Targeting of Neurotransmitter Changes in Rat Brains after Ketamine or Tetrabenazine Administration

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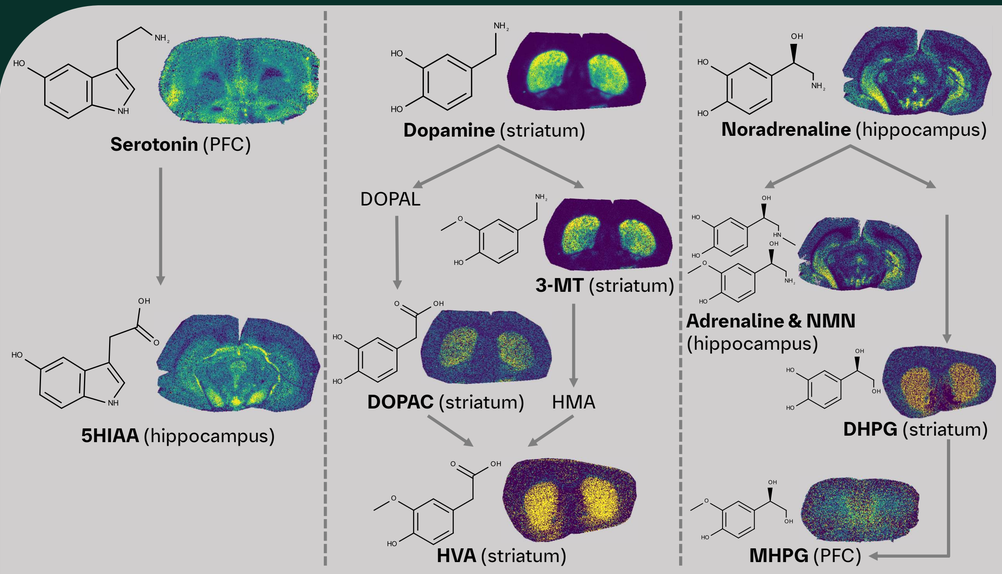


Figure 1: Neurotransmitter metabolism and corresponding MSI images

Key messages

- **In-situ investigation of the neurotransmitter metabolism is challenging but required to gain a deeper understanding of neurological disorders and the effect of drugs on the CNS.**
- **We used mass spectrometry imaging (MSI) in combination with on-tissue chemical derivatization with 2-fluoro-1-methyl pyridinium (FMP)-based reactive matrix to enhance detection of various small molecules in rat brain tissues.**
- **Rats treated with ketamine, a dissociative anesthetic, or tetrabenazine, an antihyperkinetic medication, were investigated, revealing prominent alterations in neurotransmitters and their metabolites.**
- **Changes in neurotransmitter levels in response to the treatment were examined across diverse brain regions to assess area-specific modulations.**

Introduction & Aim

Neurotransmitters are low-abundant signaling molecules involved in almost every physiological process. An imbalance in neurotransmitters is often linked to neurological disorders. MSI enables mapping of neurotransmitter spatial distribution in the brain. To overcome the challenges of low abundance, poor ionization, and spectral interferences, we used on-tissue chemical derivatization with FMP-10 (Shariatgorji, 2019). FMP-10 derivatization was used to evaluate changes in neurotransmitter levels after treatment of rats with ketamine or tetrabenazine which have shown promising potential to modulate and treat neurological disorders. Derivatization offers an unique opportunity to extract critical information from tissue and combined with MALDI-MSI represents a promising technique to visualize spatial distribution of molecules within cryosectioned brain tissues.

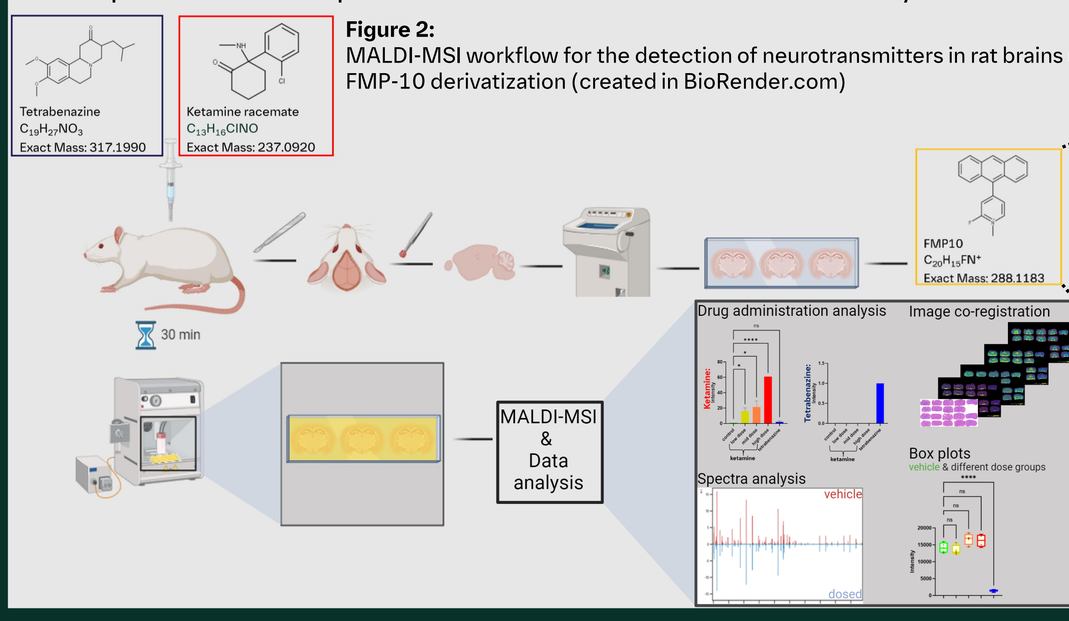


Figure 2: MALDI-MSI workflow for the detection of neurotransmitters in rat brains using FMP-10 derivatization (created in BioRender.com)

Material and Methods

- Animals: male Wistar rats (RjHan: WI), age: 4-5 months
- Sample: brain sections (12 µm) of prefrontal cortex (PFC), striatum, hippocampus
- Study design: single subcutaneous/intraperitoneal administration of ketamine or tetrabenazine, euthanasia and brain removal 30 min after administration
- Doses: ketamine: 10; 20; 30 mg/kg, tetrabenazine: 1.5 mg/kg, control: 0.9% NaCl
- Sample preparation: MSI: spray coating with FMP-10 (1.82 mg/ml in 70% ACN), LC-MS/MS: the tissues were homogenized, derivatized with benozyl chloride, and measured on a SCIEX Triple Quad™ 7500 LC-MS/MS system
- Measurement MSI: neurotransmitters and their metabolites were measured at 50 µm raster width on a timsTOF fleX (Bruker Daltonics). Digitized images of the H&E stained tissues were coregistered to MSI data and analyzed in SCiLS Lab.

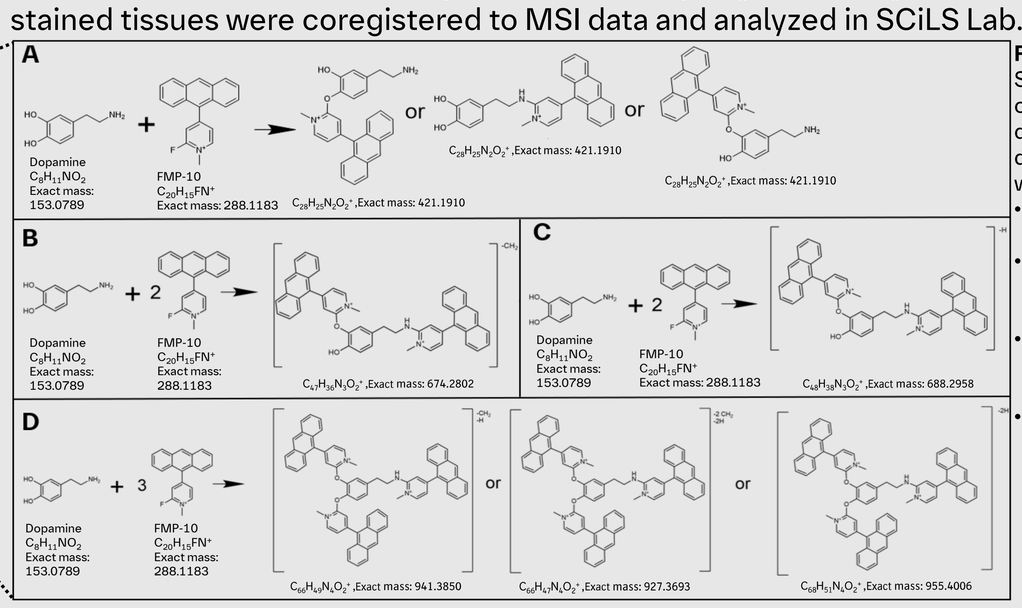
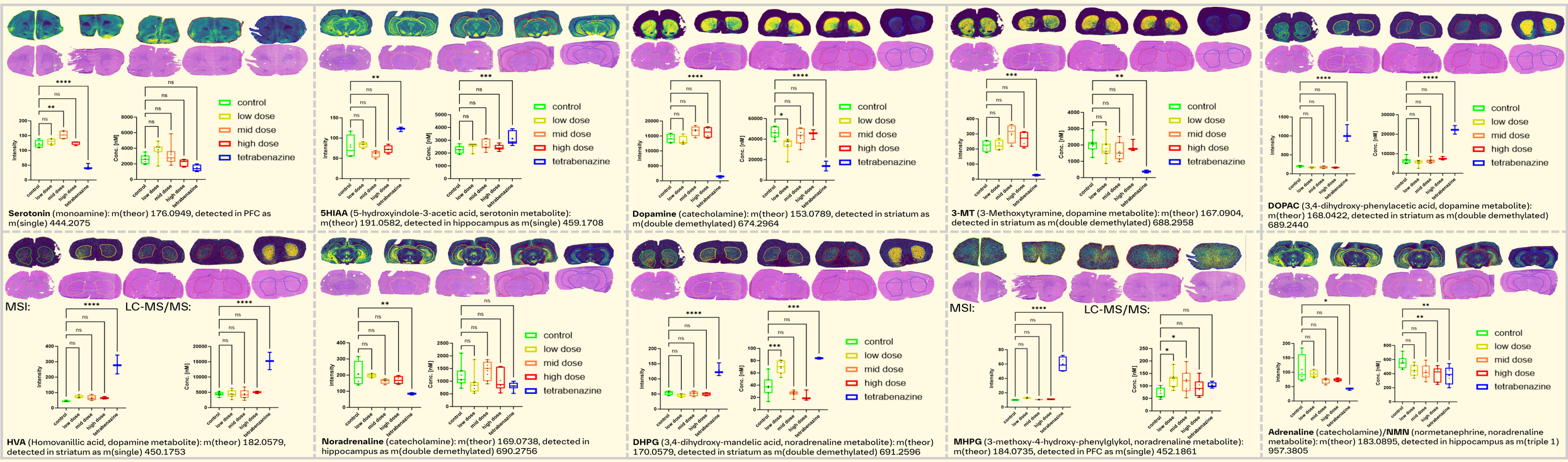


Figure 3: Schematic overview of dopamine derivatization with FMP-10:
- A: single derivatization
- B: double demethylated derivatization
- C: double deprotonated derivatization
- D: triple 1; 2; 3 derivatization

Results

Figure 4: Comparative analysis of neurotransmitters in rat brain. MALDI imaging scans showing spatial distribution of various neurotransmitters in brain sections and corresponding H&E-stained sections for histological reference. **Left:** Box plots of signal intensities from MALDI-MSI (n=1, 4 technical replicates per region). **Right:** Box plots of neurotransmitter concentrations from LC-MS/MS measurements (n=8) as a reference.



Serotonin (monoamine): m(theor) 176.0949, detected in PFC as m(single) 444.2075

5HIAA (5-hydroxyindole-3-acetic acid, serotonin metabolite): m(theor) 191.0582, detected in hippocampus as m(single) 459.1708

Dopamine (catecholamine): m(theor) 153.0789, detected in striatum as m(double demethylated) 674.2964

3-MT (3-Methoxytyramine, dopamine metabolite): m(theor) 167.0904, detected in striatum as m(double demethylated) 688.2958

DOPAC (3,4-dihydroxy-phenylacetic acid, dopamine metabolite): m(theor) 168.0422, detected in striatum as m(double demethylated) 689.2440

HVA (Homovanillic acid, dopamine metabolite): m(theor) 182.0579, detected in striatum as m(single) 450.1753

Noradrenaline (catecholamine): m(theor) 169.0738, detected in hippocampus as m(double demethylated) 690.2756

DHPG (3,4-dihydroxy-mandelic acid, noradrenaline metabolite): m(theor) 170.0579, detected in striatum as m(double demethylated) 691.2596

MHPG (3-methoxy-4-hydroxy-phenylglykol, noradrenaline metabolite): m(theor) 184.0735, detected in PFC as m(single) 452.1861

Adrenaline (catecholamine)/**NMN** (normetanephrine, noradrenaline metabolite): m(theor) 183.0895, detected in hippocampus as m(triple 1) 957.3805

control low dose ketamine mid dose ketamine high dose ketamine tetrabenazine

Summary

- Different doses of ketamine lead to divergent modulations of neurotransmitter levels in specific brain regions, rather than a uniform activating impact.
- Tetrabenazine promotes depletion of monoamines, specifically serotonin, dopamine, noradrenaline, and adrenaline.
- The anesthetic state induced by high-dose ketamine leads to a reduction in neurotransmitter levels compared to control and to the mid-dose.

Perspectives

- Advanced neurotransmitter mapping by FMP-10 derivatization and MSI.
- MALDI-MSI in combination with LC-MS analysis is a promising application in drug discovery: The ability to visualize and quantify neurotransmitter changes following drug administration can accelerate drug discovery for neurological disorders.

Life forward