receptor (ADRB1)	Rabbit	1:1000	Abcam-ab3442	A beta-adrenergic receptor
Chicken anti-GAPDH	Chicken	1:10,000	Abcam-108162	Housekeeping protein

4. Discussion

Alterations in catecholamines, along with the persistent upregulation of neuroinflammatory processes, are acknowledged to play pivotal roles in the pathophysiology of PD, and are equally implicated in the aftermath of TBI [82]. TBI is a known risk factor for the later development of PD, with risk varying based on initial injury severity [7,83,84]. To our knowledge, this was the first study to assess whether severity-dependent alterations in markers of DA and NA signaling and microglial and astrocytic reactivity within key regions implicated in PD, the PFC, STR, and SN persist up to 12 months after injury in an experimental model of diffuse axonal injury. This is significant, as such changes could potentially set the stage for the progression from TBI to PD.

Overall, the findings of this study indicate that, across the multiple DA and NA markers examined, following msTBI, only the NA pathway was disrupted, with a decrease in DβH within the SN and an increase in the NA receptor ADR2A within the STR, an effect that was not noted in animals with a milder initial injury. In comparison, only mTBI animals showed alterations in dopaminergic signaling, and this was only associated with an increase in DRD1 in the PFC, an effect not seen in any other injury group. No other changes in any dopaminergic or noradrenergic marker were noted across the three brain regions examined following injury, although it is important to note that we were not able to assess D2-like family receptors in this study. Similarly, in the evaluation of the glial response, the only significant difference relative to shams was a decrease in astrocytes, as detected by GFAP, in msTBI animals within the SN only. Taken together, the results of the current study seem to suggest that, at 12 months following the initial insult, diffuse axonal injury has minimal effect on catecholamines and the neuroimmune response, with subtle differences seen in mild compared to moderate–severe TBI.

The most notable finding in this study was that moderate–severe TBI led to an increase in ADRA2A within the STR and a decrease in DβH in SN at 12 months post-injury, while no such effects were seen in the PFC or any aspect of the DA pathway at this injury severity.

DβH is an enzyme crucial for synthesizing NA [85,86], with noradrenergic neurons known to project from the LC to the SN [87–90], raising disruption of the LC as one potential explanation for this alteration. The LC is known to be affected by TBI, with an acute increase in NA turnover in both focal and diffuse models of injury [51,52], followed by a decrease more chronically (i.e., up to 8 weeks post-injury) in the Marmarou weight drop model [52]. Consistent disruption in NA levels is also evident from clinical work; although not LC specific, plasma and cerebrospinal fluid (CSF) samples collected from severe TBI patients showed significantly increased NA levels from 1 to 14 days following injury [47], although how NA levels may change more long-term following injury has not yet been investigated. While no changes were noted in either ADRA1a or ADRB1 receptors in the current study, it may be that WB is not the most effective tool for investigating such changes. In line with this, quantitative flow cytometry (qFlow) has recently emerged as one of the best techniques for probing changes in the abundance of plasma membrane receptors (for a detailed protocol, see recent work by Fang and colleagues (2022)) [91], and may, therefore, be a useful tool for investigating alterations in these receptors in future work. This may be particularly relevant for probing potential long-term alterations in ADRB1 receptors further, which appeared to decline in the STR following both rmTBI and msTBI, although this failed to reach statistical significance using the methods employed in the current study and should, therefore, be interpreted with caution.

It is noteworthy that prior research from our own laboratory has identified mild cognitive deficits at 12 months post-moderate–severe TBI within the same animal cohort [73], suggesting a potential link between the observed alteration in NA levels following TBI and these cognitive impairments. In fact, NA is a neurotransmitter that plays a fundamental role in response to stress, mood regulation, attention, and cognitive functions, including executive function, cognitive flexibility, learning, and memory [92]. Interestingly, prior research on Alpha-2A adrenergic agonists (which activate ADRA2A receptors and inhibit NA release) has consistently demonstrated their potential to enhance spatial working memory performance and cognitive flexibility across various species, including humans [93], monkeys [94,95], and rodents [96]. While the outcomes may vary depending on the specific compound and dosage used [97–99], these agonists have also been linked with neuroprotective effects, functional restoration, and notable improvement in working memory following TBI [100–102]. Conversely, studies involving the blockade of ADRA2A receptors by using Alpha-2A adrenergic antagonists have shown negative impacts on cognitive function. For instance, young adult monkeys exhibited impaired spatial working memory [103], and aged rats displayed deficits in delayed alternation performance [104]. These collective findings suggest a pivotal role for ADRA2A receptors in cognitive function. Of note, the LC sends afferent projections to the SN and even scattered afferents to the STR [105,106]; thus, it is possible that the observed alterations within these regions in this study may be a compensatory response to alterations in NA synthesis in the LC following msTBI. In line with this, striatal NA plays an important role in cognition, with LC–striatal NA connections key for response inhibition and cognitive flexibility [107]. However, the specific mechanisms via which TBI may subtly alter noradrenergic input to the nigrostriatal pathway from the LC and its downstream effects remain to be elucidated. In order to probe this further, future investigations should investigate changes in DβH within the LC, as well as concomitant changes in NA levels within the SN and STR, at chronic time points following experimental diffuse axonal injury. Unfortunately, due to the archival nature of the tissue, such investigations were outside the scope of the current study.

Accumulating evidence also suggests that LC–SN projections and NA play an important role in maintaining dopaminergic neurons in the SN [108–111]. Studies demonstrate that disturbance of the NA system is correlated with both the onset and progression of DA neuronal loss in PD [111–114]. This is, however, contrary to our findings, where alterations in the NA system were noted, with no concomitant changes in the DA system. These discrepancies may be attributed to a delayed response within the DA system. In line with this, a post-mortem study by Zarow et al. investigating 19 idiopathic PD cases

suggests that neuronal loss in the LC-NA system is greater than in the SN-DA system, which corroborates Braak's theory, where degeneration of LC-NA neurons occurs prior to SN-DA neurons [20,115]. Thus, it may be that the 12-month time point utilized in this study is insufficient to observe changes in the DA system, but rather earlier, more subtle changes in the NA system. This would also be consistent with the relatively mild cognitive changes observed in this cohort previously [73]. It is also important to acknowledge, however, that our analysis focused on a broad characterization of DA and NA changes using Western blot analysis of total protein levels for each marker of interest. It is possible that a more detailed analysis using RNAseq or microdialysis might reveal subtle changes following TBI that are not readily apparent at the gross protein level. Future studies should also investigate longer time points following TBI, such as 15 or 18 months post-injury, to investigate progressive alteration in the DA system, particularly within the SN and STR, in the face of the aging phenotype, and incorporate additional markers associated with these pathways, particularly the D2-like receptor family and the dopamine transporter (DAT).

Another notable finding of this study was the increase in DRD1 protein levels observed in the PFC of the mTBI group at 12 months post-injury compared to the sham and rmTBI groups. This increase in DRD1 expression might be associated with the previous findings in the elevated plus maze in this cohort of animals, with mTBI animals having reduced anxiety, as indicated by them spending more time in the open arms and exhibiting a significantly higher number of open arm entries and crossings when compared to msTBI animals [73,116]. A similar phenotype of reduced anxiety and hyperactivity has also previously been reported following mTBI across multiple other studies [117–120]. Elevated DRD1 expression could provide an explanation for this effect, as increased DRD1 activity has been linked to reduced anxiety [121]. Both overexpression of DRD1 [122] and optogenetic stimulation of DRD1, but not DRD2 [121], within the PFC, have shown similar reductions in anxiety-like behavior, as indicated by a higher number of open arm entries in the elevated plus maze [121,122]. DRD1 triggers a cascade of intracellular events through the adenylate cyclase/cyclic adenylate/protein kinase A (AC/cAMP/PKA) pathway [123,124], with the activity of PKA associated with anxiety [125]. In support of this, a study utilizing PKA inhibitor (H-89) demonstrated that injecting a high dosage of H-89 into medial PFC resulted in reduced anxiety symptoms [126]. Despite these findings, the reason why increased PFC DRD1 protein expression is present in the mTBI animals, and not in the other injury groups, remains unclear. It is possible that compensatory mechanisms are at play, where mTBI may induce recovery through DRD1 signaling. Nevertheless, direct investigation into DRD1 expression and its specific effects on anxiety and hyperactivity following chronic TBI is required for a more comprehensive understanding.

Based on the findings of this study regarding the changes in the DA and NA systems after TBI, it was pertinent to also explore the protein levels of the catecholamine-metabolizing enzyme, COMT. The activity of COMT is associated with the degradation of catecholamines, where lower activity can potentially lead to elevated neurotransmitter levels, while higher activity can potentially result in decreased neurotransmitter levels [127–129]. Indeed, COMT gene variants that alter dopamine levels are associated with cognitive decline in PD [130,131] and, similarly, impaired cognitive flexibility following TBI [127,132]. The investigation of COMT protein level following TBI is limited, with one study demonstrating that following focal TBI, both sCOMT and mbCOMT were increased in the ipsilateral cortex at 3 and 14 days post-injury [133], but longer time points have not been assessed to date. Here, at 12 months post-injury, regardless of the TBI severity, no changes were seen. However, it is important to emphasize that a more comprehensive understanding of the alterations in catecholamine metabolism following TBI might be gained through the additional examination of other catecholamine-metabolizing enzymes, such as monoamine oxidase and phenylethanolamine N-methyltransferase, or the incorporation of more sensitive methods, such as high-performance liquid chromatography (HPLC), to obtain a clearer insight into catecholamine levels and levels of their metabolites in response to TBI.

Interestingly, despite the subtle changes in catecholaminergic pathways discussed above, there was very little alteration in the glial response to injury in any of the brain regions examined, regardless of injury severity. In fact, the only significant finding relative to shams was a reduction in GFAP + cells in the SN of msTBI animals. Although it did not reach statistical significance relative to sham, there was also an increase in the total population of microglial cells in the STR following msTBI compared to rmTBI, with a significant main effect of injury noted. Interestingly, these microglia appeared to be in a resting/ramified state, rather than an activated/ameboid state. The underlying mechanism driving this trend towards a higher resting microglia number within the STR at 12 months post-msTBI remains unclear; yet, it is plausible that these cells are returning to their ramified state in the STR, indicating that this may indicate recovery from a previous state of increased neuroinflammation within this region. This notion could be supported by the concurrent increase in the levels of ADRA2A receptor in the STR, as ADRA2A is known to have anti-inflammatory effects, with administration of an agonist of this receptor shown to reduce the release of pro-inflammatory cytokines and downregulate pathways like NF-kB and NLRP3 inflammasome in the acute phase following TBI [101,134]. Additionally, a study investigating adrenergic receptor signaling in microglia suggests that the process of retraction is associated with ADRA2A, mainly in activated microglia rather than resting microglia [135]. However, it is important to note that several studies have indicated that the role of microglia cannot be solely characterized by a morphological change from a ramified to an amoeboid shape or vice versa [136–138], as was done in the current work. It is, thus, crucial that future studies more comprehensively probe the neuroinflammatory response, extending the analysis to include additional markers, such as CD16/32 and CD26, and further quantification of cytokine release via ELISA, in order to identify the phenotype of microglia and their release of pro-inflammatory cytokines, respectively. Further, as IBA1 does not distinguish between microglia and infiltrating macrophages, more specific markers, such as TMEM119 and P2RY12, either alone or in combination, may have utility for more nuanced probing of the microglial response. In support of this, a recent study of aged controls and individuals with AD found that the specific combination of these markers (i.e., phenotypes) differed significantly between these two groups [139].

In addition to the increased population of microglia in the STR following msTBI, there was a notable reduction in the population of GFAP+ astrocytes in the SN. Astrocytes can function in two primary states: resting and reactive. Under normal conditions, astrocytes play a crucial role in maintaining brain homeostasis by regulating the levels of reactive oxygen species, supporting neural development and survival, and providing structural support to mitochondria to maintain the energy level and integrity of neural circuits [140–142]. Studies have shown that deficiency in astrocytes can lead to the disruption of the blood–brain barrier seen in patients with PD. Further, in a rat model genetically modified for astrocyte ablation, about 50% more loss of cortical tissue was seen compared to wild-type rats following a moderate TBI, highlighting the neuron-protective role of astrocytes following injury [141]. Conversely, reactive astrocytes undergo morphological changes in response to injury or pathological conditions, which can exacerbate neuroinflammation and worsen secondary brain injury following TBI [143]. Interestingly, our analysis of astrocyte reactivity in the SN revealed that most of these cells were in a resting state, suggesting that they were not actively responding to injury or pathology and, instead, were likely providing support in maintaining the normal function of SN. Thus, in this context, the reduction observed in the total astrocyte population in the SN may be detrimental, even without a concomitant increase in astrocyte reactivity. In line with this, impairments in cognitive flexibility [73] and decreases in locomotion [80] have previously been noted in this cohort following msTBI.

In addition to the limitations associated with the analysis of archival tissue noted above, it is also important to acknowledge that the tissue utilized in this study was collected from animals who were approximately 15 months of age (i.e., 10–12 weeks at the time of injury; follow-up time point of 12 months post-injury). While difficult to exactly equate this to

human year equivalents, based on their lifespan of ~2.5–3 years, Sengupta (2013) has offered calculations for rat–human age equivalents at each of their various "life phases" [75]. In general, across the lifespan, it is suggested that 13.2 rat days are equal to 1 human year. Thus, a 12-month-old rat would be equivalent to approximately a 30-year-old human, with an 18-month-old rat equivalent to approximately a 45-year-old human. The 15-month-old rats used within the current study would fall somewhere within this range and would, therefore, be not quite yet equivalent to true "middle age" in humans. Given that age is the biggest risk factor for PD [144], it may be that rats used in the current study were not yet old enough to observe post-injury changes in either catecholaminergic signaling or neuroinflammation that may set the stage for PD development. In line with this, the brain is known to switch to a pro-inflammatory state with increasing age, which may sensitize the brain to the effects of infection or insult (such as a TBI) (for review, see Sparkman and Johnson, 2008) [145]. Similarly, both SN/VTA-DA and LC-NA neuron density have been shown to progressively decrease with age [146], and post-mortem studies of NA extracted from homogenized brain tissue suggest that levels tend to be lower in older adults than in younger adults [147] (although, interestingly, plasma and CSF levels tend to be higher; for review, see Mather, 2022 [148]). Thus, future studies should investigate post-injury time points of 18 months or beyond in preclinical models or, alternatively, look at injury induced in older animals in order to investigate whether the aged phenotype may add a "second hit" to the system, exacerbating injury-related alterations in catecholaminergic signaling or neuroinflammation.

Similarly, in the current study, only tissue from male animals was available for analysis. This represents another potential limitation, as the markers of interest in the current study are known to vary with sex. For example, with regard to neuroinflammation, both the number and phenotype of microglia are known to differ between male and female rodents in a region- and age-dependent manner [149], with critical differences in function also noted (for review, see Han et al. 2021) [150]. Similar sex-related functional differences have also been noted for astrocytes (Santos-Galindo et al. 2011) [151]. Regarding catecholamine signaling, sex differences in LC structure and function have been widely reported to begin during puberty, with a larger LC volume, a higher number of NA neurons, and greater dendritic arbor density seen in female rodents. Sex differences have also been noted within midbrain dopaminergic regions, including both the SN and VTA, and have previously been elegantly reviewed by Glenda Gillies and colleagues [152–154]. Thus, it is plausible, and indeed likely, that there may be sex-related differences in how both catecholaminergic signaling and neuroinflammation are impacted by TBI and that this could result in different risk profiles for PD development following injury. In line with this, risk of developing PD is twice as high in males as in females [155] and there are significant differences in symptom presentation, risk factor profiles, and response to therapy in PD based on biological sex (for review, see Cerri et al., 2019 [156]). Thus, it is imperative that future work investigates these markers in females.

5. Conclusions

Taken together, this investigation represents the first comprehensive comparison of chronic alterations in catecholamine signaling and the glial response within the SN, STR, and PFC at 12 months following different severities of TBI. Although the changes were subtle at this time point, they nevertheless suggest a severity-dependent pattern. This underscores the nuanced and multifaceted nature of the brain's response to different severities of diffuse axonal injury. Further, it emphasizes the need for continued exploration to unravel the time course and precise mechanisms via which TBI may impact catecholaminergic signaling following TBI, as well as the functional consequences of this. This is particularly critical given that, despite the subtlety of the noted changes, there were nevertheless alterations in NA signaling within the nigrostriatal pathway following msTBI, which could potentially set the stage for the later emergence of PD. While further experimental evidence is clearly required, this raises the intriguing possibility that monitoring such changes in

survivors of TBI could provide an early indication of the risk of later PD development, allowing for earlier identification and potentially more effective therapeutic intervention.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules29071470/s1. Raw images of the Western blots for analysis presented in Figures S1–S8.

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Informed Consent Statement: Not applicable.

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References

1. Dewan, M.C.; Rattani, A.; Gupta, S.; Baticulon, R.E.; Hung, Y.-C.; Punchak, M.; Agrawal, A.; Adeleye, A.O.; Shrime, M.G.; Rubiano, A.M.; et al. Estimating the Global Incidence of Traumatic Brain Injury. *J. Neurosurg.* **2019**, *130*, 1080–1097. [CrossRef] [PubMed]
2. Taylor, C.A.; Bell, J.M.; Breiding, M.J.; Xu, L. Traumatic Brain Injury–Related Emergency Department Visits, Hospitalizations, and Deaths—United States, 2007 and 2013. *MMWR Surveill. Summ.* **2017**, *66*, 1–16. [CrossRef] [PubMed]
3. Faul, M.; Wald, M.M.; Xu, L.; Coronado, V.G. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalization and Deaths 2002–2006. *Cent. Dis. Control Prev. Natl. Cent. Inj. Prev. Control* **2010**. Available online: https://stacks.cdc.gov/view/cdc/5571 (accessed on 20 March 2024).
4. James, S.L.; Theadom, A.; Ellenbogen, R.G.; Bannick, M.S.; Montjoy-Venning, W.; Lucchesi, L.R.; Abbasi, N.; Abdulkader, R.; Abraha, H.N.; Adsuar, J.C.; et al. Global, Regional, and National Burden of Traumatic Brain Injury and Spinal Cord Injury, 1990–2016: A Systematic Analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* **2019**, *18*, 56–87. [CrossRef]
5. Feigin, V.L.; Theadom, A.; Barker-Collo, S.; Starkey, N.J.; McPherson, K.; Kahan, M.; Dowell, A.; Brown, P.; Parag, V.; Kydd, R.; et al. Incidence of Traumatic Brain Injury in New Zealand: A Population-Based Study. *Lancet Neurol.* **2013**, *12*, 53–64. [CrossRef]
6. Brett, B.L.; Gardner, R.C.; Godbout, J.; Dams-O'Connor, K.; Keene, C.D. Traumatic Brain Injury and Risk of Neurodegenerative Disorder. *Biol. Psychiatry* **2022**, *91*, 498–507. [CrossRef] [PubMed]
7. Gardner, R.C.; Burke, J.F.; Nettiksimmons, J.; Goldman, S.; Tanner, C.M.; Yaffe, K. Traumatic Brain Injury in Later Life Increases Risk for Parkinson Disease: TBI Increases Risk for PD. *Ann. Neurol.* **2015**, *77*, 987–995. [CrossRef] [PubMed]
8. Braak, H.; Tredici, K.D.; Rüb, U.; de Vos, R.A.I.; Jansen Steur, E.N.H.; Braak, E. Staging of Brain Pathology Related to Sporadic Parkinson's Disease. *Neurobiol. Aging* **2003**, *24*, 197–211. [CrossRef] [PubMed]
9. Dauer, W.; Przedborski, S. Parkinson's Disease: Mechanisms and Models. *Neuron* **2003**, *39*, 889–909. [CrossRef]
10. Ehringer, H.; Hornykiewicz, O. Distribution of Noradrenaline and Dopamine (3-Hydroxytyramine) in the Human Brain and Their Behavior in Diseases of the Extrapyramidal System. *Park. Relat. Disord.* **1998**, *4*, 53–57. [CrossRef]
11. Klein, M.O.; Battagello, D.S.; Cardoso, A.R.; Hauser, D.N.; Bittencourt, J.C.; Correa, R.G. Dopamine: Functions, Signaling, and Association with Neurological Diseases. *Cell. Mol. Neurobiol.* **2019**, *39*, 31–59. [CrossRef]
12. Nandhagopal, R.; Kuramoto, L.; Schulzer, M.; Mak, E.; Cragg, J.; Lee, C.S.; McKenzie, J.; McCormick, S.; Samii, A.; Troiano, A.; et al. Longitudinal Progression of Sporadic Parkinson's Disease: A Multi-Tracer Positron Emission Tomography Study. *Brain* **2009**, *132*, 2970–2979. [CrossRef]

13. Booij, J.; Tissingh, G.; Boer, G.J.; Speelman, J.D.; Stoof, J.C.; Janssen, A.G.; Wolters, E.C.; Van Royen, E.A. [^{123}I]FP-CIT SPECT Shows a Pronounced Decline of Striatal Dopamine Transporter Labelling in Early and Advanced Parkinson's Disease. *J. Neurol. Neurosurg. Psychiatry* **1997**, *62*, 133–140. [CrossRef]
14. Ziebell, M.; Andersen, B.B.; Thomsen, G.; Pinborg, L.H.; Karlsborg, M.; Hasselbalch, S.G.; Knudsen, G.M. Predictive Value of Dopamine Transporter SPECT Imaging with [^{123}I]PE2I in Patients with Subtle Parkinsonian Symptoms. *Eur. J. Nucl. Med. Mol. Imaging* **2012**, *39*, 242–250. [CrossRef]
15. Kish, S.J.; Shannak, K.; Hornykiewicz, O. Uneven Pattern of Dopamine Loss in the Striatum of Patients with Idiopathic Parkinson's Disease. *N. Engl. J. Med.* **1988**, *318*, 876–880. [CrossRef] [PubMed]
16. Fearnley, J.M.; Lees, A.J. Ageing and parkinson's disease: Substantia nigra regional selectivity. *Brain* **1991**, *114*, 2283–2301. [CrossRef]
17. Kamath, T.; Abdulraouf, A.; Burris, S.J.; Langlieb, J.; Gazestani, V.; Nadaf, N.M.; Balderrama, K.; Vanderburg, C.; Macosko, E.Z. Single-Cell Genomic Profiling of Human Dopamine Neurons Identifies a Population That Selectively Degenerates in Parkinson's Disease. *Nat. Neurosci.* **2022**, *25*, 588–595. [CrossRef]
18. Otsuka, M.; Ichiya, Y.; Kuwabara, Y.; Hosokawa, S.; Sasaki, M.; Yoshida, T.; Fukumura, T.; Masuda, K.; Kato, M. Differences in the Reduced 18F-Dopa Uptakes of the Caudate and the Putamen in Parkinson's Disease: Correlations with the Three Main Symptoms. *J. Neurol. Sci.* **1996**, *136*, 169–173. [CrossRef] [PubMed]
19. Rinne, J.O.; Ruottinen, H.; Bergman, J.; Haaparanta, M.; Sonninen, P.; Solin, O. Usefulness of a Dopamine Transporter PET Ligand [^{18}F]Beta-CFT in Assessing Disability in Parkinson's Disease. *J. Neurol. Neurosurg. Psychiatry* **1999**, *67*, 737–741. [CrossRef] [PubMed]
20. Zarow, C.; Lyness, S.A.; Mortimer, J.A.; Chui, H.C. Neuronal Loss Is Greater in the Locus Coeruleus Than Nucleus Basalis and Substantia Nigra in Alzheimer and Parkinson Diseases. *Arch. Neurol.* **2003**, *60*, 337. [CrossRef]
21. Ohtsuka, C.; Sasaki, M.; Konno, K.; Koide, M.; Kato, K.; Takahashi, J.; Takahashi, S.; Kudo, K.; Yamashita, F.; Terayama, Y. Changes in Substantia Nigra and Locus Coeruleus in Patients with Early-Stage Parkinson's Disease Using Neuromelanin-Sensitive MR Imaging. *Neurosci. Lett.* **2013**, *541*, 93–98. [CrossRef]
22. Oertel, W.H.; Henrich, M.T.; Janzen, A.; Geibl, F.F. The Locus Coeruleus: Another Vulnerability Target in Parkinson's Disease. *Mov. Disord.* **2019**, *34*, 1423–1429. [CrossRef] [PubMed]
23. Sitte, H.H.; Pifl, C.; Rajput, A.H.; Hörtnagl, H.; Tong, J.; Lloyd, G.K.; Kish, S.J.; Hornykiewicz, O. Dopamine and Noradrenaline, but Not Serotonin, in the Human Claustrum Are Greatly Reduced in Patients with Parkinson's Disease: Possible Functional Implications. *Eur. J. Neurosci.* **2017**, *45*, 192–197. [CrossRef] [PubMed]
24. Braak, E.; Sandmann-Keil, D.; Rüb, U.; Gai, W.P.; De Vos, R.A.I.; Jansen Steur, E.N.H.; Arai, K.; Braak, H. α-Synuclein Immunopositive Parkinson's Disease-Related Inclusion Bodies in Lower Brain Stem Nuclei. *Acta Neuropathol.* **2001**, *101*, 195–201. [CrossRef] [PubMed]
25. Abbott, R.D.; Ross, G.W.; White, L.R.; Tanner, C.M.; Masaki, K.H.; Nelson, J.S.; Curb, J.D.; Petrovitch, H. Excessive Daytime Sleepiness and Subsequent Development of Parkinson Disease. *Neurology* **2005**, *65*, 1442–1446. [CrossRef] [PubMed]
26. Oliveira, L.M.; Tuppy, M.; Moreira, T.S.; Takakura, A.C. Role of the Locus Coeruleus Catecholaminergic Neurons in the Chemosensory Control of Breathing in a Parkinson's Disease Model. *Exp. Neurol.* **2017**, *293*, 172–180. [CrossRef] [PubMed]
27. Zhou, C.; Guo, T.; Bai, X.; Wu, J.; Gao, T.; Guan, X.; Liu, X.; Gu, L.; Huang, P.; Xuan, M.; et al. Locus Coeruleus Degeneration Is Associated with Disorganized Functional Topology in Parkinson's Disease. *NeuroImage Clin.* **2021**, *32*, 102873. [CrossRef] [PubMed]
28. Mastroeni, D.; Grover, A.; Leonard, B.; Joyce, J.N.; Coleman, P.D.; Kozik, B.; Bellinger, D.L.; Rogers, J. Microglial Responses to Dopamine in a Cell Culture Model of Parkinson's Disease. *Neurobiol. Aging* **2009**, *30*, 1805–1817. [CrossRef] [PubMed]
29. Zhang, Y.; Chen, Y.; Wu, J.; Manaenko, A.; Yang, P.; Tang, J.; Fu, W.; Zhang, J.H. Activation of Dopamine D2 Receptor Suppresses Neuroinflammation through αB-Crystalline by Inhibition of NF-κB Nuclear Translocation in Experimental ICH Mice Model. *Stroke* **2015**, *46*, 2637–2646. [CrossRef] [PubMed]
30. Wang, T.; Nowrangi, D.; Yu, L.; Lu, T.; Tang, J.; Han, B.; Ding, Y.; Fu, F.; Zhang, J.H. Activation of Dopamine D1 Receptor Decreased NLRP3-Mediated Inflammation in Intracerebral Hemorrhage Mice. *J. Neuroinflamm.* **2018**, *15*, 2. [CrossRef]
31. Marinova-Mutafchieva, L.; Sadeghian, M.; Broom, L.; Davis, J.B.; Medhurst, A.D.; Dexter, D.T. Relationship between Microglial Activation and Dopaminergic Neuronal Loss in the Substantia Nigra: A Time Course Study in a 6-Hydroxydopamine Model of Parkinson's Disease. *J. Neurochem.* **2009**, *110*, 966–975. [CrossRef]
32. Shao, W.; Zhang, S.; Tang, M.; Zhang, X.; Zhou, Z.; Yin, Y.; Zhou, Q.; Huang, Y.; Liu, Y.; Wawrousek, E.; et al. Suppression of Neuroinflammation by Astrocytic Dopamine D2 Receptors via αB-Crystallin. *Nature* **2013**, *494*, 90–94. [CrossRef]
33. Färber, K.; Pannasch, U.; Kettenmann, H. Dopamine and Noradrenaline Control Distinct Functions in Rodent Microglial Cells. *Mol. Cell. Neurosci.* **2005**, *29*, 128–138. [CrossRef] [PubMed]
34. Evans, A.K.; Defensor, E.; Shamloo, M. Selective Vulnerability of the Locus Coeruleus Noradrenergic System and Its Role in Modulation of Neuroinflammation, Cognition, and Neurodegeneration. *Front. Pharmacol.* **2022**, *13*, 1030609. [CrossRef] [PubMed]
35. Cebrián, C.; Loike, J.D.; Sulzer, D. Neuroinflammation in Parkinson's Disease Animal Models: A Cell Stress Response or a Step in Neurodegeneration? In *Behavioral Neurobiology of Huntington's Disease and Parkinson's Disease*; Nguyen, H.H.P., Cenci, M.A., Eds.; Current Topics in Behavioral Neurosciences; Springer: Berlin/Heidelberg, Germany, 2014; Volume 22, pp. 237–270; ISBN 978-3-662-46343-7.

36. Troncoso-Escudero, P.; Parra, A.; Nassif, M.; Vidal, R.L. Outside in: Unraveling the Role of Neuroinflammation in the Progression of Parkinson's Disease. *Front. Neurol.* **2018**, *9*, 860. [CrossRef]
37. Wang, Q.; Oyarzabal, E.A.; Song, S.; Wilson, B.; Santos, J.H.; Hong, J.-S. Locus Coeruleus Neurons Are Most Sensitive to Chronic Neuroinflammation-Induced Neurodegeneration. *Brain. Behav. Immun.* **2020**, *87*, 359–368. [CrossRef] [PubMed]
38. Wang, T.-F.; Wu, S.-Y.; Pan, B.-S.; Tsai, S.-F.; Kuo, Y.-M. Inhibition of Nigral Microglial Activation Reduces Age-Related Loss of Dopaminergic Neurons and Motor Deficits. *Cells* **2022**, *11*, 481. [CrossRef]
39. McGeer, P.L.; Itagaki, S.; Boyes, B.E.; McGeer, E.G. Reactive Microglia Are Positive for HLA-DR in the Substantia Nigra of Parkinson's and Alzheimer's Disease Brains. *Neurology* **1988**, *38*, 1285. [CrossRef]
40. Mogi, M.; Harada, M.; Riederer, P.; Narabayashi, H.; Fujita, K.; Nagatsu, T. Tumor Necrosis Factor-α (TNF-α) Increases Both in the Brain and in the Cerebrospinal Fluid from Parkinsonian Patients. *Neurosci. Lett.* **1994**, *165*, 208–210. [CrossRef] [PubMed]
41. Mogi, M.; Harada, M.; Kondo, T.; Riederer, P.; Inagaki, H.; Minami, M.; Nagatsu, T. Interleukin-1β, Interleukin-6, Epidermal Growth Factor and Transforming Growth Factor-α Are Elevated in the Brain from Parkinsonian Patients. *Neurosci. Lett.* **1994**, *180*, 147–150. [CrossRef]
42. Mogi, M.; Harada, M.; Narabayashi, H.; Inagaki, H.; Minami, M.; Nagatsu, T. Interleukin (IL)-1β, IL-2, IL-4, IL-6 and Transforming Growth Factor-α Levels Are Elevated in Ventricular Cerebrospinal Fluid in Juvenile Parkinsonism and Parkinson's Disease. *Neurosci. Lett.* **1996**, *211*, 13–16. [CrossRef]
43. Butkovich, L.M.; Houser, M.C.; Tansey, M.G. α-Synuclein and Noradrenergic Modulation of Immune Cells in Parkinson's Disease Pathogenesis. *Front. Neurosci.* **2018**, *12*, 626. [CrossRef]
44. McIntosh, T.K.; Vink, R.; Noble, L.; Yamakami, I.; Fernyak, S.; Soares, H.; Faden, A.L. Traumatic Brain Injury in the Rat: Characterization of a Lateral Fluid-Percussion Model. *Neuroscience* **1989**, *28*, 233–244. [CrossRef] [PubMed]
45. Huger, F.; Patrick, G. Effect of concussive head injury on central catecholamine levels and synthesis rates in rat brain regions. *J. Neurochem.* **1979**, *33*, 89–95. [CrossRef] [PubMed]
46. Bales, J.W.; Kline, A.E.; Wagner, A.K.; Dixon, C.E. Targeting Dopamine in Acute Traumatic Brain Injury. *Open Drug Discov. J.* **2010**, *2*, 119–128. [CrossRef] [PubMed]
47. Mautes, A.E.M.; Müller, M.; Cortbus, F.; Schwerdtfeger, K.; Maier, B.; Holanda, M.; Nacimiento, A.; Marzi, I.; Steudel, W.-I. Alterations of Norepinephrine Levels in Plasma and CSF of Patients after Traumatic Brain Injury in Relation to Disruption of the Blood-Brain Barrier. *Acta Neurochir.* **2001**, *143*, 51–58. [CrossRef] [PubMed]
48. van Bregt, D.R.; Thomas, T.C.; Hinzman, J.M.; Cao, T.; Liu, M.; Bing, G.; Gerhardt, G.A.; Pauly, J.R.; Lifshitz, J. Substantia Nigra Vulnerability after a Single Moderate Diffuse Brain Injury in the Rat. *Exp. Neurol.* **2012**, *234*, 8–19. [CrossRef]
49. Wagner, A.K.; Sokoloski, J.E.; Ren, D.; Chen, X.; Khan, A.S.; Zafonte, R.D.; Michael, A.C.; Dixon, C.E. Controlled Cortical Impact Injury Affects Dopaminergic Transmission in the Rat Striatum. *J. Neurochem.* **2005**, *95*, 457–465. [CrossRef]
50. Impellizzeri, D.; Campolo, M.; Bruschetta, G.; Crupi, R.; Cordaro, M.; Paterniti, I.; Cuzzocrea, S.; Esposito, E. Traumatic Brain Injury Leads to Development of Parkinson's Disease Related Pathology in Mice. *Front. Neurosci.* **2016**, *10*, 196837. [CrossRef] [PubMed]
51. Levin, B.E.; Brown, K.L.; Pawar, G.; Dunn-Meynell, A. Widespread and Lateralized Effects of Acute Traumatic Brain Injury on Norepinephrine Turnover in the Rat Brain. *Brain Res.* **1995**, *674*, 307–313. [CrossRef]
52. Fujinaka, T.; Kohmura, E.; Yuguchi, T.; Yoshimine, T. The Morphological and Neurochemical Effects of Diffuse Brain Injury on Rat Central Noradrenergic System. *Neurol. Res.* **2003**, *25*, 35–41. [CrossRef]
53. Donnemiller, E.; Brenneis, C.; Wissel, J.; Scherfler, C.; Poewe, W.; Riccabona, G.; Wenning, G.K. Impaired Dopaminergic Neurotransmission in Patients with Traumatic Brain Injury: A SPET Study Using ^{123}I-β-CIT and ^{123}I-IBZM. *Eur. J. Nucl. Med.* **2000**, *27*, 1410–1414. [CrossRef] [PubMed]
54. Tschuor, C.; Asmis, L.M.; Lenzlinger, P.M.; Tanner, M.; Härter, L.; Keel, M.; Stocker, R.; Stover, J.F. In Vitro Norepinephrine Significantly Activates Isolated Platelets from Healthy Volunteers and Critically Ill Patients Following Severe Traumatic Brain Injury. *Crit. Care* **2008**, *12*, R80. [CrossRef]
55. Lloyd-Donald, P.; Spencer, W.; Cheng, J.; Romero, L.; Jithoo, R.; Udy, A.; Fitzgerald, M.C. In Adult Patients with Severe Traumatic Brain Injury, Does the Use of Norepinephrine for Augmenting Cerebral Perfusion Pressure Improve Neurological Outcome? A Systematic Review. *Injury* **2020**, *51*, 2129–2134. [CrossRef]
56. Dunn-Meynell, A.A.; Hassanain, M.; Levin, B.E. Norepinephrine and Traumatic Brain Injury: A Possible Role in Post-Traumatic Edema. *Brain Res.* **1998**, *800*, 245–252. [CrossRef]
57. Chien, Y.J.; Chien, Y.C.; Liu, C.T.; Wu, H.C.; Chang, C.Y.; Wu, M.Y. Effects of Methylphenidate on Cognitive Function in Adults with Traumatic Brain Injury: A Meta-Analysis. *Brain Sci.* **2019**, *9*, 291. [CrossRef]
58. Johnson, V.E.; Stewart, J.E.; Begbie, F.D.; Trojanowski, J.Q.; Smith, D.H.; Stewart, W. Inflammation and White Matter Degeneration Persist for Years after a Single Traumatic Brain Injury. *Brain* **2013**, *136*, 28–42. [CrossRef] [PubMed]
59. Loane, D.J.; Kumar, A.; Stoica, B.A.; Cabatbat, R.; Faden, A.I. Progressive Neurodegeneration after Experimental Brain Trauma: Association with Chronic Microglial Activation. *J. Neuropathol. Exp. Neurol.* **2014**, *73*, 14–29. [CrossRef] [PubMed]
60. Rusiecki, J.; Levin, L.I.; Wang, L.; Byrne, C.; Krishnamurthy, J.; Chen, L.; Galdzicki, Z.; French, L.M. Blast Traumatic Brain Injury and Serum Inflammatory Cytokines: A Repeated Measures Case-Control Study among U.S. Military Service Members. *J. Neuroinflamm.* **2020**, *17*, 20. [CrossRef]

61. Chen, W.; Man, X.; Zhang, Y.; Yao, G.; Chen, J. Medial Prefrontal Cortex Oxytocin Mitigates Epilepsy and Cognitive Impairments Induced by Traumatic Brain Injury through Reducing Neuroinflammation in Mice. *Sci. Rep.* **2023**, *13*, 5214. [CrossRef]
62. Liu, M.; Bachstetter, A.D.; Cass, W.A.; Lifshitz, J.; Bing, G. Pioglitazone Attenuates Neuroinflammation and Promotes Dopaminergic Neuronal Survival in the Nigrostriatal System of Rats after Diffuse Brain Injury. *J. Neurotrauma* **2017**, *34*, 414–422. [CrossRef]
63. Acosta, S.A.; Tajiri, N.; Shinozuka, K.; Ishikawa, H.; Grimmig, B.; Diamond, D.; Sanberg, P.R.; Bickford, P.C.; Kaneko, Y.; Borlongan, C.V. Long-Term Upregulation of Inflammation and Suppression of Cell Proliferation in the Brain of Adult Rats Exposed to Traumatic Brain Injury Using the Controlled Cortical Impact Model. *PLoS ONE* **2013**, *8*, e53376. [CrossRef]
64. Ramlackhansingh, A.F.; Brooks, D.J.; Greenwood, R.J.; Bose, S.K.; Turkheimer, F.E.; Kinnunen, K.M.; Gentleman, S.; Heckemann, R.A.; Gunanayagam, K.; Gelosa, G.; et al. Inflammation after Trauma: Microglial Activation and Traumatic Brain Injury. *Ann. Neurol.* **2011**, *70*, 374–383. [CrossRef] [PubMed]
65. Hutson, C.B.; Lazo, C.R.; Mortazavi, F.; Giza, C.C.; Hovda, D.; Chesselet, M.-F. Traumatic Brain Injury in Adult Rats Causes Progressive Nigrostriatal Dopaminergic Cell Loss and Enhanced Vulnerability to the Pesticide Paraquat. *J. Neurotrauma* **2011**, *28*, 1783–1801. [CrossRef] [PubMed]
66. Nagamoto-Combs, K.; McNeal, D.W.; Morecraft, R.J.; Combs, C.K. Prolonged Microgliosis in the Rhesus Monkey Central Nervous System after Traumatic Brain Injury. *J. Neurotrauma* **2007**, *24*, 1719–1742. [CrossRef]
67. Mouzon, B.C.; Bachmeier, C.; Ferro, A.; Ojo, J.-O.; Crynen, G.; Acker, C.M.; Davies, P.; Mullan, M.; Stewart, W.; Crawford, F. Chronic Neuropathological and Neurobehavioral Changes in a Repetitive Mild Traumatic Brain Injury Model: Chronic Effects of r-mTBI. *Ann. Neurol.* **2014**, *75*, 241–254. [CrossRef] [PubMed]
68. Mouzon, B.C.; Bachmeier, C.; Ojo, J.O.; Acker, C.M.; Ferguson, S.; Paris, D.; Ait-Ghezala, G.; Crynen, G.; Davies, P.; Mullan, M.; et al. Lifelong Behavioral and Neuropathological Consequences of Repetitive Mild Traumatic Brain Injury. *Ann. Clin. Transl. Neurol.* **2018**, *5*, 64–80. [CrossRef]
69. Molinoff, P.B.; Axelrod, J. Biochemistry of Catecholamines. *Annu. Rev. Biochem.* **1971**, *40*, 465–500. [CrossRef] [PubMed]
70. Daubner, S.C.; Le, T.; Wang, S. Tyrosine Hydroxylase and Regulation of Dopamine Synthesis. *Arch. Biochem. Biophys.* **2011**, *508*, 1–12. [CrossRef] [PubMed]
71. Weinshilboum, R.; Axelrod, J. Serum Dopamine-Beta-Hydroxylase Activity. *Circ. Res.* **1971**, *28*, 307–315. [CrossRef]
72. Grossman, M.H.; Emanuel, B.S.; Budarf, M.L. Chromosomal Mapping of the Human Catechol-O-Methyltransferase Gene to 22q11.1→q11.2. *Genomics* **1992**, *12*, 822–825. [CrossRef]
73. Arulsamy, A.; Corrigan, F.; Collins-Praino, L.E. Cognitive and Neuropsychiatric Impairments Vary as a Function of Injury Severity at 12 Months Post-Experimental Diffuse Traumatic Brain Injury: Implications for Dementia Development. *Behav. Brain Res.* **2019**, *365*, 66–76. [CrossRef]
74. Arulsamy, A.; Corrigan, F.; Collins-Praino, L.E. Age, but Not Severity of Injury, Mediates Decline in Executive Function: Validation of the Rodent Touchscreen Paradigm for Preclinical Models of Traumatic Brain Injury. *Behav. Brain Res.* **2019**, *368*, 111912. [CrossRef] [PubMed]
75. Sengupta, P. The Laboratory Rat: Relating Its Age with Human's. *Int. J. Prev. Med.* **2013**, *4*, 624–630.
76. Postuma, R.B.; Aarsland, D.; Barone, P.; Burn, D.J.; Hawkes, C.H.; Oertel, W.; Ziemssen, T. Identifying Prodromal Parkinson's Disease: Pre-Motor Disorders in Parkinson's Disease. *Mov. Disord.* **2012**, *27*, 617–626. [CrossRef]
77. Marmarou, A.; Foda, M.A.; van den Brink, W.; Campbell, J.; Kita, H.; Demetriadou, K. A New Model of Diffuse Brain Injury in Rats. Part I: Pathophysiology and Biomechanics. *J. Neurosurg.* **1994**, *80*, 291–300. [CrossRef] [PubMed]
78. Xiong, Y.; Mahmood, A.; Chopp, M. Animal Models of Traumatic Brain Injury. *Nat. Rev. Neurosci.* **2013**, *14*, 128–142. [CrossRef] [PubMed]
79. Arulsamy, A.; Teng, J.; Colton, H.; Corrigan, F.; Collins-Praino, L. Evaluation of Early Chronic Functional Outcomes and Their Relationship to Pre-Frontal Cortex and Hippocampal Pathology Following Moderate-Severe Traumatic Brain Injury. *Behav. Brain Res.* **2018**, *348*, 127–138. [CrossRef]
80. Corrigan, F.; Arulsamy, A.; Shultz, S.R.; Wright, D.K.; Collins-Praino, L.E. Initial Severity of Injury Has Little Effect on the Temporal Profile of Long-Term Deficits in Locomotion, Anxiety, and Cognitive Function after Diffuse Traumatic Brain Injury. *Neurotrauma Rep.* **2023**, *4*, 41–50. [CrossRef]
81. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition*; Elsevier: Amsterdam, The Netherlands, 2006; ISBN 0-08-047515-9.
82. Delic, V.; Beck, K.D.; Pang, K.C.H.; Citron, B.A. Biological Links between Traumatic Brain Injury and Parkinson's Disease. *Acta Neuropathol. Commun.* **2020**, *8*, 45. [CrossRef]
83. Bower, J.H.; Maraganore, D.M.; Peterson, B.J.; McDonnell, S.K.; Ahlskog, J.E.; Rocca, W.A. Head Trauma Preceding PD: A Case-Control Study. *Neurology* **2003**, *60*, 1610–1615. [CrossRef]
84. Gardner, R.C.; Byers, A.L.; Barnes, D.E.; Li, Y.; Boscardin, J.; Yaffe, K. Mild TBI and Risk of Parkinson Disease: A Chronic Effects of Neurotrauma Consortium Study. *Neurology* **2018**, *90*, e1771–e1779. [CrossRef] [PubMed]
85. Gonzalez-Lopez, E.; Vrana, K.E. Dopamine Beta-hydroxylase and Its Genetic Variants in Human Health and Disease. *J. Neurochem.* **2020**, *152*, 157–181. [CrossRef] [PubMed]
86. Robertson, D.; Goldberg, M.R.; Onrot, J.; Hollister, A.S.; Wiley, R.; Thompson, J.G.; Robertson, R.M. Isolated Failure of Autonomic Noradrenergic Neurotransmission. *N. Engl. J. Med.* **1986**, *314*, 1494–1497. [CrossRef] [PubMed]

87. Jones, B.E.; Moore, R.Y. Ascending Projections of the Locus Coeruleus in the Rat. II. Autoradiographic Study. *Brain Res.* **1977**, *127*, 23–53. [CrossRef]
88. Collingridge, G.L.; James, T.A.; MacLeod, N.K. Neurochemical and Electrophysiological Evidence for a Projection from the Locus Coeruleus to the Substantia Nigra [Proceedings]. *J. Physiol.* **1979**, *290*, 44P. [PubMed]
89. Sugama, S.; Takenouchi, T.; Hashimoto, M.; Ohata, H.; Takenaka, Y.; Kakinuma, Y. Stress-Induced Microglial Activation Occurs through β-Adrenergic Receptor: Noradrenaline as a Key Neurotransmitter in Microglial Activation. *J. Neuroinflamm.* **2019**, *16*, 266. [CrossRef] [PubMed]
90. Smits, R.P.; Steinbusch, H.W.; Mulder, A.H. Distribution of Dopamine-Immunoreactive Cell Bodies in the Guinea-Pig Brain. *J. Chem. Neuroanat.* **1990**, *3*, 101–123. [PubMed]
91. Fang, Y.; Malik, M.; England, S.K.; Imoukhuede, P.I. Absolute Quantification of Plasma Membrane Receptors Via Quantitative Flow Cytometry. *Methods Mol. Biol. Clifton NJ* **2022**, *2475*, 61–77. [CrossRef]
92. Wassall, R.D.; Teramoto, N.; Cunnane, T.C. Noradrenaline. In *Encyclopedia of Neuroscience*; Elsevier: Amsterdam, The Netherlands, 2009; pp. 1221–1230. ISBN 978-0-08-045046-9.
93. Taylor, F.B.; Russo, J. Comparing Guanfacine and Dextroamphetamine for the Treatment of Adult Attention-Deficit/Hyperactivity Disorder. *J. Clin. Psychopharmacol.* **2001**, *21*, 223–228. [CrossRef]
94. Avery, R. The Alpha-2A-Adrenoceptor Agonist, Guanfacine, Increases Regional Cerebral Blood Flow in Dorsolateral Prefrontal Cortex of Monkeys Performing a Spatial Working Memory Task. *Neuropsychopharmacology* **2000**, *23*, 240–249. [CrossRef]
95. Arnsten, A.; Cai, J.; Goldman-Rakic, P. The Alpha-2 Adrenergic Agonist Guanfacine Improves Memory in Aged Monkeys without Sedative or Hypotensive Side Effects: Evidence for Alpha-2 Receptor Subtypes. *J. Neurosci.* **1988**, *8*, 4287–4298. [CrossRef] [PubMed]
96. Carlson, S.; Tanila, H.; Rama, P.; Mecke, E.; Pertovaara, A. Effects of Medetomidine, an α-2 Adrenoceptor Agonist, and Atipamezole, an α-2 Antagonist, on Spatial Memory Performance in Adult and Aged Rats. *Behav. Neural Biol.* **1992**, *58*, 113–119. [CrossRef] [PubMed]
97. Rämä, P.; Linnankoski, I.; Tanila, H.; Pertovaara, A.; Carlson, S. Medetomidine, Atipamezole, and Guanfacine in Delayed Response Performance of Aged Monkeys. *Pharmacol. Biochem. Behav.* **1996**, *55*, 415–422. [CrossRef] [PubMed]
98. Choi, Y.; Novak, J.C.; Hillier, A.; Votolato, N.A.; Beversdorf, D.Q. The Effect of α-2 Adrenergic Agonists on Memory and Cognitive Flexibility. *Cogn. Behav. Neurol.* **2006**, *19*, 204–207. [CrossRef] [PubMed]
99. Jäkälä, P. Guanfacine, but Not Clonidine, Improves Planning and Working Memory Performance in Humans. *Neuropsychopharmacology* **1999**, *20*, 460–470. [CrossRef] [PubMed]
100. Sysoev, Y.I.; Prikhodko, V.A.; Chernyakov, R.T.; Idiyatullin, R.D.; Musienko, P.E.; Okovityi, S.V. Effects of Alpha-2 Adrenergic Agonist Mafedine on Brain Electrical Activity in Rats after Traumatic Brain Injury. *Brain Sci.* **2021**, *11*, 981. [CrossRef]
101. Wu, J.; Vogel, T.; Gao, X.; Lin, B.; Kulwin, C.; Chen, J. Neuroprotective Effect of Dexmedetomidine in a Murine Model of Traumatic Brain Injury. *Sci. Rep.* **2018**, *8*, 4935. [CrossRef] [PubMed]
102. McAllister, T.W.; McDonald, B.C.; Flashman, L.A.; Ferrell, R.B.; Tosteson, T.D.; Yanofsky, N.N.; Grove, M.R.; Saykin, A.J. Alpha-2 Adrenergic Challenge with Guanfacine One Month after Mild Traumatic Brain Injury: Altered Working Memory and BOLD Response. *Int. J. Psychophysiol. Off. J. Int. Organ. Psychophysiol.* **2011**, *82*, 107–114. [CrossRef] [PubMed]
103. Li, B.-M.; Mei, Z.-T. Delayed-Response Deficit Induced by Local Injection of the A2-Adrenergic Antagonist Yohimbine into the Dorsolateral Prefrontal Cortex in Young Adult Monkeys. *Behav. Neural Biol.* **1994**, *62*, 134–139. [CrossRef]
104. Tanila, H.; Rämä, P.; Carlson, S. The Effects of Prefrontal Intracortical Microinjections of an Alpha-2 Agonist, Alpha-2 Antagonist and Lidocaine on the Delayed Alternation Performance of Aged Rats. *Brain Res. Bull.* **1996**, *40*, 117–119. [CrossRef]
105. Ferrucci, M.; SGiorgi, F.; Bartalucci, A.; LBusceti, C.; Fornai, F. The Effects of Locus Coeruleus and Norepinephrine in Methamphetamine Toxicity. *Curr. Neuropharmacol.* **2013**, *11*, 80–94. [CrossRef]
106. Smith, Y.; Kieval, J.Z. Anatomy of the Dopamine System in the Basal Ganglia. *Trends Neurosci.* **2000**, *23*, S28–S33. [CrossRef] [PubMed]
107. Grueschow, M.; Kleim, B.; Ruff, C.C. Functional Coupling of the Locus Coeruleus Is Linked to Successful Cognitive Control. *Brain Sci.* **2022**, *12*, 305. [CrossRef] [PubMed]
108. Cragg, S.J.; Rice, M.E.; Greenfield, S.A. Heterogeneity of Electrically Evoked Dopamine Release and Reuptake in Substantia Nigra, Ventral Tegmental Area, and Striatum. *J. Neurophysiol.* **1997**, *77*, 863–873. [CrossRef] [PubMed]
109. Carboni, E.; Tanda, G.L.; Frau, R.; Chiara, G.D. Blockade of the Noradrenaline Carrier Increases Extracellular Dopamine Concentrations in the Prefrontal Cortex: Evidence That Dopamine Is Taken up In Vivo by Noradrenergic Terminals. *J. Neurochem.* **1990**, *55*, 1067–1070. [CrossRef] [PubMed]
110. af Bjerkén, S.; Stenmark Persson, R.; Barkander, A.; Karalija, N.; Pelegrina-Hidalgo, N.; Gerhardt, G.A.; Virel, A.; Strömberg, I. Noradrenaline Is Crucial for the Substantia Nigra Dopaminergic Cell Maintenance. *Neurochem. Int.* **2019**, *131*, 104551. [CrossRef] [PubMed]
111. Hassani, O.K.; Rymar, V.V.; Nguyen, K.Q.; Huo, L.; Cloutier, J.-F.; Miller, F.D.; Sadikot, A.F. The Noradrenergic System Is Necessary for Survival of Vulnerable Midbrain Dopaminergic Neurons: Implications for Development and Parkinson's Disease. *Neurobiol. Aging* **2020**, *85*, 22–37. [CrossRef] [PubMed]
112. Tong, J.; Hornykiewicz, O.; Kish, S.J. Inverse Relationship Between Brain Noradrenaline Level and Dopamine Loss in Parkinson Disease: A Possible Neuroprotective Role for Noradrenaline. *Arch. Neurol.* **2006**, *63*, 1724. [CrossRef] [PubMed]

113. Isaias, I.U.; Marotta, G.; Pezzoli, G.; Sabri, O.; Schwarz, J.; Crenna, P.; Classen, J.; Cavallari, P. Enhanced Catecholamine Transporter Binding in the Locus Coeruleus of Patients with Early Parkinson Disease. *BMC Neurol.* **2011**, *11*, 88. [CrossRef]
114. Delaville, C.; Deurwaerdère, P.D.; Benazzouz, A. Noradrenaline and Parkinson's Disease. *Front. Syst. Neurosci.* **2011**, *5*, 31. [CrossRef]
115. Braak, H.; Del Tredici, K. Invited Article: Nervous System Pathology in Sporadic Parkinson Disease. *Neurology* **2008**, *70*, 1916–1925. [CrossRef] [PubMed]
116. Walf, A.A.; Frye, C.A. The Use of the Elevated plus Maze as an Assay of Anxiety-Related Behavior in Rodents. *Nat. Protoc.* **2007**, *2*, 322–328. [CrossRef] [PubMed]
117. Tucker, L.B.; Fu, A.H.; McCabe, J.T. Performance of Male and Female C57BL/6J Mice on Motor and Cognitive Tasks Commonly Used in Pre-Clinical Traumatic Brain Injury Research. *J. Neurotrauma* **2016**, *33*, 880–894. [CrossRef] [PubMed]
118. Budinich, C.S.; Tucker, L.B.; Lowe, D.; Rosenberger, J.G.; McCabe, J.T. Short and Long-Term Motor and Behavioral Effects of Diazoxide and Dimethyl Sulfoxide Administration in the Mouse after Traumatic Brain Injury. *Pharmacol. Biochem. Behav.* **2013**, *108*, 66–73. [CrossRef] [PubMed]
119. Nolan, A.; Hennessy, E.; Krukowski, K.; Guglielmetti, C.; Chaumeil, M.M.; Sohal, V.S.; Rosi, S. Repeated Mild Head Injury Leads to Wide-Ranging Deficits in Higher-Order Cognitive Functions Associated with the Prefrontal Cortex. *J. Neurotrauma* **2018**, *35*, 2425–2434. [CrossRef] [PubMed]
120. Shultz, S.R.; MacFabe, D.F.; Foley, K.A.; Taylor, R.; Cain, D.P. A Single Mild Fluid Percussion Injury Induces Short-Term Behavioral and Neuropathological Changes in the Long–Evans Rat: Support for an Animal Model of Concussion. *Behav. Brain Res.* **2011**, *224*, 326–335. [CrossRef] [PubMed]
121. Hare, B.D.; Shinohara, R.; Liu, R.J.; Pothula, S.; DiLeone, R.J.; Duman, R.S. Optogenetic Stimulation of Medial Prefrontal Cortex Drd1 Neurons Produces Rapid and Long-Lasting Antidepressant Effects. *Nat. Commun.* **2019**, *10*, 223. [CrossRef] [PubMed]
122. Beyer, D.K.E.; Mattukat, A.; Freund, N. Prefrontal Dopamine D1 Receptor Manipulation Influences Anxiety Behavior and Induces Neuroinflammation within the Hippocampus. *Int. J. Bipolar Disord.* **2021**, *9*, 9. [CrossRef] [PubMed]
123. Zhou, J.; Peng, C.; Li, Q.; Yan, X.; Yang, L.; Li, M.; Cao, X.; Xie, X.; Chen, D.; Rao, C.; et al. Dopamine Homeostasis Imbalance and Dopamine Receptors-Mediated AC/cAMP/PKA Pathway Activation Are Involved in Aconitine-Induced Neurological Impairment in Zebrafish and SH-SY5Y Cells. *Front. Pharmacol.* **2022**, *13*, 837810. [CrossRef]
124. Jones-Tabah, J.; Martin, R.D.; Chen, J.J.; Tanny, J.C.; Clarke, P.B.S.; Hébert, T.E. Dopamine D1 Receptor Activation and cAMP/PKA Signalling Mediate Brd4 Recruitment to Chromatin to Regulate Gene Expression in Rat Striatal Neurons. *Neuroscience* **2021**. [CrossRef]
125. Keil, M.F.; Briassoulis, G.; Stratakis, C.A.; Wu, T.J. Protein Kinase A and Anxiety-Related Behaviors: A Mini-Review. *Front. Endocrinol.* **2016**, *7*, 83. [CrossRef] [PubMed]
126. Miguel, T.T.; Gomes, K.S.; Nunes-de-Souza, R.L. Tonic Modulation of Anxiety-like Behavior by Corticotropin-Releasing Factor (CRF) Type 1 Receptor (CRF1) within the Medial Prefrontal Cortex (mPFC) in Male Mice: Role of Protein Kinase A (PKA). *Horm. Behav.* **2014**, *66*, 247–256. [CrossRef] [PubMed]
127. Kurowski, B.G.; Backeljauw, B.; Zang, H.; Zhang, N.; Martin, L.J.; Pilipenko, V.; Yeates, K.; Taylor, H.G.; Wade, S. Influence of Catechol-O-Methyltransferase on Executive Functioning Longitudinally after Early Childhood Traumatic Brain Injury: Preliminary Findings. *J. Head Trauma Rehabil.* **2016**, *31*, E1–E9. [CrossRef] [PubMed]
128. Lachman, H.M.; Papolos, D.F.; Saito, T.; Yu, Y.-M.; Szumlanski, C.L.; Weinshilboum, R.M. Human Catechol-O-Methyltransferase Pharmacogenetics: Description of a Functional Polymorphism and Its Potential Application to Neuropsychiatric Disorders. *Pharmacogenet. Genom.* **1996**, *6*, 243–250. [CrossRef] [PubMed]
129. Lotta, T.; Vidgren, J.; Tilgmann, C.; Ulmanen, I.; Melen, K.; Julkunen, I.; Taskinen, J. Kinetics of Human Soluble and Membrane-Bound Catechol O-Methyltransferase: A Revised Mechanism and Description of the Thermolabile Variant of the Enzyme. *Biochemistry* **1995**, *34*, 4202–4210. [CrossRef] [PubMed]
130. Lin, C.-H.; Fan, J.-Y.; Lin, H.-I.; Chang, C.-W.; Wu, Y.-R. Catechol-O-Methyltransferase (COMT) Genetic Variants Are Associated with Cognitive Decline in Patients with Parkinson's Disease. *Park. Relat. Disord.* **2018**, *50*, 48–53. [CrossRef] [PubMed]
131. Tang, C.; Wang, W.; Shi, M.; Zhang, N.; Zhou, X.; Li, X.; Ma, C.; Chen, G.; Xiang, J.; Gao, D. Meta-Analysis of the Effects of the Catechol-O-Methyltransferase Val158/108Met Polymorphism on Parkinson's Disease Susceptibility and Cognitive Dysfunction. *Front. Genet.* **2019**, *10*, 644. [CrossRef] [PubMed]
132. Lipsky, R.H.; Sparling, M.B.; Ryan, L.M.; Xu, K.; Salazar, A.M.; Goldman, D.; Warden, D.L. Association of COMT Val158Met Genotype with Executive Functioning Following Traumatic Brain Injury. *J. Neuropsychiatry Clin. Neurosci.* **2005**, *17*, 465–471. [CrossRef] [PubMed]
133. Redell, J.B.; Dash, P.K. Traumatic Brain Injury Stimulates Hippocampal Catechol-O-Methyl Transferase Expression in Microglia. *Neurosci. Lett.* **2007**, *413*, 36–41. [CrossRef]
134. Wang, D.; Xu, X.; Wu, Y.-G.; Lyu, L.; Zhou, Z.-W.; Zhang, J.-N. Dexmedetomidine Attenuates Traumatic Brain Injury: Action Pathway and Mechanisms. *Neural Regen. Res.* **2018**, *13*, 819. [CrossRef]
135. Gyoneva, S.; Traynelis, S.F. Norepinephrine Modulates the Motility of Resting and Activated Microglia via Different Adrenergic Receptors. *J. Biol. Chem.* **2013**, *288*, 15291–15302. [CrossRef] [PubMed]

136. Vidal-Itriago, A.; Radford, R.A.W.; Aramideh, J.A.; Maurel, C.; Scherer, N.M.; Don, E.K.; Lee, A.; Chung, R.S.; Graeber, M.B.; Morsch, M. Microglia Morphophysiological Diversity and Its Implications for the CNS. *Front. Immunol.* **2022**, *13*, 997786. [CrossRef] [PubMed]
137. Wake, H.; Moorhouse, A.J.; Jinno, S.; Kohsaka, S.; Nabekura, J. Resting Microglia Directly Monitor the Functional State of Synapses In Vivo and Determine the Fate of Ischemic Terminals. *J. Neurosci.* **2009**, *29*, 3974–3980. [CrossRef] [PubMed]
138. Li, Q.; Cheng, Z.; Zhou, L.; Darmanis, S.; Neff, N.F.; Okamoto, J.; Gulati, G.; Bennett, M.L.; Sun, L.O.; Clarke, L.E.; et al. Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. *Neuron* **2019**, *101*, 207–223.e10. [CrossRef] [PubMed]
139. Kenkhuis, B.; Somarakis, A.; Kleindouwel, L.R.T.; Van Roon-Mom, W.M.C.; Höllt, T.; Van Der Weerd, L. Co-Expression Patterns of Microglia Markers Iba1, TMEM119 and P2RY12 in Alzheimer's Disease. *Neurobiol. Dis.* **2022**, *167*, 105684. [CrossRef]
140. Bélanger, M.; Magistretti, P.J. The Role of Astroglia in Neuroprotection. *Dialogues Clin. Neurosci.* **2009**, *11*, 281–295. [CrossRef] [PubMed]
141. Myer, D.J. Essential Protective Roles of Reactive Astrocytes in Traumatic Brain Injury. *Brain* **2006**, *129*, 2761–2772. [CrossRef]
142. Karve, I.P.; Taylor, J.M.; Crack, P.J. The Contribution of Astrocytes and Microglia to Traumatic Brain Injury. *Br. J. Pharmacol.* **2016**, *173*, 692–702. [CrossRef]
143. Valles, S.L.; Singh, S.K.; Campos-Campos, J.; Colmena, C.; Campo-Palacio, I.; Alvarez-Gamez, K.; Caballero, O.; Jorda, A. Functions of Astrocytes under Normal Conditions and after a Brain Disease. *Int. J. Mol. Sci.* **2023**, *24*, 8434. [CrossRef]
144. Reeve, A.; Simcox, E.; Turnbull, D. Ageing and Parkinson's Disease: Why Is Advancing Age the Biggest Risk Factor? *Ageing Res. Rev.* **2014**, *14*, 19–30. [CrossRef]
145. Sparkman, N.L.; Johnson, R.W. Neuroinflammation Associated with Aging Sensitizes the Brain to the Effects of Infection or Stress. *Neuroimmunomodulation* **2008**, *15*, 323–330. [CrossRef] [PubMed]
146. Rieckmann, A.; Johnson, K.A.; Sperling, R.A.; Buckner, R.L.; Hedden, T. Dedifferentiation of Caudate Functional Connectivity and Striatal Dopamine Transporter Density Predict Memory Change in Normal Aging. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 10160–10165. [CrossRef] [PubMed]
147. Dahl, M.J.; Bachman, S.L.; Dutt, S.; Düzel, S.; Bodammer, N.C.; Lindenberger, U.; Kühn, S.; Werkle-Bergner, M.; Mather, M. The Integrity of Dopaminergic and Noradrenergic Brain Regions Is Associated with Different Aspects of Late-Life Memory Performance. *Nat. Aging* **2023**, *3*, 1128–1143. [CrossRef] [PubMed]
148. Mather, M. Noradrenaline in the Aging Brain: Promoting Cognitive Reserve or Accelerating Alzheimer's Disease? *Semin. Cell Dev. Biol.* **2021**, *116*, 108–124. [CrossRef] [PubMed]
149. Guneykaya, D.; Ivanov, A.; Hernandez, D.P.; Haage, V.; Wojtas, B.; Meyer, N.; Maricos, M.; Jordan, P.; Buonfiglioli, A.; Gielniewski, B.; et al. Transcriptional and Translational Differences of Microglia from Male and Female Brains. *Cell Rep.* **2018**, *24*, 2773–2783.e6. [CrossRef] [PubMed]
150. Han, J.; Fan, Y.; Zhou, K.; Blomgren, K.; Harris, R.A. Uncovering Sex Differences of Rodent Microglia. *J. Neuroinflamm.* **2021**, *18*, 74. [CrossRef] [PubMed]
151. Santos-Galindo, M.; Acaz-Fonseca, E.; Bellini, M.J.; Garcia-Segura, L.M. Sex Differences in the Inflammatory Response of Primary Astrocytes to Lipopolysaccharide. *Biol. Sex Differ.* **2011**, *2*, 7. [CrossRef] [PubMed]
152. Gillies, G.E.; McArthur, S. Independent Influences of Sex Steroids of Systemic and Central Origin in a Rat Model of Parkinson's Disease: A Contribution to Sex-Specific Neuroprotection by Estrogens. *Horm. Behav.* **2010**, *57*, 23–34. [CrossRef] [PubMed]
153. Gillies, G.E.; Virdee, K.; McArthur, S.; Dalley, J.W. Sex-Dependent Diversity in Ventral Tegmental Dopaminergic Neurons and Developmental Programing: A Molecular, Cellular and Behavioral Analysis. *Neuroscience* **2014**, *282*, 69–85. [CrossRef]
154. Gillies, G.E.; Pienaar, I.S.; Vohra, S.; Qamhawi, Z. Sex Differences in Parkinson's Disease. *Front. Neuroendocrinol.* **2014**, *35*, 370–384. [CrossRef]
155. Baldereschi, M.; Di Carlo, A.; Rocca, W.A.; Vanni, P.; Maggi, S.; Perissinotto, E.; Grigoletto, F.; Amaducci, L.; Inzitari, D. Parkinson's Disease and Parkinsonism in a Longitudinal Study: Two-Fold Higher Incidence in Men. *Neurology* **2000**, *55*, 1358–1363. [CrossRef] [PubMed]
156. Cerri, S.; Mus, L.; Blandini, F. Parkinson's Disease in Women and Men: What's the Difference? *J. Park. Dis.* **2019**, *9*, 501–515. [CrossRef] [PubMed]