

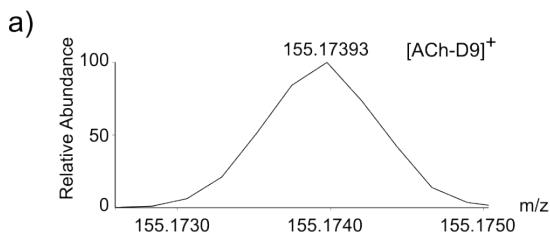
Supplementary Information

Quantitative Mass Spectrometry Imaging of Small-Molecule Neurotransmitters in Rat Brain Tissue Sections using Nanospray Desorption Electrospray Ionization

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Nano-DESI on rat brain
with internal standard



Nano-DESI on rat brain
without internal standard

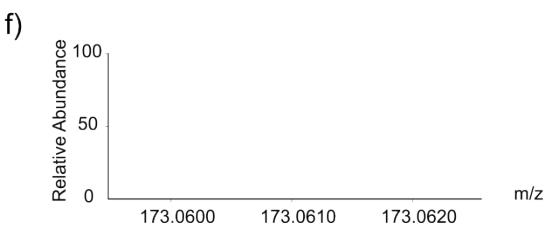
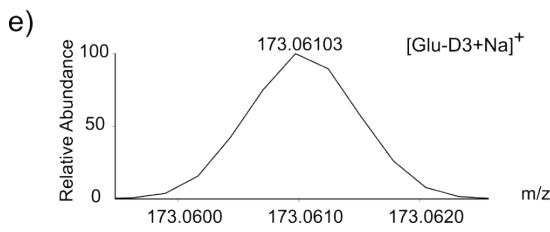
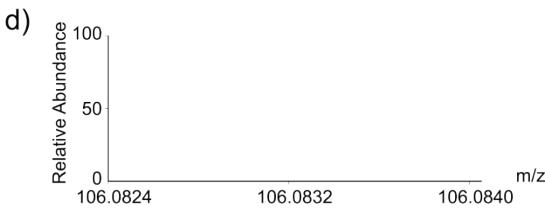
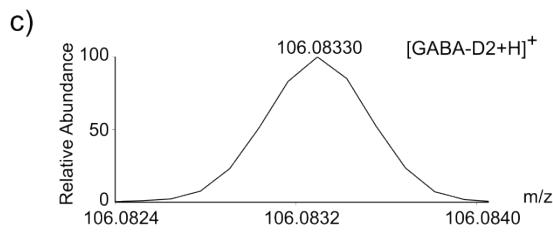
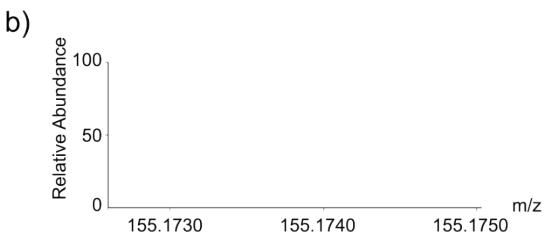


Fig. S-1 – Averaged mass spectra from rat brain tissue zoomed in at m/z corresponding to internal standards. The column to the left shows spectra recorded using a nano-DESI solvent with 10 nM ACh-D9, 2 μ M GABA-D2 and 10 μ M Glu-D3 in 80% MeOH on rat brain tissue. The column to the right shows spectra recorded using a nano-DESI solvent consisting of 90% MeOH on rat brain tissue. A minimum of 20 spectra were averaged a) Detection of $[ACh-D9]^+$, b) m/z corresponding to $[ACh-D9]^+$, c) detection of $[GABA-D2+H]^+$, d) m/z corresponding to $[GABA-D2+H]^+$, e) detection of $[Glu-D3+Na]^+$, f) m/z corresponding to $[Glu-D3+Na]^+$

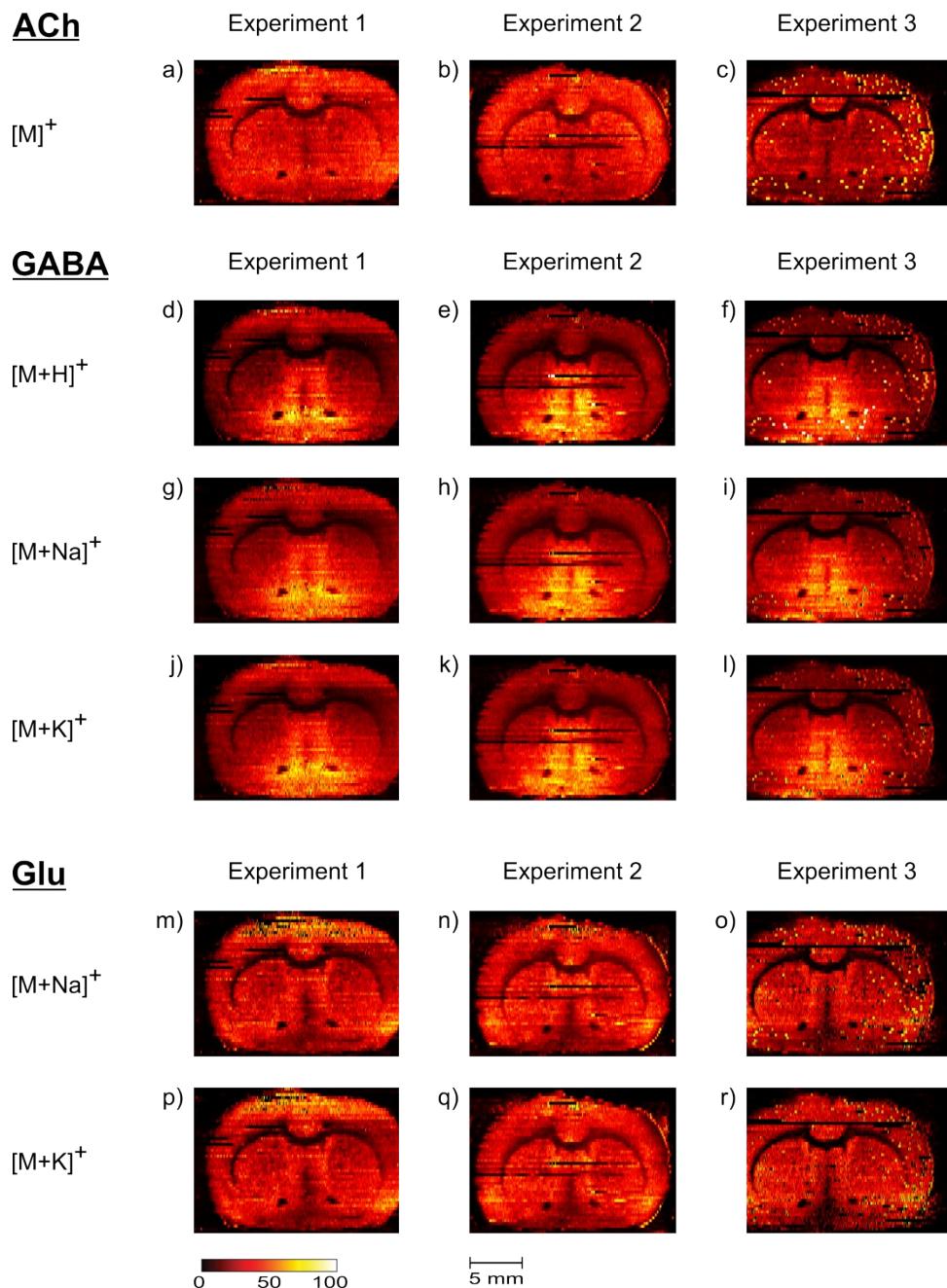


Fig. S-2 – Normalized ion images of Acetylcholine (ACh), γ -aminobutyric acid (GABA) and glutamate (Glu) in rat brain tissue sections from three nano-DESI MSI experiments. Column 1 shows ion images from experiment 1, column 2 shows ion images from experiment 2, and column 3 shows ion images from experiment 3. a-c) $[ACh]^+$, d-f) $[GABA+H]^+$, g-i) $[GABA+Na]^+$, j-l) $[GABA+K]^+$, m-o) $[Glu+Na]^+$, p-r) $[Glu+K]^+$. Scale bar: 5 mm. The signal intensity of the ion images scale from dark to bright.

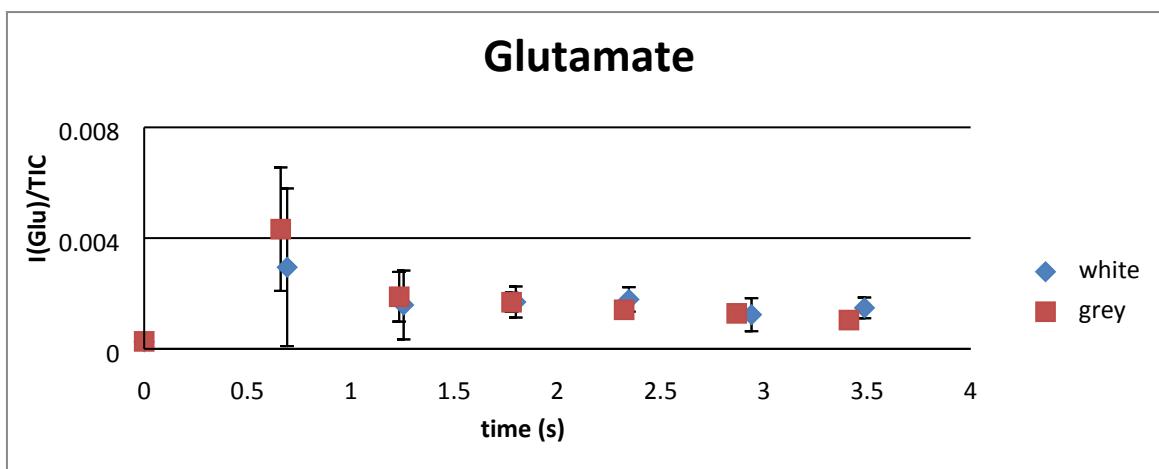
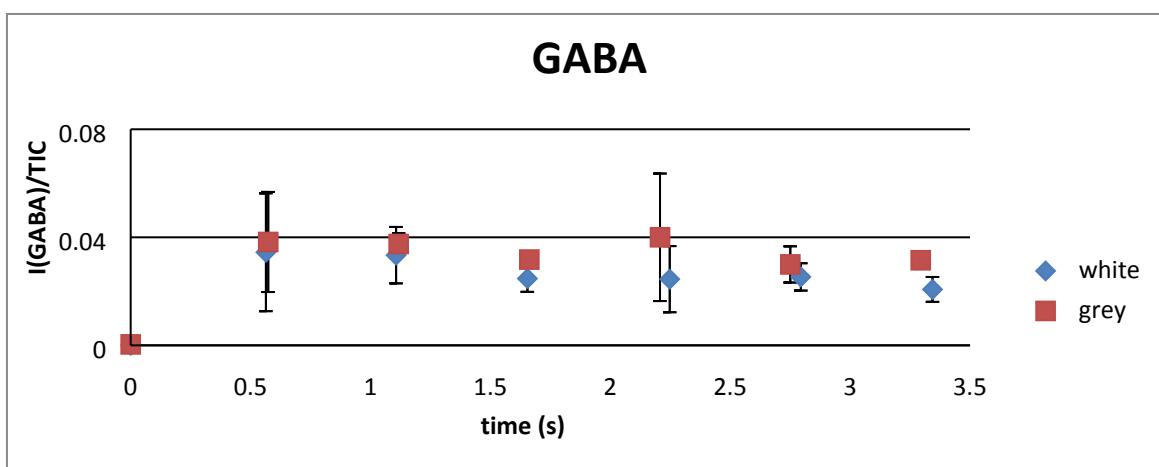
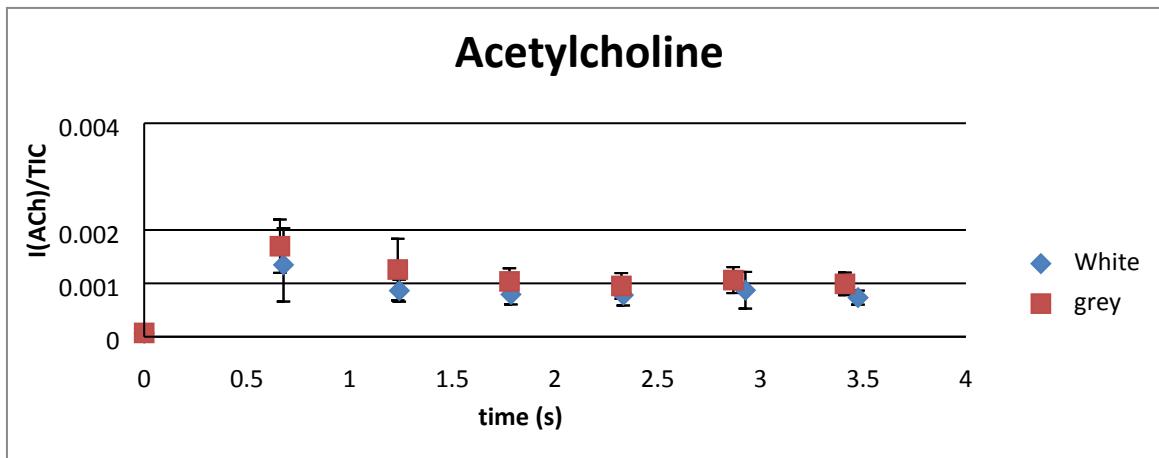
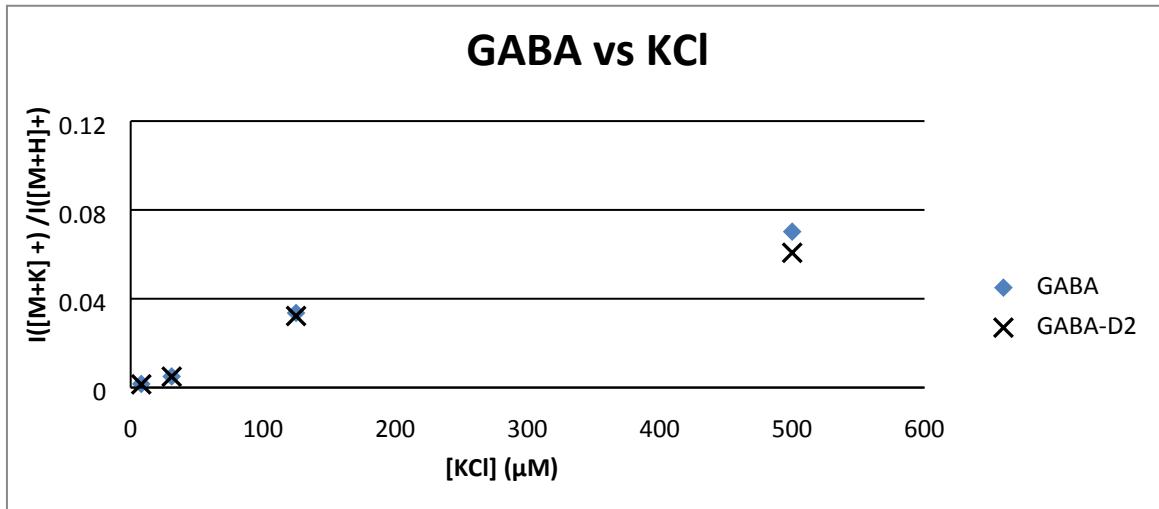


Fig. S-3 – Localized extraction of acetylcholine ($[ACh+H]^+$), γ -aminobutyric acid ($[GABA+H]^+$) and glutamate ($[Glu+Na]^+$) from white matter (blue diamonds) and grey matter (red squares) on a rat brain tissue section. The y-axis shows the relative intensity between the analyte and the total ion count (TIC) and the x-axis shows the time point for data acquisition. ($n=6$ for each data point)

a)



b)

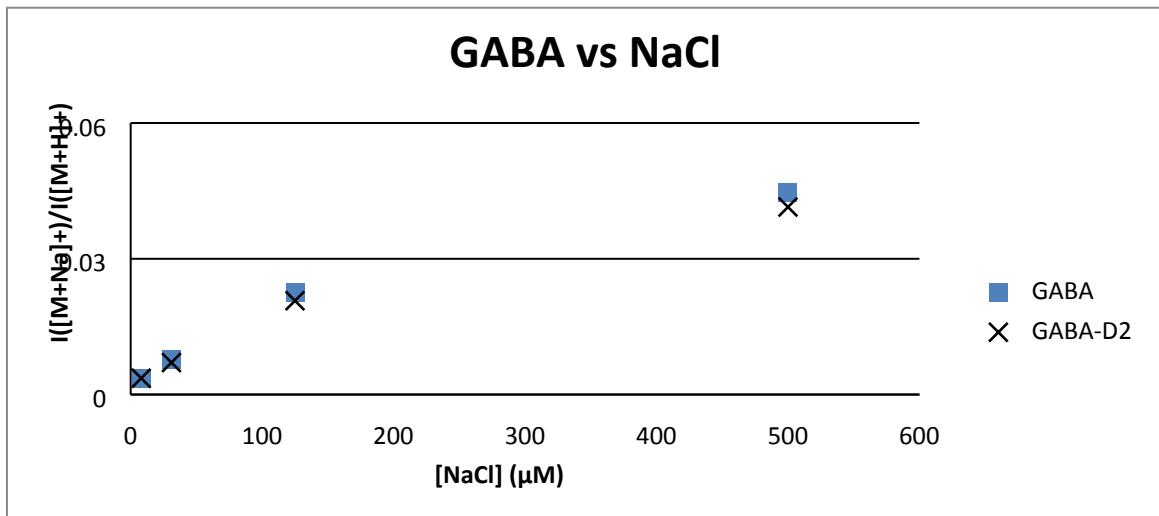
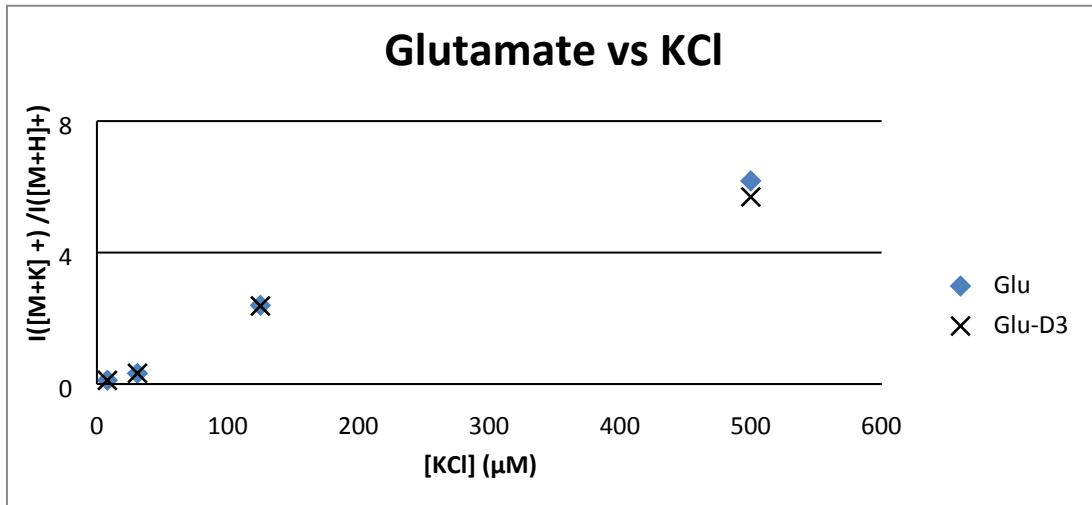


Fig. S-4 – Change in response of GABA and its deuterated standard GABA-D2 at varying salt concentrations. a) Relative responses of $[GABA+K]^+ / [GABA+H]^+$ (blue diamonds) and $[GABA-D2+K]^+ / [GABA-D2+H]^+$ (black cross) at varying concentrations of KCl and b) Relative responses of $[GABA+Na]^+ / [GABA+H]^+$ (blue diamonds) and $[GABA-D2+Na]^+ / [GABA-D2+H]^+$ (black cross) at varying concentrations of NaCl. Data for each point was averaged over 1 min.

a)



b)

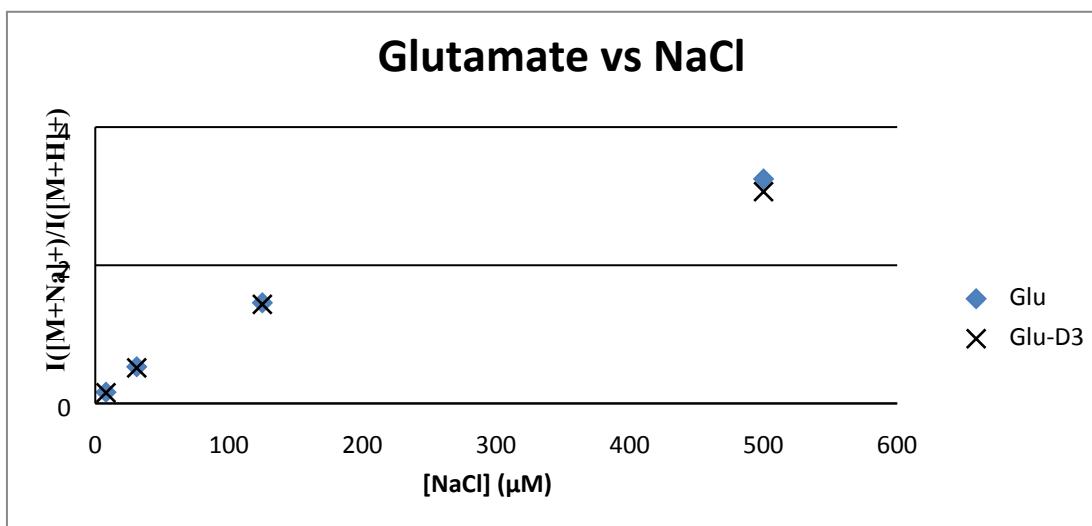


Figure S-5 – Change in response of Glutamate and its deuterated standard Glutamate-D3 at varying salt concentrations. a) Relative responses of $[Glutamate+K]^+ / [Glutamate+H]^+$ (blue diamonds) and $[Glutamate-D3+K]^+ / [Glutamate-D3+H]^+$ (black cross) at varying concentrations of KCl and b) Relative responses of $[Glutamate+Na]^+ / [Glutamate+H]^+$ (blue diamonds) and $[Glutamate-D3+Na]^+ / [Glutamate-D3+H]^+$ (black cross) at varying concentrations of NaCl. Data for each data point was averaged over 1 min.