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Simultaneous imaging of multiple neurotransmitters and neuroactive substances in the brain by desorption electrospray ionization mass spectrometry

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ABSTRACT

With neurological processes involving multiple neurotransmitters and neuromodulators, it is important to have the ability to directly map and quantify multiple signaling molecules simultaneously in a single analysis. By utilizing a molecular-specific approach, namely desorption electrospray ionization mass spectrometry imaging (DESI-MSI), we demonstrated that the technique can be used to image multiple neurotransmitters and their metabolites (dopamine, dihydroxyphenylacetic acid, 3-methoxytyramine, serotonin, glutamate, glutamine, aspartate, γ-aminobutyric acid, adenosine) as well as neuroactive drugs (amphetamine, sibutramine, fluvoxamine) and drug metabolites in situ directly in brain tissue sections. The use of both positive and negative ionization modes increased the number of identified molecular targets. Chemical derivatization by charge-tagging the primary amines of molecules significantly increased the sensitivity, enabling the detection of low abundant neurotransmitters and other neuroactive substances previously undetectable by MSI. The sensitivity of the imaging approach of neurochemicals has a great potential in many diverse applications in fields such as neuroscience, pharmacology, drug discovery, neurochemistry, and medicine.

Introduction

Small-molecule neurotransmitters are important chemical messengers that mediate signaling between neuronal cells in the brain (Greengard, 2001). Fluctuations in their concentrations are associated with numerous normal neuronal functions including sleep and aging, but they are also associated with pathological changes in neurological disorders such as Parkinson's disease (PD) (Lotharius and Brundin, 2002). To understand complex neurological processes and develop effective treatments, it is important to have reliable information on the relative abundance and distribution of neurotransmitters and their interplay with neuroactive therapeutics. Because of the great diversity of neurotransmitters and the complexity of their signaling mechanisms (and the interactions between those signaling mechanisms), it is very useful to image several neurotransmitters in the same analysis, in a way that enables simultaneous measurement and characterization of multiple neuronal networks (Shariatgorji et al., 2014a).

The most widely utilized traditional methods for mapping the distributions of small molecule neurotransmitters are indirect approaches such as histochemical, immunohistochemical and ligand-based assays (de Jong et al., 2005). The most effective of these assays involves using the localization of specific enzymes or transporters as molecular markers for the neurotransmitter of interest. For instance, the dopamine transporter (DAT) or tyrosine hydroxylase (TH) is often used as markers for dopamine (DA) (Bjorklund and Dunnett, 2007; Miller et al., 1997), and glutamate decarboxylase (GAD) is commonly used as a marker for γ-aminobutyric acid (GABA) (Storm-Mathisen et al., 1983). The few antibodies that can directly target small signaling molecules rather than associated molecular markers often suffer from major selectivity limitations. In particular, they frequently cannot distinguish a transmitter from its metabolites and precursors (de Jong et al., 2005; Jones and Beaudet, 1987). The inability to discriminate between the transmitter and its metabolites is also a complication in imaging techniques that use radiolabeled compounds because there is a risk that nuclear imaging techniques such as positron emission tomography (PET) will be unable to distinguish between the labeled parent compound and metabolites that retain the label (Badgaiyan, 2011; Pimlott and Sutherland, 2011). Consequently, there is a need for new imaging techniques that can directly map and simultaneously quantitate the

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localization of neurotransmitters and neuroactive substances in tissue samples. Label-free and molecular-specific imaging methods that use mass spectrometry (MS) to acquire data in a multiplexed and untargeted way have been introduced to circumvent these problems. Mass spectrometry imaging (MSI) makes it possible to directly map the distributions of molecular species on the surfaces of tissue sections without any need for prior information on the molecular targets that might be present.

Several MSI methodologies including secondary ion mass spectrometry (SIMS), matrix-assisted laser desorption ionization (MALDI), nano-desorption electrospray ionization (nano-DESI), liquid microjunction surface-sampling (LMJ-SSP), laser ablation electrospray ionization (LAESI) and desorption electrospray ionization (DESI) have been developed to map the distribution of ion species in biological tissue sections (Walch et al., 2008; Wu et al., 2010).

Two of the most common ionization methods in MSI are MALDI and DESI. Both technologies involve the two-dimensional acquisition of mass spectra from a sample. Ion images are then constructed by plotting the intensities of the signals for the ions of interest against their relative position on the sample surface (Cornett et al., 2007; Wiseman et al., 2006). MSI techniques are increasingly used both within academia and pharmaceutical industry. Notably, MALDI-MSI has been used to obtain qualitative and quantitative information about the localization of diverse compounds including metal ions (Shariatgorji et al., 2014b), small molecule drugs (Khatib-Shahidi et al., 2006), lipids (Jackson et al., 2007), peptides (Skold et al., 2006), and proteins (van Remoortere et al., 2010; Zavalin et al., 2015) in a variety of samples and tissues. DESI-MSI methods were developed more recently, and enable ionization to be achieved under ambient conditions (Fig. 1). They have been used to image lipids (Eberlin et al., 2011), metabolites and small molecules (Liu et al., 2014; Vismeh et al., 2012; Wiseman et al., 2006) e.g., epinephrine and norepinephrine in adrenal gland (Wu et al., 2010). Although less widely used than MALDI-MSI, DESI-MSI has the important advantage of obviating the need for matrix deposition before analysis. However, MALDI-MSI can be used to analyze larger biomolecules than can be detected with DESI-MSI, and can currently achieve higher spatial resolutions. The two ionization techniques also have different sensitivities for different analytes, and are thus complementary. Both achieve ion formation with crude samples such as biological tissue sections with either no or minimal sample preparation, concentration or purification. This limits the sensitivity of MALDI- and DESI-MSI because tissue samples also contain signal-suppressing components such as highly abundant cell debris, lipids, and salts, which can interfere with the ionization and/or desorption of the analyte of interest.

We recently developed an innovative MALDI-MSI approach for the simultaneous imaging and quantitation of multiple neurotransmitters, precursors, and metabolites in histological tissue sections at high spatial resolutions. The method was utilized to directly measure changes in the absolute and relative levels of neurotransmitters in specific brain structures in animal disease models and in response to drug treatments (Shariatgorji et al., 2014a).

Here, we extend our earlier work and describe the novel use of DESI-MSI to map the localization of neurotransmitters, their metabolites and neuroactive drugs directly in brain tissue sections. Initial experiments showed that DESI-MSI could be used to image analytes in native rodent brain tissue sections from animals that had been subjected to a range of treatments. However, many neurotransmitters exhibited poor sensitivity under the initial imaging conditions and were not readily ionized. Such problems can be overcome by selective on-tissue chemical derivatization procedures that add or stabilize a charge on the target molecules. In the case of DESI-MSI, this could render the charge competition in the electrospray mechanism insignificantly and hence enhances the sensitivity of the analysis. It has been shown that pyrylium salts react readily and specifically with primary amines to form pyridinium cations, (Johannesen et al., 2012; O'Leary and Samberg, 1971) and we have previously reported that this phenomenon can be exploited to dramatically increase the sensitivity of MALDI-MSI analyses towards monoamine and amino acid neurotransmitters, drugs and metabolites in brain tissue sections (Fig. S1) (Shariatgorji et al., 2014a; Shariatgorji et al., 2015).

This work also increases the utility of DESI-MSI by demonstrating the use of pyrylium-on-tissue-derivatization for the analysis of low abundance and/or difficult to ionize target molecules. We demonstrate how the charge-tagging achieved through the chemical derivatization reaction increases the sensitivity of the DESI-MSI analysis, enabling the detection of low abundance neurotransmitters and other neuroactive substances that were previously undetectable by MSI. This method is also compatible with quantitative DESI-MSI analysis. The results presented herein demonstrate that strategies for improving the

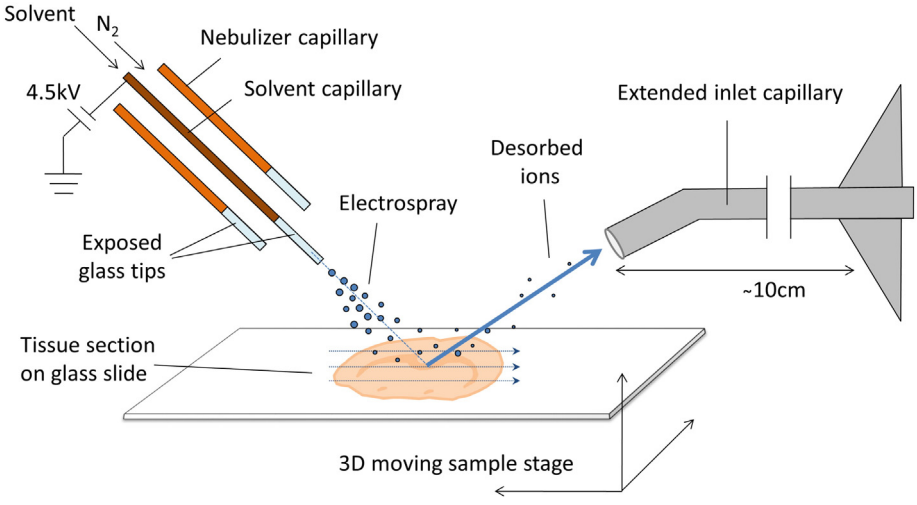

Fig. 1. Schematic diagram of the DESI-MSI experiment. A pneumatically-assisted electrospray is directed at the tissue section surface, where it forms a liquid film that dissolves analytes from the sample surface. On impact of further primary droplets, secondary droplets containing the analyte ions are ejected and ionized in an electrospray-like mechanism. Ions are sampled by the mass spectrometer using an extended mass spectrometer inlet (sniffer). Each step of this process is performed under ambient conditions. For imaging applications, the sample position relative to the position of the DESI sprayer assembly is changed in horizontal continuous movements while spectra are acquired for each pixel of the resulting image.

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