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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.					
n/a	a Confirmed				
\boxtimes		The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement			
\boxtimes		A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly			
\boxtimes		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.			
\boxtimes		A description of all covariates tested			
\boxtimes		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons			
\boxtimes		A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)			
\ge		For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.			
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings			
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes			
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated			
Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.					

Software and code

Policy information about <u>availability of computer code</u> Data collection FTICR MALDI-MSI data: flexControl (Bruker Daltonics, version 4.0) and FTMS control (Bruker Daltonics, version 2.2.0). DESI-MSI data: Omnispray 2D (Prosolia, version 2.0.1), Tune instrument control software (Thermo Scientific, version 2.9), Xcalibur Sequence Manager (Thermo Scientific, version 4.1.31.9)

Data analysis MS data: FlexImaging (Bruker Daltonics, version 4.1), DataAnalysis (Bruker Daltonics, version 4.2), msIQuant 2.0.1.15. SCiLS Lab Pro (Bruker Daltonics, version 2020b)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data sets generated during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Behavioural & social sciences

Ecological, evolutionary & environmental sciences For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

All studies must dis	close on these points even when the disclosure is negative.
Sample size	Animal sample size was selected to minimize the number of sacrificed animals. The present protocols do not present any statistical tests. Ethical concerns, sample availability and acquisition time were limiting factors for the number of samples.
Data exclusions	No data was excluded in this work.
Replication	MALDI-MSI experiments were replicated on consecutive or adjacent tissue sections. MALDI-MSI is a destructive technique meaning that experiments are difficult to replicate on the same tissue section. MALDI-MSI experiments were also performed on different coronal or sagittal brain levels. All experiments were successfully replicated.
Randomization	Data acquisition was always randomized for multiple tissue sample experiments.
Blinding	Blinding is not relevant since data in this study was generated only to present the protocols for improved detection of neurotransmitters.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
\boxtimes	Antibodies
\boxtimes	Eukaryotic cell lines
\boxtimes	Palaeontology and archaeology
	Animals and other organisms
\boxtimes	Human research participants
\boxtimes	Clinical data
\boxtimes	Dual use research of concern

Methods

n/a Involved in the study \boxtimes ChIP-seq ☐ Flow cytometry \boxtimes \boxtimes MRI-based neuroimaging

Animals and other organisms

Policy information about s	udies involving animals; ARRIVE guidelines recommended for reporting animal research
Laboratory animals	Male Sprague-Dawley rats (Scanbur, mean age: 8 weeks)
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Rat experiments were performed in agreement with the European Communities Council Directive of November 24, 1986 (86/609/ EEC) on the ethical use of animals and were approved by the local ethical committee at the Karolinska Institute, Stockholm, Sweden (N350/08 and N105/16).

Note that full information on the approval of the study protocol must also be provided in the manuscript.