Supplementary Methods

Chemicals, Reagents and Materials

The LC-MS-grade solvents methanol (MeOH), acetonitrile (ACN), isopropanol (IPA) and water, as well as the acetylcholinesterase inhibitor phenylmethylsulfonyl fluoride (PMSF) were purchased from Fisher Scientifc. Formic acid, polyacrylonitrile (PAN), N,N-dimethylformamide (DMF) and the standards of neurotransmitters: γ-aminobutyric acid (GABA), glutamic acid (Glu), acetylcholine (ACh), histamine (Hist), serotonin (5-HT), dopamine (DA) and choline (Cho) as well as their deuterated analogues were purchased from Millipore Sigma (Oakville, ON, Canada). Epinephrine (Epi), norepinephrine (NE) and their deuterated analogues were obtained from Cerilliant Corporation (Round Rock, TX, USA). Choline-D9, was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). The reagents used for synthesis of hydrophilic-lipophilic balance polymer particles functionalized with strong cation exchange groups, as well as compounds for preparation of PBS were purchased from Millipore-Sigma. The stainless steel wire (stainless steel grade AISI 304, 150 μm diameter) used for manufacturing of SPME probes was purchased from Unimed S.A. (Lausanne, Switzerland). The stainless steel tubing used as guiding cannulas (270 μm O.D.; 200±5 μm I.D.) was obtained from Vita Needle (Needham, MA, USA).

LC-MS/MS Analysis

On the day of analysis, the SPME probes were defrosted and desorbed into 40 μL of water/ACN/MeOH 40:30:30 (v/v/v) mixture containing 1 % of formic acid and a mixture of deuterated isotopologues of targeted neuromodulators (used as internal standards, IS) at 20 ng/mL. The desorption was carried out for 1 h with agitation at 1500 rpm. The extracts were injected into the LC-MS system for targeted neurotransmitter analysis within a few hours after desorption. All experiments were carried out using an Ultimate 3000RS HPLC system coupled to TSQ Quantiva triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, California, USA). Data processing was performed using Thermo software Xcalibur 4.0 and Trace Finder 4.1. For chromatographic separation of the target compounds, a modified method of what is previously reported (Cudjoe and Pawliszyn 2014) was used, adjusted to include more

targeted neuromodulators and their corresponding IS. A Kinetex® PFP LC column (100 x 2.1 mm, 1.7 μm; Phenomenex, Torrance, CA, USA) was held at 30°C with the mobile phase flow rate at 400 μL/min. The mobile phase A consisted of water/MeOH/ACN 90:5:5 (v/v/v) with 0.1 % formic acid, and mobile phase B was ACN/water 90:10 (v/v) with 0.1% of formic acid. The chromatographic gradient was applied starting from 100% B for 1 min and increasing the aqueous mobile phase A to 100% for 3 min with convex gradient function, held for 0.5 min and subsequent linear return to initial conditions and re-equilibration for 1 min, yielding total time of 6.5 min. The injection volume was 10 μL. MS/MS analysis was performed with electrospray ionization in positive mode under selected reaction monitoring conditions, with two MS/MS transitions for each neuromodulator (quantifier and qualifier) and one for each IS.

Quantitation of neuromodulators

Individual stock standard solutions of all targeted neuromodulators were prepared in methanol or water with 0.1% formic acid at a concentration of 1 mg/mL and stored at −80 °C for maximum of one month. In order to calculate the amounts of neuromodulators extracted by each probe, calibrator standards prepared in the same desorption solvent as real samples were analyzed in the same batch. This instrumental calibration curve was prepared in the range of 0.1-200 ng/mL by a serial dilution of the stock standard mixture of all compounds at 1μg/mL. The IS concentration was kept constant at 20 ng/mL in all calibrators, identically as in the real samples. The amounts extracted were calculated based on linear regression equation obtained from the analytical signal (the ratio of chromatographic peak areas of analytes and their corresponding IS) plotted against the concentration.

In order to calculate the concentrations of neuromodulators in brain, matrix-matched external calibration approach was used. The surrogate matrix consisted of 2% agar gel mixed with brain homogenate in the ratio 1:1 (*v/w)*. The homogenized brain tissue was earlier incubated with 1 mM PMSF for 1 h at 37°C to prevent enzymatic digestion of acetylcholine in the calibrator samples. Due to several target compounds being present in brain homogenate at high concentrations (e.g. for glutamate and choline the “blank” brain homogenate matrix doesn’t exist), their quantitation was based on signals of their deuterated isotopologues. The calibrator samples were prepared in the surrogate matrix with concentrations of neuromodulators ranging

from 5 to 3000 µg/mL for the isotopically labelled compounds or from 10 to 2000 ng/mL for the remaining compounds.

The extractions were carried out with SPME probes manufactured and pre-treated identically to the probes used for *in vivo* sampling and using the same 20 min extraction time and desorption conditions as for the real samples. The amounts of neuromodulators extracted from the calibrator samples were determined in the same way as described above and plotted against concentrations of calibrators. The resulting weighted linear regression equations were applied to the amounts of neuromodulators extracted from the *in vivo* samples, yielding values of concentrations of the compounds of interest in brain.

The limits of detection (LOD) were estimated as the levels corresponding to the signal to noise ratio of 3 and were calculated based on the signal of blank calibrator sample (considered as the noise).

Supplementary Results

Observed serotonin concentrations. Overall, serotonin concentrations were not observed as reliably as the other reported neurochemicals and therefore were excluded from the main analyses. However, in one of the animals, serotonin was observed near the LOD in the prefrontal cortex as well as the caudate in a subset of probes. Within the 9 probes placed in both areas, serotonin was detected in 22% of samples (2/9 probes) in the prefrontal cortex and 33% of samples (3/9 probes) in the caudate nucleus. Serotonin observations ranged from 149 ng/mL to 232 ng/mL with a median of 171 ng/mL (±18.5 ng/mL; standard error of the median) with a LOD of 100 ng/mL. The proximity of the measurements to the LOD suggest that the other probes likely collected concentrations of serotonin below the detection threshold. No serotonin observations were made in the second animal.

Supplementary Discussion

Future adjustments to the SPME neurochemical sensing. Several improvements to the protocol can be made in order to make SPME more informative. Using a more sensitive MS would reduce detection thresholds and increase sensitivity in detecting compounds not

successfully measured here such as GABA, serotonin or norepinephrine. This would be very impactful, especially in the case of Serotonin for reasons discussed above. Additionally, efforts could be made to improve the extracting phase synthesis and functionalization protocols and increase the extracting capabilities of SPME probes. Moreover, in order to decrease the MS background and interferences in the range of small molecules, derivatization strategy could be considered.

For GABA, the most promising strategy is post-desorption derivatization by reagent increasing the compound's hydrophobicity resulting in better MS signal such as benzoyl chloride (Wong 2016). Catecholamine and acetylcholine detection may be improved by optimizing the post-sampling analysis pipeline. Improvements can be made at several steps in order to better preserve and quantify catecholamines: (1) faster coupling of sampling to desorption (2) trying various antioxidant solutions and other preservatives for maintaining catecholamine integrity and (3) faster analysis of the desorbed analytes. Faster coupling of sampling to desorption could be achieved through a more stream-lined process of fibre placement and retraction involving a static cannula maintained through several sampling events. This mechanism would also allow for a faster replacement of SPME probes resulting in a shorter delay between consecutive measurements.

A preliminary experiment using spiked artificial cerebra-spinal fluid (aCSF) with very high, known concentrations of all target compounds yielded detectable concentrations of all target compounds indicating that the extracting phase of the SPME probe is capable of capturing all compounds of interest. Furthermore, simultaneously collected replicates displayed very little loss of collected compounds over several days when placed in a -80°C freezer (data not shown). Such findings neither support nor antagonize the suggested strategies for improving SPME yield and sensitivity in primates as detection properties within the aCSF do not seem to accurately reflect those observed in vivo in NHPs. This is likely contributed to by the dynamic and highly regulated nature of target compounds within the extracellular space of the brain and the dynamic equilibrium between the extracting phase of the SPME probes and the extracellular environment. Further tests of the possible differences between these two mediums and improvement strategies to SPME probes are subject for future studies.

Supplementary Figures

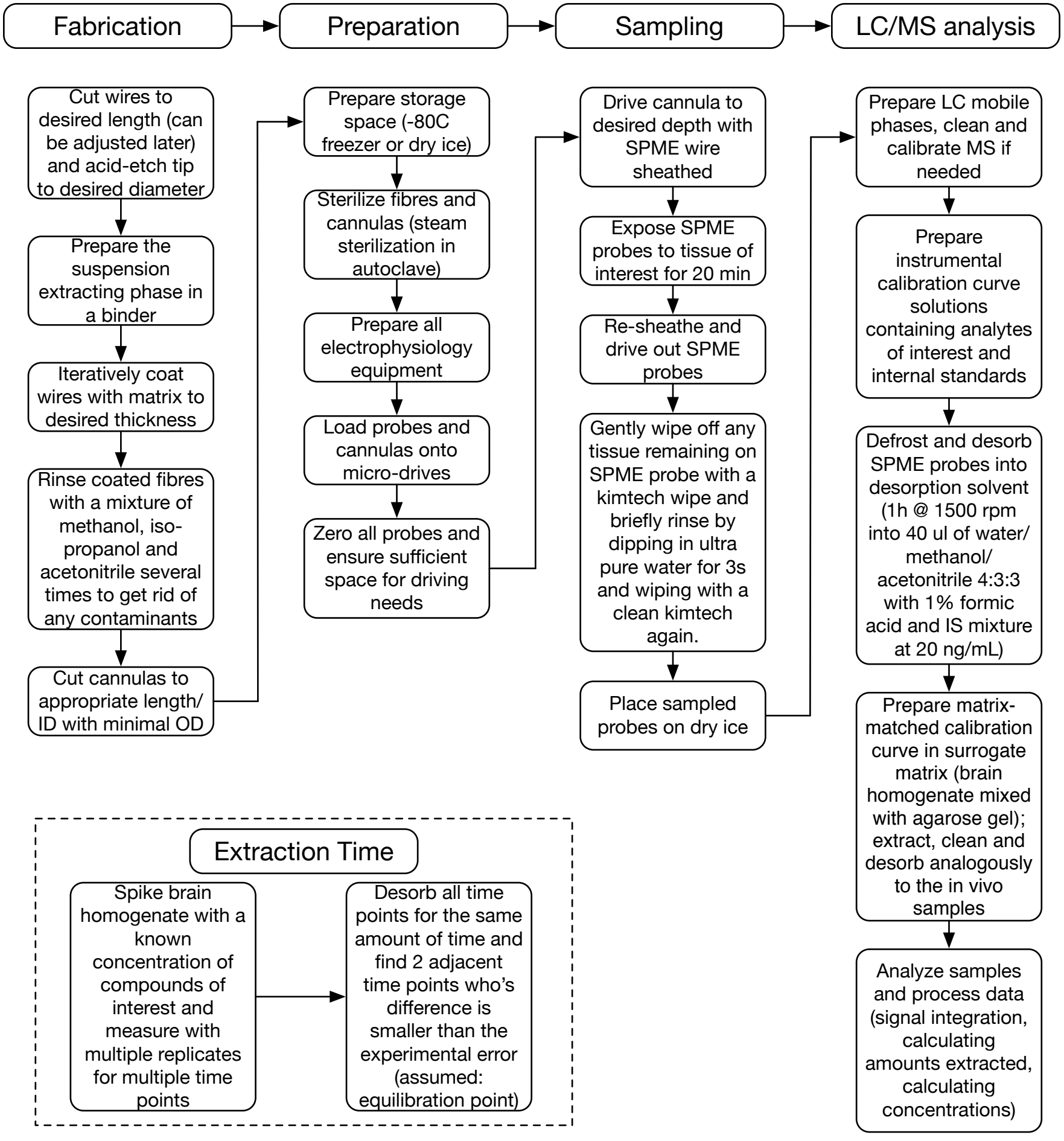
Fig S1. Experimental procedure from SPME probe fabrication to quantitation. Fabrication described the in-house procedure to prepare SPME probes. Preparation describes experimental set-up. Sampling describes the actual data collection process. LC/MS analysis describes the chemical quantitation of collected data samples.

Supplementary Tables

Table S1. Overview of in vivo neurochemical measurement methods

	PET imaging	**Electro-chemistry**	**Micro-dialysis**	**Solid phase micro-extraction**
Temporal Resolution	Minutes	Highest (millisecond range)	1-30 minutes (usually 20 minutes); dependent on MS sensitivity, target etc.	<5-30 minutes; dependent on MS sensitivity, coating thickness, target etc.
Spatial Resolution	Voxel	High; surface area may vary (relevant for enzyme based methods)	Diffusion based; surface area may vary	Diffusion based; surface area may vary
Sensitivity	Indirect measurement via competitive radiolabeled species	High	Depending on post-hoc methods (i.e. MS)	Depending on post-hoc methods (i.e. MS)
Neuro-active targets	A few at most	A few at most	Many	Many
Non-neuro-active targets	No	No	Yes, greater efficacy for hydrophilic compounds	Yes, greater potential efficacy for hydrophobic compounds
In vivo feasibility	Difficult in awake, behaving animal models; movement highly restricted	Good (low reliability in NHPs)	Good	Best
Cost	High	Requires special equipment	Requires special equipment	Easy to port to an acute micro-electrode setup; requires a chemistry core

Table S1. A comparison of methods capable of measuring single or multiple neurochemicals in vivo. Temporal resolution, spatial resolution, sensitivity, capability to measure neuro-active and non-neuro-active compounds, in vivo feasibility and cost. PET: positron emission tomography; NHP: non-human primate; MS: mass spectrometer.


Fabrication
Preparation
Sampling
LC/MS analysis
Cut wires to desired length (can be adjusted later) and acid-etch tip to desired diameter
Prepare the suspension extracting phase in a binder
Iteratively coat wires with matrix to desired thickness
Rinse coated fibres with a mixture of methanol, iso-propanol and acetonitrile several times to get rid of any contaminants
Cut cannulas to appropriate length/ ID with minimal OD
Prepare storage space (-80C freezer or dry ice)
Sterilize fibres and cannulas (steam sterilization in autoclave)
Prepare all electrophysiology equipment
Load probes and cannulas onto micro-drives
Zero all probes and ensure sufficient space for driving needs
Drive cannula to desired depth with SPME wire sheathed
Expose SPME probes to tissue of interest for 20 min
Re-sheathe and drive out SPME probes
Gently wipe off any tissue remaining on SPME probe with a kimtech wipe and briefly rinse by dipping in ultra pure water for 3s and wiping with a clean kimtech again.
Place sampled probes on dry ice
Prepare LC mobile phases, clean and calibrate MS if needed
Prepare instrumental calibration curve solutions containing analytes of interest and internal standards
Defrost and desorb SPME probes into desorption solvent (1h @ 1500 rpm into 40 ul of water/ methanol/ acetonitrile 4:3:3 with 1% formic acid and IS mixture at 20 ng/mL)
Prepare matrix-matched calibration curve in surrogate matrix (brain homogenate mixed with agarose gel); extract, clean and desorb analogously to the in vivo samples
Analyze samples and process data (signal integration, calculating amounts extracted, calculating concentrations)
Extraction Time
Spike brain homogenate with a known concentration of compounds of interest and measure with multiple replicates for multiple time points
Desorb all time points for the same amount of time and find 2 adjacent time points who's difference is smaller than the experimental error (assumed: equilibration point)