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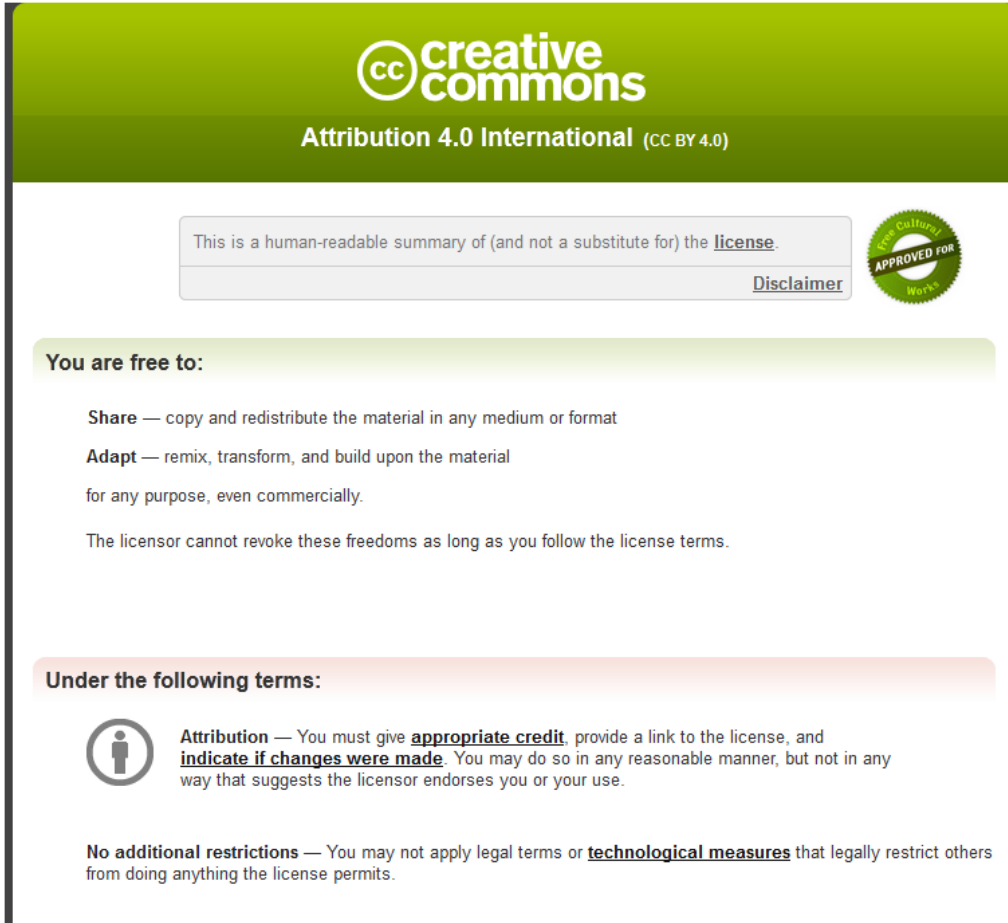
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REVIEW

The use of MALDI-MSI in the investigation of psychiatric and neurodegenerative disorders: A review

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Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is a mass spectrometry technique used for the analysis of macromolecules on an intact tissue of interest, thereby allowing the assessment of molecular signatures in health and disease in the anatomical context. MALDI-MSI is increasingly used to investigate neurodegenerative and psychiatric disorders at the molecular level, including Alzheimer's disease (AD), Parkinson's disease (PD), and schizophrenia (SCZ). These illnesses are characterized by complex neuropathological processes, and conventional proteomic techniques investigating brain tissue homogenates have inherent limitations in determining the precise anatomical or cellular location of proteomic findings. In this article, we review MALDI-MSI studies on neurodegenerative and psychiatric disorders, and explore whether the technique could accelerate the translation of proteomic information into improved understanding and ultimately better therapeutic applications.

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1 Introduction

Neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD), and psychiatric disorders

such as schizophrenia (SCZ), are thought to represent the largest contributor to all-cause disease burden in developed countries [1]. For many sufferers, these illnesses take a chronic course and lead to functional cognitive decline and loss of independence.

Despite decades of scientific inquiry, the molecular mechanisms driving the aetiopathology of these devastating disorders remain incompletely understood, and treatments remain largely non-specific and palliative rather than curative (for example, see [2, 3]). One of the reasons for this relative lack of scientific progress is that the CNS is a most complex structure, with an extraordinarily high degree of inter-connectivity and interaction within and between brain regions. Native and pathological processes in the CNS rely on translocation and concentration gradient formation of molecules in time. Therefore, in studying changes of these analytes in the CNS in relation to psychiatric disorders and neurodegenerative disorders, it is pivotal to ensure a reliable representation of their spatio-temporal distribution in health and disease.

Proteomic, metabolomic, and lipidomic techniques offer a potential avenue towards better understanding of processes

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Abbreviations: 6-OHDA, 6-hydroxydopamine; ACh, acetylcholine; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; CSF, cerebrospinal fluid; DA, dopamine; DHA-PC, docosahexaenoic acid containing phosphatidylcholines; DPP-TFB, 2,4-diphenyl-pyranilium tetrafluoroborate; FAD, familial Alzheimer's disease; FFPE, formalin-fixed paraffin embedded; GABA, γ -amino butyric acid; GLU, glutamate; hSOD1, human superoxide dismutase 1; LBs, Lewy inclusion bodies; L-DOPA, L-3,4-dihydroxyphenylalanine; LID, L-DOPA-induced dyskinesia; MAO-B, monoamine oxidase B; MPAQ, 2-methyl-3-phenyl-6-amino-quinoline; MPP+, N-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ND, neuropsychiatric disorders; PC, phosphatidylcholine; PD, Parkinson's disease; PEP-19, Purkinje cell protein 4; SCZ, schizophrenia; SNpc, substantia nigra pars compacta; SOD, superoxide dismutase; Ubc-T, truncated ubiquitin

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Color Online: See the article online to view Figs. 1–3 in colour.

underpinning disorders of the brain. Through their ability to simultaneously quantify large numbers of molecules in a given biological substrate, these techniques can uncover disease-related pathways and processes previously invisible to purely hypothesis-driven research. Consequently, a large number of studies have been undertaken that profile post-mortem brain tissue, cerebrospinal fluid (CSF), and peripheral body fluids such as blood serum or plasma of patients with neurodegenerative and psychiatric disorders. However, for post-mortem brain investigations in particular, the challenge remains that most studies have worked with tissue homogenates of brain areas of interest, which inherently cannot provide information of the exact anatomical or cellular location of the detected molecular abnormalities. Further, a comparison of common proteomic post-mortem findings in various neurodegenerative and psychiatric conditions indicates that there is a large overlap in the molecular pathways and processes apparently implicated in disease pathophysiology. For example, aberrant proteins and pathways involved in oxidative stress, mitochondrial function, energy metabolism, the cytoskeleton, and the synapse have been reported in PD [4, 5], AD [6, 7], and in psychotic illnesses including schizophrenia and bipolar disorder [8–12]. It is not clear whether these overlapping findings reflect true mechanistic commonalities relevant to chronic brain conditions [13, 14], or whether they are a consequence of “*déjà vu* proteomics” where similar findings for diverse disorders may be a reflection of technical shortcomings and biases rather than actual biological discoveries [15, 16]. Because of these difficulties, it is of little surprise that the development of clinical diagnostics based on proteomic findings in neurodegenerative and psychiatric disorders has remained relatively unproductive and controversial [17].

One established *in vitro* technique that could contribute to improving this situation is matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). MALDI-MSI provides molecular mass determination of analytes from complex mixtures (e.g. tissue) while keeping their spatial distribution intact [18]. This allows for the creation of *m/z* images, where the location and intensity of distinct *m/z* values correspond to morphological and/or pathological features of the analysed sample (for a recent review see [19]). This constitutes the advantage of MALDI-MSI in comparison with “classical” proteomic, metabolomic, and lipidomic techniques, which normally involve a homogenization step and therefore the loss of spatial information within the analysed samples. Further, MALDI-MSI has the potential to represent a true -omics technique that is independent of *a priori* specified target molecules, in contrast to other *in vitro* imaging methods such as antibody-based immunohistochemistry. Additionally, MALDI-MSI can be used to replace immunohistochemistry-based quantitative analyses of proteins of interest, providing a faster and reliable method that does not require stereologic cell counting procedures.

Proteomic investigation of CNS tissue samples in neurodegenerative and psychiatric disorders by MALDI-MSI has been

an advancing field over the last decade, and the technique has been applied to human post-mortem tissue as well as to established mouse models of these diseases. In this article, we review how MALDI-MSI has been used to advance and complement neuropathological knowledge and previous proteomic, metabolomic, and lipidomic investigations in the major neurodegenerative and psychiatric illnesses. We also point out the potential of MALDI-MSI for future studies.

2 MALDI-MSI: the technique in a nutshell

MALDI-MSI is a technique where tissue sections get mounted onto electrically conductive slides, spray coated with matrix and rastered by a laser beam to acquire mass spectra of analytes of interest (most commonly proteins, peptides, metabolites, small molecules or lipids [20]) (see Fig. 1). This technique was firstly introduced by Caprioli et al. in 1997 [18]. Depending on the tissue and analyte of interest, different matrices and sample preparation steps have to be applied. Protein imaging is normally being conducted on fresh frozen tissues, using sinapinic acid as a matrix and only minimal sample preparation is necessary (for a review see [21]). This basically also applies for lipid imaging [21]. Peptide imaging can be conducted on fresh frozen, but most importantly on formalin-fixed paraffin embedded (FFPE) tissues [22]. The

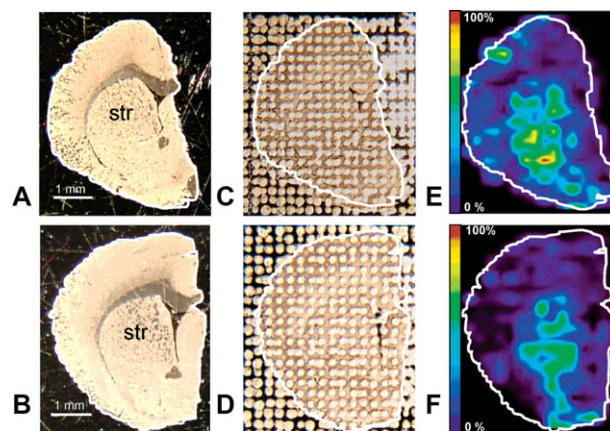


Figure 1. MALDI-MSI of brain tissue sections following MPTP treatment. Panels a-b show the photographs of mouse brain hemispheres from one control and one MPTP treated animal, respectively, mounted on a gold-coated stainless steel plate (str = striatum). Panels c-d display the same tissue sections spotted with matrix solution (SA, 50:50:0.1 ACN:H₂O:TFA). The diameters of the matrix spots are about 190 μ m and the distance from center to center is 280 μ m. Panels e-f show the ion density of PEP-19. The distribution and relative intensity difference between control and MPTP treatment is representative for the analysed tissue sections. Reprinted with permission from Skold, K., Svensson, M., Nilsson, A., Zhang, X. et al., Decreased striatal levels of PEP-19 following MPTP lesion in the mouse. *J. Proteome Res.* 2006, 5, 262–269. Copyright 2006 American Chemical Society.

ability to investigate FFPE tissue makes vast libraries of clinical tissue specimen available for analysis by MALDI-MSI (reviewed in [23]). However, sample preparation for peptide imaging from FFPE tissues is complex. Generally, an antigen retrieval step has to be applied to reverse protein cross-linking caused by formalin [24] (for a review see [23]), followed by a tryptic digest. Advantageous is the application of internal calibrants to increase mass accuracy of detected m/z features, as this improves matching of peptides between MALDI-MSI and LC-MS/MS for identification purposes [25]. In recent years, MALDI-MSI has been extended to the analysis of N-glycans [26], which enables investigation of differentially expressed glycan species in relation to tissue type [26–29].

3 Applications of MALDI-MSI in neurodegenerative and psychiatric disorders research

MALDI-MSI is an emerging technique and therefore only a limited number of specialist laboratories apply this technique. This is evidenced by the small number of studies in the field of ND research featuring this technology. Further, ND research faces unique challenges: Human samples of ND are very rare as they can only be acquired post-mortem; therefore animal models have to be applied. This further limits the number of ND which can be investigated, as development of animal models of complex psychiatric disorders such as schizophrenia [30, 31] or depression [32] are a challenging task [33]. This is reflected in the number of publications regarding the type of ND, PD being the most studied, followed by AD. Apart from MALDI-MSI, other imaging technologies like laser-ablation inductively coupled plasma MS or secondary ion MS have been applied in ND, however these investigations were considered out of scope for this review. For comprehensive reviews of these technologies for neuroscience applications see [34] and [35].

3.1 Parkinson's disease

PD is a neurodegenerative disorder leading to progressive motor disability through body tremors, slowed movement, muscle rigidity, and an irregular posture [36]. Genetically, PD appears to differentiate into rare familial forms that show associations with autosomal dominant and autosomal recessive loci and into more common 'idiopathic' illness types with a complex polygenetic signature [37]. Neuropathologically, all forms of PD are characterized by the loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc), and by the development of intracellular Lewy inclusion bodies (LBs) containing α -synuclein [38]. This is pointing to an impairment of a selected group of neurons in the handling of abnormally processed cellular proteins and a subsequent deposition of those proteins as insoluble and toxic aggregates. Molecular and proteomic investigations of human SNpc and LBs have, in summary, implicated oxidative stress,

mitochondrial dysfunction, protein aggregation, and the ubiquitin-proteasome system as potential pathways relevant to PD pathophysiology [5, 39–43] (reviewed in [4]). However, how these candidate cellular processes drive LB development and dopaminergic degeneration within the relevant brain areas and neural networks, and how they are affected by common treatments such as L-3,4-dihydroxyphenylalanine (L-DOPA), is not well understood. Proteomic studies utilizing MALDI-MSI have begun to provide additional information on these questions.

3.1.1 Animal models of PD and MALDI-MSI

In proteomic research using MALDI-MSI, a number of PD animal models have been studied to date. One model investigated by MALDI-MSI studies uses unilateral injection of 6-hydroxydopamine (6-OHDA) by stereotaxic surgery into SNpc, where it causes degeneration of dopaminergic neurons following uptake via dopamine and noradrenaline transporters [44–46]. The toxicity of 6-OHDA is derived from its oxidation by monoamine oxidase B (MAO-B), which produces hydrogen peroxide [47]. Further, 6-OHDA undergoes auto-oxidation, which produces hydrogen peroxide, reactive oxidative species and catecholamine quinonones [48, 49]. Mitochondrial function is affected as well by 6-OHDA, as the function of complex I of the electron transport chain is impaired [45].

Another model of PD investigated by MALDI-MSI uses systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which reproduces all clinical manifestations of PD in primates, however without the development of Lewy inclusion bodies (reviewed in [50]). In contrast to 6-OHDA, MPTP easily crosses the blood-brain barrier and is subsequently oxidized in astrocytes [51] into N-methyl-4-phenylpyridinium (MPP+) by MAO-B [51, 52]. MPP+, as the actual neurotoxic compound, is accumulated intracellularly by the presynaptic re-uptake system [53, 54], and causes inhibition of the electron transport by mitochondrial complex I and, ultimately, cell death [55, 56].

MALDI-MSI studies of PD animal models have addressed the following research questions: (i) to validate animal models of PD; (ii) to complement targeted investigations on the differential expression of PD candidate proteins in different brain areas of PD rodents [57]; (iii) to identify differential proteomic effects of L-DOPA in various brain regions of PD animals [58, 59]; (iv) to map changes in neurotransmitters in PD; (v) to identify the spatial distribution of novel PD drugs in rodent brain.

(a) Targeted MALDI-MSI for the validation of PD animal models

Investigating the clinical validity of the MPTP model, Kadar et al. applied MALDI-MSI to investigate the distribution of the neurotoxic component MPP+ in mouse brain

after intranasal application of MPTP [60]. Ion images for the m/z of 170.1, corresponding to MPP⁺, recorded with a spatial resolution of 70 μm showed that the compound could be detected in almost the entire brain 10 min following MPTP administration, whereas after 90 min MPP⁺ was found predominantly concentrated in dopaminergic areas, including the SNpc, thus strongly supporting intranasal MPTP administration as a valid model for PD research.

(b) Targeted MALDI-MSI investigations of PD-associated proteins in animal models

Nilsson et al. addressed the question whether the immunophilin protein FKBP-12, a binding partner of the experimental PD drug FK506 and thought to possess neuroprotective and neuroregenerative properties in experimental PD models [61], shows an anatomically differential expression pattern in the striatum of 6-OHDA unihemispherically lesioned rat brain [57]. Using MALDI-MSI, the authors reported that FKBP-12 (m/z 11 791) ion intensity was elevated in the dorsal and medium part of lesioned rat striatum, but unchanged in the ventral part [57].

Demonstrating similar anatomical differences with regards to striatal protein changes following 6-OHDA lesion, Pierson et al. reported increased levels of ubiquitin (m/z 8565), a protein that accumulates in LBs, in the dorsal but not the ventral rat striatum affected by 6-OHDA [59]. The authors speculate that these anatomical differences could be either due to lower dopaminergic innervation of the ventral striatum, or to lower efficacy of the 6-OHDA lesion in this area. It is of value to note that in previous studies using homogenized samples from human SNpc [39] and from 6-OHDA treated rats [62], changes in brain ubiquitin levels could not be detected, highlighting the potential advantages of MALDI-MSI over conventional proteomic techniques.

A similar study using MALDI-MSI investigated the expression levels of Purkinje cell protein 4 (PEP-19), a protein thought to possess anti-apoptotic properties in neurodegenerative disorders [63–65] in the brains of C57BL/6 after subcutaneous injection of MPTP [66]. Anatomical and quantitative analysis of PEP-19 ion intensity by MALDI-MSI (spatial resolution = 280 μm) revealed that PEP-19 expression is mostly restricted to the striatum, with a significant decrease in expression area in case of MPTP treated mice [66] (Fig. 1).

(c) MALDI-MSI experiments to identify differential proteomic effects of L-DOPA on various brain regions of PD animals

Pierson et al. conducted a MALDI profiling study investigating the effects L-DOPA on 12 brain regions of interest in 6-OHDA treated rats [58]. Seven m/z features exhibited differential expression (student's t -test $p < 0.05$) between corresponding locations in the two hemispheres in the L-DOPA treated group but not in the saline control. Five of these differentially expressed peptides were located to the

6-OHDA-lesioned striatum, and three striatal features could be identified as under-expressed calmodulin and cytochrome c oxidase subunit VIIa-L and as cytochrome c oxidase. Further, an increased ratio of PTM such as acetylations was found in the striatum of proteins in the dopamine depleted side of the brain, an effect that was attenuated following L-DOPA treatment.

Two MALDI-MSI studies have investigated a common and serious side-effect of L-DOPA treatment seen in PD patients, L-DOPA-induced dyskinesia (LID). In a targeted approach, the spatial ion distribution of peptide masses corresponding to endogenous opioid neuropeptides (endogenous dynorphins), thought to mediate LID in humans and rodents, was examined in the brains of 6-OHDA- and L-DOPA treated rats [67, 68]. Both studies applied DHB as matrix using an inkjet printer on 12 μm brain slices, however the resolution differed between 250 [68] and 300 μm [67]. Both studies report the up-regulation of dynorphin B and alpha neoeendorphin in the lesioned striatum [67] and substantia nigra [68] in animals showing high dyskinesia behaviours compared to the low dyskinesia and saline control groups. Additionally, a linear correlation between expression levels of the differentially expressed neuropeptides and the severity of dyskinesia is reported in both studies. In the striatum, substance P was additionally up-regulated in the high dyskinesia group [67], while in the substantia nigra Leu-Enk-Arg was increased [68].

(d) MALDI-MSI to map changes in neurotransmitters in PD

A further study by Shariatgorji et al. applied a novel approach to detect low molecular weight compounds such as dopamine (DA), γ -amino butyric acid (GABA), glutamate (GLU) and acetylcholine (ACh) in the brains of sham lesioned, 6-OHDA lesioned and D₃-L-DOPA (after 6-OHDA lesion) treated rats [69]. The novel approach was to derivatise primary amines with 2,4-diphenyl-pyranilium tetrafluoroborate (DPP-TFB) to enable their ionization by laser energy without the further assist of a matrix and achieves a remarkable spatial resolution of 15 μm which achieves visualization of the nuclei of the facial nerves and pons [69] (Fig. 2). In 6-OHDA lesioned animals, the authors found decreased striatal levels of DA and increased striatal levels of GABA, mirroring findings in humans with PD. This approach was further investigated using a primate model of MPTP lesion, where differential distribution of DA, 3-methoxytyramine and GABA in coronal brain tissue sections were detected [69]. The quantification was achieved by comparing the signal intensities of analytes of interest with known quantities of deuterated calibration standards. Additionally, it was shown that the use of deuterated HCCA matrix can be successfully applied to overcome masking effects of matrix compounds with the same m/z as analytes of interest such as ACh, which lack a primary amine [69].

(e) MALDI-MSI experiments to detect the spatial distribution of novel PD drugs in rodent brain.

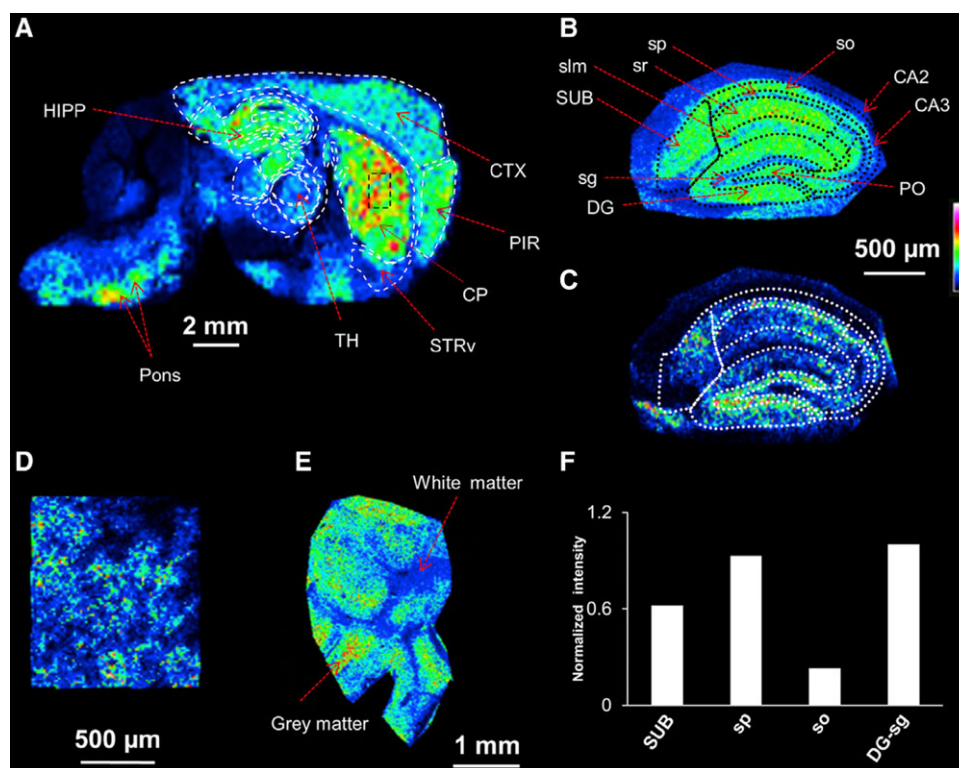


Figure 2. High-resolution MALDI-MS Images of ACh in a Sagittal Rat Brain Section (A–F) MALDI-MS images of ACh in a rodent brain sagittal tissue section. The relative distribution and abundance of ACh determined by MS imaging are consistent with existing data on the major cholinergic pathways in the brain (A). ACh-rich structures such as the HIPP, CP, CTX, and even small structures such as the nuclei of the facial nerves and pons are readily apparent in the MALDI-MS image. High-resolution MALDI-MS images (15 μm spatial resolution) of various structures in consecutive brain tissue sections were also acquired. Substructures of the hippocampus were delineated by high-resolution imaging of the distribution of a lipid with m/z 721.2 (B). The relative abundance and distribution of ACh in the HIPP (C), a subregion of the CP indicated by a dashed rectangular border in (A) (D), and CB (E) were determined by high-resolution imaging. The concentration of ACh in the gray matter of CB is around five times greater than that in the white matter. In the HIPP, high ACh concentrations are apparent in DG-sg and sp regions (F). MS images were acquired using a MALDI TOF/TOF mass spectrometer. Data are shown using a rainbow scale and are normalized against the total ion counts; scale bars, 2, 1, and 500 mm; spatial resolutions = 100 and 15 mm. Caudate-putamen, CP; hippocampus, HIPP; cerebellum, CB; granule cell layer of the dentate gyrus, DG-sg; pyramidal layer of CA1, sp; cerebral cortex, CTX; piriform area, PIR; striatum (ventral region), STRv; thalamus, TH; subiculum, SUB; stratum lacunosum-moleculare, Slm; stratum radiatum, Sr; stratum oriens, So; cornu ammonis (field 2), CA2; cornu ammonis (field 3), CA3; polymorph layer of dentate gyrus, PO. Reprinted from *Neuron* 84 (2014), Shariatgorji, M., Nilsson, A., Goodwin, R. J., Kallback, P. et al., Direct targeted quantitative molecular imaging of neurotransmitters in brain tissue sections. Pages 697–707, Copyright 2014, with permission from Elsevier.

MALDI-MSI has been used to investigate the localization of novel drugs thought to counteract PD development or progression in the brain. Quinoxaline derivatives such as 2-Methyl-3-Phenyl-6-Amino-Quinoxaline (MPAQ) reportedly possess such neuroprotective properties for dopaminergic CNS neurons [70]. A MALDI-MSI experiment in MPAQ treated mice was carried out, using a HCCA matrix applied onto 14 μm thick brain sections, with a distance of 70 μm between acquisition sites [70]. MPAQ (m/z 237.1) was localized mainly to striatum and the ventral mesencephalon, which contains the SNpc [70]. Findings support MPAQ as a suitable candidate for neuroprotective therapy in PD.

There is currently no published study investigating PD in human post-mortem brain using MALDI-MSI, and given the relatively extensive use of the technique in studies of

PD mouse models, future translational opportunities in this disorder are obvious.

3.2 Alzheimer's disease

AD is a common neurodegenerative disorder causing progressive decline of cognitive functions, particularly memory. Neuropathologically, AD is characterized by the formation of senile plaques, neurofibrillary tangles, and by synaptic disruption and neuronal loss. According to the amyloid cascade hypothesis of pathological origin, aggregation and deposition of amyloid precursor protein cleavage products (amyloid- β peptides) are causative for neurotoxicity and subsequently affect the protein tau, which in its hyperphosphorylated form

is a major constituent of neurofibrillary tangles (reviewed in [71]).

Similar to the situation in PD research, MALDI-MSI has been mostly limited to studying mouse models in preclinical marker research or for drug imaging, and the technique is yet to realize its full potential in complementing the rich proteomic literature of 2D gel electrophoresis and LC-MS/MS (reviewed in [7] and [72]) in post-mortem AD brain.

The following research questions in AD research have been addressed by MALDI-MSI studies to date: (i) localization of amyloid- β isoforms; (b) changes in the lipidome related to AD in humans; (c) changes in the lipidome related to AD in mouse models and (d) investigation of drug distribution in the brain.

(a) Localization of amyloid- β in mouse models of AD

The general suitability of MALDI-MSI to contribute to AD research was established by Stoeckli et al., using fresh frozen brain sections of APP23 transgenic mice [73], a frequently used AD model based on the overexpression of human amyloid precursor protein (APP) exhibiting the Swedish mutation [74, 75]. APP23 mice show first deposits of amyloid- β plaques in neocortex and hippocampus after 6 months [75]. Further, hyper-phosphorylated tau is located only in congophilic plaques [75]. This mouse model is therefore an ideal candidate to study over-expression of amyloid- β peptides as an Alzheimer's model. Sinapinic acid was used as a matrix to identify m/z features corresponding to amyloid- β peptides. By slow crystallization at low temperature, matrix crystals with a size of up to 200 μm were grown and rastered by a laser every 100 μm . Subsequently, m/z features of interest corresponding to amyloid- β isoforms (1-37, 1-38, 1-39, 1-40 and 1-42) were compared with sections immunostained with NT11 antiserum, detecting amyloid- β residue 1-40 [76]. m/z features of amyloid- β isoforms and immunostaining signals could be reasonably well spatially matched.

(b) MALDI-MSI of lipids in human AD post-mortem tissue

Although generally applied for protein and peptide analysis, changes in the distribution of lipids can also be detected by MALDI-MSI. Yuki et al. investigated the distribution of hydroxylated and non-hydroxylated sulphatide species of the sphingolipid sulphatide in post-mortem human brain [77]. Reductions of sulphatide, a major component of the myelin sheath, has been reported as a potential early pathological characteristic in AD and may be one molecular mechanism underlying the cortical demyelination and oligodendrocyte degeneration seen in the disorder [78]. Detected by MALDI-MSI applying a spatial resolution of 100 μm , the ratio of hydroxylated to non-hydroxylated sulphatide species differed most at the border of white to grey matter AD and healthy control brains, with no detectable differences in species distribution between the two groups [77]. Whilst in need of replication, these results suggest that mechanisms other than

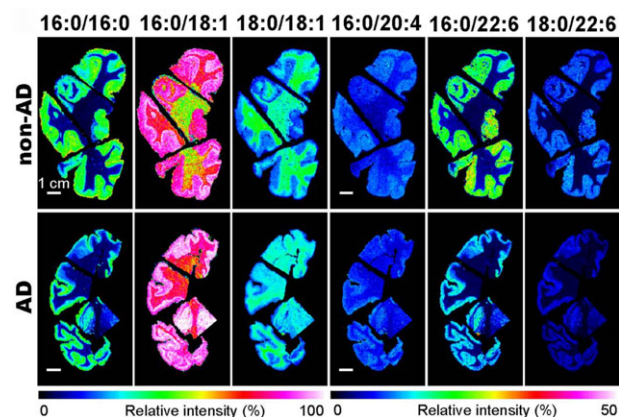


Figure 3. The distributions and intensities of PC species in coronal sections of non-AD and AD brains as analysed by MALDI-MSI. Scale bar equivalent to 1 cm. Adapted from Yuki, D., Sugiura, Y., Zaima, N., Akatsu, H. et al., DHA-PC and PSD-95 decrease after loss of synaptophysin and before neuronal loss in patients with Alzheimer's disease. *Sci Rep* 2014, 4, 7130. Under Creative Commons license (CC BY 4.0) (<http://creativecommons.org/licenses/by/4.0/>).

sulphatide species generation through hydroxylation underlie sulphatide abnormalities in AD.

In a subsequent study, the authors investigated the distribution of species of docosahexaenoic acid containing phosphatidylcholines (DHA-PC) in AD patients brains compared to normal controls (spatial resolution = 50 μm) [79]. Reduced levels of DHA-PCs, which are major brain lipid constituents regulating membrane fluidity, had previously been reported in AD cerebral tissue [80]. Lower relative intensities of DHA-PC (16:0/22:6) and (18:0/22:6) in grey matter in brains affected by AD was detected in the temporal, parietal and frontal lobe, while relative intensities of DHA-PC (16:0/16:0) were not changed (Fig. 3). Further, a correlation of reduced DHA-PC (18:0/22:6) with the disease duration in AD as well as earlier age of death was identified [79]. It was speculated by the authors that the lower intensity of DHA-PC in AD patients brain influences neuronal cell death, as cleavage products of DHA-PC can act as anti-apoptotic factors [81].

(c) Detection of lipid changes in mouse models of AD

A further study by Hong et al. investigated changes in phospholipids in 5XFAD mice [82]. These mice are a very rapid on-set Familial Alzheimer's disease (FAD) model exhibiting three mutations in amyloid precursor protein (Swedish, Florida and London) and two mutations in presenilin1 (M146L and L286V) [83]. The rapid on-set is reflected in the large deposits of A β -42, overexpression of p25 and neuronal loss these mice show after 2 months [83]. MALDI-MSI was conducted on fresh frozen brain slices of three mice at age of 3 and 9 months and compared against age matched control mice. As matrix a 1:1 mixture of HCCA and DHB was

applied and spectra were acquired in both positive and negative ion mode with a lateral resolution of 150 μm . Brain regions first to accumulate amyloid plaques showed marked decrease in phospholipid abundance of PC32:0 in frontal cortex and, PC32:0, SMD34:1 and PC34:1 in the subiculum, while LPC16:0 was increased in both frontal cortex and subiculum of 9 months of 5XFAD mice [82]. However, a co-localization of these features with amyloid plaques was not shown.

(d) MALDI-MSI experiments to detect the spatial distribution of novel AD drugs in transgenic rodent brain

As described previously, MALDI-MSI is a powerful approach to test the localization of low molecular weight compounds that have possible therapeutic potential. Spatial distribution of Clioquinol, a “metal attenuating compound” with proposed therapeutic properties in AD, was investigated in the brains of treated TgCRND8 mice [84]. These mice exhibit the Swedish and Indiana mutation of the amyloid precursor protein 695, accumulate cerebral amyloid- β plaques and show marked working memory deficits at a young age, thereby representing a suitable model of early onset AD [85]. Clioquinol, in this model, reverses the behavioural deficits and reduces amyloid- β plaque burden in the cortex and hippocampus. MALDI-MSI visualization of the clioquinol diagnostic signal using a HCCA matrix that was air-brushed onto 10 μm brain sections; data was acquired with a resolution of 250 μm in reflectron positive mode. Detection of the clioquinol signal (m/z 305.9) revealed that the drug was enriched specifically in the cortex and hippocampus [84], two brain areas prominently involved in the cognitive function affected by AD pathology. Hence the authors interpreted these findings as support for the potential of metal attenuating agents in AD therapy, and for the role of biometals such as zinc, copper, and iron such in the formation of amyloid- β plaques. The study thus complements previous findings that these ions are enriched in plaques in human AD brain as well as in AD mouse models [86–89].

3.3 Schizophrenia

Only one pilot MALDI-MSI study to date has examined post-mortem brain tissue of patients with schizophrenia [90]. In a two-step approach, the authors first characterized and compared lipid species in homogenized brain tissue of a schizophrenia case and healthy control. Subsequently, lipid species differentially expressed in the homogenized control and diseased brains were screened for in the mass spectra obtained from a MALDI-MSI experiment imaging the prefrontal cortex. The authors reported abnormal distributions of a phosphatidylcholine (PC) molecular species particularly in the cortical layer of the frontal cortex in schizophrenia, compared to a matched healthy control brain. Additionally, a PC containing arachidonic acid was increased in the frontal cortex of the schizophrenia patient.

Whilst it is difficult to draw scientific conclusions from this experiment, due to an experimental sample size of two and several potential confounders that had not been controlled for, the study complements previous findings that implicate abnormal brain lipid compositions in schizophrenia [91, 92]. The demonstrated ability of MALDI-MSI to detect such lipid changes in an anatomical context represents a major technological advance.

3.4 Amyotrophic lateral sclerosis (ALS)

ALS is a fatal progressive, degenerative motor neuron disease (reviewed in [93]). The origin of the disease is unknown and current consensus is that underlying genetic causes are due to rare traits leading to a common disease pathomechanism [93]. Common mutations encountered in familial ALS affect superoxide dismutase (SOD)1 [94], which gave the incentive for Acquadro et al. to investigate the effects of mutant human SOD1 (G93A hSOD1), wild type human SOD1 (hSOD1), and murine SOD1 in the brain of transgenic and non-transgenic mice [95]. Applying MALDI-MSI, G93A hSOD1 was detected to be restricted to facial nuclei, while hSOD1 and murine SOD1 showed no preferential localization. Within the facial nuclei, 40S ribosomal protein S19 was detected to be up-regulated in G93A hSOD1 compared to hSOD1 and non-transgenic mice [95]. This protein is of special interest, as it has been shown to interact with fibroblast growth factor 2 [96, 97], which is involved in motor neuron development, maintenance and repair [98, 99].

The only study, published to date in the field of neurodegenerative disorders, that employed MALDI-MSI technology for hypothesis-free profiling of human post-mortem tissue, investigated human spinal tissue from patients with ALS [100]. Here, post-mortem spinal cord sections of four ALS patients and three healthy controls were analysed with a spatial resolution of 350 μm for global differential protein expression. A truncated form of ubiquitin (Ubc-T) and an unidentified m/z feature of 8429 were found to be under-expressed in ALS spinal cord compared to healthy controls. The authors additionally describe that full-length ubiquitin localizes to the dorsal horn of human spinal cord, while the truncated form Ubc-T is evenly distributed throughout the grey matter, pointing to a region-specific defect of specific ubiquitin isoforms in ALS. This finding, specific to a distinct protein isoform in a distinct anatomical region, represents a major advance towards the understanding of potential ubiquitin pathology in the disease, and highlights the opportunities of MALDI-MSI investigations in neurodegenerative and psychiatric disorders.

4 Concluding remarks

Over the last decade, biomedical research into neurodegenerative and psychiatric disorders has gradually begun to

integrate MALDI-MSI into studies aiming at the targeted detection of proteins, lipids, neuropeptides, and small molecule therapeutics in human and animal CNS tissue. Our review of published studies indicates that the technique is still at an early stage in the neurosciences, and that its full potential remains to be realized for neurodegenerative and psychiatric disorders. Nevertheless, a small number of research groups have made considerable inroads into the investigation of disorders of the brain using MALDI-MSI, particularly in the fields of PD and AD. Our overview of the current literature highlights the opportunities for this novel technology in neuroscience research.

In the targeted studies summarized in this article investigating expression levels of selected proteins, lipids and drugs in human or animal brain, MALDI-MSI has provided a considerable methodological advantage over conventional quantitative techniques. Examining protein, lipid or drug distribution in intact tissues by immunohistochemistry or radiography for radiolabelled drugs are time consuming (additionally to their synthesis), require stereological counting of immunolabelled structures for proteins, and have a limited dynamic range. Further, metabolites of radiolabelled drugs might escape detection. Additionally, mass spectrometry can distinguish between metabolic fragments, conjugated proteins and post-translational modifications, an ability that immunological techniques generally do not possess.

While other state-of-the-art proteomic methods like LC-MS/MS of homogenized tissues can identify thousands of proteins and are capable to detect significant differential expression in hundreds of them, MALDI-MSI can only provide this for a couple of hundred proteins/peptides. However, being able to accurately access the proteome in a spatially resolved point of the investigated tissue is a major advantage, allowing identification and quantification of changes in miniscule, restricted parts of tissue/cells corresponding to disease. This stands in contrast to classical proteomic methods applying tissue homogenization, which can only detect gross changes, affecting large parts of the tissue which avoid normalization of expression changes by the unaffected parts of the sample. Although smaller affected areas can be sampled by e.g. laser micro-dissection, the resolution of this method is limited by the protein amount that can be extracted for subsequent analysis. Therefore, MALDI-MSI offers the unique chance to identify molecular mechanisms of changes in early stages of disease, where only small parts of tissue are affected, while avoiding protein/peptide losses due to extensive sample preparation applied in non in-situ methods. This renders MALDI-MSI a prime methodology to be used in neuroscience not only in a targeted fashion, but as a true –omics technology.

It is important to mention that there have been considerable barriers towards a more widespread use of MALDI-MSI in neurology and psychiatry. Firstly, the technology is expensive and extremely labour and computationally intense, thereby severely limiting experimental sample sizes that can be assessed. In the studies published to date, experimental groups routinely consist of one to five specimen, which

is hardly enough to serve as a representative model of the clinical population. Secondly, the technique requires considerable expert knowledge and access to state of the art MS instruments; both are not routinely available to biomedical researchers. Thirdly, as mentioned above, the depth of the coverage of the proteome reflecting disease is limited compared to other –omics technologies. As fourth consideration, MALDI-MSI investigations have focused on the analysis of fresh frozen brain tissue, which is however hard to preserve and is therefore difficult to obtain from human brain banks. Lastly, animal model systems can ultimately only be approximations of human neurodegenerative and psychiatric diseases in terms of their genetic complexity and subsequent consequences in terms of transcriptomics, peptidomics, proteomics, lipidomics and resulting morphological changes. Therefore, special care and critical consideration have to be taken into account when drawing conclusions from these models.

A major step towards the expansion of sample sizes and the increased use of post-mortem human tissue is the applicability of MALDI-MS to FFPE brain tissues for detection of differential protein expression by MALDI-MSI [22], which has long faced considerable technical difficulties. Proof of principle was demonstrated by Stauber et al., who investigated FFPE brain samples stored for 9 years at room temperature [22]. Specimens were dewaxed, and 10 μm sections were digested using trypsin and analysed. m/z features corresponding to morphological brain structures could be successfully extracted. Recently, this approach saw a major extension as access to metabolites in FFPE tissues was demonstrated by Buck et al. [101]. As the vast majority of human brain tissue specimen is stored by FFPE, this opens up medical research archives for the investigation by MALDI-MSI and dramatically increasing the number of samples available for analysis. Further, with the gradual increase in spatial resolution offered MALDI-MSI, which now are typically around 50 μm for peptides from on-tissue digests and down to 5 μm for analytes not requiring preliminary preparation, such as lipids, neuropeptides and drug compounds ([21]), single cell layers can be visualised and limited subcellular information produced.

In conclusion, we identified a range of studies using MALDI-MSI technology in the fields of AD, PD, schizophrenia, and ALS research, with other neurodegenerative and psychiatric disorders (such as depression) completely untapped. Whilst sample sizes have been small and the focus of many investigations has been on proving the technique's applicability to neurological and psychiatric research questions, this body of literature clearly demonstrates the potential of MS imaging for future research programmes. Applied to validated animal models of neurodegenerative and psychiatric disorders as well as to human samples, the technique should provide a much needed extension of the neuropathological toolbox.

Peter Hoffmann, Bernhard T. Baune, Klaus Oliver Schubert and Florian Weiland declare no conflict of interest.

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