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Advances and Applications of Mass Spectrometry Imaging in Neuroscience: An Overview

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Abstract : Understanding the chemical composition of the brain helps researchers comprehend various neurological processes effectively. Understanding of the fundamental pathological processes that underpin many neurodegenerative disorders has recently advanced thanks to the advent of innovative bioanalytical techniques that allow high sensitivity and specificity with chemical imaging at high resolution in tissues and cells. Mass spectrometry imaging [MSI] has become more common in biomedical research to map the spatial distribution of biomolecules in situ. The technique enables complete and untargeted delineation of the in-situ distribution characteristics of proteins, metabolites, lipids, and peptides. MSI's superior molecular specificity gives it a significant edge over traditional histochemical methods. Recent years have seen a significant increase in MSI, which is capable of simultaneously mapping the distribution of thousands of biomolecules in the tissue specimen at a high resolution and is otherwise beyond the scope of other molecular imaging techniques. This review aims to acquaint the reader with the MSI experimental workflow, significant recent advancements, and implementations of MSI techniques in visualizing the anatomical distribution of neurochemicals in the human brain in relation to various neurogenerative diseases.

Keywords : mass spectrometry imaging, neuroscience, neurodegenerative disorders, ambient mass spectrometry, Alzheimer's disease, Epilepsy, Parkinson's disease

Introduction

The extraordinary mechanism that directs the body's sensory activities is the human brain, which is regarded as the nervous system's control station. It is crucial for processing sensory data, producing hormones, regulating motor control, and preserving cognition and memory. The brain's chemical makeup and the participation of intricate molecular machinery with the spatial and temporal orientation of multiple kinds of neurotransmitters and other chemicals in it during cognitive disorders were revealed by remarkable advancements in neurochemical studies in recent years.[1] Multiple neurodegenerative disorders, such as Alzheimer's disease, Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis, are characterized by the buildup of misfolded proteins into toxic deposits inside or outside of cells as well as progressive neuronal degeneration.[2] The lack of biochemical tools with the required responsiveness, selectivity, and spatial and temporal resolution to characterize molecular mechanisms at cellular scales is a component that furthers our comprehension of the biological processes driving neurodegenerative diseases. Therefore, accurate molecular visualization of the human brain is critical to understanding biology and constructing effective diagnostic, predictive, therapeutic, and monitoring methods for various neuro disorders.[3] The advancement of modern healthcare imaging modalities like MRI, PET, computed tomography, as well as their variations, has had significant effects on brain analysis. Even though these methods have transformed medical imaging techniques since their inception, none of them can directly analyze the tissue at a sufficiently high resolution to reveal the cellular and molecular traits related to the subtype and severity of the disorder.[1] To fully comprehend molecular mechanisms at the subcellular level, chemical imaging modalities are pivotal bioanalytical techniques. *In vivo* and *in situ* spatial changes of different molecular targets could potentially be investigated using a variety of biological imaging modalities.[4] Specifically, immunohistochemistry and in situ hybridization, along with the employment of chemical probes[5] and spectroscopic techniques.[6,7] Nevertheless, maintaining a

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high degree of molecular adaptability, sensitivity, and precision while obtaining enough resolving power is a substantial issue.[4] Ex vivo tissue imaging methodologies have undoubtedly improved recently, providing strong channels for clinical research.[1] Many of these are anticipated to soon move from a discovery-based approach to normative clinical settings. An evolving label-free analytical technique, MSI can offer spatially distributed chemical data by detecting the mass-to-charge ratio of various molecular species throughout the tissue section.

Mass Spectrometry Imaging (MSI)

It has been shown that MSI is a highly effective tool for examining *in situ* the spatial variation of biological molecules. MSI enables label-free assessment without requiring any prior understanding of the possible target species, in contrast to other, more well-established biochemical imaging technology. Even though MSI assessments are untargeted, sample preparation can be customized for distinct classifications of the intended molecules of interest.[4]

MSI is an ex vivo molecular imaging modality that outlines significant biomolecules and increases comprehension of the biochemical events occurring in the tissue. In contrast to the traditional imaging modalities, which structurally image the brain, these methodologies do not involve imaging the brain. Many neurological illnesses exhibit molecular downregulation as a defining feature.[8-11] This opens new opportunities for management and therapy thanks to MSI studies of the biomarkers associated with such diseases. Analysis of tissue chemicals usually provides more quantifiable data on the disease, and this is especially useful when the morphological signifiers in conventional histopathology are uncertain.[1] It is not necessary to have prior information about the target species to employ the MS-targeted technique, in contrast to the traditional molecular visualization through chemical discoloration, immunostaining, fluorescent, and radiolabeling.[1] With the help of this MSI characteristic, researchers can examine the chemical components of tissues more thoroughly to comprehend the workings of various biochemical processes that are essential to living systems and spot any irregularities that may be connected to them.[1]

Another thing to keep in mind is that MSI looks at how chemicals are distributed on the tissue in space, which is noticeably different from the typical MS-based omics methodologies using concentrates of tissues or fluids.[12] By predicting the transition from the time-consuming LC-MS methodology to an effective and spatially resolved in situ strategy, Thus, MSI has opened new opportunities in the discipline of clinical MS. In fact, MSI has been shown to be highly useful in evaluating the distribution of biomarkers in the tissue section to evaluate the margins of malignancy.[13-16] Mass spectrometric (MS) data are captured pixel-by-pixel after scanning the specimen surface in 2D for imaging (Figure 1). Thus, a large amount of MS data is gathered across the sample surface, and chemical species' ion signals, whichever ones are found, can be incorporated with their spatial data to create their ion images.[17] Given that each ion signal can be spatially mapped, MSI can produce a huge collection of molecular images during a single scan. As a result, the spread of numerous endogenous and foreign species in the tissue specimen is continuously visualized, which can help with the identification of biomarkers, the diagnosis of diseases, the emergence of novel therapies, and pharmaceutical research.[18]

Typically, two parameters are prioritized: (i) spatial resolution and (ii) m/z span, for evaluating the effectiveness and appropriateness of MSI for the analysis of diverse

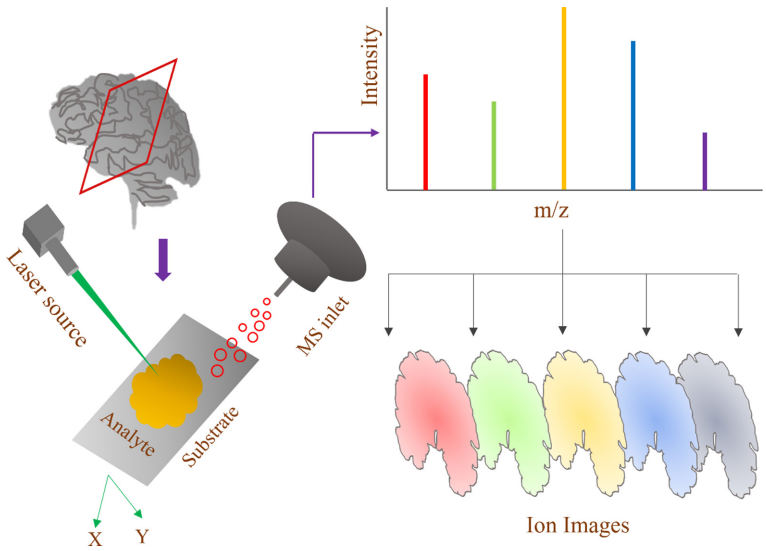

Figure 1. Schematic illustration of a typical MSI workflow.

Table 1. Empirical studies on MSI in neuroscience.

Domain	Ionization	Authors	Key metabolites	Metabolic pathway	MS analyzer
pharmacokinetics and pharmacodynamics	AFADESI-MSI	Liu et al. (2022)	adenosine, histamine, GABA, taurine, glutamine	drug metabolism	Q-orbitrap
Alzheimer's disease	MALDI-TIMS-MSI	Michno et al. (2021)	lysophosphatidicacid,glycerolipids, glycerophospholipids, phosphatidylcholine, sphingolipids,phosphoinositol	lipid metabolism	TIMS-TOF
Quantification of Dopamine	nano-SIMS	Rabasco et al.(2021)	NA	drug metabolism	NR
Neuroinflammation & N-glycosylation	MALDI-MSI	Rebelo et al. (2021)	sialylated and fucosylated structures	N-glycosylation	Q-TOF
Alzheimer Plaque Heterogeneity	MALDI-MSI	Wehrli et al. (2022)	lipids and peptides	NA	TOF
Glioblastoma multiforme	MALDI-TOF-MSI	Kampa et al. (2020)	lactate, glutamine, N-acetylaspartate, taurine, ascorbic acid	Purine & pyrmidine metabolism	TOF
N-Glycosylation	MALDI-MSI	Heijs et al. (2020)	N-glycans	N-glycosylation	QTOF
Temporal Lobe Epilepsy	DESI-MSI	Ajith et al. (2021)	phosphatidylcholine, phosphatidyl- ethanolamine	Lipid metabolism	Ion-trap
Glioblastoma tumor	MALDI-FTICR-MSI	O'Neill et al. (2022)	Cardiolipins, phosphatidylinositol, ceramide-1-phosphate, and gangliosides	Lipid metabolism	FT-ICR
L-DOPA, Parkinson's disease	MALDI-MSI	Fridjonsdottir et al. (2021)	L-DOPA, 3-o-methylDOPA	L-DOPA metabolism	FT-ICR
Alzheimer's disease	MALDI-MSI	Strnad et al. (2020)	gangliosides,phosphatidylinositols	Lipid metabolism	TOF
blood brain barrier & antipsychotic drug	MALDI-qMSI	Luptakova et al. (2021)	risperidone, clozapine, and olanzapine	drug metabolism	FT-ICR
glycoproteomics & N-glycosylation	MALDI-MSI	Malaker et al. (2022)	HexNAc4-Hex5-NeuAc2	N-glycosylation	TOF
Neurotransmitters	LDI-MSI	McLaughlin et al. (2020)	acetylcholine, dopamine, epinephrine, glutamine, norepinephrine, and serotonin	NA	TOF
Parkinsons diesease- neuropeptides alteration	MALDI-MSI	Hulme et al. (2020)	dynorphins, enkephalins, tachykinins, and neurotensin	NA	TOF & FT-ICR
glioblastoma	MALDI-MSI	Duhamel et al. (2022)	protein biomarkers	NA	TOF
Ganglioside metabolism -Alzheimers disease	MALDI-MSI	Kaya et al. (2020)	monosialogangliosides	Ganglioside metabolism	TOF
Amyloid plaque-Alzheimer's disease	MALDI-MSI	Kaya et al. (2020)	galactosylceramides, phopshatidylethanolamines, sulfatides, lysophosphatidylethanolamines, lysophosphatidylcholines	sphingolipid metabolism	TOF
Amyloid plaque-Alzheimer's disease	MALDI-MSI	Michno et al. (2018)	lysophospholipids, phospholipid	lipid metabolism	TOF
Amyloid plaque-Alzheimer's disease	MALDI-TIMS-MSI	Michno et al. (2021)	gangliosides, phosphoinositols, phosphoethanolamines, phosphatidic acids, sulfatides , cardiolipins	lipid metabolism	TIMS-TOF
Glioblastoma	MALDI-MSI	Panitz et al. (2021)	N-formylkynurenine, kynurenine, hydroxy-tryptophan, anthranilic acid, tryptophan	Tryptophan metabolism	FT-ICR
Chlolestrol brain tissue imaging	DESI-MSI & MALDI-DESI	Angelini et al. (2021)	Cholestrol	NA	QTOF, L-TOF & Orbitrap

NA – not applicable; NR – not reported; TOF – time of flight; FT-ICR – fourier transform ion cyclotron resonance; QTOF – quadrupole time of flight; TIMS-TOF – trapping ion mobility spectrometry time of flight

molecular species in tissues. Both variables are influenced by the desorption technique, which includes the probe's size, the design of the mass detector, and most notably, the sample preparation.[4] According to McDonnell and Heeren (2007), the various ionization techniques used in MSI are each distinguished by complementary advantages and disadvantages that primarily relate to spatial resolution, mass accuracy and resolution, chemical sensitivity, specificity, and m/z span.[19] The sample ionization is essential for transferring the analyte from the condensed to the gas phase when analyzing gaseous ionic species in a MS.[4] The efficiency and molecular coverage of the various techniques that could be used in MSI studies can vary.[4]

The most popular clinical MSI techniques are [SIMS] secondary ion mass spectrometry imaging, [DESI-MSI] desorption electrospray ionization, and [MALDI-MSI] matrix-assisted laser desorption ionization, which inherently vary in ionization.[20] The type of molecular data that each of these various MSI methods gathers and their spatial resolution are different. As a result, the MSI should be selected according to the application.[20] Although MALDI-MSI is the MSI technique that is most frequently used in clinical research, [21] recent advancements in DESI-MSI also look promising for use in neurological research. This review aims to inform the reader about the MSI workflow, significant recent advancements, and application of MSI techniques in visualizing the anatomical distribution of chemicals in the human brain in relation to various neurodegenerative diseases. Table 1 lists empirical studies, the neuro-domain, key metabolites, metabolic pathways, the ionization tools, and the MS-Analyzer.

Sample Preparation

Between collecting samples to surface pretreatment before analysis, there are numerous aspects of MSI sample preparation that must be considered. The most important factors affecting the integrity of the data for all MSI research are tissue extraction from human or animal sources and tissue preservation.[4] To prevent molecular breakdown and spatial rearrangement, tissue must be treated properly and promptly following surgical removal.[22] Although fresh-frozen tissue is the most frequently utilized type, MSI may evaluate both chemically preserved tissue, including FFPE fixed, and fresh-frozen tissue. This is primarily because MS-based analyses do not readily accommodate chemical infusion and fixation techniques since these polymeric fixations and embedding agents compete with MS detection. Nevertheless, recent research has demonstrated that lipids, peptides, and even metabolites can be successfully detected in FFPE-fixed specimens,[23-25] which is creating enormous opportunities for MALDI MSI-based histology studies on sizable unhealthy specimen sizes that are accessible through tissue banks.[26-27] For MSI, fresh-frozen tissue continues to be the most frequently used method for molecular evaluation of ex vivo tissue specimens.[4] Rapid freezing is essential for maintaining protein functionality at the cellular and subcellular levels as well as overall tissue architecture, which would otherwise be affected by cell wall ripping. This causes water to solidify into an amorphous, vitreous state as opposed to a crystalline structure.[4] The most rapid rate of cooling is theoretically possible when dissected tissues are flash frozen in liquid nitrogen, which increases the amount of tissue that comes in contact with the cooling agent.[4] Nevertheless, inconsistent freezing may occur if a vapor barrier forms on the outermost layer of warm tissue. For larger samples, such as complete rat brains, liquid nitrogen cooled isopentane is also utilized, as well as occasionally dry ice cooled isopentane.[4]

For MSI analysis, thin, flat substrates are necessary. On a microtome, specimens are often prepared by cryo-sectioning, where the temperature of sectioning varies according to the kind of tissue (fatty versus water-based tissues). For comprehensive tissue imaging, the separated tissue slices can be put directly on a conductive surface. The sample stretching method is an alternative way. The tissue slice is placed in this instance onto glass beads imbedded in parafilm, stretched uniformly in two dimensions, and the parafilm is stabilized by being fastened to a glass slide. Without having to worry about diffusion, the stretching approach enhances spatial resolution and analyte extraction.[28,29] Following the mounting of the tissue segment onto a target in either approach, the sample may need to be further prepared. Depending on the MS type, ionization technique, and desired molecule kind, one may use washing processes, enzymatic digestion, or matrix deposition. If the target molecules are lipids or salts, washing is not advised because these molecules are frequently destroyed by typical washing solvents. Prior to protein or peptide matrix deposition, more than one wash procedure is advised.[30] The matrix can homogeneously crystallize when salts that might react with it are removed, which improves the ionization efficiency of peptides and proteins. Sophisticated multistep rinsing techniques are frequently used before applying the matrix for neuropeptide MSI. These involve organic and aqueous washes with pH optimization that are meant to precipitate peptides and proteins, removal of lipids, and wash away ions that can obstruct MSI signal.[31-33] Nevertheless, harsh cleaning techniques might cause damage to the sample and eliminate peptides of analytical interest. To determine the appropriate sample processing protocol to apply for each sample type, it is advised to optimize the matrix and washing operations on neighboring slices. There is normally no sample pretreatment for DESI-MSI.

Matrix deposition needs to be uniform, repeatable, sensitive enough, and simple to utilize. The maximal spatial resolution that may be achieved is also determined by the size of the matrix crystals. For biological samples, matrix deposition can be accomplished with or without earlier cleans-

ing processes.[30] For protein and peptide analysis, commonly used matrices are 2,5-dihydroxy-benzoic acid (DHB),[22] sinapinic acid (SA),[34] 2,5-dihydroxy acetophenone (2,5-DHA)[35] and 4-hydroxy-alpha-cyano-cinnaminic acid (HCCA).[36] DHB-based MALDI IMS has been proven to be a dependable method for robust and repeatable neuropeptide imaging.[32] The crystal size, which affects spatial resolution and necessitates careful tuning, is a characteristic shared by all matrices. A few matrices, such as DHB, also produce bigger crystals than others. However, this problem can be managed with the help of ideal solvent settings and optimum nebulizer configuration parameters. According to previous studies,[37,38] very small crystal sizes are produced by dry matrix application techniques such dry coating and sublimation. While this enables MSI at high spatial resolution, there are restrictions because these techniques often exhibit insufficient analyte extraction efficiency and limited applicability for protein imaging. The type of application sought determines the selection of the matrix deposition. For big samples and high-throughput research, automated procedures are preferable. Matrix deposition techniques are the subject of intensive research because they could become the bottleneck for emerging high-throughput biomedical experiments.

Data Evaluation

The use of appropriate programs for investigating data is a key component of the MSI process due to the enormous amount of detail of MSI information sets. MSI data sets can be examined for fair classification using multivariate analysis (MVA) methods.[39,40] Essentially, this method views every single pixel of the spectra as an independent sample in a multivariate space, and it employs multivariate statistics to determine the predominant variation and co-variation, accordingly, which are then included in the corresponding component of the analysis. The factors, i.e., mass peaks and their magnitude, that are most important for capturing the variation in the component are extracted from the relevant loading numbers.

A popular multivariate data analysis approach that is uncomplicated to employ with MSI datasets is principal component analysis "PCA".[41,42] For MSI, PCA, an autonomous technique, is utilized for separating sets of variables with strong correlations, such as spatial coordinates and mass. Unlike PCA, which may have non-negative elements, probabilistic latent semantic analysis (pLSA), also known as digital staining, decomposes imaging data into their non-negative components after applying noise reduction and a computerized tissue categorization method.[43,44] MSI data acquired via various ways can be correlated using PCA in conjunction with canonical correlation analysis (CCA). Through the correlation of the spatial and spectral elements from every data set, the technique produces more tailored outcomes.

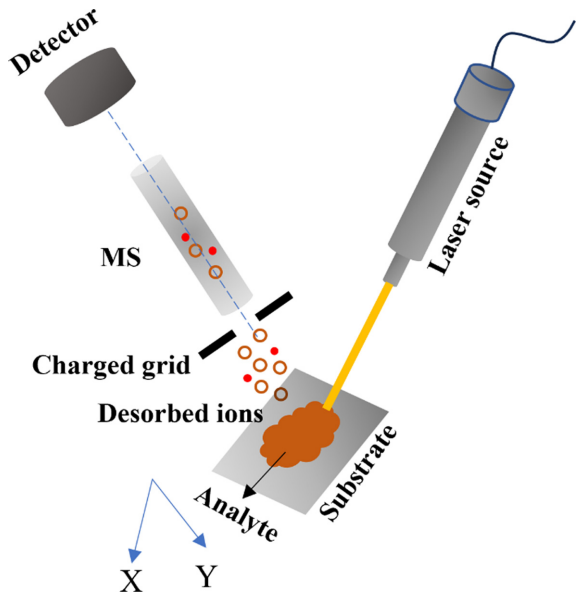

Figure 2. Illustration of MALDI ionization source

MSI Ionization

MALDI-MSI

In MALDI (Figure 2), the specimen spot on the target plate that has been blended with a matrix material is illuminated by a laser pulse.[20] The result is a hot gaseous plume where the analytes are ionized by the protonation or deprotonation procedure. This creates ablation and desorption of the from the solid substance.[20] The analytes should either be extracted into the matrix or relatively close to the matrix for efficient desorption and ionization to take place. The chemical composition of the mixture and how it is distributed throughout the sample determine which analytes can be detected.[20] The imaging investigation is carried out by uniformly applying the intended matrix to the specimen surface, which makes it easier for charged ions to dissipate from the tissue.[1] The spatial resolution of MALDI-MSI varies from 5 to 50 μm, depending on the matrix composition, laser beam, and the dispersion of analytes in the sample.[1]

DESI-MSI

The DESI-MSI technique visualizes small molecules (metabolites, lipids, drugs, etc.) in tissues that have been resected[45] or unresected[46] in a living system. DESI was created in Professor Graham Cooks' lab in 2004 (Figure 3).[45] As DESI-MSI can operate in ambient conditions and requires minimal prior sample preparation, it is superior to all counterpart ionization techniques (e.g., MALDI, SIMS, etc.). The method, unlike MALDI or SIMS, is also simple to operate because it only needs a cheap solvent spray and doesn't need a vacuum environment for ionization. The DESI probe can instantly capture molecular fingerprints from live organs, tissues, etc.[45] The DESI-MSI is typically

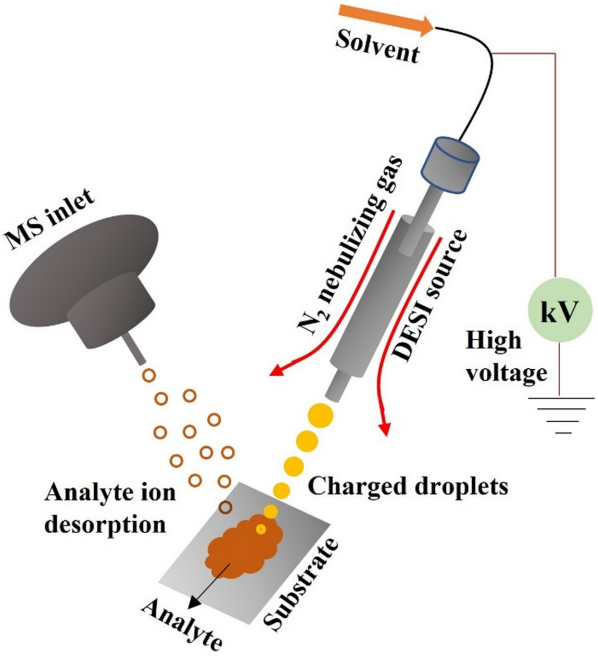

Figure 3. Illustration of DESI ionization source.

carried out on a tissue section with a thickness of about 10-15 μm. To electrospray a solvent (such as water, methanol, acetonitrile, dimethylformamide, etc.) at high voltage while using nitrogen as the nebulizing gas, this tissue section was subjected to a stream of fast moving charged microdroplets.[46,47] The droplet of solvent wets the tissue surface as a result, dissolving the biochemical species (mostly metabolites and lipids) that are present in the tissue. The mechanism of the desolvation process for generating ionic species in the gas phase is identical to that of the ionization by electrospray process, involving solvent evaporation and Coulomb fission of the secondary microdroplets that contain the analyte species.[46] The bioimaging experiment collects mass spectra across the sample and scans the tissue surface in the x and y directions while bombarded by a spray of charged microdroplets. The two-dimensional image created from the pixel-to-pixel MS spectra can be employed to map the tissue's biochemical composition.[48]

SIMS-MSI

A primary ion beam that is intensely focused is employed in SIMS imaging to strike the specimen surface and dislodge secondary ions. The sample is ionized in a vacuum environment, and the desorbed ion flight paths are transmitted to the mass analyzer for analysis by being guided via ion optics.[1] Due to the possibility that the primary ion pulse may cause molecular dissociation, the detection of SIMS is restricted to ions below *m/z* 1500.[3] In fact, SIMS is a destructive technique for analyzing surfaces, and one way to minimize sample surface destruction is to minimize the ion strength. SIMS analysis performed below the static threshold is referred to as static SIMS, and analysis performed above the threshold is dynamic SIMS.[3] Static SIMS can be utilized to identify atoms, lipids, peptides, and metabolites despite having a low ionization performance.[3] Consequently, in SIMS imaging studies, a trade-off between sample deterioration and responsiveness is pivotal.

Mass Spectrometry Imaging (MSI) in Neuroscience Research

Alzheimer's Disease

Alzheimer's disease (AD) is characterized by the formation of amyloid plaques, which are a key factor in the pathogenesis of the disease. It appears that the development of plaques is predominantly associated with cognitive impairment and the pathogenesis of AD, as evidenced by the fact that Aβ deposits have diffuse morphological characteristics in patients who express amyloid pathology but maintain cognitive normality.[49] In the brain, Aβ oligomers accumulate to form diffuse and neuritic plaques, which have been associated with neuronal dysfunction and synaptic loss.[50,51] The degradation of oligodendrocytes and lipid-rich myelin sheath observed in the brains of AD patient groups and transgenic AD mouse models may be impeded by this process, according to emerging research.[52-54] As a result, the relationship between intracellular lipid deposits, lipid biochemistry, and myelin disruption in relation to amyloid pathology in AD pathogenesis has received considerable attention.[55-60]

In their research on lipid alterations in double mutant mice, a model of ADs like pathology, Strnad et al. examined paraformaldehyde-fixed free-floating segments for the measurement of lipids in mice's brains.[61] The research additionally explored liraglutide, a type 2 diabetes medication, and palm11-PrRP31, an anorexigenic and glucose-lowering variant of prolactin-releasing peptide.[61] In numerous mouse models of dementia, liraglutide, a stimulant of the incretin

hormone glucagon-like peptide 1 (GLP-1)[62], has demonstrated neuroprotective benefits.[64-67] The hormone prolactin releasing peptide [PrRP] controls how much food is consumed and how much energy is expended.[68] Natural PrRP has no core effects following peripheral delivery because it cannot pass the blood-brain barrier. Nevertheless, following peripheral injection, lipidated PrRP analogs, such as palm11-PrRP31, showed anorexigenic actions and decreased glucose concentrations in mice and rats.[69-72] Researchers showed that free-floating segments may be employed for the MSI of lipids and that lipid images could be acquired without any disruption or delocalization.[61] Findings revealed that, in comparison to controls, the spread of different lipids had changed.[61] In areas where senile plaques accumulated, gangliosides and phosphatidylinositols saw the most notable lipid alterations in mouse brains in contrast to wild-type specimens.[61] In addition,

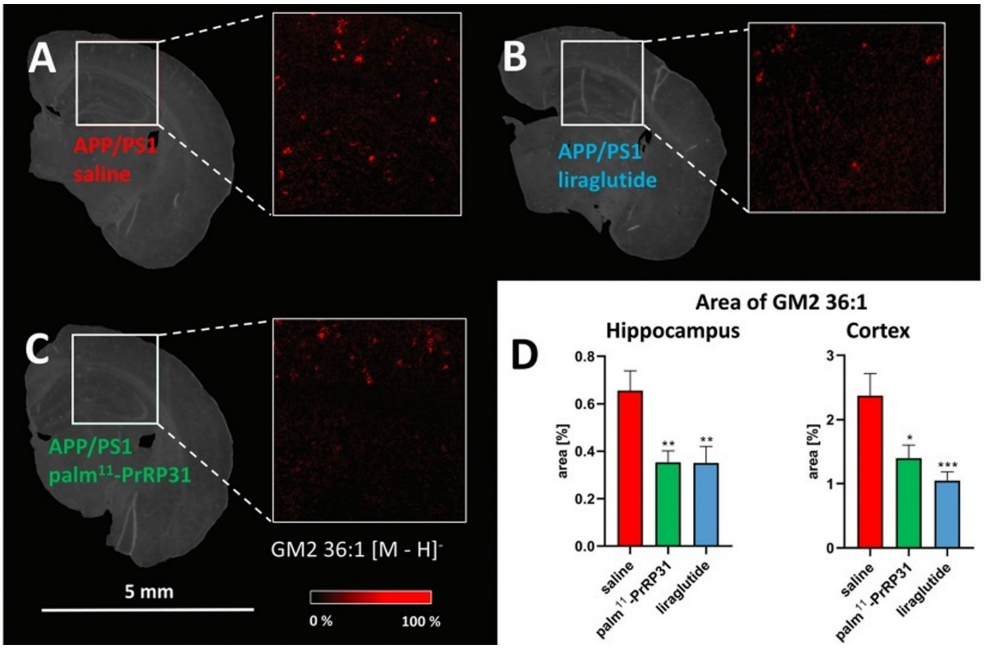

Figure 4. MALDI MSI analysis of 8-month-old APP/PS1 mice treated with (A) saline, (B) liraglutide or (C) palm11-PrRP31. (A, B, C) Optical image of coronal brain tissue section of APP/PS1 model and treated models with measurement regions. Ion images of ganglioside (GM2 36:1, m/z 1382.7) were obtained in negative ion mode ([M − H]−) at spatial resolution 15 μm. (D) Data are mean ± SEM, n = 4 (5–6 sections per one mouse). Statistical analysis was performed with Student t-test. Significance is *P < 0.05, **P < 0.01. (Reproduced with permission from Strnad et al., 2020, RSC).

studies on mice given a two-month period of treatment with palm11-PrRP31 or liraglutide revealed that both peptides decreased the volume and surface area populated by lipids, which were associated with senile plaques.[61] According to these findings (Figure 4), both liraglutide and palm11-PrRP31 is perhaps successful in treating neurodegenerative disorders.

Determining the etiology of AD needs molecular evaluation of how important pathogenic components, particularly A plaques, affect the local microenvironment.[73] Neuronal lipids are of special importance in this context since they are connected to pathogenic and neurodegenerative events.[73] However, the precise molecular properties of the cellular medium immediately surrounding Aβ plaques are still unknown, in part because of the elevated molecular intricacies of lipid types and in part because analytical methods lack spatial resolution, sensitivity, and specificity.[73] Additionally, it has been difficult to determine whether such micro environmental changes vary between structurally polymorphic Aβ characteristics, such as disperse, undeveloped, and mature fibrillary components. This has required complementary, multidimensional imaging techniques.[73]

Considering the intricacies of the various lipid categories, the capacity of MSI has been substantially enhanced because of the addition of ion mobility to the MS system, which permits further separation of structurally comparable and even isomeric substances.[74-78] In a recent work, Michno et al. used MALDI-TIMS (trapped ion mobility spectrometry)-TOF in conjunction with hyperspectral imaging to examine the lipidomic microenvironment correlated to the structural polymorphism of Aβ deposit in mutant mouse models of AD. Analysis of multivariate imaging information showed that several lipid species had been altered, with overall enrichment or depletion patterns associated with Aβ.[73] A distinctive spread of phosphatidicacid, phophoethanolamine, and phosphoinositol lipids to a greater or lesser extent clustered Aβ fibrillary structures occurring inside individual A plaques at various timepoints of developing plaque disease was further characterized by the hyperspectral imaging method (Figure 4). The researchers also discovered a distinct cardiolipin deficiency that would not have been effectively resolved in a conventional MALDI-TOF configuration.[73] The investigation additionally revealed that increased phosphoinositol buildup is a hallmark of plaque progression.[73] Collectively, these findings show how multidimensional imaging techniques have the capacity to overcome the drawbacks of traditional MS imaging techniques.[73] This enabled the linkage of both unique lipid elements to disease related amyloid plaque polymorphs in addition to their variation in an intricate microenvironment.

In another investigation, MSI-based spatial lipid analysis of AD plaque pathogenesis targeting TIMS-MSI was used.[79] Hyperspectral microscopy information obtained

from conformation-sensitive amyloid probes recorded on identical tissues is merged with the imaging modalities. The unique aspect of the present study is how thoroughly the multidimensional imaging information was included into the multivariate data processing workstream to recognize and delineate the structurally various amyloid shapes inside each deposit and the accompanying lipid trends, respectively.[79] Gangliosides were discovered to be localized specifically to plaques at the overall plaque threshold by TIMS MSI, whereas cardiolipin, phosphoserines, and sulfatides were reported to be reduced at plaque areas. Cardiolipin deficiency associated with plaques is probably a side effect of neuronal degeneration. Therefore, CL reduction suggests neuronal degeneration, probably brought on by oxidative stress on the mitochondria.[80] Furthermore, due to the phosphatidic acids and phosphoethanolamines' core-specific localizations, it was discovered that sulfatides, ST (d36:1), had become elevated at the plaque's exterior edge.[79] The researchers evaluated the top loadings utilizing a univariate evaluation of their portioning ratio between central and peripheral among the two age groups to gauge comparative variations in spatial lipid distribution.[79] However, a key finding for the age assessments was a very static pattern of distributions of lipids linked with plaque architecture that progressed with age in plaque disease. This shows that Aβ absorption, and plaque growth have plateaued for many plaques.[79]

In a recent work, Wehrli et al. utilized a spatial chemometrics technique to analyze multidimensional MSI and microscopy information thoroughly to investigate molecular histopathology in complicated tissue samples.[81] Effective data mining and programmed image authentication are used in the workflow, enabling multivariate statistical simulation of chemical parameters across several modalities while minimizing human bias.[81] The study's use of the technology to identify chemical characteristics of AD pathology illustrated the method's promise.[81] The spatial distribution variations in lipids and proteins related to A dysfunction in mutant AD mice were examined by trimodal MALDI-MSI and hyperspectral fluorescence microscopy.[81] Using multiblock complementary component assessment and unattended cross-modal modality chemometrics simulation, it was possible to identify covariance structures and particular chemical deviations during the acquired image resolution, which was otherwise impossible to do using conventional techniques.[81] The provided methodologies lay the foundation for correlative molecular imaging, which will help researchers comprehend how the underlying biochemistry interacts with a multidimensional methodology.[81]

Brain Tumor

The WHO (World Health Organization) classifies glioblastoma multiforme (GBM), the most prevalent major cancerous brain tumor in adults, as a level 4 neoplasm.[82] The outlook for treatment for glioblastoma is poor despite surgery and treatment efforts, with a typical survival rate of up to fifteen months after diagnosis.[83] The intratumor variability in glioblastoma is blamed for the treatment intervention's failure. There are few therapy options for glioblastomas since they are difficult to completely remove and become resilient to modern therapeutic regimens,[84] which results in an elevated likelihood of relapse. High intratumoral[85] and intertumoral variability,[86,87] glioblastoma cell malleability,[88,89] and an extremely immune-suppressive tumor microenvironment (TME)[90] made up of phagocytes, the microglia, and MDSCs[90-93] all influences on the high resistance to therapies of this cancer entity. To produce medicines that may alleviate illness, it is crucial to understand the glioblastoma cellular diversity inside the TME.

In glioblastoma as well as other tumor types, tryptophan (Trp) metabolism plays a significant role in regulating therapeutic resistance.[94] Tryptophan catabolic enzymes (TCEs) have been linked to the development of tumors and immunodeficiency through two different processes. a) Trp degradation can, deprive cells and their surroundings of this vital amino acid, causing immunosuppressive outcomes in the TME, and b) the breakdown of Trp results in the production of bioactive secondary metabolites. AHR, a ligand-activated transcription factor that stimulates the replication of AHR target genes, can be triggered by several metabolites, including kynurenine (Kyn) and kynurenic acid.[95] High tryptophan-2,3-dioxygenase (TDO2) translation in glioblastomas causes the synthesis of Kyn, which stimulates AHR and increases cancer cell mobility while reducing immune cell growth and functionality in the TME.[96] Therefore, a deeper comprehension of Trp breakdown in glioblastoma is necessary to combat therapeutic resistance and identify the patients who will respond best to therapies that focus on TCEs and AHR.

Panitz et al. used MALDI-MSI to investigate tryptophan breakdown in the tumor and blood of individuals with glioblastoma multiforme.[97] Glioblastoma patients have reduced serum Trp levels than the general population. Interestingly, there was a decrease in Trp concentrations of metabolites as well. Increased enzymatic interactions between Trp and its metabolites resulted in fewer drops, indicating that Trp supply regulates the concentrations of its systemic metabolites.[97] Poorer life expectancy correlates with elevated tumor size, reduced systematic metabolite levels, and reduced systemic kynurenine concentrations.[97] Trp degradation was shown to be heterogeneous among glioma tissues by MALDI-MSI. The investigation of scRNA-seq data showed that practically every kind of cell in glioblastoma displayed genes involved in Trp metabolism, and that numerous kinds of cells, particularly macrophages and T cells, had AHR stimulation.[97] In the glioblastoma TCGA data, increased AHR activity was also linked to a lower life expectancy rate.

Glioblastoma has only been partially subclassified at the

epigenetic, transcriptomic, and genomic stages. The categorization of brain malignancies based on DNA methylation has in the meantime developed into a thorough machine learning techniques that has influenced the current WHO classification and led to the identification of additional unusual methylation categories of glioblastoma.[98] Even though they can detect and assess the results of modified genomes and transcriptomics and might more accurately describe the triggering of pathways,[99-101] proteomic techniques have not been studied as frequently. To determine proteomic variations between grades and genetic changes, proteomic investigations of gliomas have been carried out.[102] In more recent times, categorizing glioblastoma patients using proteogenomic methods has shown a greater correlation between protein expression and the survival of patients than RNA transcripts.[103] Glioblastomas are very diverse tumors; however, a spatially detailed proteomics method may offer fresh perspectives on the biology of glioblastomas to enhance categorization. The identification of proteomic markers may enhance the ability to distinguish between the many glioblastoma subgroups and provide treatment guidance.[104]

Duhamel et al. investigated a spatially defined proteomic method. 96 glioblastoma patients with various odds of surviving were evaluated as a group.[104] The molecular variability among these tumors was highlighted by a non-targeted MALDI-MSI investigation accompanied by spatial division using several techniques.[104] Contrary to the malignancies of patients with short and moderate survival, RPS14 and PPP1R12A have been detected at greater concentrations in cancer patients with a longer survival rate. Relative to patients with prolonged survival, tumors from patients with short and moderate survival had higher levels of ANXA11 expression.[104] For ALCAM, no statistically significant variations in expression were found, as was the case for the first batch of patients.[104] Except for ALCAM, these findings support the usefulness of the discovered predictive indicators. The study's discovery of proteomic markers illustrates the intratumoral molecular variability of glioblastoma tumors.[104] Although these symptoms have been correlated with survival in other studies, the findings of the current investigation revealed that multiple instances of these characteristics can be found in a single tumor, precluding their application as predictive biomarkers.[104] Considering this great variability, researchers have demonstrated that, with confirmation in a separate patient group, several common indicators might be discovered for cancer patients with a shorter lifespan and, conversely, for tumors of individuals with more prolonged survival.[104]

Lipids are essential governing chemicals with functions involving proliferation,[105] differentiation,[106] and apoptosis.[107] They are closely related to cell survival through both direct and secondary signaling processes. Consequently, it is not unusual that changes in lipid breakdown and cellular composition have been connected to cancer cell progression, aggressiveness, metastasis, and treatment resistance. Previous studies have demonstrated that GSCs have elevated amounts of long-chain polyunsaturated fatty acids (PUFA) that enhance oncogenic EGFR signaling.[108] In glioblastoma, the sphingolipid rheostat, a factor in cell fate determination, is also disrupted, which results in a decrease in growth inhibition routes and a spike in growth pathways.[109] Several of these irregular lipid pathways are thought to be conceivable targets for treatment.[110]

It is critical to have a greater comprehension of the aberrant lipidomic profiles inside the glioblastoma TME to fully utilize these cutting-edge therapies and create a treatment plan that enhances clinical effectiveness. In clinical glioma specimens the spatial distribution of lipids had already been examined using MSI.[111-113] These investigations have concentrated on the differentiation among various anatomical slices of tissue, such as tumor vs. stroma,[114,115] and alterations between glioblastoma and less severe gliomas based on lipid profiles.[111] In a recent study, high resolution MALDI-FTICR-MSI was utilized to identify the various kinds of microvascular forms and the initial intratumoral lipid variability among cancer cell subdivisions.[116] Studies have shown that the glioblastoma stem cell indicator, GD3, as well as cardiolipins, phosphatidylinositol, ceramide-1-phosphate, and gangliosides, differentially aggregate in cancer and endothelial cell subsections. Sphingomyelins and sulfatides, on the other hand, were shown (Figure 5) to be decreased in cancerous cell areas.[116] Recognizing the links between lipid structure as well as function is crucial since the makeup of every lipid category fatty acid residue determined how much of each category's cellular aggregation o occurs.[116] The identification and correlation of distinguishing ions with histopathological information. The results of this study revealed several lipids in the glioblastoma TME that call for additional research to provide biomarkers for prediction and lipid-based treatments.[116]

Epilepsy

Approximately 45 million individuals globally suffer from epilepsy, a neurological condition with definite signs, an origin, and a likely outcome.[117] The most common kind of epilepsy, known as temporal lobe epilepsy [TLE], affects roughly 20% of all diagnosed epilepsy occurrences.[118] TLE, which is frequently accompanied by hippocampal neurodegeneration. TLE is anticipated to disturb lipid homeostasis, just like various other neurological disorders. Unprovoked repetitive focal epileptic seizures occur often in the temporal lobe, which causes a variety of clinical symptoms.[119] TLE is anticipated to disturb lipid homeostasis, just like other neurological disorders. The etiology of TLE has been associated with a wide range of potential causes, involving hereditary, physiological, and biochemical elements.[120] The bulk of TLE instances still have unidentified reasons.

According to current trends in epilepsy research, the origin and evolution of various epilepsies may be attributed to

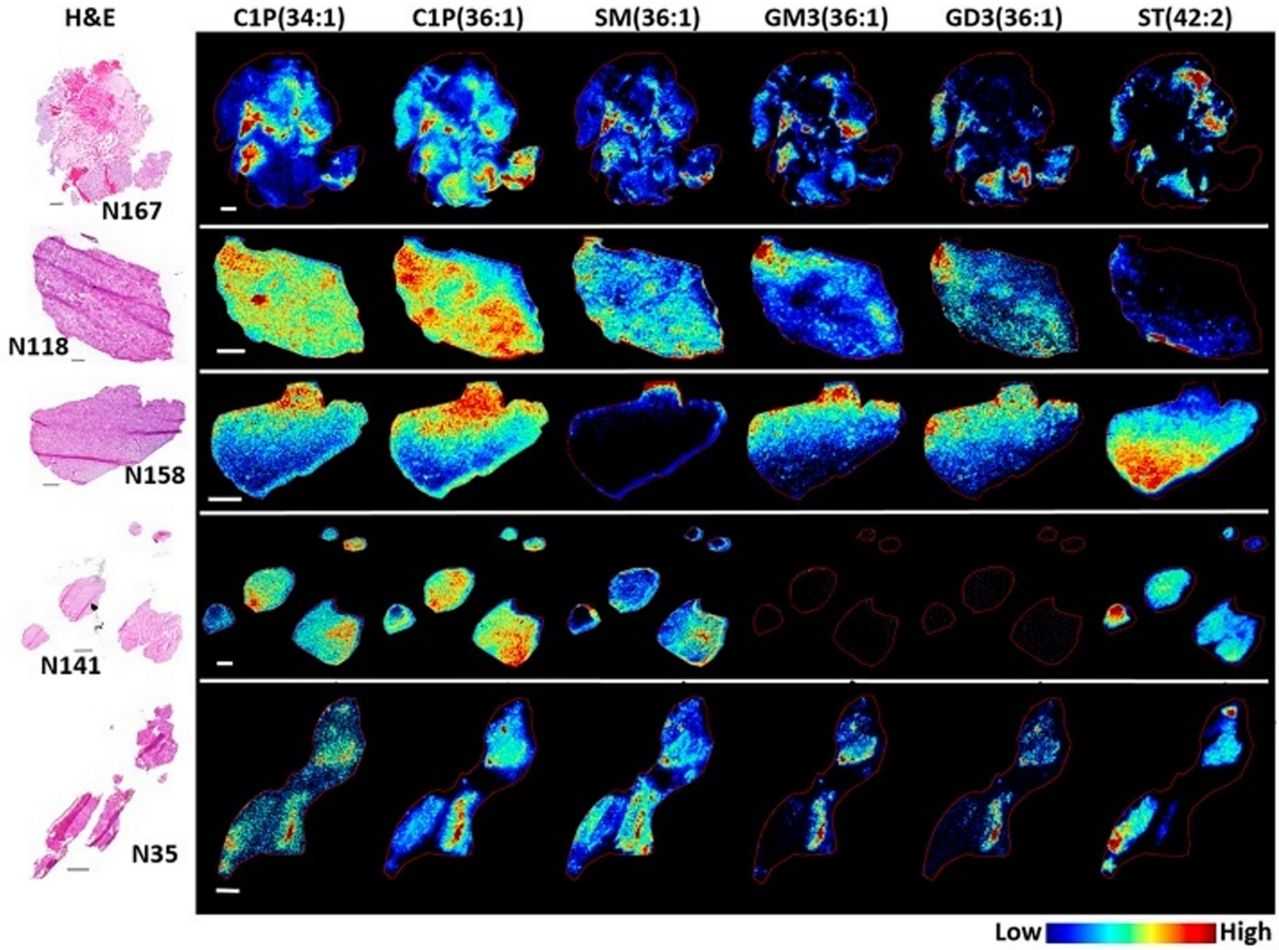

Figure 5. Representative MS images for several sphingolipid species. Sphingolipid subclasses include ceramide1-phosphate (C1P), sphingomyelin (SM), mono- and di-sialodihexosylganglioside (GM3 and GD3 respectively) and sufatide (ST). C1P(34:1) and (36:1) correspond to m/z 616.471 and 644.502, respectively. SM(36:1) corresponds to m/z 715.578. GM3(36:1) and GD3(36:1) correspond to m/z 1179.741 and 1470.830, respectively. ST (42:2) corresponds to m/z 888.624. Images were acquired at 30 μm pixel resolution from 5 μm thick sections; the H&E stained section of each sample is shown in the lef panel. Scale bars are 1 mm. [Reproduced with permission from O'Neill et al. (2020), Nature Portfolio].

a series of biochemical happenings. There is an increase in the levels of excitatory amino acids, which is followed by excessive stimulation of NMDA receptors, an elevation in calcium entry into neurons, and the participation of pro-inflammatory processes.[121-123] The precise pathogenic pathways causing this severe pathological condition are not completely comprehended.[123] In contrast to individuals with other temporal lobe lesions, TLE-HS (TLE- hippocampal sclerosis) individuals' human hippocampi exhibited a different lipid composition with lower triglyceride concentrations.[124] As a result, discovering lipids and the processes of metabolism that link to them in the initiation and development of various neurological illnesses opens up new opportunities for creating new therapeutic approaches and indicators for improved outcomes as well as early disease identification.[125]

By analyzing the spatially defined tissue metabolome, DESI-MSI has been widely employed to identify various cancers.[126] Its therapeutic applications for lipidomics assessment have been supported by numerous investigations over the last ten years.[127,128] Ajith et al. used DESI-MSI in a recent study to examine the metabolic alterations in the hippocampus of TLE patients in contrast to those in non-TLE patients.[129] 39 fresh frozen surgical tissues of the human hippocampus were examined using DESI-MSI to determine the lipid compositions of the experimental populations with TLE-HS (n = 14) and control subjects (n = 25) (Figure 6).[129] In comparison to various other investigations on animal models of epilepsy, the human TLE-HS displayed decreased levels of a number of significant lipids, especially phosphatidylcholine (PC) and phosphatidylethanolamine (PE).[129] The Kennedy pathway may have been

Figure 6. Representative positive ion mode DESI-MS images showing the spatial distribution of 27 lipid species in the human hippocampal section obtained from a TLE (shown on the left-hand side) and a non-TLE (shown on the right-hand side) patient. The upper left box shows optical images of the corresponding H&E-stained tissues. Image data are total ion current (TIC) normalized. [Reproduced with permission from Ajith et al. (2021), ACS].

suppressed by TLE, which would have caused a dramatic reduction in PC and PE thresholds, according to metabolic pathway analysis.[129] This finding makes it possible to do more thorough research on the related biological pathways and potential clinical TLE targets.[129]

Parkinsons Disease

Throughout both the core and distal neural systems, neuropeptides are crucial signaling molecules. Neuropeptides communicate through distant signaling mechanisms such as hormonal circulation, chemical synapses, volumetric trans-

mission by diffusion, and other mechanisms. According to Hallberg and Nyberg, these tiny neuroactive peptides play a role in a variety of neurological processes, including discomfort, anxiety, emotion, memory, motion, and dietary intake.[130] Numerous neurological disorders, including Parkinson's disease (PD), have been associated with aberrant neuropeptide production and biotransformation. Movement disorder, bradykinesia, stiffness, and a decline in balance are used to identify PD, which is also characterized by symptoms that are not related to movement.[131] The deterioration of neurons producing dopamine in the substantia nigra pars compacta (SNc) which extends to the stria is a key pathological characteristic of PD. According to Lang and Lozano, disruption of SNc-striatum signaling impacts interactions with other descending regions of the basal ganglia neuronal circuitry, notably the exterior and interior portions of the globus pallidus.[132] Opioid, tachykinin, and neurotensin neuropeptide production or concentration changes have been connected to PD.[133,134] Due to measurement issues, it is unknown exactly what the transformed peptides are and the way their synthesis is modified in PD. These altered peptides serve as endogenous ligands for the transporters in the basal ganglia.

Featuring the capacity to identify uncharacterized neuropeptides, MALDI-MSI can discriminate between neuropeptides with identical sequences of amino acids and PTMs. The relatively small concentrations of these compounds in the human brain make it difficult to detect neuropeptides by MSI, despite the success of MALDI-MSI in several studies.[135-138] In this study, researchers found that 6-hydroxy dopamine (6-OHDA) lesioning and L-3,4-dihydroxyphenylalanine (L-DOPA) therapy generated substantial modifications in several neuropeptides.[139] The CPu (caudate-putament) neuropeptides are particularly impacted by the 6-OHDA lesioning, but the GP (globus pallidus) neuropeptides are significantly impacted by the L-DOPA therapy. MALDI-MSI approach, which effectively demonstrated the impact of these treatments on neuropeptides in particular areas of the brain.[139] In the 6-OHDA lesioned CPu, elevated levels of many PENK (proenkephalin) generated neuropeptides were found, supporting the results of other mRNA investigations.[133,140,141] Similarly saline and L-DOPA administered mice had higher levels of many PENK generated peptides in the GP in the lesioned half relative to the undamaged half (Hulme et al., 2020).[139] According to Bissonnette et al., the rises in PENK neuropeptide concentrations during PD are regarded as preventive and might be the consequence of corrective reactions to dopamine neuron depletion.[142] Additionally, the researchers discovered that L-DOPA therapy changed the concentrations of the neuropeptide enkephalin. In both the lesioned and unlesioned halves of the GP and LH of L-DOPA treated rats, reduced concentrations of many PENK-derived peptides were found.[139] Although there have been several studies on the effects of L-DOPA therapy on neuropeptides, according to our understanding, significant decreases in PENK-derived neuropeptide levels following L-DOPA therapy have never been described.[139] The unique dorsal sub-region of the GP showed the greatest rise in neurotensin concentrations following 6-OHDA lesioning.[139] Using alternative techniques that require the removal and homogenization of brain materials, it may be challenging to identify this unexpected finding. In models of PD, neurotensin amounts are probably elevated as a protective measure against dopamine depletion. Thus, previous investigations examined neurotensin as a therapy to reduce effects in PD models and revealed positive outcomes.[143,144] In conclusion, the technique described here significantly increased the threshold for detection and the variety of neuropeptides that may be examined by MALDI-MSI.[139]

L-DOPA has been utilized in the management of PD and is still the best treatment for easing PD symptoms.[145] The enzyme aromatic L-amino acid decarboxylase is thought to convert L-DOPA into dopamine (DA) after decarboxylation, which is what gives L-DOPA its curative qualities. Despite being quite effective in the early phases of PD, prolonged therapy causes L-DOPA-induced dyskinesia (LID), which affects 10% of individuals every year while being treated.[145] LID results in the most severe motor side effect of DA substitution treatment, and it significantly lessens the therapeutic effectiveness of L-DOPA administration. The 5-hydroxytryptamine (5-HT) system appears to be specifically linked to dyskinesia. Studies have shown that 5-HT neurons can discharge DA as a fake neurotransmitter when treated with L-DOPA.[146-149] Modification may not only contribute to LID but additionally play a role in nonmotor disturbances brought on by L-DOPA.[150] It is yet unclear exactly what these neurochemical changes are or how extensive they are.

The spatial distribution of L-DOPA in the brains of dys and non-dyskinetic monkeys was mapped using MALDI-MSI.[151] LID mice had very high levels of L-DOPA, which led to a spike in DA and DA metabolites in extrastriatal regions. Additionally, L-DOPA's primary metabolite, 3-OMD (3-methyl dopamine), was shown to be significantly higher in LID, suggesting that L-DOPA's breakdown is disrupted in this condition.[151] Because of the elevated concentrations of L-DOPA in LID, extrastriatal areas had a greater concentration of DA, which was mostly processed by COMT (catechol-O-methyl transferase).[151] The elevated concentration of DA in extrastriatal areas may influence biological processes by changing signaling across the entire brain, leading to a variety of negative consequences from L-DOPA treatment.[151]

Other Neurological Disorder

Most neurological disorders have neurological inflammation as an underlying disorder, which is currently being understood. Glycosylation is one biochemical route that has been disregarded in neurological inflammation.[152] N-glyco-

sylation is a particular kind of glycosylation that plays an essential part in the central nervous system [CNS]. Congenital glycosylation illnesses that cause neuropathological manifestations including seizures and cognitive impairment.[152] The actions of glycoproteins might ultimately be affected by changes in N-glycosylation, which will eventually influence cellular machinery.[152] Identifying N-glycosylation changes in a neuroinflammatory setting can therefore offer a possible strategy for future treatments.[152] Although little has been learned about N-glycans' function in brain physiology, it is understood that they play an essential part in neuronal growth and distinction, synaptic development, and myelinogenesis.[153] This emphasizes the importance of thoroughly characterizing the brain's N-glycome, particularly in neurological disorders like neuroinflammation, to comprehend how glycosylation may be contributing to the etiology of neurological conditions.[152]

In a recent study, Rebelo et al. discussed the function of N-glycosylation in an example of neurological inflammation brought on by lipopolysaccharide (LPS).[152] The cell membrane of gram-negative bacteria contains LPS, a strong immunostimulant that primarily attaches to the numerous TLR-4 receptors found in microglia[154,155] as well as to a lesser degree, in neurons and astrocytes.[156-158] The production of those aforementioned harmful inflammatory compounds is then activated by the stimulation of transcription variables by TLR-4, as well as other subsequent pathways in microglia.[159,160] Following LPS injection, there was a considerable reduction in sialylation and fucosylation (7.5% and 8.5%, respectively), indicating that this downregulation may be an important potentiator of inflammatory cascades. The presence of bisected N-glycan structures and an overall rise in oligomannose (13.5%) in the inflamed tissue could further point to their involvement in this phenomenon.[152] The brain's glycomic profile can be fully understood using a variety of glyco-analytical methodologies, which creates opportunities for additional study in this area.[152]

In the process of drug discovery and chemical sensor development, tracking variations in concentrations of drugs during an experiment and their ultimate concentration near the location where they act has long been an issue. Additionally, an objective for determining the potential for therapy when it comes to effectiveness as well as safety is dosage estimation.[161,162] The administration of drugs aimed at compartments inside cells may assist in enhancing drug absorption and function in addition to transport mechanisms. The cellular distribution of drugs is another crucial characteristic.[163-165] Therefore, it is crucial to create and enhance diagnostic modalities for high resolution and precise measurement of substances within cells.[166-168]

According to a recent clinical study, CNS barriers are believed to perform an impact in the onset and course of various neurological illnesses, including schizophrenia.[169-171] Recent investigations by scholars studying the blood brain barrier (BBB) have revealed significant unresolved inquiries regarding (a) the possibility of an association between antipsychotic drugs and BBB function impairments, (b) targeting BBB transport processes to enhance the performance of antipsychotic medication, and (c) the importance of treatment options intended for safeguarding or restoring the BBB in psychosis.[170] Recognizing that solely loose and nonionized particles can traverse the barrier to communicate with receptors in the brain and start a pharmacological reaction is crucial to understanding the biochemical processes of low molar mass drug delivery through the BBB.[172-175] Drugs nevertheless remain in the blood and brain regions in both their loose and bonded forms that are combined with blood proteins or elements of cells.[173] To explore the quantitative influence of several connected mechanisms on unattached antipsychotic drug transport over the BBB at various levels, wherein small variances may exist, particular and highly sensitive analytical techniques are required.

To measure and assess the degree of free drug BBB transport as well as the post-BBB cerebral dispersion of medications at regional as well as subregional stages, Luptakova et al. (2021) introduced a technique that combines in vivo and in vitro neuropharmacokinetic research with MALDI-MSI.[176] Using this method, direct visualization of the antipsychotic medications' allowed distinction of BBB transport features at a resolution of 20 micron.[176] For precise characterization and imaging of the volume of localized unbound medication movement across the BBB, the technology offers a label-free and multimodal methodology. It also offers superior lateral resolution, concomitant drug metabolite recognition, and localization.[176]

Discussion

According to Taylor et al. (2002),[177] many neurodegenerative disorders are linked to alterations in protein dynamics that cause misfolded proteins to gradually accumulate as intracellular and/or extracellular aggregates. On numerous occasions, the absence of biochemical techniques that concurrently provide specificity and sensitivity, while preserving the requisite spatial and temporal resolution, makes it difficult to identify the molecular mechanism behind these pathochemical alterations.[4] This becomes especially interesting when prospective targets are unclear or when variable protein and peptide synthesis and enzymatic processing may be responsible for these species' propensity to aggregate as well as their function at various phases of illness and disease.[4]

The studies listed above (Table 1) has expanded our understanding of molecular neuroanatomy and helped us to identify the underlying molecular causes of a number of neurological ailments. Additionally, the spatially detailed in situ study of metals and biochemicals in tissue using MSI holds enormous promise for neuropathology. Even if the effectiveness of image-guided therapy and treatment is continuously being leveraged by the incorporation of new

modalities and sophisticated imaging techniques, the analysis of tissue specimens is frequently the gold standard in determining the condition's subgroup and severity.[4] Therefore, by interpreting the neurobiomarkers, MSI can be a useful auxiliary to neurohistochemistry. MS has already become the gold standard in many common clinical procedures due to its superiority to conventional immunoassays, but MSI has not yet made the transfer from bench to bedside. The speed, extremely high sensitivity, specificity, and multiplex molecular detection along with the preservation of spatial information in MSI, which is otherwise difficult by the time-consuming LC-MS-based techniques, are expected to make this shift successful.[4]

The application of MALDI-MSI in neuroscience has received the most attention against all MSI techniques. Instantaneous examination of biological samples with minimal or no sample preparation is now possible because of recent advancements in atmospheric ionization methodologies (DESI, AFADESI, IR-MALDESI). Due to this benefit, MSI is now more frequently used for quick in situ examinations that are compliant with intraoperative procedures. Even though MSI has attracted a lot of interest with regard for investigations on brain tumors and Alzheimer's disease, including some research being conducted on Parkinson's and Huntington's disorder, the expected uses of this approach are never restricted.

Applying mouse models for MSI in both malignancies of the brain and neurological disorders has been extensively reported. These investigations ought to be expanded to include people. There exist brief accounts of MSI investigations on the human brain for psychological disorders including schizophrenia.[1] Most research studies have too small of a sample size to draw an authoritative conclusion or make a prognosticating statement. Additionally, a neuro-biomarker may exhibit the condition's early symptoms or show signs of a related illness.[1] Currently, an important component of MSI is improving image resolution to localize cellular and subcellular characteristics. In that regard, the use of gold nanoparticles in MSI methods is growing in popularity.[178] To solve the problem of clarifying isomeric species or isolating the poorly ionized species from MSI noise, post-ionization isolation techniques must be developed. To encompass a variety of species in one MSI scan, it is very desirable to create unique and effective preparation of samples and ionization procedures.[1] To broaden the use of MSI, research must be done on the accurate characterization and complete quantification of analytes from tissue samples.

As mentioned above, the various MSI modalities can be used to target diverse chemical classes and highlight their function in associated biomechanisms thanks to their complementing application profiles. MSI can be a potent tool for probing biological mechanisms and advancing our scientific comprehension of the pathophysiology of neurodegenerative diseases when utilized effectively and in conjunction with an established and controlled research protocol. Additionally, the discovery and confirmation of mechanistically entailed novel molecular species can help in accelerating the discovery of pathology-associated biofluid- and imaging-based indicators for neurodegenerative disorders. These biomarkers are essential for both everyday clinical surveillance of neurodegenerative abnormalities and the advancement of new pharmacotherapy strategies.

Conclusions

MSI is a prominent and developing discipline in biomedical studies. Since experiments are conducted in natural environments, it is straightforward to see the distribution of an extensive array of chemicals in various specimens, such as tissue sections. It is uncomplicated to evaluate the native material for information on surface chemical makeup because these methods are safe and ambient. In contrast with numerous other popular analytical modalities, MSI seems to be an effective technology capable of supplying the complex molecular information of the tissue of interest in an impartial manner. By directly examining metabolic processes in brain samples, MSI investigations have aided in understanding the biological causes and clinical traits of several brain illnesses. These advantages ensure that scientific curiosity in biochemical MSI via ambient conditions will greatly increase in the future. In the areas outlined below, MSI can be strengthened as a multimodal molecular visualization tool.

Isobaric ion detection

It is difficult to distinguish isobaric ions with MSI's in-situ detection method. Ions with a comparable chemical composition cannot be distinguished from one another. The invention of [IMS] ion mobility spectrometry provides a practical solution to this challenge due to its application in gas-phase separation by chromatographic methods. Chemometrics based on a variety of complementary elements, such as isotope abundance and highlighted segments, can enhance ion characterisation.[179-181] Along with the post-acquisition dry method, online wet experiments also provide additional chemical traits to help distinguish isobaric ions, such as hydrogen-deuterium exchange during ionization in the liquid state or the post-ionization process in the gaseous phase.[182-183]

Sensitivity

It is necessary to establish an enhanced sensitivity in situ benign ionization technology to collect chemical fingerprints, especially from a sub-micron-sized material, without experiencing a major sensitivity loss. The development of innovative nanomaterials for analyte attachment, meticulously developed secondary ionization, intricate ion focusing lens systems, and sample-friendly preparation processes should all be consistently pursued. This represents a signifi-

cant field of research that might advance developments on MSI research. The field of relevant neuroscience research will expand along with advancements in technology.

Spatial resolution

The precision by which an ion map may be collected to acquire more accurate spatial information can be evaluated using this MSI data, which is an additional significant factor. This is often a conflicting situation when paired with the degree of sensitivity because a reduction in sample size typically results in a concentration of extremely small molecules and the associated signal output offers a uniform ionization efficiency. Currently, spatial resolution can be improved through multi-modular picture blending. Co-registering pixels from a rough MS scan with additional cell-resolved digital representations from H&E, IF, and MRI allows one to predict the molecular data inside a more constrained area.[184-187] Such an image with a high resolution is strategically anticipated as opposed to being observed.

Data acquisition

For automatic processing of tissue samples, accelerated MSI data collecting, and quality verification, a fully automated robotic device is expected to produce a less complicated and more trustworthy method. The initiatives that have been taken to develop this component of commercial design are outstanding.[188] For larger samples or when a higher image resolution is required, the current standard approach for gathering MSI data uses a microprobe-oriented spot-by-spot or a single line at a time mode, which may take several hours or even an entire day to complete. A rapid, small snapshot-mode data collection will surely boost the MSI's operating efficiency.[189] This calls for a micro-arrayed ion detection strategy and a more complicated MS setup.

Artificial intelligence and omics database

For comprehending the chemical profile sequence and direct accurate spatial recognition, machine learning has been successfully introduced.[190] Additional sophisticated data science tools and computational methods are projected to be implemented with the objective to simplify the integration of spatial data from various omics, particularly spatial transcriptomics, and genomics. An open-access MSI data repository must be created so that MSI researchers can collaborate and conduct research without restriction.[191] In addition, it is planned that a spatial omics library with free access will contain biochemical phenotypic atlases from cancer cells and vital organs. The larger MSI and omics societies must undoubtedly work together and come to a consensus on this subject.

Nature of specimen and functional imaging

Less biological materials that are used more commonly in pathological research can be rendered MSI compliant. This may be particularly true for imaging studies that target neuroscience and use MSI. The mass spectral fingerprint generated by an MSI study is not merely a list of single, inconsequential peaks; rather, it contains multiple biological relationships beneath the peaks. For instance, the relationship between a drug's (treated for a neurological disorder) characteristics and its spatially defined molecular makeup and functionality may be better understood because of these biologically significant connections. It is envisaged that MSI may develop into a functional imaging system to view biological phenomena like growth, spread, autophagy, iron metabolism, and enzyme catalysis by analyzing and organizing the data gathered.[192-196]

Given all the advantages outlined in the studies mentioned above (Table 1), MSI ought to be used only when conventional modalities, particularly liquid chromatographic (LC) procedures employed in conjunction with MS, are unable to deliver the necessary analytical data. LC techniques used in conjunction with MS are frequently more precise and quantifiable than MSI. Therefore, unless fine spatial details are necessary, such as when verifying or quantifying the presence of a metabolite in a specimen, LC-MS is probably a better option. This implies that if a molecule cannot be identified by LC-MS after careful specimen extraction and workup, there is little reason to believe that it will be recognized by MSI, which lacks the capacity for pre-concentration and separation as in conventional bioanalysis.

In conclusion, compared to numerous popular analytical modalities, MSI seems to be an effective technology capable of supplying the complex molecular information of the tissue of interest in an impartial manner. With its extremely high levels of selectivity and sensitivity for locating indicators, assessing disease etiology, simplifying pharmaceutical trials, etc., MSI continues to develop as a viable tool for neuroscience research.

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Conflict of Interest

The authors declare no conflict of interest.

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