The relationship between cognitive reserve and neuroplasticity in older adults.

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School of Psychology

Faculty of Health and Medical Sciences

University of Adelaide

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Contents

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Abstract

Background: Cognitive Reserve (CR) is suggested to explain the difference between the expected impact of levels of age-related neuropathology and the real deficits which people experience. Neuroplasticity is speculated to be the neurophysiological mechanism underlying the cognition-protective effects of CR; however, this has not previously been experimentally demonstrated. **Aim:** To identify whether neuroplasticity mediates the relationship between CR and cognitive ability. **Method:** 23 healthy older adults participated in this study, which comprised 3 brain stimulation sessions: (1) continuous theta-burst stimulation (cTBS) applied to left dorsolateral prefrontal cortex, (2) cTBS applied to left motor cortex, and (3) a sham session. Resting electroencephalography (EEG) was used to calculate change in the aperiodic slope of neural power spectra (a novel measure of neuroplasticity) following cTBS. Participants were also assessed with measures of CR (lifetime of experiences; crystallised intelligence) and cognitive ability (fluid intelligence; paired associates learning). **Results:** We induced a neuroplasticity-like effect in both of the active cTBS conditions. This was not observed in the sham condition. We did not observe a significant relationship between neuroplasticity and CR or cognitive ability. This meant mediational analysis was not justified. **Conclusions:** We successfully demonstrated that analysis of the aperiodic slope is an effective means of identifying neuroplasticity with EEG. While we did not identify a significant relationship between our neuroplasticity measure and CR, we recommend further studies investigate other forms of neuroplasticity. Continued investigation of the neurophysiology underlying CR may facilitate the development of early interventions which could reduce the prevalence of age-related cognitive impairment.

Declaration

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September 2020

Contribution Statement

In writing this thesis, XXXX, XXXX and I collaborated to generate research questions of interest and design the appropriate methodology. I conducted the literature search, EEG data processing, statistical analysis and thesis write up. XXXX provided some of the apparatus specifications which were used in the method section. XXXX and XXXX conducted tutorials and provided example scripts for MATLAB, which I adapted for my data set during the EEG data processing and statistical analysis. XXXX assisted me with RStudio programming for the Principal Component Analysis. As this thesis was based on a pre-existing data set, XXXX, XXXX, XXXX, XXXX and XXXX were responsible for participant recruitment and data collection.

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This research could not have been completed by a single person, so thank you to everyone who contributed to this thesis.

Introduction

1.1 Cognitive Ageing

Declining cognitive ability in later life is considered a natural consequence of the ageing process. It is well documented that increasing age is associated with decrements in several cognitive functions critical for maintaining independence and quality of life; however, the emergence of these deficits can differ substantially between individuals. The factors determining these individual differences in cognitive ageing are currently not fully understood. This impedes our ability to predict which individuals are at risk of faster cognitive decline, thus preventing the development of early interventions which may slow down or prevent impairment in older age. The current paper explores neuroplasticity as a factor which may explain individual differences in cognitive ageing.

Research within the cognitive ageing literature often involves measurement of cognitive ability with standardised measures of intelligence. Horn and Cattell (1967) identified two intelligence factors which demonstrate distinct trends of change with advancing age. The first is fluid intelligence (Gf), which refers to the individual's capacity to solve novel problems through the use of operations such as inductive and deductive reasoning, concept formation, and classification. Several studies have demonstrated significantly higher Gf scores in younger adults compared to older adults (Bugg et al., 2006; Bors & Forin, 1995; Verhaeghen & Salthouse, 1997). Evidence examining cognitive processes thought to be involved in fluid ability show similar age discrepancies. Processing speed, attention, inhibitory control, executive functions and working memory all experience gradual decline throughout adulthood (Park & Schwartz, 2000, Verhaeghen & Salthouse, 1997). The second factor identified by Horn and Cattell was crystallised intelligence (Gc), which refers to the breadth and depth of knowledge an individual accumulates over the course of their life (e.g. general knowledge, language proficiency). Horn and Cattell observed that Gc follows an opposite trend of age-

related change, with older adults scoring significantly higher than their younger counterparts. Further studies have replicated these findings; however, evidence suggests Gc may also experience decline after the age of 70 (Wardlaw et al., 2017; Ritchie et al., 2016).

Age-associated changes in brain structure are often implicated in reductions in cognitive functioning. For example, increasing age is associated with reductions in the volume of the hippocampus and entorhinal cortex, resulting in poorer episodic memory (Rosen et al., 2003; Rodrigue & Raz, 2004). Furthermore, prefrontal white matter lesions commonly developed in older age are associated with reduced performance on working memory tasks (Head et al., 2004). A higher prevalence of neurodegenerative diseases such as Alzheimer's Disease (AD) also results in poorer functioning across a range of cognitive abilities (McKhann et al., 1984; Gilman, 1999). These findings suggest brain pathology is a major contributor to cognitive impairment in later life.

While there is clear evidence neurodegeneration contributes to cognitive ageing, two individuals with comparable brain pathology can have considerably different cognitive outcomes. Katzman et al. (1989) described 10 cases of elderly women judged to be 'cognitively normal' during life as having advanced AD brain pathology following post-mortem examination. Similar findings were demonstrated in a later study which found 25% of individuals with AD brain pathology were not clinically diagnosed with the disorder during life (Ince, 2001). Moreover, two individuals who endure a stroke of comparable magnitude can have considerably different outcomes. One individual may have substantial impairment, while others may experience little disruption (Scarmeas & Stern, 2003). The discrepancy between expected impairment from brain damage and observed cognitive function suggests additional factors influence susceptibility to age-related cognitive decline.

1.2 Cognitive Reserve

The concept of cognitive reserve (CR) has been proposed to explain the difference between the expected impact of underlying levels of neuropathology and the real cognitive deficits that individuals experience (Stern, 2002). These discrepancies are suggested to result from exposure to different environmental factors which influence susceptibility to cognitive impairment. Some of these environmentally driven CR proxies include education, crystallised intelligence, cognitively engaging work/leisure and socioeconomic status. Since the development of the CR theory, several studies have investigated the relationship between CR proxies, cognitive functioning and neurophysiology.

The impact of CR on cognitive functioning has been demonstrated by studies investigating biomarkers of brain pathology. In a study of older adults with AD, CR was observed to moderate the relationship between neuropathology and cognitive performance, with more highly educated individuals showing a weaker association (Yaffe et al., 2011). A Parkinson's disease study also demonstrated that higher levels of education delays the transition from mild cognitive impairment to dementia in individuals with comparable levels of pathological burden (Poletti et al., 2011). These studies support the notion that CR has a cognition-protective influence in the presence of neurodegenerative disease.

Insights into the brain profiles of individuals with differing levels of CR have been provided by various brain imaging studies. Functional magnetic resonance imaging (fMRI) has particularly good utility for identifying these differences, because of its ability to measure brain activation with a high degree of spatial precision. Solé-Padullés and colleagues (2009) observed that elderly individuals with high CR had lower functional activation in the right inferior frontal cortex during a working memory task, suggesting CR is associated with increased neural network efficiency. An additional fMRI study suggests CR is also characterised by an improved ability to counter the effects of brain pathology (Bosch et al.,

2010). In a group of cognitively impaired older adults, higher CR was associated with increased functional activity in task-specific brain areas during a speech comprehension task. Conversely, in a group of healthy adults this relationship was inverted. This suggests CR modulates functional brain activity to compensate for the cognitive impact of age-related neurodegeneration.

Patterns of brain activity associated with CR suggest the brain undergoes physical changes in response to environmental factors. Brain changes such as these are suggested to be driven by mechanisms involved in neuroplasticity (Lerch et al., 2011; Davidson & McEwen, 2012; Leger et al., 2015). The details of this phenomenon will be discussed, followed by presentation of evidence linking neuroplasticity to CR in older adults.

1.3 Neuroplasticity, ageing and cognitive reserve

The term neuroplasticity is derived from the Greek words *neuro* meaning "nerve or central nervous system" and *Plastikos* meaning "able to be moulded". Neuroplasticity therefore refers to the ability of the brain to change its function and structure throughout life in response to different experiences (Cramer et al., 2011). Investigations into the biological basis of neuroplasticity have revealed multiple mechanisms by which neuroplasticity occurs, including long-term potentiation (LTP) (the strengthening of neural connections), long-term depression (LTD) (the weakening of neural connections), synaptogenesis (the production of new synapses) and other structural changes (e.g. neurogenesis and angiogenesis) (Pascuale-Leone, 2011).

1.3.1 Neuroplasticity across the lifespan in animals

Animal studies investigating age-related changes in neuroplasticity suggest these mechanisms may follow a similar trajectory of age-related decline to that of cognitive functioning. For example, hippocampal LTP becomes less efficient in older rats and is

associated with a greater degree of memory loss (Detoledo-Morrell et al., 1988). Age-related deficits in the balance between LTP and LTD mechanisms have also been shown to contribute to learning and memory impairment in rodents (Rosenzweig & Barnes, 2003). These findings suggest synaptic plasticity becomes less effective in older age.

There is limited literature examining whether there is age-related decline in other neuroplastic mechanisms. Synaptogenesis has been observed to continue into late adulthood in rats, indicated by a significant increase in dendritic tree complexity following a spatial learning paradigm (Ambrogini et al., 2010). Additionally, thousands of newly generated neurons have been observed in the hippocampi of several different adult mammals, suggesting neurogenesis could continue into adulthood (van Praag et al., 2002). However, there is a lack of consensus as to whether this occurs in humans, as a recent large study suggests neurogenesis may cease after childhood development (Sorrells et al., 2018).

These animal studies provide a useful reference when seeking to identify the age-related trajectory of neuroplastic mechanisms in humans.

1.3.2 Indirect evidence of neuroplastic changes across the human lifespan

Evidence of large-scale neuroplastic reorganisation resulting in maintained cognitive functioning has been provided primarily by fMRI studies. One of the most notable age-related patterns of change associated with cognitive task performance is a reduction in prefrontal hemispheric asymmetry, referred to as the HAROLD model (hemispheric asymmetry reduction in older adults) (Cabeza et al., 2002). Bilateral prefrontal activation during working memory task execution is more pronounced in high performing older adults, suggesting these individuals are better able to counteract age-related cognitive decline through reorganisation of neurocognitive networks (Reuter-Lorenz et al., 2000). Evidence of neuroplastic brain reorganisation in later life is further supported by the PASA model (posterior-anterior shift in

ageing) (Davis et al., 2008). Elderly individuals demonstrate a preference for prefrontal, rather than occipital-temporal functional activation during execution of episodic retrieval and visual perceptual tasks. This is indicative of neuroplastic compensation for reduced functionality of posterior regions.

fMRI studies have also revealed age-related differences across large-scale brain networks, most notably within the default mode network (DMN). In later life, there is a tendency towards lower activity and reduced functional connectivity in the DMN, with poorer working memory performance associated with these deficits (Koch et al., 2010; Gradey et al., 2010; Sambarto et al., 2010). DMN areas have some of the highest levels of neuroplasticity in the cerebral cortex, leading researchers to suggest dysfunctional DMN connectivity in older age may emerge from a breakdown in neuroplastic efficiency (Vidal-Piñeiro et al., 2014; Pascuale-Leone, 2011).

1.3.3 Cognitive Reserve and Neuroplasticity

It is often speculated that the mechanisms of neuroplasticity play a role in determining neural profiles of older adults with higher levels of CR; however, there is limited literature directly investigating whether such a relationship exists. One animal study observed that biomarkers of neuroplasticity were associated with cognitive resilience in enriched environments (Sun et al., 2010). Rats that spent time in an 'enriched' cage (cages with more opportunity for activities and socialisation) demonstrated higher non-spatial memory performance following surgically induced chronic cerebral hypoperfusion (CCH) in comparison to rats that spent time in standard laboratory cages. Concurrent observation of higher brain-derived neurotrophic factor (BDNF) protein and N-methyl-D-aspartate (NMDA) receptor levels in the enriched group, indicated both may contribute to the protective effects of environmental enrichment following CCH. BDNF and NMDA play mutually important roles

in modulating synaptic plasticity, suggesting neuroplasticity may be involved in this CR-like phenomenon.

Evidence for an association between neuroplasticity and CR in humans has been primarily provided by studies of individual differences in brain morphology. Gaser & Schlaug (2003) observed differences in the grey matter volume of motor, auditory and visuospatial brain regions when comparing professional musicians to amateurs. This suggests the brain may undergo neuroplastic change in response to specialised occupation. Additionally, Bartrés-Faz and colleagues (2019) observed higher cortical thickness in medial frontal, anterior cingulate and orbitofrontal areas in individuals with more years of education. These regions exhibited a distinct gene expression profile, with upregulation of gene sets implicated in synaptic transmission and plasticity. This points to a higher capacity for neuroplastic changes in response to lifetime intellectual enrichment, potentially facilitating resilience to brain pathology.

1.3.4 Direct methods of measuring neuroplasticity in humans

The findings from neuroimaging studies provide evidence of functional activation, connectivity and morphology influencing cognitive function in old age. The compensatory nature of these findings suggest neuroplasticity may play a role in preventing the cognitive impact of age-related neurodegeneration and pathology. However, given the mechanisms of neuroplasticity were not manipulated in these studies, it is speculative to conclude that these neurophysiological profiles emerge as a result of some neuroplastic process. The studies presented above rely on observation of large-scale functional or morphological change, which take years to decades to occur, and could be confounded by physiological changes unrelated to neuroplasticity (e.g. hemodynamic factors in fMRI) (Arthurs & Boniface, 2002).

The emergence of non-invasive brain stimulation methods over the past two decades provides an opportunity for some of these limitations to be overcome. Methods such as transcranial magnetic stimulation (TMS) allow for direct stimulation of focal cortical areas and provide opportunities to non-invasively induce and measure neuroplasticity with a higher degree of resolution. This allows the mechanisms of neuroplasticity to be identified and studied on substantially shorter time scales, while precisely targeting cortical areas of interest.

1.4 Transcranial Magnetic Stimulation

TMS is a non-invasive form of brain stimulation performed by positioning a magnetic coil over the scalp. The coil produces a magnetic field which travels through the brain's protective layers, thereby creating an electric current that stimulates neurons in the targeted area of the cortex. Technological advancements have produced repetitive TMS (rTMS) machines, which have the capability to stimulate the brain at frequencies observed to elicit synaptic plasticity-like phenomena (Huang et al., 2005).

1.4.1 Inducing synaptic plasticity with theta-burst stimulation

Theta-burst stimulation (TBS) is one rTMS protocol with extensive evidence for inducing both LTP-like and LTD-like responses in humans. The TBS pattern was based on animal models, which initially demonstrated LTP could be experimentally induced at synapses by applying repeated bursts of electrical stimuli at the theta frequency (~5 Hz). Huang and colleagues (2005) conducted the first study of TBS in humans, testing several protocols including continuous TBS (cTBS) and intermittent TBS (iTBS) applied to the motor cortex. Motor-evoked potentials (MEPs) in the targeted muscle (first dorsal interosseous) were measured with electromyography (EMG) to identify changes in motor cortical excitability as a marker of neuroplasticity. The cTBS protocol produced an LTD-like phenomenon, as MEP

amplitude tended to reduce following stimulation. Conversely, the iTBS protocol produced an LTP-like phenomenon, inferred from an increase in MEP amplitude. A further study demonstrated that these responses were blocked following administration of an NMDA antagonist, suggesting these effects are driven by the cellular mechanisms of synaptic plasticity (Huang et al., 2007). The outcomes of these studies demonstrate that neuroplasticity can be manipulated with an external non-invasive stimulator. This facilitates experimental research of neuroplasticity in humans, where previously, evidence had been largely correlational, and thus vulnerable to confounding by other physiological variables.

1.4.2 cTBS-induced plasticity in older adults

Brain stimulation research investigating neuroplasticity across the lifespan suggests the efficiency of plasticity may decrease in older age. A cross-sectional cTBS study observed a progressive linear decline in LTD-like corticomotor plasticity in a sample of 36 adults between the ages of 19 and 81 (Freitas et al., 2011). The results indicated advancing age was negatively correlated with the duration of the effect of cTBS and the overall amount of corticomotor suppression. This reduction in neuroplastic efficiency across the lifespan is suggested to contribute to the motor and cognitive decline associated with ageing.

The outcome of this study supports the findings of previous animal research that synaptic plasticity tends to decline across the lifespan (Detoledo-Morrell et al., 1988; Rosenzweig & Barnes, 2003). However, this study does not provide evidence for neuroplastic decline in non-motor regions, where the majority of higher-order cognitive processing takes place (Bressler & Menon, 2010). Examination of alternative plasticity measurement methods suggests pairing rTMS with electroencephalography (EEG) can enable non-motor plasticity to be measured.

1.5 Measuring neuroplasticity with EEG

EEG is a method of brain imaging where electrodes are placed on various locations of the scalp. The electrodes measure voltage fluctuations (i.e. oscillations) caused by the synchronous firing of large populations of neurons (Jackson & Bolger, 2014). Neuroplasticity induced by rTMS can be identified through various methods of EEG data analysis which quantify changes in cortical excitability (Rogasch & Fitzgerald, 2013). Given pairing TMS with EEG is a relatively new methodology for identifying neuroplasticity, optimal data analysis methods are still under investigation.

EEG data are commonly analysed through examination of periodic oscillations, which can be identified by conducting a spectral density estimation to convert raw EEG data (comprising amplitude at particular timepoints) into a power spectrum (comprising power within frequency bands). Changes in the amount of power within these frequency bands have been associated with a diverse range of physiological phenomena, including changes in cortical excitability. For example, increased power in delta (~1-4 Hz) and theta (~4-7 Hz) bands is associated with cortical inhibition (Woźniak-Kwaśniewska et al, 2014).

While analysis of periodic oscillations can be useful in identifying changes in brain activity, the aperiodic non-oscillatory component of the power spectrum is often overlooked. This is problematic, as measurable differences in aperiodic activity have been observed depending on age, task demands and cognitive state (Haller et al., 2018). The aperiodic signal can be identified by fitting a linear model to the power spectrum in log-log space:

$$L(log_{10}(F)) = aF + b$$

where b = the 'offset', a = the slope of the power spectrum (aperiodic slope), and F = the array of frequency values.

Recent evidence suggests changes in the aperiodic slope may be indicative of a change in the excitation/inhibition (E:I) balance of neural activity. Gao and colleagues (2017) observed

an increase in aperiodic slope when macaques were administered with a general anaesthetic. Given this anaesthetic increases cortical inhibition, the findings suggest a steeper aperiodic slope is indicative of inhibition dominance. This relationship was also demonstrated in the same study through computational modelling and theta-modulated shifts in the E:I ratio of rat hippocampi.

The findings of Gao and colleagues suggest analysis of the aperiodic EEG signal may be an effective data analysis method for identifying LTP-like or LTD-like changes in brain excitability following rTMS. Furthermore, an advantage of this method is that it considers the entire frequency spectrum of brain activity, rather than a small frequency range which may not capture the full effect of the stimulation protocol. Most importantly, this also enables researchers to identify plasticity-like responses following rTMS to any area of the cortex, including those important for CR and cognitive ability.

1.6 The present study

Review of the current literature indicates the relationship between neuroplasticity and cognitive reserve in older adults is not fully understood. The findings of fMRI and brain morphology studies suggest a relationship exists; however, further investigation with alternate methods of plasticity measurement are required to state this with a higher degree of certainty. Experimentally manipulating neuroplasticity with rTMS enables the phenomenon to be more directly assessed, as merely observing individual differences in brain structure or function can be confounded by factors unrelated to plasticity (e.g. larger blood vessels influencing fMRI). Evidence indicates that rTMS coupled with EEG is a promising methodology for inducing neuroplasticity and measuring its effect on brain activity. Furthermore, incorporating analysis of the aperiodic EEG signal to infer neuroplasticity holds promise as an effective data analysis methodology.

In the present study, we aimed to assess the relationship between cognitive ability, cognitive reserve and cTBS-induced neuroplasticity in older adults. Given the novelty of the present study, our working hypotheses were designed with an exploratory approach to understanding the relationship between these variables. We hypothesised that (1) there would be a significant decrease in excitation following the cTBS protocol (indicated by a steepening of the aperiodic slope), (2) the change in excitation (neuroplasticity) would be positively associated with cognitive reserve and cognitive ability, and (3) this neuroplasticity measure would mediate the relationship between cognitive reserve and cognitive ability.

Method

2.1 Participants

Participants were recruited from an existing database of 115 older adults aged 60-85 who had participated in the Social and Neurocognition in Adulthood study (Lavrencic et al., 2016). Eligibility criteria were: (1) native English speaker; (2) right handed; (3) no uncorrected hearing or visual impairment; (4) no uncontrolled high blood pressure; (5) no history of cancer within the past 5 years; (6) no medications targeting the central nervous system within the past month; (7) no alcohol or substance abuse within the past year, or recreational drugs within the past month; (8) no psychiatric or brain disorder within the past 5 years; and (9) no learning disability.

The sample for the present study consisted of 23 healthy older adults (mean age: 71.3 ± 6.8 years; range: 62-83 years; 16 female). Lifetime of Experiences Questionnaire (LEQ) scores [see below] were used to selectively recruit the participants, with priority given to either end of the distribution to achieve a wide range of CR scores. Additional eligibility criteria included: (1) no contradictions to TMS or MRI; (2) no diagnosis of HIV or Hepatitis C [See Appendix 5].

2.2 Materials and Apparatus

2.2.1 Lifetime of Experiences Questionnaire

The Lifetime of Experiences Questionnaire (LEQ) was administered to measure cognitive lifestyle (Valenzuela & Sachdev, 2007). The scale consists of 42 5-point Likert scale questions which quantify participation in cognitively stimulating activities undertaken at three life stages: (1) Early life (13-30 years), (2) Mid-life (30-65 years), (3) Later life (65+ years). Cognitive engagement in Early life was measured by the number of years spent in education and level of the education's intellectual complexity. For Mid-life, it was quantified by

occupation complexity, with jobs falling into 9 categories set out in the Australian Standard Classification of Occupations (Australian Bureau of Statistics, 1997). For Later life, it was quantified by ongoing social and intellectual activity e.g. diversity of reading materials, membership of social groups. There are also non-specific questions about intellectual pursuits at each life stage. These refer to hobbies, physical activity, travel, social engagement and learning a second language. The LEQ has moderate internal consistency reliability (a = 0.66).

2.2.2 Weschler Abbreviated Scale of Intelligence

The Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-II) Full-scale IQ-2 (FSIQ-2) was administered to measure fluid and crystallised intelligence (Weschler, 2011). The first section of the scale tested vocabulary by requiring the participants to define 31 words. Accuracy on this task corresponds to word knowledge and verbal concept formation, both of which are crystallised abilities. The second section tested perceptual reasoning by requiring participants to correctly solve a set of 30 incomplete matrices through multiple choice. Accuracy on this task is assumed to reflect fluid intelligence, visual intelligence, simultaneous processing and perceptual organisation. The FSIQ-2 has excellent reliability, indicated by an average reliability coefficient of 0.94 for adults (Maccow, 2011).

2.2.3 Paired Associates Learning

The Cambridge Neuropsychological Test Automated Battery (CANTAB) – Paired Associates Learning (PAL) test (Cambridge Cognition Ltd., England) was administered as a second measure of cognitive ability, specifically directed at quantifying visuospatial learning and memory. The task was presented on a computer screen which displayed between 6 and 8 white boxes arranged in a circle. Figures were then consecutively presented in each box in random order. At the end of each stage, the participants were randomly presented with the

figures in the centre of the circle and were required to select the box in which they were located. The number of figures in each box increased with each stage of the test. Errors were recorded when participants selected boxes that did not contain the correct figures.

2.2.4 Electroencephalography

EEG was recorded using 62 sintered Ag/AgCl electrodes, mounted on a flexible cap in a standard 10-10 layout (Waveguard, ANT, Netherlands) and connected to a TMS-compatible, DC-coupled amplifier (ANT, Netherlands). EEG signals were filtered online (DC-552 Hz) and sampled at 2048 Hz using an average reference, with the ground electrode located at AFz. Impedance for all electrodes was kept below 5 kΩ.

2.2.5 Electromyography

MEPs were recorded using EMG in order to identify the resting motor threshold (RMT) of each participant [see below]. This was only performed to calibrate cTBS intensity, and MEPs were not measured following RMT identification. EMG was recorded using Ag/AgCl surface electrodes positioned in a bipolar montage on the right first dorsal interosseous muscle. The signals were amplified ×1000, sampled at 5 kHz, and online filtered (20-1000 Hz) (Cambridge Electrical Design 1401, Cambridge, UK).

2.2.6 Theta burst stimulation

cTBS was applied with a biphasic current waveform using an air-cooled figure-of-eight coil, connected to a Magstim Super Rapid magnetic stimulator (Magstim, Whitland, UK). cTBS intensity was set at 70% of the RMT value. RMT is the minimum TMS intensity required to elicit a MEP from the relaxed right first dorsal interosseous muscle with peak-to-peak amplitude >50 μV in at least 5 of 10 consecutive trials. Following intensity calibration, cTBS

was applied in two trains separated by 15 minutes, with the coil positioned over the scalp with the handle pointing posteriorly at a 45° angle to the midline. The cTBS parameters consisted of short bursts of three stimuli at 30 Hz, repeated at a frequency of 6 Hz for a total of 600 stimuli (Nyffeler et al., 2006; Goldsworthy et al., 2012).

2.3 Procedure

The CR and cognitive performance measures were administered to the participants at the time of the Social and Neurocognition in Adulthood study. However, given the rTMS testing occurred three years after the original data collection, the later life LEQ questions were updated in the present study.

The participants were initially invited to attend two rTMS sessions, with active cTBS applied to the left dorsolateral prefrontal cortex (DLPFC) in one session (N=21) and the left primary motor cortex (M1) in the other (N=22). 20 participants attended both sessions (mean age: 71.6 ± 7.0 years; range: 62-83 years; 14 female). A subset of 15 participants also attended a third session, in which sham cTBS was applied to left DLPFC using the same stimulation intensity and pattern as the active condition, but with the coil tilted away from the scalp at 90° (mean age: 72.3 ± 6.6 years; range: 63-83 years; 12 female). Sessions were randomised and counterbalanced, with each session separated by a minimum of one week to prevent carryover effects.

During each session, cTBS-induced after-effects were measured using resting-state EEG for three minutes at four time points: at baseline (BL), immediately after the first cTBS train (MID), immediately after the second train (P1) and 30 minutes after the second train (P2) [See Figure 1]. During resting EEG, the participants were asked to keep their eyes open, avoid blinking if possible, remain still and avoid tensing facial muscles to avoid artefacts. A single pulse TMS-EEG protocol was also conducted immediately after resting EEG at each timepoint

(two blocks of 50 stimuli, interstimulus interval 4.5-5.5 s); however, this data was not used in the present study.

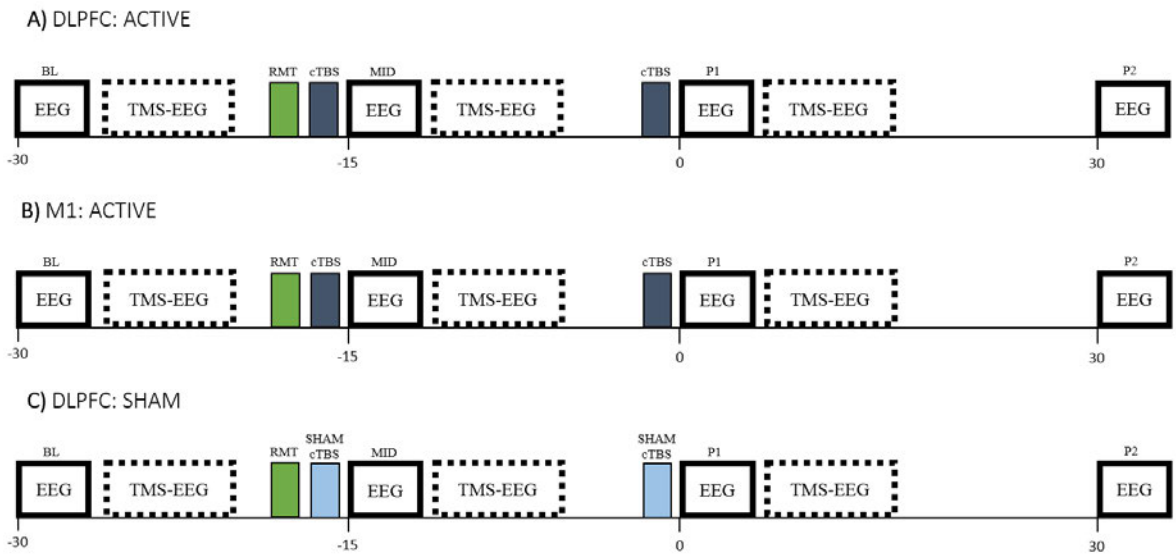

Figure 1. Schematic overview of the timeline of the experiment. Resting EEG blocks: BL = baseline, MID = immediately after the first cTBS train, P1 = immediately after the second cTBS train, P2 = 30 minutes after the second cTBS train. RMT = resting motor threshold. TMS-EEG data were not used in the current study.

2.4 Data Analysis

2.4.1 EEG data pre-processing

EEG data were pre-processed using the EEGLAB toolbox (Delorme & Makeig, 2004) in conjunction with customised scripts in MATLAB (R2019b, MathWorks, USA). Each block of resting state EEG data (BL, MID, P1, P2) was sequentially imported into EEGLAB, bandpass filtered (1-100 Hz), notch filtered (48-52 Hz), epoched at a 2 second interval and merged into a single data frame for each participant. The data was then visually inspected using the 'eegplot' function to identify and remove channels that were turned off during data collection or had been contaminated by noise. During the visual inspection, any epochs which had been contaminated by artefacts (e.g. muscle activation or movement from the participants)

were also identified and removed. Independent component analysis (ICA) was then conducted using the TMS-EEG signal analyser (TESA) Fast ICA algorithm (Rogasch et al., 2017). The independent components identified in this process were then visually inspected using the TESA component selection GUI to ensure components were being accurately categorised. Components identified as containing muscle activity, eye movement or blinks were removed. The final step of the data cleaning process involved interpolating channels which had been removed earlier in the process, re-referencing the data to the average of all channels and splitting the merged data back into their respective blocks.

2.4.2 Calculation of the power spectrum and the aperiodic signal

Following data pre-processing, Welch's method was used to calculate the power spectral density (PSD) for each timepoint and channel. This was conducted using MATLAB's 'pwelch' function. The Hamming window was set at 2 times the sampling rate (4096) and the number of overlapped samples was set at 2048. These PSD estimates were then exported to be used in the calculation of the aperiodic signal.

The aperiodic signal was calculated using the FOOOF (fitting oscillations & one over f) toolbox (Haller et al., 2018) on the Spyder platform (running Python 3.7). The 'FOOOFGroup' function was used to calculate the aperiodic slope value for each EEG electrode. These values were then exported in data frames to be re-imported into MATLAB for statistical analysis.

2.4.3 Statistical analyses

All statistical analyses of EEG data were performed using the FieldTrip toolbox (Oostenveld et al., 2011) with customised scripts on MATLAB. The aperiodic slope values for

the blocks after cTBS (MID, P1 and P2) were compared with baseline (BL) using non-parametric cluster-based permutation statistics (Maris & Oostenveld, 2007). This process was conducted to (1) indicate whether a change in aperiodic activity was induced by cTBS and (2) to correct for multiple comparison errors. Clusters were defined as two or more neighbouring electrodes for which the difference between BL and MID/P1/P2 exceeded the *a priori* threshold of $p < 0.05$ (dependent samples *t*-test). A reference distribution was generated using 5000 random permutations (Monte Carlo method), and significance of the cluster-statistic was accepted at $p < 0.05$. Clusters identified in the cluster-based permutation test were used as a proxy of plasticity induction. The cluster-based permutation methodology was used because no *a priori* assumptions were made about the location of electrophysiological response to cTBS. This methodology enabled us to control for the number of spatial comparisons made between EEG electrodes, thus reducing the probability of making type I errors.

A second cluster-based permutation test was conducted comparing delta (Δ) aperiodic slope values (calculated by subtracting post cTBS blocks from baseline) between conditions (DLPFC, M1 and Sham). The test was conducted using the same settings as previously specified. This analysis was performed for two reasons: (1) comparison of the two active conditions was performed to assess whether the nature of the aperiodic response was dependent on the site of stimulation, and (2) comparisons between the active and sham conditions were performed to assess whether changes in aperiodic slope occurred in the active conditions, above and beyond any potential confounding by extraneous variables introduced during data collection.

Prior to statistical analysis of the relationships between the behavioural and neurophysiological data, the behavioural data were assessed as to whether composite CR and cognitive ability factors could be produced. Pearson's method was used to correlate the CR measures (LEQ total scores and WASI-II vocabulary scores) and the cognitive ability measures

(WASI-II matrices scores and CANTAB-PAL total errors adjusted). If significant associations were observed, principal component analysis (PCA) was conducted to generate the composite factors. This was performed using the 'principal' function in the 'psych' package in R 3.4.4.

Cluster-based correlation statistics were used to assess the relationship between the electrophysiological and behavioural data. Clusters were defined as two or more neighbouring electrodes for which the difference in aperiodic slope (Δ) was correlated with the respective behavioural measure at a level exceeding the *a priori* threshold of $p < 0.05$ (Spearman's Rho correlation). A reference distribution was generated using 5000 random permutations (Monte Carlo method), and significance of the cluster-statistic was accepted at $p < 0.05$. These settings were used for cluster correlations between the Δ aperiodic slope values; and CR and cognitive ability scores.

If significant correlations were observed between delta aperiodic slope values, CR factor scores and the cognitive ability measures, multiple regression modelling would have been used to conduct a mediational analysis. As this criterion was not fulfilled by the results, this analysis was not justified.

Results

3.1 Behavioural Data Characteristics

The sample demonstrated above average performance on all behavioural measures. These are presented in Table 1.

	Sample Distribution	Estimated Population Distribution
LEQ: Total Score	102.86 ± 26.3	75.5 ± 20*
WASI-II – FSIQ-2	111.13 ± 15.42	100 ± 15^
CANTAB-PAL: Total Errors Adjusted	28.39 ± 22.2	34.3 ± 16.7#

*Table 1. Distribution of sample and estimated population scores on: Lifetime of Experiences Questionnaire, Weschler Abbreviated Scale of Intelligence 2nd edition – Full Scale Intelligence Quotient 2, Cambridge Neuropsychological Test Automated Battery – Paired Associative Learning (note that fewer errors indicate better performance). Data are presented as mean ± SD. See *Valenzuela et al., 2007, ^McCrimmon & Smith, 2012, #Abbott et al., 2019*

3.2 Principal Component Analysis

As the LEQ and WASI-II vocabulary scores were significantly correlated (r = 0.53, p < 0.01), a PCA was conducted on these two measures of CR. Scores on the first unrotated component were used as the composite CR factor, accounting for 87% of the variance in the CR measures. However, the WASI-II matrix scores and CANTAB-PAL total errors adjusted scores were not significantly correlated (r = –0.28, p = 0.1), so the two measures of cognitive ability were not combined into a single cognitive ability factor. Instead, they were analysed separately.

3.3 Neuroplastic response to cTBS

To determine the effects of cTBS on aperiodic activity, we performed cluster-based permutation analyses comparing the post cTBS aperiodic slopes to baseline for each condition.

For the DLPFC condition, aperiodic slopes were steeper immediately after the second cTBS train (consistent with an LTD-like effect), indicated by a positive cluster over the right posterior electrodes with a moderate-large effect size (P1 vs BL: Cluster statistic = 29.88, p = 0.038, Cohen's d = 0.69). For the M1 condition, aperiodic slopes were steeper immediately after the first cTBS train, indicated by a positive cluster comprising the left temporal, left frontal, right frontal and right midline electrodes with a moderate-large effect size (MID vs BL: Cluster Statistic = 86.67, p = 0.014, Cohen's d = 0.75). These clusters are topographically represented in Figure 2A and the change in aperiodic slope of these clusters are presented in Figure 2B and 2C. No significant differences in aperiodic slope were observed during the sham condition or other blocks in the active conditions.

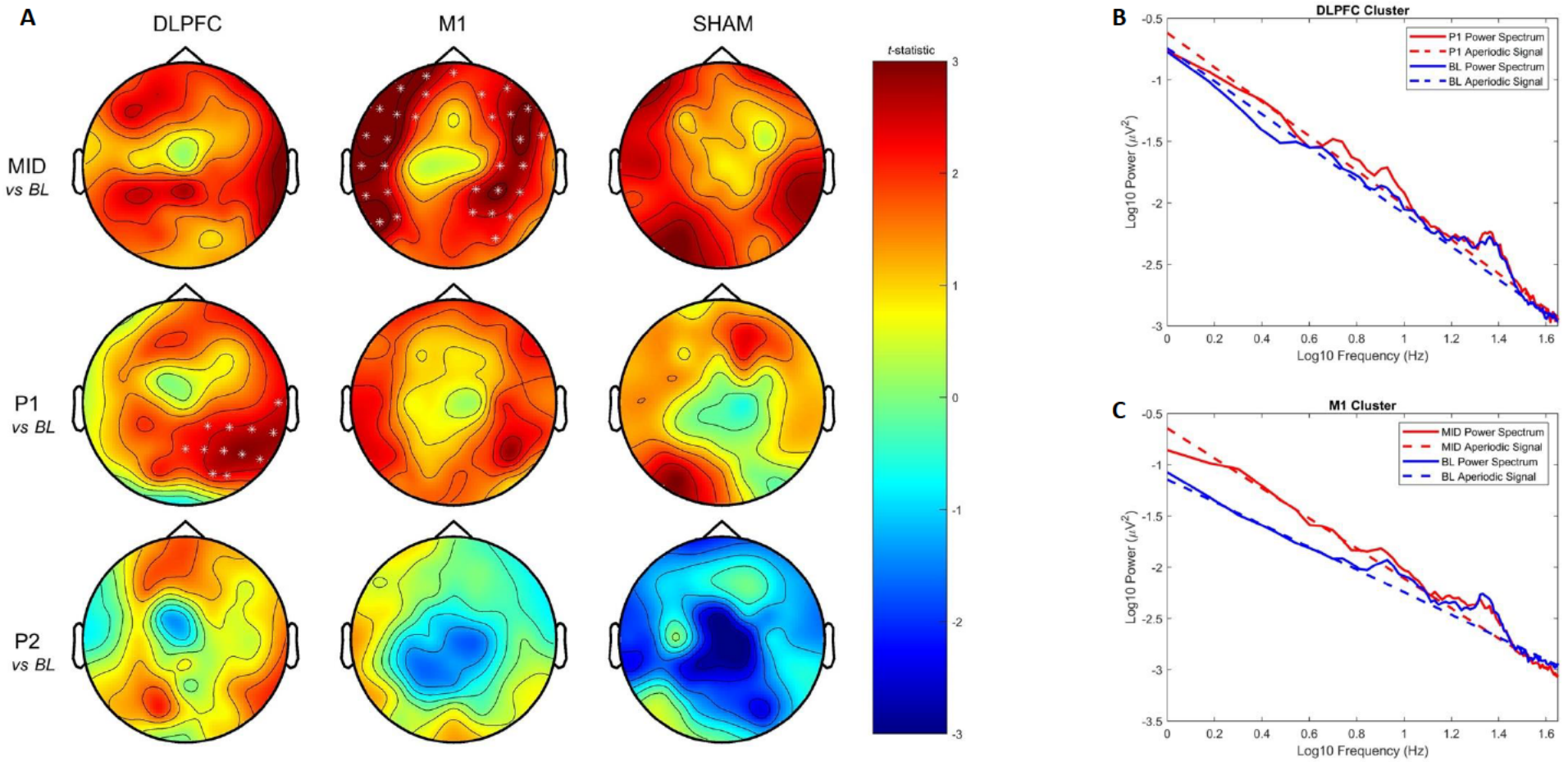

*Figure 2A: Topographical representation of the cluster-based permutation analyses comparing post cTBS aperiodic slopes to baseline (colour coded by t-statistic). Red indicates a steepening of the slope; blue represents a flattening slope. BL: aperiodic slopes prior to cTBS protocol, MID: aperiodic slopes immediately after first cTBS period, P1: aperiodic slopes immediately after second cTBS period, P2: aperiodic slopes 30 minutes after second cTBS period. *p < 0.05. Figure 2B and 2C: Linear modelling of the aperiodic signal from positive clusters identified by the cluster-based permutation tests. (B) Dorsolateral prefrontal cortex: P1((log10)F) = –1.4F–0.62, BL((log10)F) = –1.3F–0.74. (C) Motor Cortex: MID((log10)F) = –1.46F–0.64, BL((log10)F) = –1.1F–1.14.*

A second cluster-based permutation analysis was conducted comparing differences in in aperiodic slope between conditions. No significant differences were observed when comparing the two active conditions, suggesting there was no clear discrepancy in neurological response dependent on the site of stimulation. When comparing the DLPFC and sham conditions, the change in slope from baseline was larger for the DLPFC condition 30 minutes after the second cTBS train, indicated by a positive cluster over the right posterior electrodes with a large effect size (Δ (P2): Cluster Statistic = 30.04, p = 0.04, Cohen's d = 0.90). This suggests extraneous variables are less likely to be responsible for changes in aperiodic activity observed during the DLPFC condition. When comparing the M1 and sham conditions, the change in slope from baseline was not significantly different at any of the three timepoints. This suggests changes in aperiodic activity observed during the M1 condition should be conservatively interpreted. When considering these findings, it should be noted that the sham condition was administered to a subset of 15 participants, reducing the power of these comparisons. The results of these cluster-based permutation analyses are topographically represented in Figure 4.

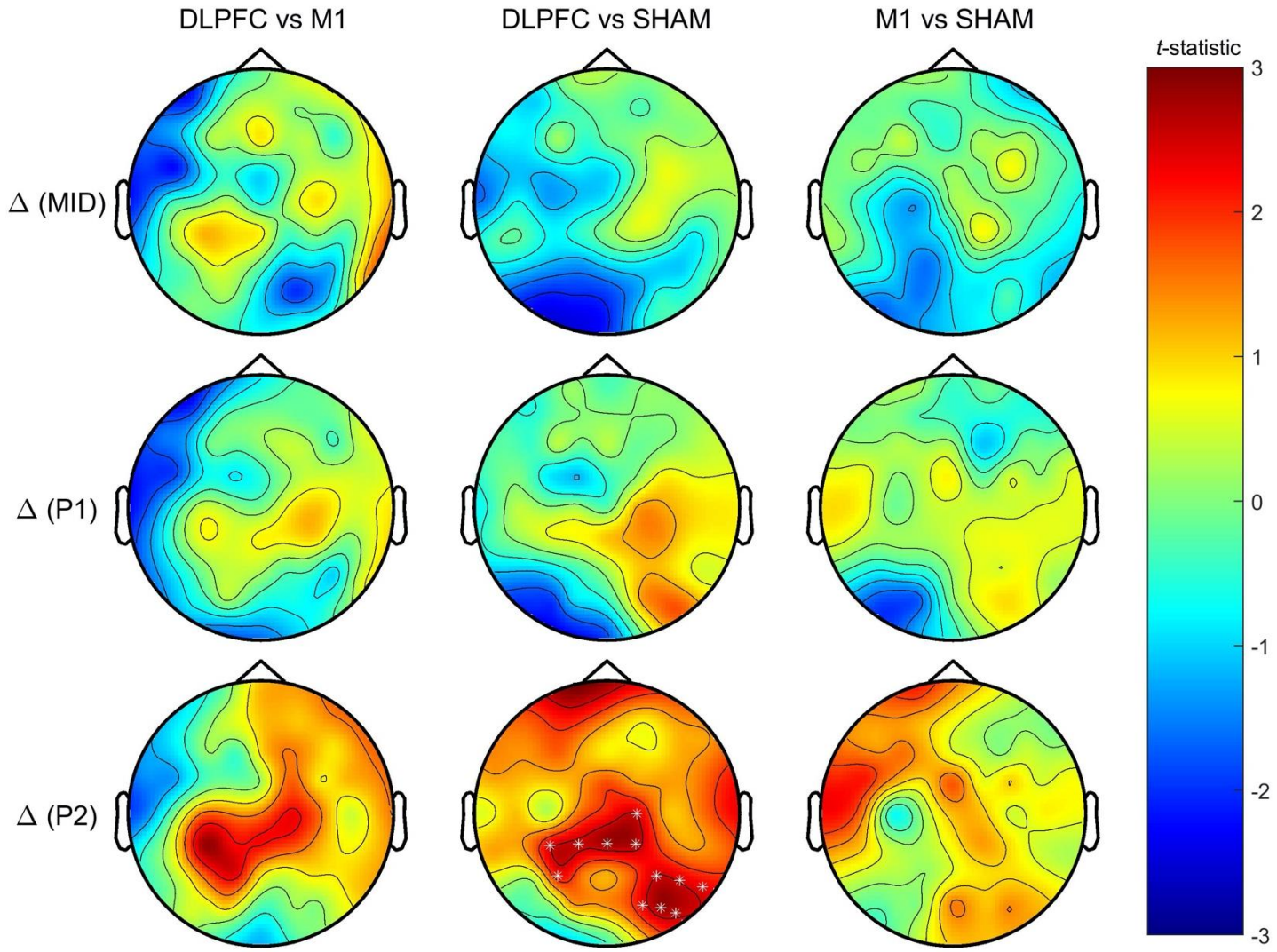

*Figure 3. Topographical representation of the cluster-based permutation analyses comparing differences in in aperiodic slope between conditions. Red at the positive cluster (DLPFC vs SHAM) indicates aperiodic slopes were significantly steeper in the DLPFC condition. Δ = difference in aperiodic slope between post cTBS block and baseline. *p < 0.05. [For MID, P1, P2 descriptions see Figure 2]*

3.4 Correlations between the behavioural measures and neuroplastic response to cTBS

Cluster-based correlation analyses were conducted to assess the relationship between changes in aperiodic slope and the behavioural data (CR factor; WASI-II; CANTAB-PAL). There were no significant associations between changes in aperiodic slope and CR factor in any of the experimental conditions. In the DLPFC condition, a positive cluster was observed in the left frontal electrodes immediately after the second cTBS train (P1); however, this did not meet the significance criterion (Cluster Statistic = 18.55, $p = 0.068$, Rho = 0.52). A positive cluster was also observed during the sham condition immediately after the second cTBS train (P1); however, this also did not meet the significance criterion (Cluster Statistic = 14.22, $p = 0.11$, Rho = 0.61). These results are topographically presented in Appendix 1. There were no significant associations between change in aperiodic slope and either of the cognitive ability measures. The results of the cluster correlations indicate a relationship was not observed between cTBS-induced neuroplasticity and CR; or cTBS-induced neuroplasticity and cognitive ability. The results of the cluster-based correlation tests averaged across the three cTBS blocks are topographically presented in Figure 5.

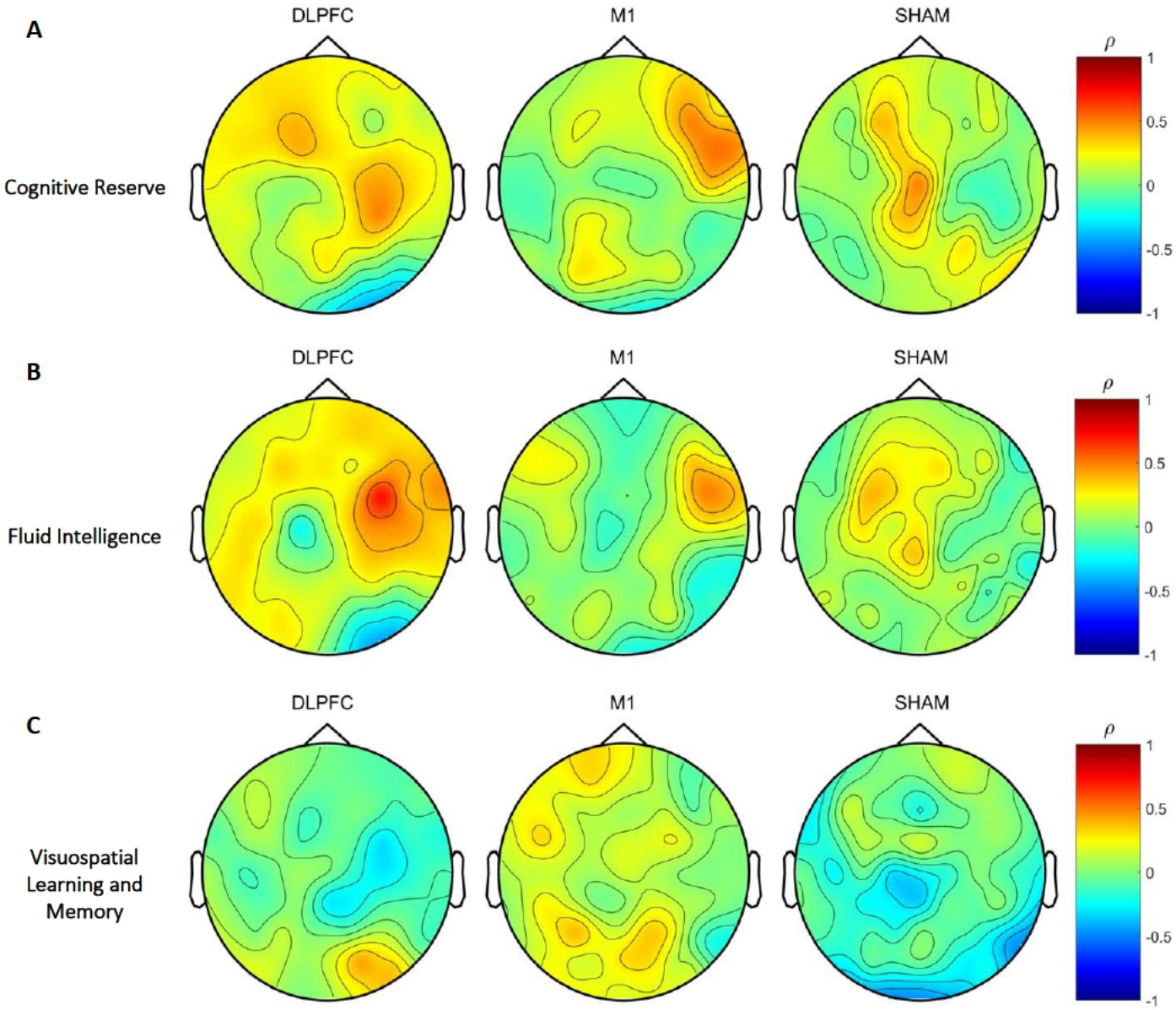

Figure 4. Topographical representation of the cluster-based correlation analyses comparing (A) cognitive reserve (LEQ total; WASI-II – vocabulary), (B) fluid intelligence (WASI-II – Matrices), and (C) visual learning and memory (CANTAB-PAL) with average differences in in aperiodic slope across post cTBS blocks. Heatmaps are colour coded by Spearman's Rho values. No clusters observed.

Discussion

This study aimed to determine whether neuroplasticity, as induced by cTBS, could be identified through analysis of changes in the aperiodic slope of the neural power spectrum. Consistent with our first hypothesis, here we demonstrate that our cTBS protocol induced an LTD-like effect, indicated by a steepening of the aperiodic slope at post cTBS timepoints. We also aimed to identify whether cTBS-induced plasticity was associated with CR and cognitive ability. Counter to our second hypothesis, we did not find evidence of a significant relationship between these variables. The outcome of this analysis meant it was not justified to assess whether neuroplasticity mediated the relationship between CR and cognitive ability. These findings are discussed in detail below.

4.1 Neuroplasticity induced by theta burst stimulation

The finding of steeper aperiodic slopes in the active cTBS conditions (indicating reduced cortical excitation) is consistent with the wider literature. Several corticomotor TMS studies have demonstrated a decrease in the size of MEPs following cTBS, indicative of reduced cortical excitability (Huang et al., 2005; Goldsworthy et al., 2012; Di Lazzaro et al., 2005). Furthermore, research has demonstrated that the effects of cTBS in modulating cortical excitation can be identified through various methods of EEG data analysis (Vernet et al., 2013; Rocchi et al., 2018). Given both animal and human models have identified that the after-effects of TBS paradigms are blocked by NMDA antagonists, this suggests our findings are indicative of an LTD-like effect (Capocchi et al., 1992; Huang et al, 2007).

We observed a steepening of the aperiodic slope in both the active DLPFC and M1 conditions, but not in the sham condition. The electrode clusters that indicated these effects demonstrated differing spatial and temporal patterns of response. In the DLPFC condition, the response was observed in a small group of electrodes located contralaterally to the site of

stimulation. In the M1 condition, the response was more diffuse, located bilaterally in the frontal and temporal areas of the scalp. While it may appear odd that these responses were not observed at the site of stimulation, this is not unusual given the poor spatial resolution of EEG. EEG relies on voltage conduction to identify brain activity, meaning positive or negative deflections in the EEG recording are influenced by the orientation of dendritic arbours (Jackson & Bolger, 2014). This means the response identified at a particular electrode may in fact be caused by brain activity located beneath a different area of the scalp. Given this knowledge, we refrained from drawing any conclusions about the location of the brain in which the response was most likely to have occurred. Nonetheless, these spatial patterns may serve as a useful reference for future research which utilises EEG and rTMS to induce and measure neuroplasticity.

The time at which these cTBS-induced responses were identified also differed between the DLPFC and M1 conditions. The DLPFC cluster was observed after the second cTBS train, whereas the M1 cluster was observed after the first cTBS train. Prior research suggests the application of multiple cTBS trains results in stronger and longer lasting LTD-like after-effects compared with those induced by a single train (Goldsworthy et al., 2015). Given these findings, our results may suggest the motor cortex is more sensitive to cTBS than the DLPFC, as a single train was sufficient to induce a steepening of the aperiodic slope in the M1 condition, whereas the DLPFC condition required two trains for a response to be identified. However, as our second cluster-based permutation analysis did not indicate a significant difference in aperiodic slope between DLPFC and M1, this prevents us from making any substantive conclusions about site-dependent differences in the character of neuroplasticity.

Comparisons of changes in aperiodic slope between the active and sham conditions were also conducted to identify whether the effects being measured were reliable indicators of neurophysiological change. We observed a significant difference between DLPFC and sham

30 minutes after the second cTBS train, suggesting the DLPFC neuroplasticity measures were less likely to have been confounded by extraneous variables. However, a difference was not identified between M1 and sham, suggesting it is possible the effect observed in M1 was influenced by factors unrelated neurophysiological effects of cTBS. While this suggests the effect in M1 may not be as robust as the effect identified in the DLPFC, it should be noted that the sham comparisons only comprised 15 participants. This reduced the statistical power, leaving these findings more vulnerable to noise than the prior within-condition comparisons.

The method we used to identify neuroplasticity is novel in the context of previous brain stimulation research, as rTMS-induced after-effects have not previously been identified through analysis of the aperiodic slope. Gao and colleagues (2017) identified that a steepening of the aperiodic slope was indicative of a reduction in the neural excitation/inhibition ratio (i.e. reduced cortical excitation or increased inhibition). Given our cTBS paradigm has been shown to induce LTD-like responses in previous research, our findings of steeper aperiodic slopes in the active cTBS conditions support the notions from Gao and colleagues' paper. This highlights the physiological relevance of the aperiodic signal, which has historically been neglected in favour of periodic/oscillatory components of the neural power spectrum (Haller et al., 2018). Studies which have paired EEG with rTMS have commonly identified changes in brain activity through analysis of TMS-evoked oscillations (Rogasch & Fitzgerald, 2013), which consider changes in the power of narrow frequency bands as evidence of physiological change. Our findings suggest these power differences can be confounded by the fact that oscillations are embedded within the aperiodic signal. For example, an increase in alpha power may be caused by an increase in the aperiodic slope, rather than oscillatory change. This suggests conflating these two aspects of the power spectrum may influence the validity of the findings from these older EEG data analysis methods. The benefit of analysing the aperiodic slope is that it considers the characteristics of the entire frequency spectrum, thus preventing confounds

associated with considering a narrow frequency range. Another benefit of this methodology is its ability to identify neuroplastic changes at any location of the cortex. As demonstrated by our findings in the DLPFC condition, this methodology enables researchers to identify neuroplastic change in areas of the brain relevant for cognition and other non-motor functions, where historically this has been difficult or unfeasible.

4.2 Cognitive reserve and cTBS-induced plasticity

Prior evidence for a relationship between CR and neuroplasticity has largely been indirect. Here, we manipulated a mechanism of neuroplasticity (LTD) and correlated the electrophysiological response with a CR factor to provide a more direct assessment of the relationship. We did not find sufficient evidence to suggest such a relationship was present. Given previous findings of distinct neural profiles associated with lifelong cognitive engagement, we are hesitant to suggest there is no relationship between neuroplasticity and CR (Solé-Padullés et al., 2009; Bosch et al., 2010; Gaser & Schlaug, 2003; Bartrés-Faz et al., 2019). There are three potential explanations for our findings which will be explained in more detail below: (1) the relationship between neuroplasticity and CR is expressed over a longer period of time to what was assessed in the present study; (2) the specific mechanism of neuroplasticity we assessed may be less relevant to CR than other neuroplastic mechanisms; (3) the limitations of the study prevented us from observing an effect.

We modulated synaptic plasticity for a short period of time (twice for 33 seconds, with the stimulation period separated by 15 minutes) in each experimental condition. The electrophysiological response induced by these paradigms provided a measure of the short-term neuroplastic efficiency of the participants. Given the neural mechanisms of CR are theorised to reorganise cognitive networks in response to lifetime exposures, this short-term measure of neuroplasticity may not have been appropriate for identifying the relationship we

hypothesised. Stern (2006) suggested there are two neural mechanisms which underly CR: neural reserve and neural compensation. The neural reserve concept suggests individuals with higher cognitive network capacity will be less susceptible to impairment from brain damage. Additionally, the neural compensation concept suggests particular individuals are able to recruit areas of the brain unaffected by pathology better than others. Given both neural reserve and neural compensation likely accumulate over a long period of time, it is possible our short-term plasticity measure did not capture neuroplasticity on the correct time scale. It may be necessary to measure neuroplasticity on several occasions to accurately quantify longer-term neuroplastic changes, which may be more closely related to CR.

The lack of a significant relationship between our measure of plasticity and CR may also be explained by the form of neuroplasticity we assessed. LTD is a form of synaptic plasticity, which differs from other structural neuroplastic mechanisms such as synaptogenesis and neurogenesis. These forms of structural neuroplasticity may be more appropriate in explaining previous findings of distinct brain morphology associated with CR. Animal studies have demonstrated that synaptogenesis continues into adulthood and is modulated through cognitive engagement (Ambrogini et al., 2010). Given synaptogenesis occurs alongside axonal growth, increases in dendrite complexity and subcortical myelination, this may explain the individual differences in brain volume observed in CR studies (Huttenlocher & Dabholkar, 1997). Additional animal studies have demonstrated that complex mental activity enhances BDNF and nerve growth factor, both of which increase synaptogenesis and neurogenesis in rats (Sachdev & Valenzuela, 2009). Assuming the findings of this animal research translates to human subjects, this may indicate that CR is explained by the formation of new synapses or neurons, rather than the strengthening or weakening of previously established synaptic connections.

Conversely, synaptic plasticity may still be related to CR; however, LTP may play a more central role in the relationship than LTD. Animal models have demonstrated that LTP experiences a decline in efficiency with advancing age, while the threshold for LTD decreases (Rosenzweig & Barnes, 2003; Hsu et al., 2002). This change in the balance between LTP and LTD is suggested to be responsible for the memory impairments in many aged mammals, potentially also in humans. Thus, it is possible the cognitive resilience observed in individuals with high CR might be better explained by the preservation of LTP in older age. Future brain stimulation studies utilising iTBS will be better suited for investigating whether LTP is responsible for the neuroplasticity-CR relationship (Huang et al., 2005).

While we did not find evidence of a significant relationship between CR and neuroplasticity, it is important to mention that we observed a positive cluster immediately after the second cTBS train in the DLPFC condition [See Appendix 1]. This cluster had an alpha value that was just above our significance threshold ($p = 0.068$). Given this value was close to the significance level we specified, we considered the possibility that retaining the null hypothesis was a type II error. There are certain aspects of the results which are in favour of the effect being noteworthy. The cluster was observed in the same time period as the neuroplastic response identified in the first cluster-based permutation test, which is consistent with what would be expected if the association was driven by a real physiological effect. Additionally, the cluster was observed in the frontal area of the scalp, which is where we expected to see an effect for a cognition related concept. However, the poor spatial resolution of EEG means a high degree of weight should not be attributed to these observations. Conversely, there are other aspects of the results suggesting the effect is a result of random error. A positive cluster was also observed in the sham condition during the same block as the DLPFC cluster ($p = 0.11$), suggesting the likelihood of identifying a cluster unrelated to the cTBS paradigm was possible. Furthermore, the low statistical power of this study due to a low

number of participants suggests the probability of our results being influenced by error is already high. Given the quantity of evidence suggesting the cluster could have been influenced by extraneous variables, we determined the result was more likely to be a result of random error than a real effect.

4.3 Cognitive ability and cTBS-induced plasticity

A large number of animal studies suggest neuroplasticity is involved in cognition; however, direct evidence of this relationship in humans is still limited. We initially intended to create a single cognitive ability factor by combining our fluid intelligence (Gf) measure with the visuospatial learning and memory measure. We assumed these measures would be positively associated because of the positive manifold typically observed between cognitive functions (Schneider & Newman, 2015); however, this phenomenon was not replicated in our current study. Inspection of a scatter plot of the cognitive ability test scores did not indicate there were any outliers that may have explained the weak association [See Appendix 4B]. Given our sample was high performing in comparison to the general population and the CANTAB-PAL distribution was skewed towards higher performance [See Appendix 4E], ceiling effects may be responsible for the non-significant correlation between the two cognitive ability measures.

As Gf performance relies on proficiency in several cognitive domains, we used scores on this measure as a proxy of the general cognitive ability of our sample. We correlated this measure with changes in aperiodic slope to identify whether the cognitive deficits experienced in later life were associated with an age-related decline of neuroplastic efficiency. Neural networks characterised by a high degree of life-long plasticity (e.g. the DMN) are suggested to be particularly susceptible to this form of impairment (Koch et al., 2010; Gradey et al., 2010; Sambarto et al., 2010). This is because neuroplasticity is considered to be an adaptive

mechanism that reduces the impact of neurodegeneration in areas responsible for cognitive functioning (Vidal-Piñeiro et al., 2014). If a relationship had been observed between Gf and neuroplasticity in the DLPFC, this would have supported these notions. However, as a significant effect was not observed, it is possible the form of plasticity we measured is not implicated in the relationship. Given the age-related breakdown in neuroplastic efficiency observed in animal studies appears to affect LTP more strongly than LTD (Rosenweig & Barnes, 2003; Hsu et al., 2002), this may explain our non-significant results.

We also correlated performance on a visuospatial learning and memory task with our measures of cTBS-induced neuroplasticity. Prior research suggests neuroplastic changes in brain connectivity are the major physiological basis of learning and memory formation (Malenka & Bear, 2004). Furthermore, a recent study by Goldsworthy and colleagues (2020) demonstrated that LTP-like plasticity induced by iTBS to the lateral prefrontal cortex was associated with visuospatial learning. Our study was novel in that the relationship between LTD-like plasticity and visuospatial learning had not previously been investigated. Given we did not observe a significant relationship, this may suggest LTD plays a less important role in visuospatial learning and memory than LTP. However, this study may have been underpowered, and further research replicating these findings will be required to substantiate these claims. Future studies may elect to consider the relationship between cognitive function and the LTP-LTD balance to provide a more comprehensive assessment of neuroplasticity's role in this phenomenon.

4.4 Strengths of the study

The most notable strength of this study is that EEG enabled us to measure cTBS-induced plasticity in non-motor areas of the brain. The vast majority of prior rTMS research has identified modulations in neuroplasticity in the motor cortex through analysis of motor-

evoked responses measured from peripheral hand muscles (Chung et al., 2016). This limited understanding of cortical neuroplasticity to the motor cortex and did not provide opportunity for plasticity to be investigated in cognition-specific brain areas. Furthermore, motor evoked responses can be confounded by characteristics of non-cortical physiology (e.g. spinal cord excitability), whereas EEG provides a more direct measure of cortical brain activity (Chung et al., 2015).

As previously discussed, there were also strengths attributed to using the aperiodic slope in conjunction with cluster-based permutation statistics. Firstly, accounting for multiple comparison errors with cluster-based permutation statistics enabled us to identify changes in cortical excitation across the entire scalp (Maris & Oostenveld, 2007). This was particularly useful considering we did not have any *a priori* assumptions about where the response would be located. Secondly, analysing the aperiodic slope enabled us to consider a characteristic of the entire neural power spectrum, rather than changes in single predefined frequency bands which can be vulnerable to misinterpretation (Haller et al., 2018).

Another strength was that we used the LEQ to provide a comprehensive assessment of CR across all life stages. Evidence for a relationship between CR and neuroplasticity was previously limited in that CR had typically been quantified by a single proxy – often years of education or specialised occupation (Solé-Padullés, 2009; Gaser & Schlaug, 2003). The total LEQ score encapsulated 6 proxies of CR and weighted these sub scores through empirically grounded means.

4.5 Limitations of the study

There are several limitations which must be considered when interpreting our findings. Firstly, there are multiple documented challenges associated with using rTMS to induce neuroplasticity. Hamada and colleagues (2013) demonstrated the effects of rTMS are highly

variable, and populations of cortical neurons are more easily stimulated in different people at different times. This variability may explain the time discrepancies for when we observed the LTD-like effects between the DLPFC and M1, given the sites were stimulated on different days. Other studies have demonstrated that the effectiveness of TMS protocols can depend on the state of the individual. Physical exercise, cortisol levels, genes and history of synaptic activity can all influence the character of the phenomenon being induced by TMS (Kramer & Erickson, 2007; Sale et al., 2008; Cheeran et al., 2008; Todd & Ridding, 2010). It is possible our results were influenced by intraindividual state factors, rather than the interindividual traits we were seeking to quantify.

We were also limited by our behavioural data, as our sample demonstrated above average performance on the measures of cognitive ability and CR. Given our sample contained a limited number of individuals on the lower end of the performance spectrum, this suggests we may not have captured the diversity of neuroplasticity in the general population. This reduced variance may have also prevented significant relationships from being identified. There may also be limitations associated with using Gf as our only measure of general cognitive ability, as some have argued that Gf is so highly correlated with working memory capacity that they could be deemed isomorphic (Kyllonen, 2002; Jensen, 1998). A more diverse range of cognitive measures (e.g. measures of attention, processing speed) would have provided a more comprehensive assessment of the general cognitive performance our sample. We initially intended to produce general cognitive ability scores by combining Gf scores with the visuospatial learning and memory scores; however, we did not observe the expected positive manifold between these measures.

The results of our correlations between plasticity and the behavioural data also meant we could not conduct all the analyses we intended to perform. We initially hypothesised neuroplasticity would mediate the relationship between CR and cognitive ability. If this

hypothesis had been supported, this would have provided evidence of a neurophysiological mechanism underlying the cognition-protective effects of CR. However, the lack of significant results in the prior analyses indicated this investigation was not appropriate.

Finally, perhaps the most obvious limitation was the size of our sample. Given this was an exploratory study, the current findings should only be used as a point of reference for future research with larger samples. Currently, there is not a method of conducting a power analysis for cluster-based permutation statistics that we are aware of. Nonetheless, as our sample only consisted of 23 participants, it is clear the power of this study is not large enough for our findings to be considered reliable.

4.6 Future Directions

The number of studies which have paired EEG with rTMS is still very limited, and the characteristics of responses to stimulation at different brain areas is still unclear. We identified several discrepancies in the times and locations of neuroplastic responses during the active cTBS conditions. This suggests there may be site-dependent differences in the sensitivity of particular brain areas to cTBS, as well as differing spatial dynamics of electrophysiological change measured at the scalp. Mapping these characteristics in future EEG studies will enable researchers to refine rTMS paradigms, so neuroplasticity can be more precisely induced and measured.

While we did not observe a relationship between our measure of neuroplasticity and CR, other mechanisms of plasticity which were not assessed in this study should be considered in future research. Evidence suggests LTP may play a more central role in driving the cognition-protective effects of CR in older age (Rosenweig & Barnes, 2003; Hsu et al., 2002). The relationship between LTP and CR could be assessed with a similar methodology to the current study, with the exception of using iTBS instead of cTBS. Alternatively, both protocols

could be used to induce and measure the LTP-LTD balance, providing a more comprehensive assessment of synaptic plasticity. Furthermore, we recommend using a longitudinal study design when quantifying neuroplasticity, as a long-term measure of plasticity is more consistent with the theorised neural mechanisms of CR (Stern, 2006). A more diverse range of cognitive ability measures should also be included in these studies, as the neurophysiological and environmental determinants of cognitive ageing are still not fully understood.

Future studies should also investigate whether particular CR proxies are more related to neuroplasticity than others. A large proportion of the indirect evidence for a relationship between these factors has involved an association between a single CR proxy and some structural or functional brain characteristic (e.g. specialised occupation associated with cortical thickness). As these studies generally have not considered multiple CR proxies, differences in the neurophysiological impact of these environmental influences are still unknown. Given neuroplasticity is suggested to become less efficient across the lifespan, early life proxies such as years of education may be more related to plasticity than those in later life. Understanding these differences may be particularly useful when devising early interventions to slow down or prevent cognitive decline in later life.

4.7 Conclusions

The findings of this study hold several implications for areas of neuroplasticity and cognitive ageing research. The novel EEG data analysis methodology used in this study has demonstrated effectiveness in identifying neuroplasticity following rTMS to motor and non-motor areas of the cerebral cortex. Furthermore, the patterns of brain activity we identified contribute useful insights for future studies which seek to map the character of cTBS-induced neuroplasticity. An improved understanding of these characteristics will increase the precision of rTMS research methodologies, thus facilitating the investigation of neurophysiological

phenomena which could not previously be explored. Additionally, the results indicating that LTD-like plasticity was not significantly associated with cognitive reserve and cognitive ability raise the possibility that other forms of neuroplasticity may be more related to these concepts. We recommend future studies investigate the relationship of LTP to these variables and consider measuring neuroplasticity over a longer period of time. The neurobiological mechanisms underlying the cognition-protective effects of cognitive reserve are yet to be empirically demonstrated. Continued investigation of these mechanisms may facilitate the development of early interventions which could reduce the prevalence of age-related cognitive impairment.

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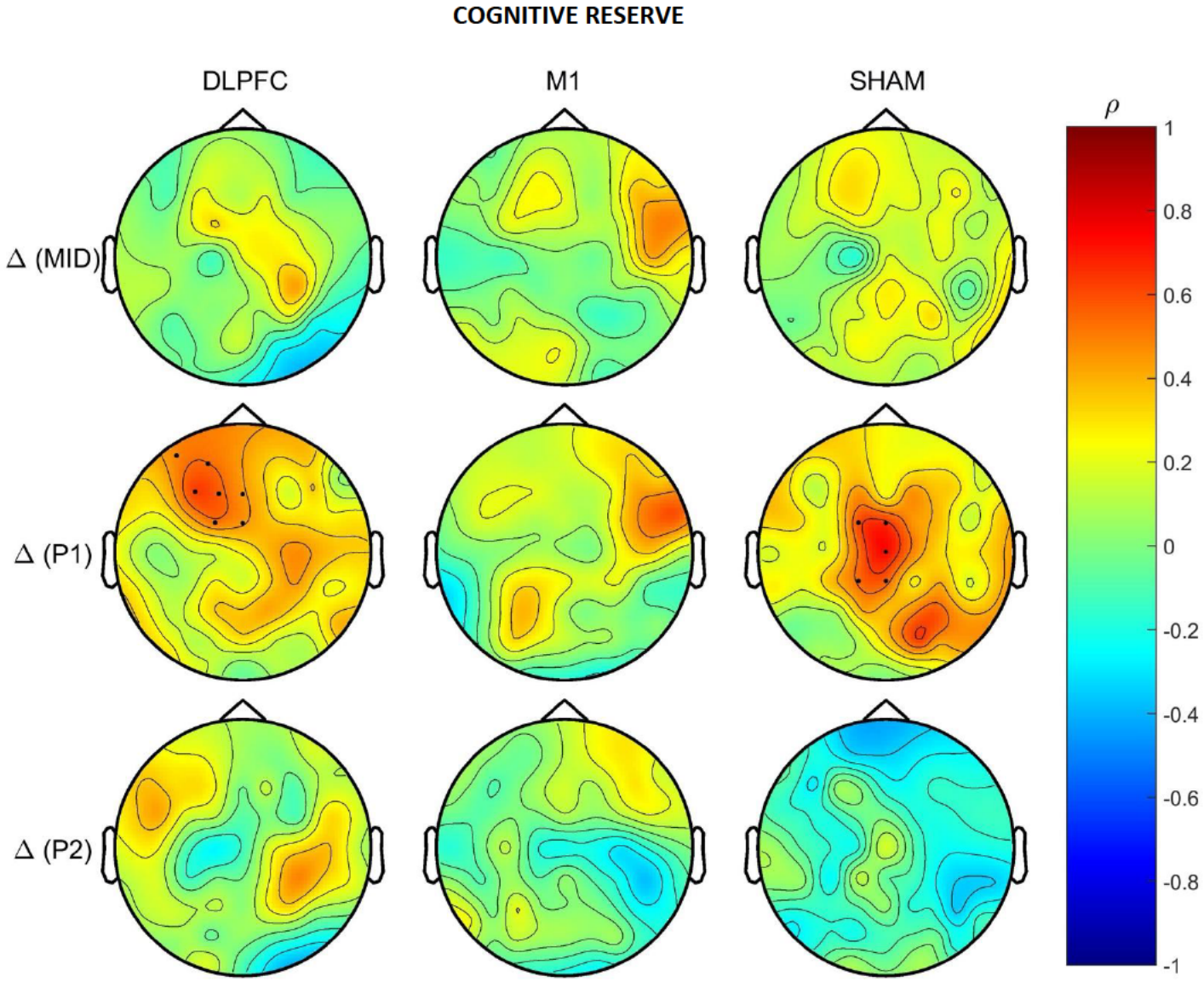
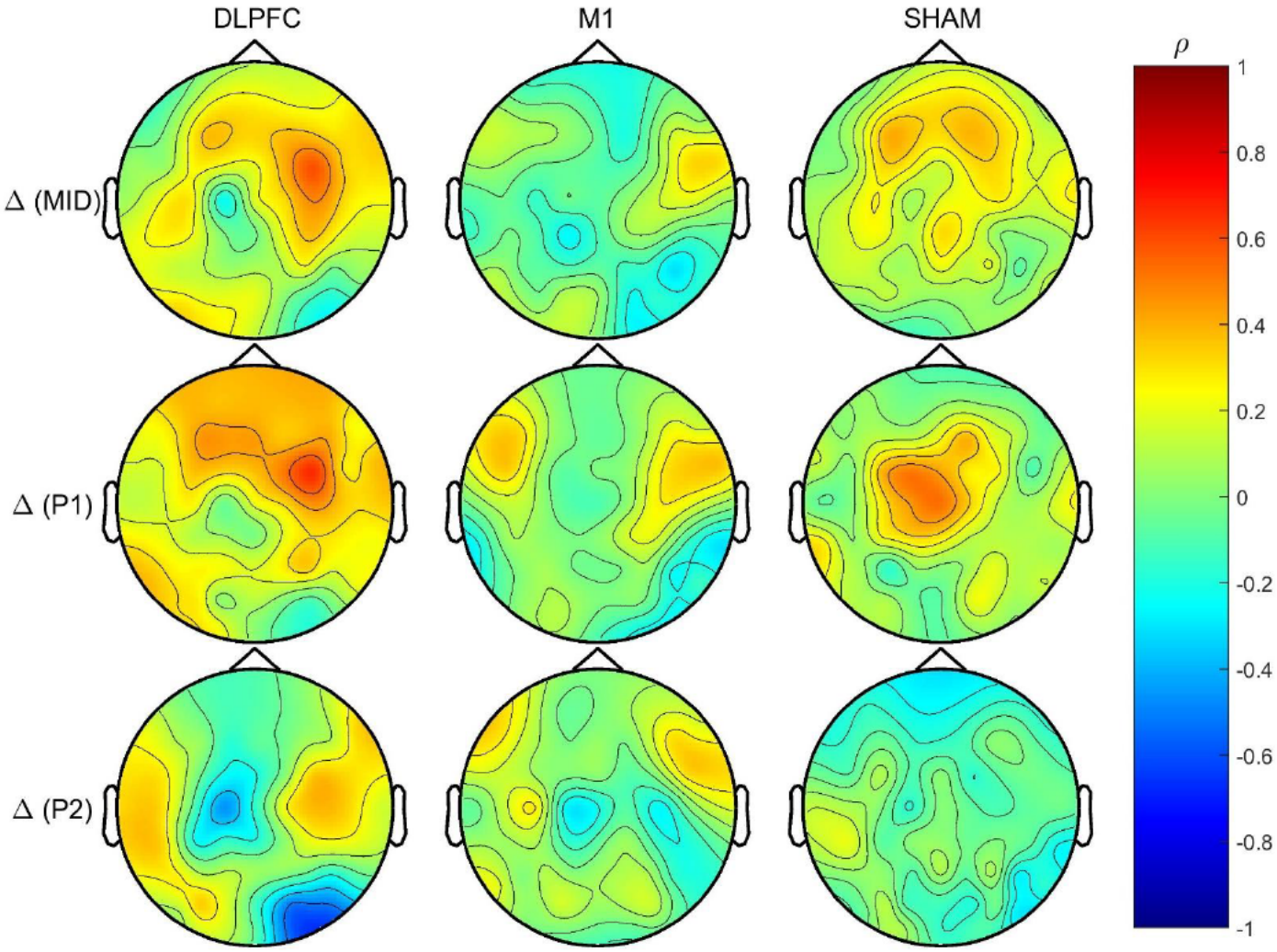
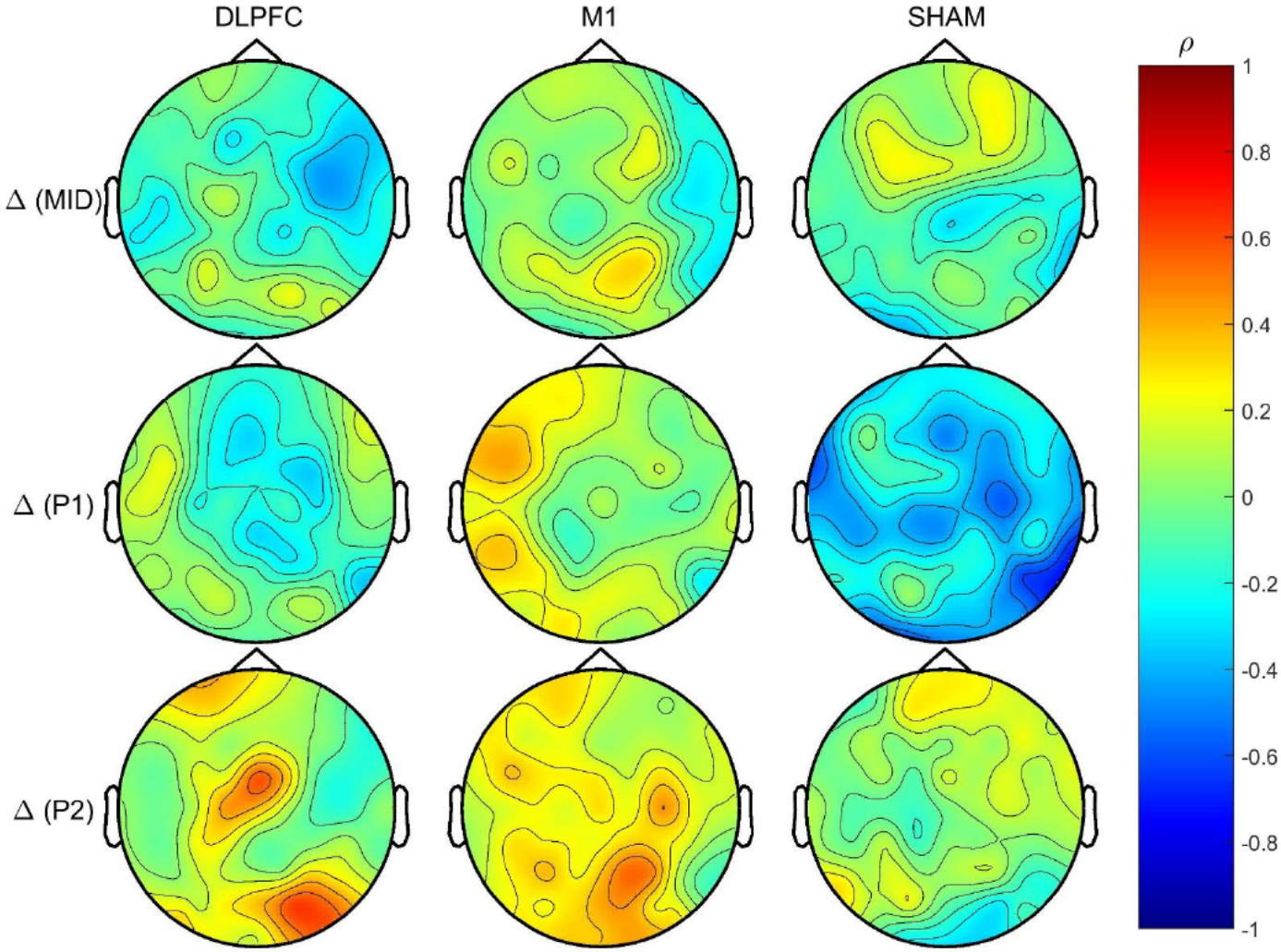
Appendices



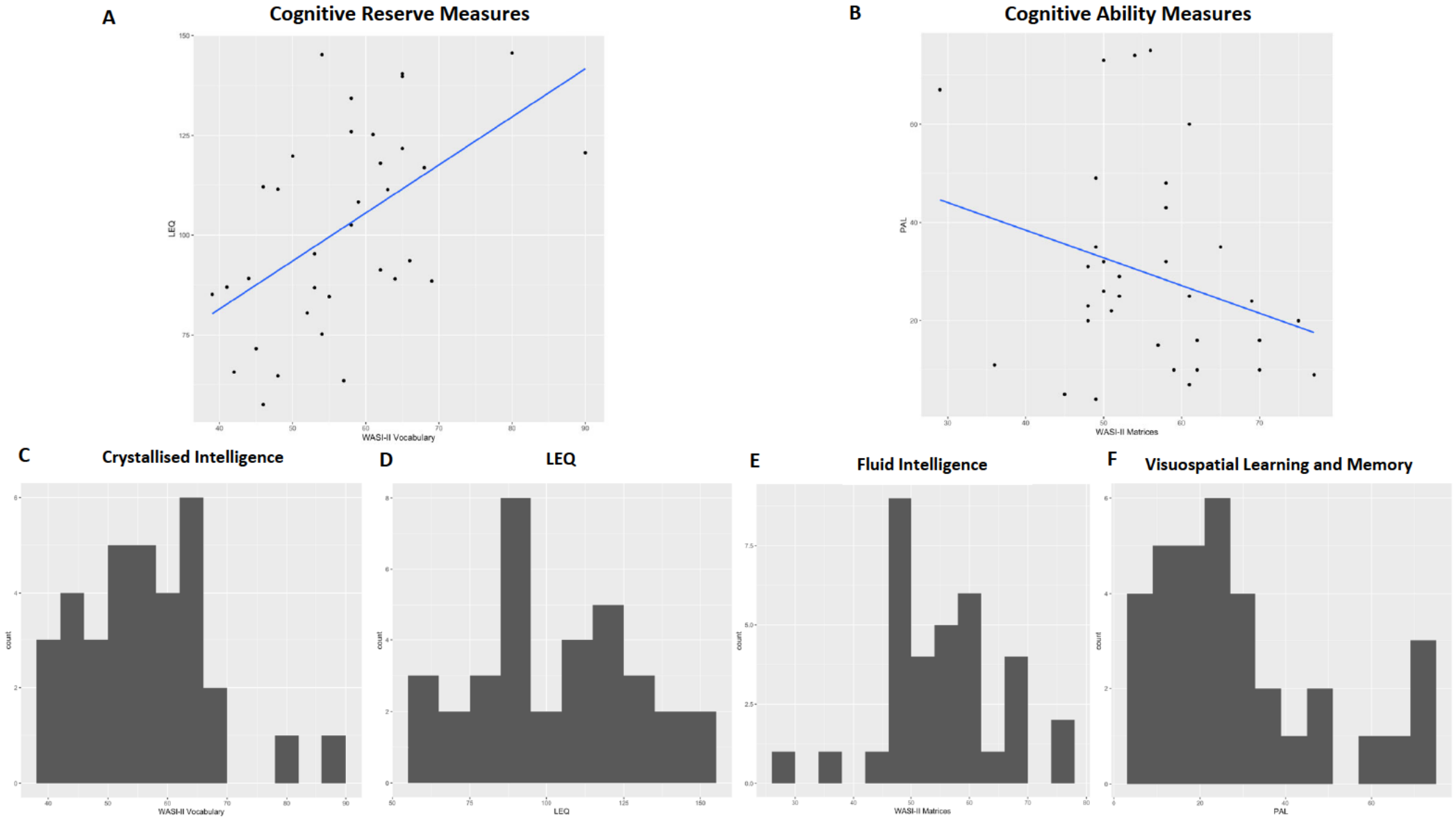
Appendix 1: *Topographical representation of the cluster-based correlation analyses comparing change in aperiodic slope with cognitive reserve factor scores (LEQ; WASI-II vocabulary) for each of the post cTBS timepoints. "." denote electrodes in positive clusters that did not exceed the significance threshold.*



Appendix 2: *Topographical representation of the cluster-based correlation analyses comparing change in aperiodic slope with WASI-II matrices scores for each of the post cTBS timepoints. No clusters observed.*



Appendix 3: *Topographical representation of the cluster-based correlation analyses comparing change in aperiodic slope with CANTAB-PAL Total Errors Adjusted scores for each of the post cTBS timepoints. No clusters observed.*



Appendix 4: *(A,B) Scatter plots of the scores on the cognitive reserve (WASI-II Vocabulary, LEQ) and cognitive ability (WASI-II Matrices, CABTAB-PAL Total Errors Adjusted) measures with regression lines. (C,D,E,F) Histograms of behavioural data. (note lower scores on CANTAB-PAL (F) are indicative of higher performance).*

Appendix 5: Participant handouts. (Note: as this study was based on a pre-existing data set, I did not produce any of these documents. The documents used in the prior Social and Neurocognition in Adulthood study were not available, as this was conducted by the University of South Australia).

PARTICIPANT INFORMATION

PROJECT TITLE: Investigating the role of brain plasticity in cognitive reserve

HUMAN RESEARCH ETHICS COMMITTEE APPROVAL NUMBER: H-2016-131

PRINCIPAL INVESTIGATOR: XXXX

Dear Participant,

You are invited to participate in the research project described below.

This information sheet is intended to provide you with sufficient information to make an informed decision about participating in this study. If there is any aspect that is not clear to you, please discuss this with one of the investigators.

What is the project about?
The human brain is capable of undergoing changes in how it is connected (plasticity) throughout life. Plasticity is important for learning and other cognitive function such as memory. These modifications allow you to improve your performance. Cognitive reserve describes the varying ability of individuals to retain normal cognitive function in the presence of brain pathology (damage). Many life influences (such as education) affect cognitive reserve. However, we don't have a good understanding of what brain functions and processes are involved in determining cognitive reserve. This project will examine the role of brain plasticity in cognitive reserve.

Who is undertaking the project?
This project is being conducted by XXXX

Why am I being invited to participate?
You are invited to participate in this follow-up study to the Social and Neurocognition in Adulthood study (being conducted by XXXX at the University of South Australia) because you were involved in it between 2013 and 2014 and consented to being contacted for follow-up studies.

What will I be asked to do?
You are invited to participate in this testing session that will investigate how neuroplasticity in the brain is related to cognitive reserve and cognition. You will be asked to attend the laboratory on two separate occasions for this session. At both times, we will use non-invasive

brain stimulation called transcranial magnetic stimulation (TMS). TMS is used to send a magnetic pulse from a coil placed near the scalp. It is completely painless and safe. We will also use a special form of TMS known as theta burst stimulation to produce a short lasting plastic change in your brain. Again, this is painless and you will not be aware of any effects from the stimulus

In addition, we will non-invasively measure your brain activity using electroencephalography (EEG). This will involve us fitting a cap (like a swimming cap) to your head, and putting a small amount of conductive gel in various positions under the cap. You will also be asked to complete a short questionnaire at the beginning of the first testing session.

How much time will the project take?
You will be asked to attend for testing. Testing session will last approximately 2.5 hours. You will be reimbursed at $20 to cover expenses associated with study visits.

Are there any risks associated with participating in this project?
We wish to make it clear that although the TMS techniques are used both diagnostically and in research laboratories around the world, all studies involve a small but finite risk. It is not expected that there will be any side-effects as a result of receiving the theta burst stimulation in this study. Some people have, however, reported mild headaches in other studies that have used this technique. In the many thousands of studies using TMS there have been two reported seizures. These were in non-screened participants who would normally have been excluded from study (and will be in this study). Participants are asked to advise researchers if they experience headaches or any other side effects.

A full list of possible side effects and their likelihood is given below:

Seizure induction - (very rare, however some medications for depression are thought to lead to a greater risk of seizures.)

Fainting (possible secondary effect – not related to direct brain effect)

Temporary headache or neck pain (possible, ~3%)

Temporary hearing changes (possible)

Temporary change in thinking and memory skills (not reported)

Burns to scalp (none)

Structural brain changes (not reported)

There may be other risks which are not known at this time.

If you believe you are experiencing any of these effects after you leave a testing session, please contact the researchers (details at the end of this information sheet).

What are the benefits of the research project?
You will not benefit directly from this study. However, the study may provide very useful information for our understanding of the ability of the brain to change its connectivity throughout life and how this contributes to cognitive reserve, or the ability of the brain to compensate for damage. The more we know about brain plasticity the more likely we will be

to harness and take advantage of this ability to improve outcomes for people with brain injury or damage.

Can I withdraw from the project?
Your participation is completely voluntary and you may withdraw your consent at any time.

What will happen to my information?
Initially your information will be recorded on paper that will be stored in a locked filing cabinet in the Neuromotor Plasticity and Development area within the Robinson Research Institute. Only the research team listed on this document will have access to this confidential information that will be stored securely for 10 years and then disposed of using confidential bin disposal. Electronic files containing your data will have a code used in place of your name to ensure confidentiality. You will not be identified in any published/publically presented data or reports. The results from this study will also be shared with the research team at the University of South Australia (headed by XXXX) but your data will not be personally identifiable.

Who do I contact if I have questions about the project?

If you have concerns or questions either before, or following, the study, please contact:

XXXX

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at the University of Adelaide (approval number H-2016-131). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the Principal Investigator. Contact the Human Research Ethics Committee's Secretariat on phone +61 8 8313 6028 or by email to hrec@adelaide.edu.au if you wish to speak with an independent person regarding concerns or a complaint, the University's policy on research involving human participants, or your rights as a participant. Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

If I want to participate, what do I do?
If you would like to participate please contact XXXX who is listed above. Should you decide to participate in the study, the first stage is basic screening that we will conduct over the phone. If it is determined you are suitable to participate in this study an appointment will be arranged for testing conducted by one of the research team. You will be asked to attend for two sessions separated by at least 1 week. Testing sessions will be conducted at the NeuroPAD laboratories at the Robinson Research Institute, located in the Norwich Centre, opposite the Women's & Children's Hospital in North Adelaide. The sessions can be arranged at a time to suit you.

Yours faithfully, XXXX

Human Research Ethics Committee (HREC)

CONSENT FORM

1. I have read the attached Information Sheet and agree to take part in the following research project:

Title:	**Investigating the role of brain plasticity in cognitive reserve**
Ethics Approval Number:	[redacted]

2. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

3. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

4. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

6. I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

7. I agree to the interview being audio/video recorded. Yes ☐ No ☐

8. I am aware that I should keep a copy of this Consent Form, when completed, and the attached Information Sheet.

Participant to complete:

Name: _____ Signature: _____ Date:

_____

Researcher/Witness to complete:

I have described the nature of the research to

_____

(print name of participant)

and in my opinion she/he understood the explanation.

Signature: Position: Date:

Transcranial Magnetic Stimulation† (TMS) Adult Safety Screen

Name:
Date:
Age:

Please answer the following:

Do you have epilepsy or have you ever had a convulsion or a seizure?	❍Yes	❍No
Have you ever had a fainting spell or syncope? If *yes*, please describe in which occasions in the space provided below.	❍Yes	❍No
Have you ever had severe (i.e., followed by loss of consciousness) head trauma?	❍Yes	❍No
Do you have any hearing problems or ringing in your ears?	❍Yes	❍No
Are you pregnant or is there a chance you might be?	❍Yes	❍No
Do you have cochlear implants?	❍Yes	❍No
Do you have an implanted neurostimulator? (e.g., DBS, epidural/subdural, VNS)	❍Yes	❍No
Do you have a cardiac pacemaker or intracardiac lines or metal in your body	❍Yes	❍No
Do you have a medication infusion device?	❍Yes	❍No
Are you taking any medications? *(Please list)*	❍Yes	❍No
Have you had a surgical procedure to your spinal cord?	❍Yes	❍No
Do you have spinal or ventricular derivations?	❍Yes	❍No
Did you ever undergo TMS in the past?	❍Yes	❍No
Did you ever undergo MRI in the past?	❍Yes	❍No

Subject signature:	
Experimenter name:	**Signature:**

*If you answered **yes** to any of the above, please provide details (use reverse if necessary):*

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