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## Supplementary information

# Spatial visualization of comprehensive brain neurotransmitter systems and neuroactive substances by selective in situ chemical derivatization mass spectrometry imaging

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# Supplementary Information

# Spatial visualization of comprehensive brain neurotransmitter systems and neuroactive substances by selective in situ chemical derivatization mass spectrometry imaging

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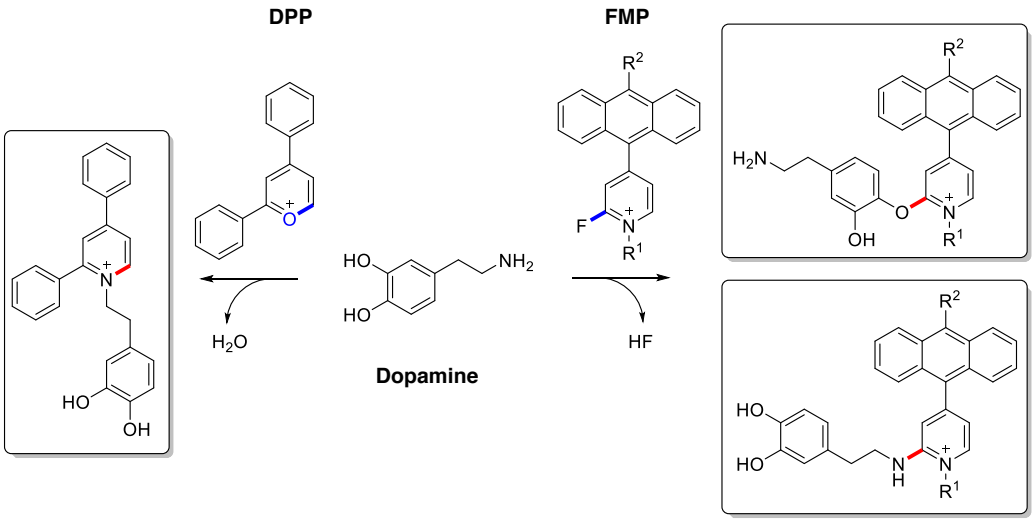
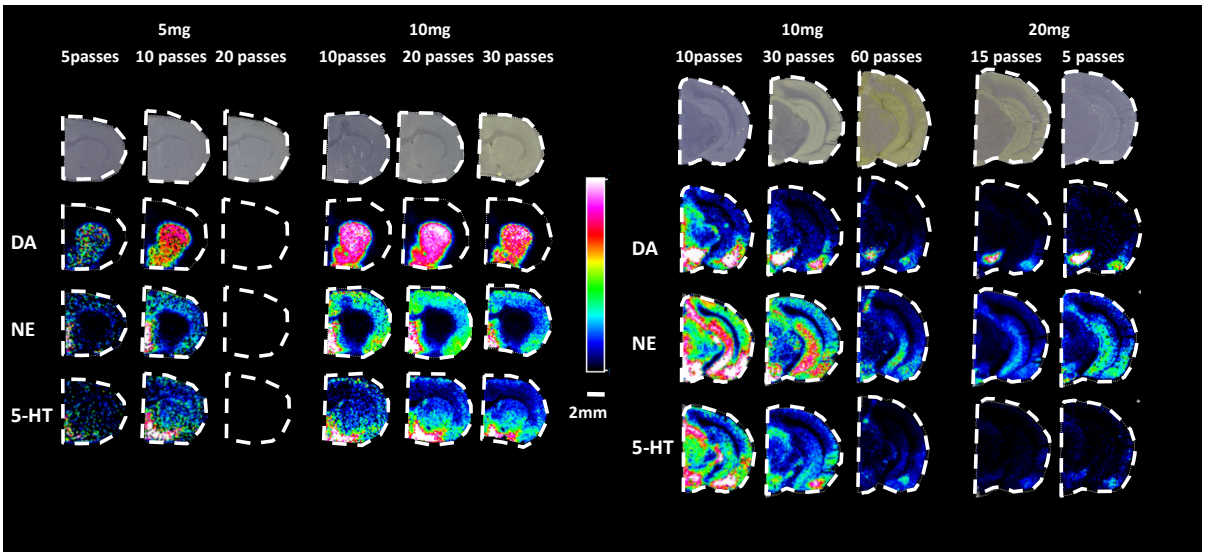
# Supplementary Figures



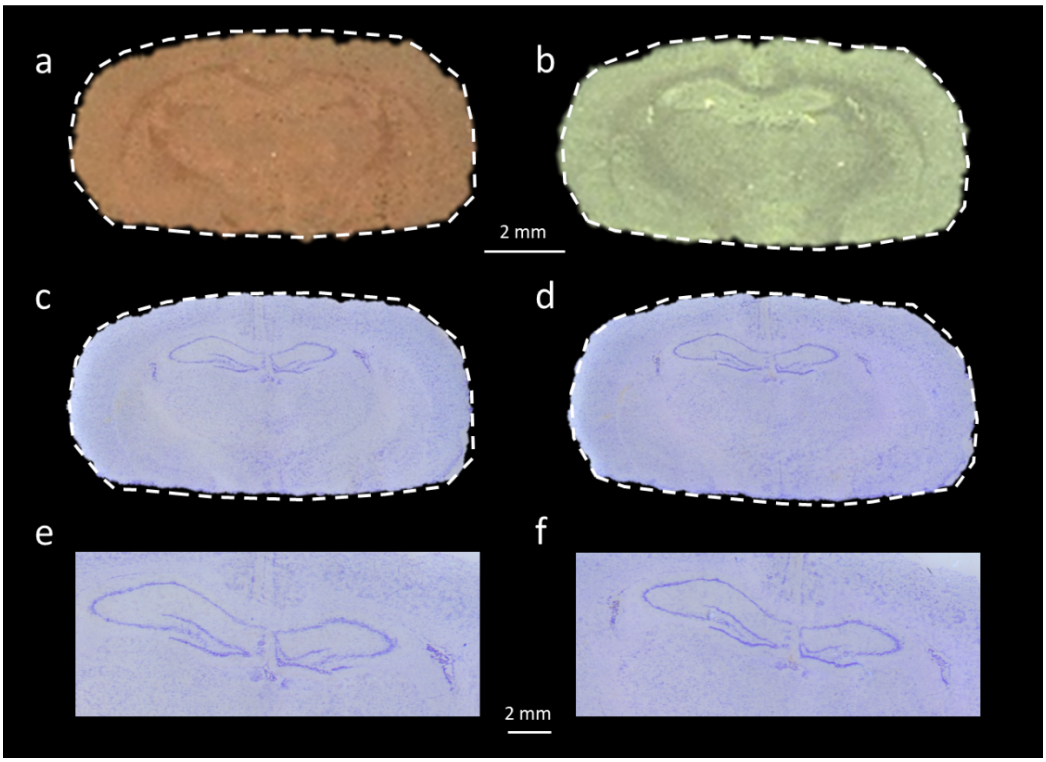

**Supplementary Figure 1. Derivatization reaction scheme of the DPP and FMP matrices.**

Overview of the derivatization reactions using DPP (left side) that reacts with primary amines and FMP (right side) that reacts with primary amines and/or hydroxy phenols. Dopamine (DA) is used as an example for the reactions. FMP-8 ($R^1$ = Me, $R^2$ = Br), FMP-9 ($R^1$ = Et, $R^2$ = H) and FMP-10 ($R^1$ = Me, $R^2$ = H).




**Supplementary Figure 2. Optimization of FMP-10 application protocol.**

Detection of the neurotransmitters dopamine (DA), norepinephrine (NE), and serotonin (5-HT) was optimized using different number of spray passes and different concentrations of FMP-10. FMP-10 (5 mg, 10 mg, or 20 mg) was added to 5.5 ml of 70 % ACN and different number of passes (5, 10, 15, 20, 30 or 60) were applied using the TM sprayer. Left panels show ion distribution images of DA (scaled to 80% of max intensity), NE (scaled to 40% of max intensity) and 5-HT (scaled to 40% of max intensity) from coronal rat brain tissue sections (distance from bregma 0.48 mm) [1] using different optimization parameters. Right panels show ion distribution images of DA (scaled to 40% of max intensity), NE (scaled to 30% of max intensity), and 5-HT (scaled to 40% of max intensity) from coronal rat brain sections (distance from bregma 5.6 mm) [1] using different optimization parameters.




**Supplementary Figure 3. Brain tissue integrity after chemical derivatization.**

Rat brain tissue sections are derivatized by **a**, DPP and **b**, FMP-10. Optical images of Nissl-stained tissue sections show preservation of the tissue integrity after washing off **c**, DPP and **d**, FMP-10. Substructures of hippocampus are clearly preserved after derivatization by **e**, DPP and **f**, FMP-10.

## Supplementary Tables

**Supplementary Table 1. Overview of the mass tags added to molecules containing a primary amine and/or hydroxy phenols upon derivatization using DPP, FMP-8, FMP-9, and FMP-10.**

| Reactive matrix | Elements added (Da) | Elements added (Da) | Elements added (Da) |
|---|---|---|---|
| FMP-8 | $C_{20}H_{13}NBr^+$ (346.0226) | $C_{39}H_{23}N_2Br_2^+$ (677.0223)[a] | $C_{40}H_{25}N_2Br_2^+$ (691.0379)[b] |
| FMP-9 | $C_{21}H_{16}N^+$ (282.1277) | $C_{40}H_{27}N_2^+$ (535.2169)[c] | $C_{42}H_{31}N_2^+$ (563.2482)[b] |
| FMP-10 | $C_{20}H_{14}N^+$ (268.1121) | $C_{39}H_{25}N_2^+$ (521.2012)[a] | $C_{40}H_{27}N_2^+$ (535.2169)[b] |
| DPP | $C_{17}H_{11}^+$ (215.085526) | N/A | N/A |

[a] +2[FMP-8]-$CH_3^+$ or +2[FMP-10]-$CH_3^+$, [b] +2[FMP-8]-$H^+$ or +2[FMP-9]-$H^+$ or +2[FMP-10]-$H^+$,
[c] +2[FMP-9]-$C_2H_5^+$

**Supplementary Table 2. The linearity and limit of detection for different neurotransmitters and neurotransmitter metabolites using DPP and FMP-10.**

| Matrix | Analyte | Normalization | Linear regression ($r^2$) | LOD (pmol/mg) |
|---|---|---|---|---|
| FMP-10 | HVA | RMS | 0.997994 | 12.24 |
| FMP-10 | HVA | IS | 0.999429 | 6.52 |
| FMP-10 | DA (single) | RMS | 0.999269 | 2.78 |
| FMP-10 | DA (single) | IS | 0.999969 | 0.58 |
| FMP-10 | DA (double) | RMS | 0.998928 | 3.36 |
| FMP-10 | DA (double) | IS | 0.999748 | 1.63 |
| DPP | GABA-$d_6$ | RMS | 0.987321 | 6.83 |
| FMP-10 | GABA-$d_6$ | RMS | 0.99622 | 132.82 |
| DPP | DA-$d_4$ | RMS | 0.998613 | 5.08 |
| FMP-10 | DA-$d_4$ (single) | RMS | 0.995424 | 2.31 |
| FMP-10 | DA-$d_4$ (double) | RMS | 0.981629 | 4.66 |
| DPP | 3-MT-$d_4$ | RMS | 0.999383 | 3.06 |
| FMP-10 | 3-MT-$d_4$ (single) | RMS | 0.999661 | 2.33 |

Data were extracted using RMS or IS normalization. LOD was calculated using the LINEST function in Excel where LOD = 3.3*(Sy/S), where Sy is the standard deviation of the response of the curve and S is the slope of the calibration curve. Data were extracted using msIQuant [2,3]. The calibration curves were prepared as described previously [4]. Raw data is available in Supplementary Data. Abbreviations: 3-MT, 3-methoxytyramine, DA, dopamine; DPP, 2,3-diphenyl-pyranylium; FMP-10, 4-(anthracen-9-yl)-2-fluoro-1-methylpyridin-1-ium iodide; GABA, γ-aminobutyric acid; HVA, homovanillic acid; IS, internal standard; LOD, limit of detection; RMS, root-mean-square.

**Supplementary Table 3. Summary of detected neurotransmitters, metabolites and amino acids in brain tissue sections following derivatization with DPP or FMP-10.**

| Compound | M | M+DPP | M+FMP | M+2FMP | Derivatization compound | |
|---|---|---|---|---|---|---|
| | Monoisotopic mass (*m/z*) | | | | | |
| **Dopaminergic pathway** | | | | | | |
| Tyrosine | 181.0739 | 396.1594 | 449.1860 | 702.2751 | - | FMP |
| L-3,4-dihydroxyphenylalanine (L-DOPA) | 197.0688 | 412.1543 | 465.1809 | 718.2700 | DPP(*) | FMP(*) |
| Dopamine (DA) | 153.0790 | 368.1645 | 421.1911 | 674.2802 | DPP | FMP |
| 3-Methoxytyramine (3-MT) | 167.0946 | 382.1802 | 435.2067 | 688.2959 | DPP | FMP |
| 3,4-Dihydroxyphenylacetaldehyde (DOPAL) | 152.0473 | - | 420.1594 | 673.2486 | - | FMP |
| Homovanillin | 166.0624 | - | 434.1745 | - | - | FMP |
| 3,4-Dihydroxyphenylacetic acid (DOPAC) | 168.0423 | - | 436.1543 | 689.2435 | - | FMP |
| Homovanillic acid (HVA) | 182.0579 | - | 450.1700 | - | - | FMP |
| 3-O-Methyldopa | 211.0845 | 426.1700 | 479.1965 | 732.2857 | - | FMP(*) |
| Norepinephrine (NE) | 169.0739 | 384.1594 | 437.1860 | 690.2751 | DPP | FMP |
| Epinephrine (EP) | 183.0895 | - | 451.2016 | 704.2908 | - | FMP |
| 3,4-Dihydroxyphenylglycol (DOPEG) | 170.0579 | - | 438.1700 | - | - | FMP |
| 3-Methoxy-4-hydroxyphenylglycol (MOPEG) | 184.0736 | - | 452.1856 | - | - | FMP |
| **Serotonergic pathway** | | | | | | |
| Tryptophan | 204.0899 | 419.1754 | 472.2020 | - | - | FMP |
| Serotonin (5-HT) | 176.0950 | 391.1805 | 444.2070 | 697.2962 | DPP | FMP |
| 5-Hydroxyindoleacetic acid (5-HIAA) | 191.0582 | - | 459.1703 | - | - | FMP |
| 5-Hydroxytryptophol (5-HTOL) | 177.0790 | - | 445.1911 | - | - | FMP |
| 5-Hydroxyindole aldehyde (5-HIAL) | 175.0633 | - | 443.1755 | - | - | FMP |
| **Amino acids and other metabolites** | | | | | | |
| Ethanolamine | 61.0528 | 276.1383 | 329.1648 | - | DPP | FMP |
| Glycine | 75.0320 | 290.1176 | 343.1441 | - | DPP | FMP |
| Alanine | 89.0477 | 304.1332 | 357.1598 | - | DPP | FMP |
| γ-aminobutyric acid (GABA) | 103.0633 | 318.1489 | 371.1754 | - | DPP | FMP |
| Serine | 105.0426 | 320.1281 | 373.1547 | - | - | FMP |
| Valine | 117.0790 | 332.1645 | 385.1911 | - | DPP | FMP |
| Threonine | 119.0582 | 334.1438 | 387.1703 | - | DPP | FMP |
| Phenethylamine | 121.0891 | 336.1747 | 389.2012 | - | DPP | FMP |
| Taurine | 125.0147 | 340.1002 | 393.1267 | - | DPP | FMP |
| Leucine/Iso-leucine | 131.0946 | 346.1802 | 399.2067 | - | DPP | FMP |
| Aspartate | 133.0375 | 348.1230 | 401.1496 | - | - | FMP |
| Tyramine | 137.0841 | 352.1696 | 405.1961 | 658.2853 | DPP | FMP |
| Glutamine | 146.0691 | 361.1547 | 414.1812 | 667.2704 | DPP | FMP |
| Lysine | 146.1055 | 361.1911 | 414.2176 | 667.3068 | DPP | FMP |
| Histidine | 155.0695 | 370.1550 | 423.1816 | 676.2707 | DPP | FMP |

| | | | | | | |
|---|---|---|---|---|---|---|
| Tryptamine | 160.1000 | 375.1856 | 428.2121 | - | DPP | FMP |
| Phenylalanine | 165.0790 | 380.1645 | 433.1911 | - | DPP | FMP |
| Arginine | 174.1117 | 389.1972 | 442.2238 | - | - | FMP |
| 6-Hydroxymelatonin | 248.1161 | - | 516.2282 | - | - | FMP |
| Agmatine | 130.1218 | 345.2074 | 398.2339 | - | DPP | FMP |
| Creatine | 131.0695 | 346.1545 | 399.1815 | - | DPP | FMP |
| Creatinine | 113.0589 | 328.1444 | 381.1710 | - | - | FMP |
| Cystein | 121.0197 | 336.1053 | 389.1318 | - | - | FMP |
| Formiminoglutamic acid | 174.0641 | 389.1496 | 442.1761 | - | - | FMP |
| Histamine | 111.0796 | 326.1652 | 379.1917 | - | DPP | FMP |
| Putrescine | 88.1000 | 303.1856 | 356.2121 | - | DPP | FMP |
| Spermidine | 145.1579 | 360.2434 | 413.2670 | 666.3592 | DPP | FMP |
| Homocarnosine | 240.1222 | 455.2078 | 508.2343 | 761.3235 | DPP | FMP |
| Spermine | 202.2157 | 417.3013 | 470.3278 | - | DPP | FMP |
| Adenine | 135.0545 | 350.1400 | 403.1666 | - | DPP | FMP |
| Alpha-tocopherol | 430.3811 | - | 698.4932 | - | - | FMP |
| Carnosine | 226.1066 | - | 494.2187 | - | - | FMP |
| Vanillactic acid | 212.0685 | - | 480.1805 | - | - | FMP |
| Vanilpyruvate | 210.0528 | - | 478.1649 | - | - | FMP |

(*) only detected after *in vivo* L-dopa administration.

## Supplementary References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