Multi-Neuromodulator Measurements across Fronto-Striatal Network Areas of the Behaving Macaque using Solid-Phase Microextraction

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Author contributiosn:
Seyed-Alireza Hassani and Sofia Lendor together collected all data and conducted statistical analysis of the collected data. Seyed-Alireza Hassani trained and handled the animals, prepared the MRI mapping and managed sampling equipment. Sofia Lendor prepared all SPME probes and conducted all chemical analyses/quantification of collected compounds. Ezel Boyaci contributed to the preparation of SPME probes and quality control. Janusz Pawliszyn and Thilo Womelsdorf contributed to the collection of data and analyses. All authors contributed to the writing, visualization and editing of the manuscript.

Abstract

Different neuromodulators rarely act independent from each other to modify neural processes but are instead co-released, gated, or modulated. To understand this interdependence of neuromodulators and their collective influence on local circuits during different brain states, it is necessary to reliably extract local concentrations of multiple neuromodulators in vivo. Here we describe results using solid phase microextraction (SPME), a method providing sensitive, multi-neuromodulator measurements. SPME is a sampling method that is coupled with mass spectrometry to quantify collected analytes. Reliable measurements of glutamate, dopamine, acetylcholine and choline were made simultaneously within frontal cortex and striatum of two macaque monkeys (*Macaca mulatta*) during goal-directed behavior. We find glutamate concentrations several orders of magnitude higher than acetylcholine and dopamine in all brain regions. Dopamine was reliably detected in the striatum at tenfold higher concentrations than acetylcholine. Acetylcholine and choline concentrations were detected with high consistency across brain areas, within monkeys and between monkeys. These findings illustrate that SPME microprobes provide a versatile novel tool to characterize multiple neuromodulators across different brain areas in vivo to understand the interdependence and co-variation of neuromodulators during goal directed behavior. Such data will be important to better distinguish between different behavioral states and characterize dysfunctional brain states that may be evident in psychiatric disorders.

Introduction

Extracellular concentrations of neuromodulators influence firing regimes, input-output relationships and neural interactions in local circuits and long-range brain networks (Marder 2012; Thiele and Bellgrove 2018), and are dysregulated in virtually all psychiatric disorders (Avery and Krichmar 2017; Millan et al. 2012). Accumulating evidence suggests that these fundamental roles of neuromodulators for circuit functioning are unlikely realized by single neuromodulators operating in isolation. Rather, neuromodulatory systems are heavily intertwined (Avery and Krichmar 2017; Gobert et al. 1998; Moehle et al. 2017), and operate simultaneously on individual cells and circuits (Arnsten et al. 2010; Hassan et al. 2015; Marder 2012; Santana et al. 2018). In each circuit, local mechanisms exert control over the release of neuromodulators from terminals of brainstem-originating projection neurons. This local control proceeds through activation of pre-synaptic glutamatergic receptors (Ghersi et al. 2003; Grilli et al. 2009; Luccini et al. 2007; Pittaluga 2016; Pittaluga et al. 2006; Wang et al. 1992). These insights suggest that an understanding of the contribution of neuromodulators to circuit functioning requires measuring, simultaneously, multiple neuromodulators in conjunction with ongoing glutamatergic neurotransmitter concentrations and action. Consistent with this conclusion single neuromodulator theories often fail to account for all observable symptoms in psychiatric diseases (Bohnen et al. 2015; Halliday et al. 2014; Remy et al. 2005).

Despite the accumulating evidence for the interdependence of neuromodulator actions, few methods exist for their simultaneous measurement in vivo and across multiple brain areas (**Table S1**). Most of these existing neurochemical sensing methods have a high barrier to entry by requiring specialized equipment and trained experts preventing data collection by scientists who are otherwise interested in the role of endogenous and exogenous neuroactive chemicals in

73 cognition and psychiatric disorders. Electrochemical methods such as fast scan cyclic
74 voltammetry (FSCV) and amperometry have sub-second temporal resolution, but are limited to
75 the measurement of a few compounds (Dale et al. 2005; Jacobs et al. 2010) and are not robust for
76 in vivo application in nonhuman primates (Vartak et al. 2017). Imaging techniques such as
77 positron emission tomography (PET) are also limited to the measurement of one or a few
78 compounds simultaneously (Fisher et al. 1995). Microdialysis (MD) paired with mass
79 spectrometry is the most commonly used method for measuring multiple neuromodulators in
80 awake behaving animals. MD provides a data collection method which is then analyzed post hoc
81 to identify and quantify collected analytes. It operates with a semi-permeable membrane which
82 allows for the continuous collection of the available extracellular neuromodulators through
83 passive diffusion, and can even be used to locally release pharmacological agents (Anderzhanova
84 and Wotjak 2013; Kennedy 2013; Watson et al. 2006). However, MD does have several
85 disadvantages including disrupting the tissue during its initial placement of the probe or a
86 guiding cannula resulting in damage-induced release of neuromodulators that can last several
87 hours before stable measurements become possible. Moreover, MD has low affinity for
88 hydrophobic compounds and comparatively broad spatial and temporal resolution that is in the
89 range of 200-400 μm in diameter and 10-20 minutes, respectively. These values are dependent
90 on the surface area of the permeable membrane, the exact method of MD, flow rate, resolution of
91 detection methods for analytes of interest, tissue tortuosity and more (Anderzhanova and Wotjak
92 2013; Kennedy 2013; Watson et al. 2006).

93 In light of these limitations of available measurement techniques, we set out to
94 characterize a novel protocol for measuring multiple neuromodulators in vivo using probes
95 optimized for solid phase microextraction (SPME) (Pawliszyn 2012). SPME probes are thin (200

96 μm) wires of arbitrary length coated with an inert porous polymeric matrix using biocompatible
97 binder on one end where molecules with appropriate size and affinity migrate via passive
98 diffusion and are retained by chemical interactions (*see* Methods). SPME provides an alternate
99 method for data collection which can then be analyzed by tools such as mass spectrometers. This
100 method has been shown to extract in neural tissue dynamic changes in dopamine (DA) and
101 serotonin (5-HT) levels with comparable precision to MD (Cudjoe et al. 2013; Cudjoe and
102 Pawliszyn 2014). Additionally, due to the similarity of SPME probes to commonly used
103 microelectrodes in electrophysiological recordings, relatively minor adjustments will allow for
104 the adaptation of conventional microelectrode driving systems for SPME use. This, combined
105 with post collection analysis through standard chemistry facilities makes SPME an attractive and
106 easy-to-use tool for electrophysiology labs.

107 The ability of SPME to report major neuromodulators as well as glutamate and GABA
108 were tested in two behaving rhesus macaques. Probes were repeatedly and simultaneously
109 inserted into two cortical regions and the striatum to observe inter-areal differences between
110 extracellular neuromodulator concentrations. We found that extracellular concentrations of
111 glutamate, dopamine, acetylcholine and choline could be reliably distinguished and differed
112 systematically between brain regions.

113

114 **Methods**

115 **Animals**

116 Data was collected from two 8 year-old male rhesus macaques (*Macaca mulatta*) weighing 8-12
117 kg. All animal care and experimental protocols were approved by the York University Animal
118 Care Committee and were in accordance with the Canadian Council on Animal Care guidelines.

119 Details regarding the experimental setup, recording procedures, and reconstruction of recording
120 sites have been described previously (Oemisch et al. 2015). Briefly, animals were implanted with
121 a 20 mm by 28 mm recording chamber over the frontal region of the right hemisphere guided by
122 stereotaxic coordinates (Paxinos et al. 2000) and MR images. The animals were seated in a
123 custom made primate chair and head stabilized with their eyes 65cm away from a 21' LCD
124 monitor refreshed at 85 Hz. Eye traces were collected by a video-based eye-tracking system
125 (Eyelink 1000 Osgoode, Ontario, Canada, 500 Hz sampling rate). The animals were engaged in
126 an over-trained attention tasks in which they would use saccadic eye movements to acquire juice
127 reward. The specifics of the task are described elsewhere (Hassani et al. 2017). Both animals
128 showed stable performance and acquired similar reward volumes on all recording days. In both
129 tasks, stimulus presentation and reward delivery was controlled through MonkeyLogic
130 (http://www.brown.edu/Research/monkeylogic/).

131 **SPME protocol and Fabrication of SPME Probes**

132 We provide a visual overview of the complete SPME protocol used here in **Fig S1** and delineate
133 the chemical materials, LC-MS/MS analysis, and detailed quantitation of neuromodulators in the
134 ***Supplementary Methods*** and in a companion paper (Lendor et al., submitted). All measurements
135 used miniaturized SPME probes that were manufactured by repeated dip-coating of stainless
136 steel wires in a suspension of extracting phase in a binder. This general procedure reported
137 previously (Gómez-Ríos et al. 2017), has been modified to fit the purpose of *in vivo* brain
138 sampling (Lendor et al., submitted). The 3 mm long tip of the wire was acid-etched down to
139 approx. 100 μm to create a recession capable of accommodating thicker layer of coating without
140 significantly increasing the total probe diameter. The extracting phase was an in-house
141 synthesized hydrophilic-lipophilic balance polymer functionalized with benzenesulfonic acid to

142 introduce strong cation exchange properties (HLB-SCX). The monodispersed polymeric particles
143 with diameter of approx. 1 μm were suspended in the binder consisting of 7% polyacrylonitrile
144 dissolved in N,N-dimethylformide (w/v), ensuring the particles-to-binder ratio at 15% (w/v). The
145 extracting phase suspension was prepared one day before the probe coating, homogenized by
146 sonication and stirred at 800 rpm overnight. Several layers of coating were deposited on the
147 modified wires, resulting in total coating thickness of ≈50 μm and total probe diameter of 195
148 μm. The average pore size of the extracting phase was measured at 1.2nm. Before the use, the
149 probes were cleaned with the mixture of methanol, acetonitrile and 2-propanol (50:25:25, v/v/v),
150 activated in the mixture of methanol and water (50:50. v/v) and sterilized in steam for 15 min at
151 121°C. During the sampling, SPME probes were inserted into sheathing cannulas to protect the
152 coating and prevent it from extracting compounds on the way to the target brain area. The
153 cannulas underwent the same cleaning and sterilization procedure as the probes and then the
154 lengths of both components of the SPME assembly were adjusted to 60-70 and 70-80 mm for the
155 probes and cannulas, respectively.
156

157 **MRI Guided Electrophysiological Mapping of Target Tissue**

158 The anatomical coordinates of the brain regions of interest were first identified through 3 T MR
159 images. The MR images were then verified with extracellular electrophysiological recordings of
160 the target areas, which provided the gray and white matter boundaries for the cortical sites and
161 the dorsal most aspect of the head of the caudate nucleus. Tungsten microelectrodes were 200
162 μm thick with an impedance of 1-2 MΩ. All electrodes, SPME probes and their accompanying
163 guiding cannulas were driven down into the brain and later out using software-controlled
164 precision microdrives (Neuronitek, ON, Canada). Electrodes were connected to a multichannel

165 acquisition processor (Neuralynx Digital Lynx system, Inc., Bozeman, Montana, USA) which
166 was used for data amplification, filtering and acquisition of spiking activity. Spiking activity was
167 obtained by applying a 600-8000 Hz bandpass filter, with further amplification and digitization
168 at a 32 KHz sampling rate. For every recording day, electrodes were lowered until the first
169 detection of spiking activity (indicative of gray matter) at the depth suggested by the MR images.

170

171 **SPME Sampling and Post-Processing Procedures**

172 All three SPME assemblies (example of sampling using one assembly in **Fig 1A-C**) were
173 simultaneously driven to 200 μm above the point of first spiking detection. SPME assemblies
174 were located ~1 mm away from the electrode penetration location. Then, only the SPME probes
175 were inserted 3 mm into the areas of interest. On average macaque cortical thickness is only 2
176 mm while the head of the caudate at the point of sampling was well over 3 mm. An extraction
177 duration of 20 min was selected to reflect stable extraction time profiles for all compounds of
178 interest. Extraction time profiles reflect the detectable concentration of collected analyte over
179 time, which is captured in sequence by a linear "kinetic" regime, a "dynamic" regime and an
180 "equilibrium" regime over extraction time. We illustrate in **Fig 1D-E** these regimes for two main
181 compounds measured with the SPME probe we used in vivo. After the 20 min extraction event,
182 all SPME probes were driven back 3 mm into the guiding cannulas and all SPME assemblies
183 were withdrawn from the brain (**Fig 1A-C**).

184 The microdrives were removed from the chamber to enable unclamping of the SPME
185 probes, a brief wash and then storage in glass vials surrounded by dry ice until placing them into
186 a -80°C freezer. One entire sampling event (one extraction in 3 different brain areas together
187 with assembling the SPME probes and cannulas, driving into and out of brain, washing and

188 preparing for storage) was performed within 50 min, except for the first sampling event in each
189 sampling day. The first sampling event, with the area identification using electrophysiology
190 recording, was performed within 75 min.
191
192 **Neuromodulators detection and quantitation**
193 Detection and quantitiation of neuromodulators followed previously established procedures
194 described in the **Supplementary Material**. In brief, SPME probes were defrosted and desorbed
195 in an aqueous solution containing water, acetonitrile, methanol, formic acid and deuterated
196 isotopologues of target neuromodulators as internal standards (IS). Chromatographic separation
197 of target compounds was conducted as previously reported (Cudjoe and Pawliszyn 2014).
198 Tandem mass spectrometry (MS/MS) was performed with electrospray ionization with two
199 MS/MS transitions for each neuromodulator and one for each IS (Lendor et al., submitted).
200 Stock solutions of all target neuromodulators were prepared and used to generate
201 standard calibration curves and to calculate the amounts of neuromodulator extracted by each
202 experimental SPME probe. Conversion of the amount of neuromodulator extracted to in-brain
203 concentrations was done using matrix-matched external calibrations. LOD is defined as 3 times
204 the signal obtained from blank samples.
205
206 **Results**
207 During all measurements animals were engaged in a cognitively demanding task with stable
208 behavioral performance to minimize state dependent fluctuations of basal extracellular
209 concentrations of neuromodulators. Three brain regions were selected to provide a sample of
210 extracellular neuromodulatory concentrations. Two cortical regions: prefrontal cortex (PFC) area

211 8, premotor cortex (PreMC) area 6 and the head of the caudate nucleus (CD) were selected (**Fig**
212 **1F**). In 3 daily sessions, we sampled 3 times per session simultaneously from all three areas.
213 Monkey As had an additional 3 days of recording with one sample collected on each day.
214 Overall we collected and analyzed 12 probes in monkey As and 9 probes in monkey Ke. We
215 were specifically interested in major neuromodulators and neurotransmitters but successfully
216 measured other compounds such as amino acids (e.g. glutamine, taurine, phenylalanine etc) that
217 we do not discuss here.

218 To allow comparison of the SPME extraction results to those typically reported in
219 microdialysis studies, we calculated the relative change in measured concentrations across the
220 three successive sampling events per session pooled across monkeys to enhance the statistical
221 power of the analysis (**Fig 2**). Relative to the end of the first sampling event, the second and third
222 sampling events started after 40 minutes and 100 minutes respectively. We expected that the
223 variability of measurements (indexed as standard error of the median) is comparable to
224 repeatedly measured microdialysis of an identical, active brain state. We found that measured
225 concentrations did not change significantly across sampling events for any compound area
226 combination (Wilcoxon rank sum test; **Fig 2**).

227 We found that four target neurotransmitters and neuromodulators were reliably detected
228 in each animal: glutamate, dopamine, acetylcholine, and choline. Serotonin was also detected on
229 several probes but always near the limit of detection (LOD) and therefore was excluded from
230 analyses here (a report of serotonin is provided in the **Supplementary Results**) (Lendor et al.,
231 submitted). Glutamate concentrations were several orders of magnitude higher than all other
232 observed compounds of interest in all areas and both animals. Relative to glutamate, choline

233 concentrations were >15 times lower, DA concentrations were >700 lower and ACh
234 concentrations were >8300 times lower (**Fig 3**).

235 Glutamate concentrations (across areas), measured as median ± standard error, ranged
236 from 159,147 (±19,395) to 233,659 ng/mL (±33,917) in monkey As, and from 135,523
237 (±22,945) to 184,333 ng/mL (±33,516) in monkey Ke. Neither animal showed a significant
238 difference in glutamate concentrations between areas (As: kruskal-wallis test, p = 0.063; Ke:
239 kruskal-wallis test, p = 0.48). However, in both monkeys, glutamate concentrations were highest
240 in the head of the caudate, which showed a trend to be higher in the caudate than in premotor
241 cortex in monkey As (Wilcoxon rank sum test, p = 0.0226, not significant at the Bonferroni
242 corrected alpha level of p= 0.0167). Comparisons between monkeys showed no significant
243 differences between the glutamate concentrations in any of the measured areas (**Fig 3**).

244 In addition to glutamate, dopamine could be reliably detected in both monkeys in the
245 caudate at concentrations of 232 ng/mL (±47) in monkey As, and 226 ng/mL (±31) in monkey
246 As. These concentrations were similar between monkeys (Wilcoxon rank sum test p = 1, n.s.).
247 Dopamine was not found above detection limits in frontal cortex.

248 Acetylcholine measurements in the three brain areas ranged from (across areas) 25 (±2)
249 to 36 ng/mL (±5) in monkey As, and from 29 (±2) to 36 ng/mL (±4) in monkey Ke. Extracellular
250 ACh concentrations were significantly different between areas in monkey As (kruskal-wallis test,
251 p = 0.008). The caudate showed significantly higher concentrations than premotor cortex and a
252 trend to higher concentrations than PFC (PFC8a: Wilcoxon rank sum test, p = 0.04; PreM6:
253 Wilcoxon rank sum test, p = 0.005); only the difference between PreM6 and caudate survived
254 multiple comparisons correction (Bonferroni alpha = 0.0167). Cortical areas were not
255 significantly different from one another (Wilcoxon rank sum test, p = 0.17). In monkey Ke, only

256 one cortical region had concentrations of ACh above detection limits, which approached
257 significant difference to the concentrations measured in the caudate (Wilcoxon rank sum test, p =
258 0.08). Measurements of extracted ACh in the pre-motor cortex of monkey Ke were consistently
259 below detection limits (**Fig 3**).

260 We next measured choline which is a main product of the enzymatic breakdown of the
261 highly regulated ACh and which is a main indicator of attentional modulation of cholinergic
262 activity in frontal cortex (Parikh et al. 2007). Choline concentrations ranged across areas from
263 2,914 (±590) to 5,078 ng/mL (±675) in monkey As, and 3,408 (±3,705) to 5,533 ng/mL (±860)
264 in monkey Ke. Choline concentrations between areas were significantly different in monkey As
265 (kruskal-wallis test, p = 0.0262), but not monkey Ke (kruskal-wallis test, p = 0.25). Pairwise
266 comparisons in monkey As showed that concentrations after multiple comparison correction
267 were significantly different between PreM6 and the caudate (Wilcoxon rank sum test, p = 0.01;
268 Bonferroni alpha = 0.0167), and approached significance for PFC8 *versus* caudate (Wilcoxon
269 rank sum test, p = 0.06; Bonferroni alpha = 0.0167). Comparisons of choline concentrations
270 between monkeys were not significant in any of the measured areas.

271

272 **Discussion**

273 We demonstrated the reliable measurements of glutamate, dopamine, acetylcholine and choline
274 simultaneously within cortical and subcortical regions of awake and behaving macaques using
275 coated microprobes optimized for solid phase microextraction. Glutamate concentrations were
276 several orders of magnitude higher than dopamine, acetylcholine and choline across brain
277 regions. Extracellular concentrations of choline, dopamine and acetylcholine were detected at
278 >15, >700, and >8,300 times lower than glutamate respectively. Dopamine was readily detected

279 in the caudate but not observed at detectable concentrations in the cortical regions measured.
280 Acetylcholine concentrations showed a statistical trend of being different between the caudate
281 and measured cortical areas and with high consistency between monkeys. Choline
282 concentrations, a product of acetylcholine degradation as well as a precursor for its synthesis,
283 were negatively correlated with acetylcholine ($R = -0.355$; $p = 0.01$). Together, these findings
284 provide new and rare insights about the neurochemical circuit profiles during an active brain
285 state in three areas of the primate fronto-striatal network.

286 The high consistency of measured concentrations within animals, between brain areas
287 and between monkeys suggests that SPME microprobes could provide a versatile neuro-
288 technique for understanding how variations of the neurochemical milieu relate to local and long-
289 range circuit operations and ultimately cognitive functioning.

290

291 **Extracellular Concentrations of Glutamate, Dopamine, Acetylcholine and Choline.** Our
292 results revealed particularly high levels of glutamate within all measured brain regions (PFC,
293 PreMC, and CD) relative to other neuromodulators. This finding is consistent with its ubiquitous
294 role as major excitatory neurotransmitter. This holds true even in the caudate which consists
295 exclusively of GABAergic neurons, but receives glutamatergic inputs from the cortex (Haber
296 and Knutson 2010). Tonic concentrations of glutamate within the striatum have been previously
297 reported to be comparable to that of the hippocampus, prefrontal cortex and other cortical areas
298 due to glutamatergic inputs, extra-synaptic release and glial release (Ascenzo et al. 2006;
299 Moghaddam 1993; Moussawi et al. 2011). In contrast, classical neuromodulators such as DA and
300 ACh, are released in extracellular space through volume transmission where they are highly
301 regulated and may bind to receptors on multiple neurons (Santello et al. 2012; Schwartz and

302 Javitch 2013; Syková 2004; Vizi 2000). This regulation may help explain the relatively low
303 observed concentrations of DA and ACh and may be a large source of extracellular choline
304 through the enzymatic breakdown of ACh.

305 Extracellular DA was measured well above the detection threshold in the striatum. In
306 combination with glutamatergic and cholinergic concentrations, our results show promise in the
307 application of SPME to further our understanding of the relationship between glutamatergic
308 inputs to the striatum and the neuromodulatory ACh and DA signals impinging on striatal
309 circuits. Such circuits are strongly involved in hypo- and hyperkinetic diseases (Halliday et al.
310 2014; Millan et al. 2012). Dopaminergic signals did not exceed the detection threshold in either
311 cortical regions. The presence of DA in the PFC is well documented (Goldman-Rakic et al.
312 1990) and thus has failed to exceed our detection limit. A possible explanation for the lack of
313 detectable cortical DA is the susceptibility of catecholamines to oxidation. This may be partially
314 addressed by faster coupling of sample collection to quantitation by MS (see **Supplementary**
315 **Discussion**).

316 Few previous studies have documented ACh concentrations in vivo due to its rapid
317 breakdown by acetylcholinesterase (Kreitzer 2009; Schwartz and Javitch 2013; Zapata et al.
318 2013). ACh plays a major role in organizing local circuits, deployment of attention, locomotion
319 and reward through different receptors present in cortical and subcortical regions (Granger et al.
320 2016; Howe et al. 2017; Moehle et al. 2017). It has been difficult to discern which ACh
321 concentration corresponds to efficient endogenous circuit operations. Our finding of
322 measurements in all sampling locations except for premotor cortex in monkey Ke, is therefore a
323 promising starting point for future studies comparing ACh concentrations, in conjunction with
324 other neuromodulators, during different cognitive states. The fast turn-around of ACh due to

325 AChE however, likely leads to an underestimation of ACh concentrations by SPME. Although
326 AChE cannot traverse the porous SPME coating due to its size (~7.6nm diameter), preventing
327 any enzymatic degradation of trapped ACh (**Supplementary Material**), the dynamic
328 equilibration process continuously leads to a transfer of ACh out of the SPME coating following
329 the concentration gradient of the rapidly depleted ACh into the extracellular space as discussed
330 in the case of calibration in the coating approach (Alam and Pawliszyn 2016; Zhang et al.
331 2007a).

332 Amongst other sources, choline is created as a byproduct of ACh degradation by AChE.
333 Choline is also a precursor for the synthesis of ACh. Given this relationship, a negative
334 correlation between choline concentrations and ACh is expected which we indeed observed in
335 our dataset. Despite the difference in the measured concentrations of choline and acetylcholine,
336 other studies with similar differences have demonstrated the relationship of ACh and choline to
337 behavior within the cortex (Parikh et al. 2007). Future studies may focus on quantifying this
338 relationship across a wider range of brain states.

339

340 **Reliable Measurement of Individual Differences of State Specific Neuromodulatory Tone.**
341 Our results suggest that SPME probes provide reliable and sensitive measurements in
342 consecutive sampling events within an experimental session and between sessions on
343 consecutive days. These results were obtained while we controlled brain states across
344 measurement events by engaging animals in a cognitive task. This experimental control could
345 have contributed to the comparable extracellular levels of neuromodulators from sample to
346 sample within *and* between days from similar brain locations (**Fig 2**) (Marder et al. 2014;
347 McGinley et al. 2015; Reimer et al. 2014, 2016). The reproducibility of multiple measurements

348 at the same anatomical location within and between days suggests that the SPME penetrations
349 did not significantly disturb tissue (Cudjoe et al. 2013) (**Supplementary Information**). This
350 conclusion contrasts with reported experience from microdialysis measurements, where the
351 initial placement of the probe or guiding cannula causes transient measurement instabilities that
352 can require several hours of settling time before reliable, steady state neuromodulator
353 concentrations are measurable (Kodama et al. 2002, 2015, 2017; Moussawi et al. 2011;
354 Watanabe et al. 1997). These waiting periods must be taken into account when designing
355 microdialysis experiments for in vivo tracking of neuromodulators during behavioral tasks in
356 awake and restrained animals. Furthermore, the reliability of observations for the reported
357 neurochemicals with SPME is extremely high and outperforms many reported microdialysis
358 studies. We present 100% reliability in reporting glutamate and choline concentrations in all
359 brain regions and subjects. Acetylcholine concentrations were reported with 100% reliability in
360 one animal and 92% (11/12 probes) in the other within the prefrontal cortex, 0% reliability
361 (below detection threshold) in one animal and 83% (10/12 probes) in the other within the
362 premotor cortex and 100% reliability in one animal and 92% (11/12 probes) in the other within
363 the caudate. Dopamine concentrations were reported with 100% reliability in both animals in the
364 caudate and were not observed above detection thresholds in the cortex.
365
366 **Qualities and Advantages of SPME.** Our results illustrate several inherent advantages of using
367 SPME to measure neurochemical profiles in brain circuits (Lendor et al., submitted). As
368 illustrated in **Table S1**, SPME complements or outperforms alternative techniques in various
369 domains. Practically, the arbitrary length of the wire where the SPME coating is placed allows
370 for robust placement within the brain. The narrow diameter of the SPME probe that is within the

371 range common to electrodes for electrophysiological recordings, allows for repeated,
372 simultaneous sampling at multiple sites without observable changes to detected extracellular
373 neuromodulator levels using 20-minute sampling times.

374 *Spatial Resolution.* In principle, the spatial resolution of the SPME measurements are
375 capable of reflecting laminar concentration gradients across areas as small as several tens of
376 micrometers. In our protocol we coated 3mm in length with 50 μm of thickness and desorbed the
377 entire area into an organic solvent mixture for subsequent LC-MS/MS analysis. However, with
378 the use of other techniques, such as desorption electrospray ionization (DESI), which represents
379 a direct-to-MS approach (Deng et al. 2014), much smaller areas of the SPME matrix may be
380 analyzed at a time allowing for laminar or near-laminar resolution. Direct coupling of SPME
381 fibres to MS is the preferred mode, as it provides the most rapid analysis thus preventing
382 diffusion of compounds within the coating and smearing the imprinted layer information. If
383 diffusion of molecules within the fibre is high, it can be prevented by, for example, the inclusion
384 of a divider between two halves of the coating. This would allow the simultaneous measurement
385 of separated compartments of a cortical column. The absolute spatial resolution of SPME is
386 difficult to estimate as SPME operates through diffusion and dynamic equilibration similar to
387 MD. As a consequence, the sampled volume will depend on sampling time, the compound's
388 diffusion coefficient, as well as the target tissue and its properties affecting the rate of diffusion
389 such as tortuosity (Syková 2004).

390 *Temporal Resolution.* Several factors determine the appropriate extraction time using
391 SPME. Depending on the goals of an experimenter, resolutions below 5 minutes may be
392 achieved as the detection limit is reached within the first few minutes for most compounds tested
393 (**Fig 1D-E**). As sampling continues, the SPME coating will eventually reach an equilibrium point

394 with the external environment (**Fig 1D-E**). In principle, sampling times beyond a stable
395 equilibrium point will result in the same extracted values of compounds. However, in the brain
396 there is no single stable equilibrium point and thus, even with long sampling times, there will be
397 some variation in extracted concentrations. Within the dynamic regime, we can still calculate
398 environmental concentrations from which sampling occurred given knowledge of the extraction
399 time and the dynamic range. The lower limit of this dynamic range that can be informative is
400 determined by the coating's physicochemical characteristics and sample's properties and in
401 practice also volume of desorption and sensitivity of the MS. The analytical technique's
402 sensitivity also practically determines the thickness of the SPME coating as thinner coatings
403 could in principle increase temporal resolution but would extract less compounds overall
404 requiring higher sensitivity to detect. With higher sensitivity, less time is required for the thin
405 coating to extract sufficient amounts to exceed limits of detection and quantitation. This then
406 means that the compound with regards to which the sensitivity is the lowest, limits the temporal
407 resolution because it will have the slowest-to-reach detectable concentration. However, making
408 measurements in the dynamic range requires consistency of extraction times as differences in
409 time will lead to variations of extraction yields in a range that can be estimated from the SPME
410 extraction time profiles (**Fig 1D-E**). Given the dynamic nature of brain networks and the lack of
411 a stable equilibrium point, we expect consistency in brain states to be one of the biggest factors
412 in reducing replicated variability. Moreover, SPME measurements are inherently an average of
413 the temporal dynamics of the target tissue milieu. This means that due to the bi-directional
414 exchange of neuromodulators from the extracellular space and the SPME coating, longer periods
415 of stable concentration gradients will be more strongly reflected in the extracted measurements.

416 *Utility and Ease of Use.* SPME based microprobe extraction allows for multiple
417 simultaneous measurements that can be reliably repeated within the same measurement sessions
418 at the same locations with no evidence of damage induced disruption of the neurochemical
419 environment. Another critical advantage of SPME over comparable MS-analysis based methods
420 such as MD is its ease of use and accessibility. Due to their similar size to recording
421 microelectrodes used for electrophysiology, little adjustment is required for conducting SPME
422 measurements using existing acute microelectrode positioning systems. Measurements in deeper
423 structures should use an accompanying guide tube (**Fig 1C**) as travelling through non-target
424 tissue will result in unwanted chemical collection. Beyond the collection, a chemistry core could
425 apply LC-MS/MS to the probes allowing for relatively easy quantitation of extracellular
426 compounds of interest.

427 *Detection Methods.* SPME is ultimately a sampling method, much like MD, that provides
428 data for analytical tools such as chromatography and mass spectrometry. In fact, once SPME
429 analytes have been desorbed from the probe into a solvent, data provided by SPME and MD are
430 treated very much the same. Thus, the advancement in detection limits and reliability of post-hoc
431 analytical methods utilized by MD also benefit SPME, and vice versa. However, SPME sample
432 analysis is more reliably and conveniently coupled to LC-MS/MS as it lacks issues such as high
433 salt content of collected dialysate that MD suffers from (Guihen and Connor 2009).

434 *Extension of SPME measurements beyond classical neuromodulators*. A unique
435 advantage of SPME over alternative methods of in vivo detection of compounds within the brain
436 is its potential affinity for hydrophobic compounds. Although MD is capable of collecting
437 metabolomics data similarly to SPME (Anderzhanova and Wotjak 2013; Li et al. 2012; Zhang et
438 al. 2007b), the artificial cerebra-spinal fluid that is commonly traversing the semi-permeable

439 membrane better facilitates the collection of hydrophilic molecules. This means that molecules
440 that cross the semi-permeable membrane and are therefore collected and quantified through LC-
441 MS are much more likely to be hydrophilic. SPME, in contrast, provides balanced analyte
442 coverage, which means that in principle certain SPME matrix coatings have similar affinity for
443 both hydrophilic or hydrophobic compounds (Reyes-garcés et al. 2018). Data collected here
444 includes other neurochemicals of interest such as amino acids (e.g. glutamine, taurine,
445 phenylalanine, etc) that we reliably detected but do not discuss here. In practice, the detection of
446 very polar molecules such as monoamines and catecholamines requires coating chemistry that
447 facilitates hydrophilic compound extraction. Comparatively many lipids play important roles in
448 intracellular signaling and have been suggested to provide biomarkers for psychiatric disorders
449 (Tamiji and Crawford 2011; Yehuda et al. 1999), therefore making SPME a potentially versatile
450 and comprehensive tool for brain neurochemistry studies.
451
452 **Implications for Understanding and Treating Psychiatric Disease States.** Most psychiatric
453 disorders are accompanied by neuromodulatory dysregulation (Millan et al. 2012). However, this
454 fact is seldom studied in a multi-modulatory manner with a single or few nuclei or
455 neurochemicals being observed at a time. This is an increasingly evident problem because
456 models attributing symptoms of a disorder to a single neuromodulator often fall short in
457 explaining many symptoms (Bohnen et al. 2015; Remy et al. 2005). For example, in Parkinson's
458 disease, outside of the well characterized dopaminergic deficits, there is evidence for deficits in
459 noradrenergic, cholinergic and serotonergic systems as well (Halliday et al. 1990, 2014; Moehle
460 et al. 2017). Many cognitive deficits observed within Parkinson's disease are linked to such non-
461 dopaminergic deficits. Such findings emphasize the need to simultaneously observe multiple

462 neuromodulatory systems. Thus the simultaneous measurement of multiple neuromodulators, in
463 multiple brain regions within healthy and clinical populations will allow for a better
464 understanding of the underlying causes of symptoms and progression of psychiatric disorders
465 (Anderzhanova and Wotjak 2015; Avery and Krichmar 2017; Millan et al. 2012).

466 A better understanding of psychiatric disorders through multi-modulator methods may
467 also lead to more accurate understanding of the action of pharmacological agents. Previously,
468 many studies aimed at identifying the locus of action of a pharmacological agent have used local
469 injection methods such as iontophoresis or microinjections (Lapiz and Morilak 2006; Wang et al.
470 2007). Although highly informative about the role of neuromodulators in modulating the activity
471 of individual cells and circuits, this approach does not allow physiologically realistic exploration
472 of a systemically administered pharmacological agent. Pharmacological agents are often
473 administered in some systemic fashion and even with highly specific receptor affinities, may
474 interact with multiple neuromodulatory systems through the actions of heteroreceptors (Avery
475 and Krichmar 2017; Gobert et al. 1998; Millan et al. 2015). Multi-modulator measurements, as
476 described here, will allow for a better understanding of pharmacological agents, as well as
477 provide novel insights into the development of more effective drugs or combinations of drugs to
478 better treat the clinical population.

479

480 **Conclusion**. We described a novel SPME protocol capable of simultaneous, multi-modulator
481 measurements of multiple brain regions. Our results suggest that SPME both supplements
482 current methods of neuromodulator detection and allows for novel measurements previously not
483 possible for the investigation and dissection of neuromodulatory systems, their role in
484 physiological brain processes and their modulation by pharmacological agents.

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Conflicts of interest

None of the authors have any conflict to disclose.

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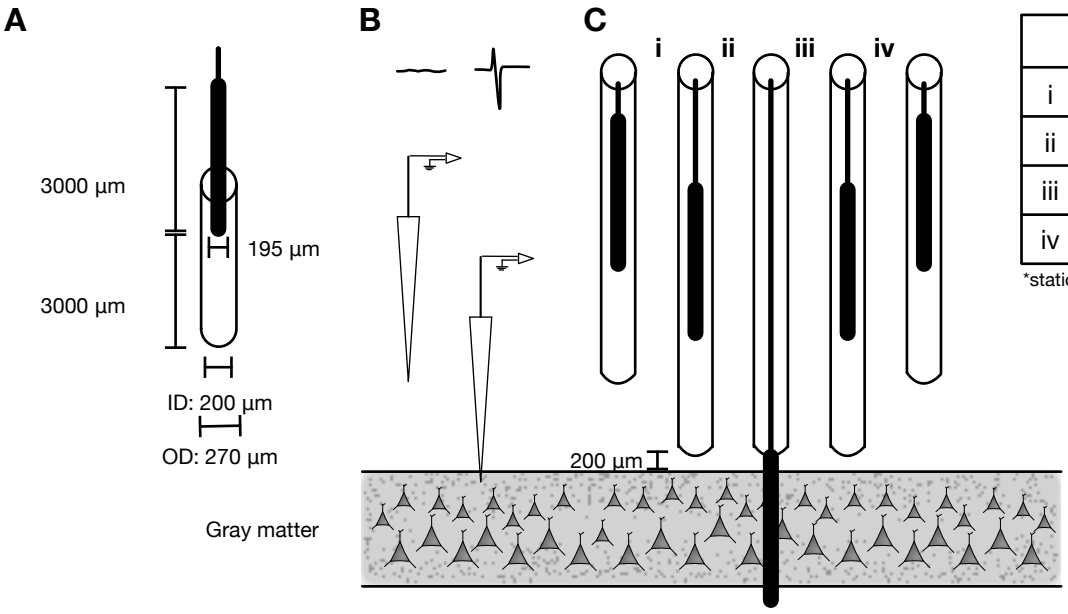
Fig 1. SPME sampling procedure, extraction time profiles and measurement locations.
(A) Dimensions of the SPME probe and the accompanying cannula. There was a 3000 µm buffer from the opening of the cannula and the start of the SPME coating. The SPME coating was 195 µm at the tip. The SPME coating made up the terminal 3000 µm of the entire probe and was placed in a cannula with an internal diameter of 200 µm and an external diameter of 270 µm. **(**B) Tungsten microelectrodes were lowered into the brain guided by 3 T magnetic resonance (MR) images in order to map the depth at which detectable spiking was observed matching expectations from the MR images. (C) SPME probes and their accompanying cannulas in all brain areas were then simultaneously lowered to 200 µm above the point of first observable firing in the target brain region and the SPME probes were lowered to expose only the 3000 µm SPME coating. After 20 minutes of extraction, the SPME probes were then retracted 3000 µm back into the cannula and the probes plus their accompanying cannulas were removed from the brain. The table inset into the figure provides the velocities and times of each probe and cannula as they transitioned between the extraction steps. (D) Extraction quantities of Glutamate in ng and (E) Dopamine in ng over time. The linear "kinetic" regime and "equilibrium" regime are labelled with the dynamic range being in between. (F**)** Anatomical locations of SPME sampling events in the right hemisphere of two Rhesus Macaques. Target brain regions are highlighted in red and include two cortical regions, prefrontal cortex (PFC) area 8 and premotor cortex (PreM) area 6 as well as a subcortical region: the head of the caudate.

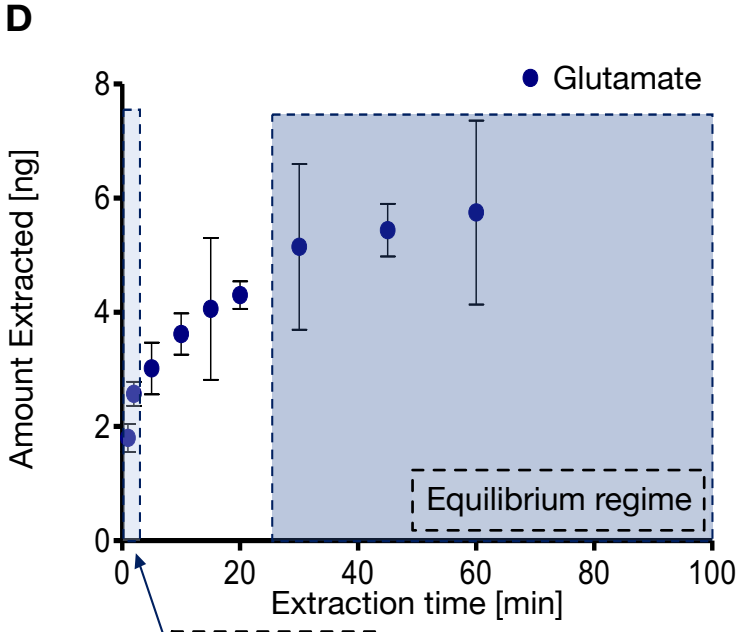
Fig 2. Changes in SPME sampling over time.
Changes in the normalized concentrations of the observed neuromodulators across daily sampling sessions in each sampled brain region. The median concentration of (A) Glutamate (B) acetylcholine (C) choline and dopamine (D) with standard error of the median plotted for each sampling time and location. Data was normalization for each subject separately and for the respective neurochemical and area combinations. Sampling locations are represented by different lines: solid line for the prefrontal cortex, dashed line for the premotor cortex and dotted line for the caudate nucleus. Sampling events were 20 minutes in length and the time between the end of one sampling event to the start of the next sampling event was 40 minutes (STD 2 minutes) making the second sampling event 40 minutes and the third 100 minutes from the initial measurement. No significant change was observed for any neuromodulator in any brain region (Wisconsin rank sum test). Note that a single prefrontal choline data point was excluded in this analysis for being >4 standard deviations from the median (data point is present in **Fig 3a**).

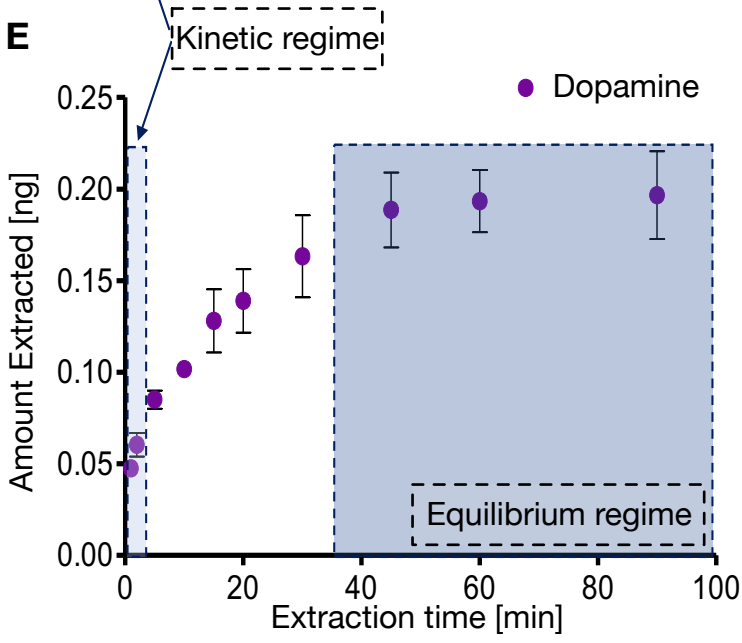
Fig 3. SPME sampling of the macaque brain.
SPME probes extract from the cortex (PFC and PreM6) as well as the caudate of two adult macaques after 20 minutes of extraction within the respective brain region. Median and standard error of the median of n= 12 probes for animal As and n= 9 probes for animal Ke with no data excluded. Four neuromodulators were reliably measured: glutamate, dopamine, acetylcholine, and choline (orange, green, purple, red respectively). All brain regions had high concentrations of glutamate and relatively much lower concentrations of acetylcholine and choline extracted (with the exception of animal Ke PreM6 lacking detectable acetylcholine). The only tested region to yield measurable dopamine was the caudate. The dashed blue line depicts the limit of detection (LOD).

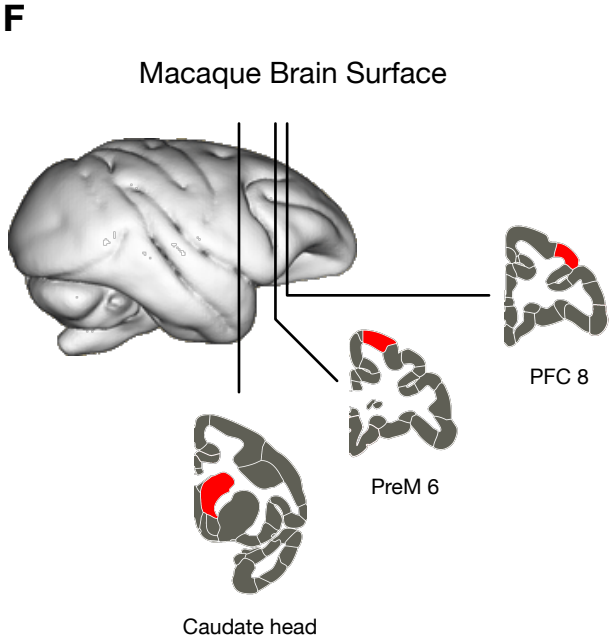


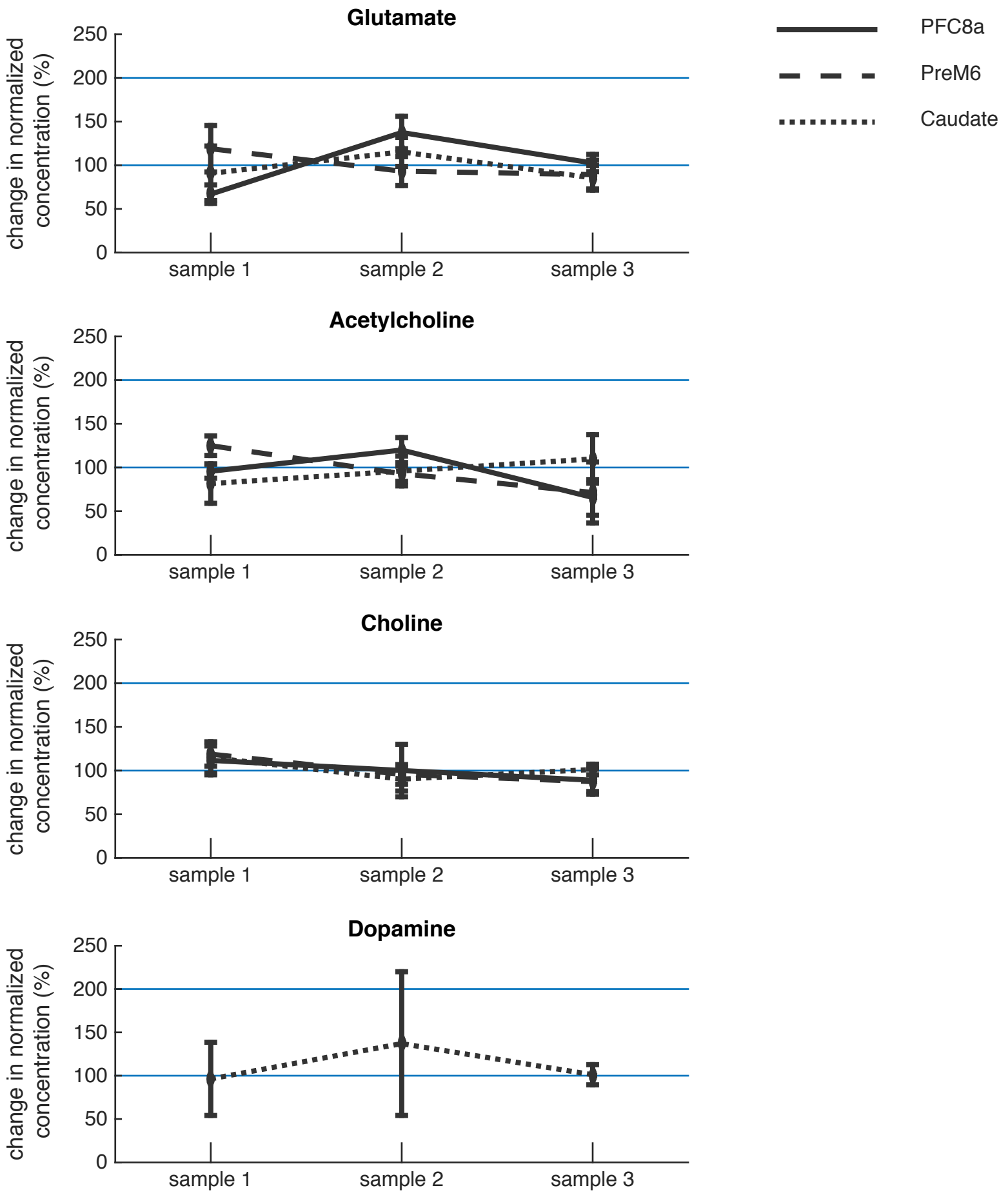
	fibre	cannula	time
i	30 µm/s	30 µm/s	variable
ii	30 µm/s	-	200s
iii	30 µm/s	-	1200s* + 200s
iv	30 µm/s	30 µm/s	variable

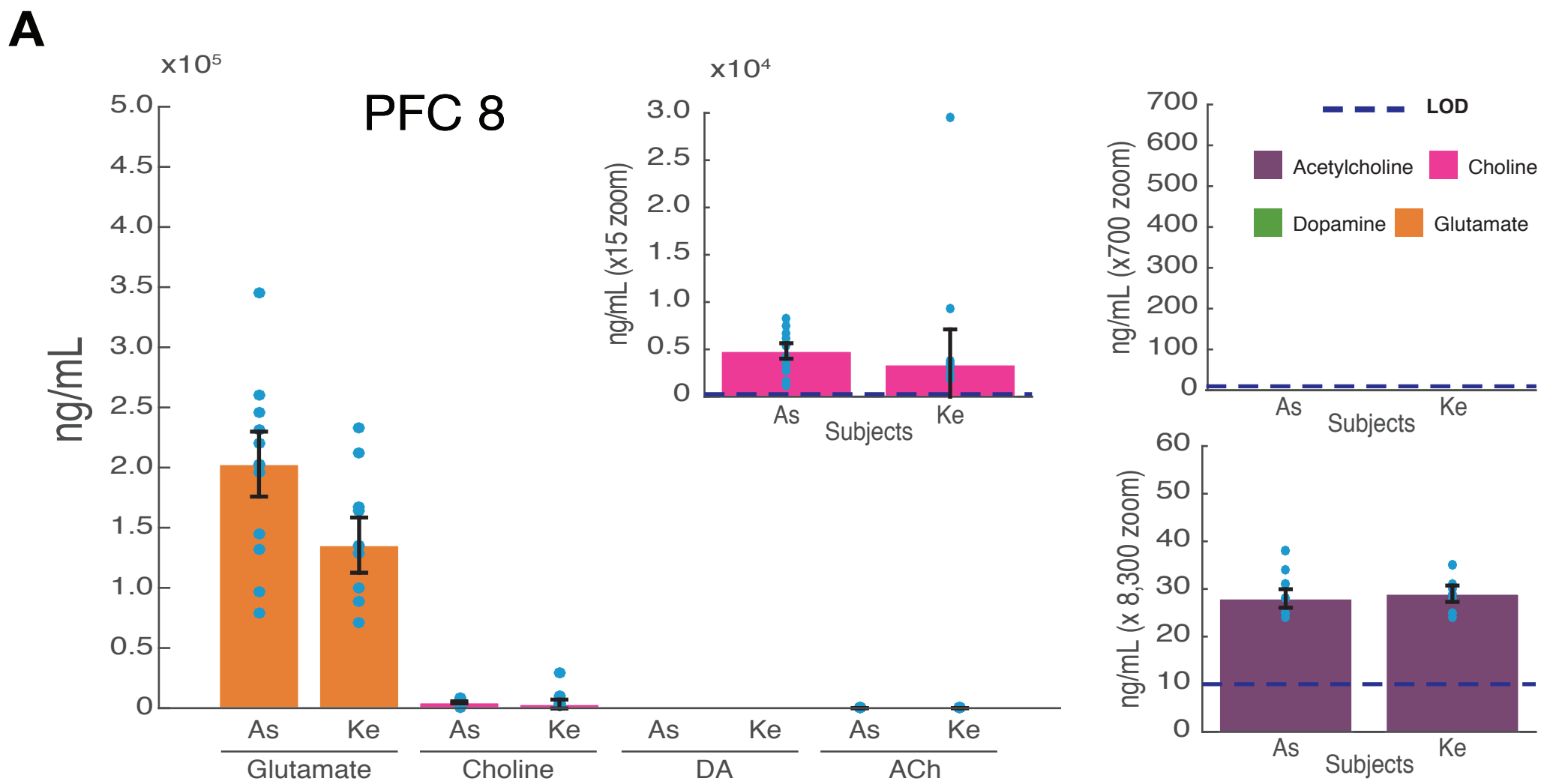
*stationary: extraction period of 20 min

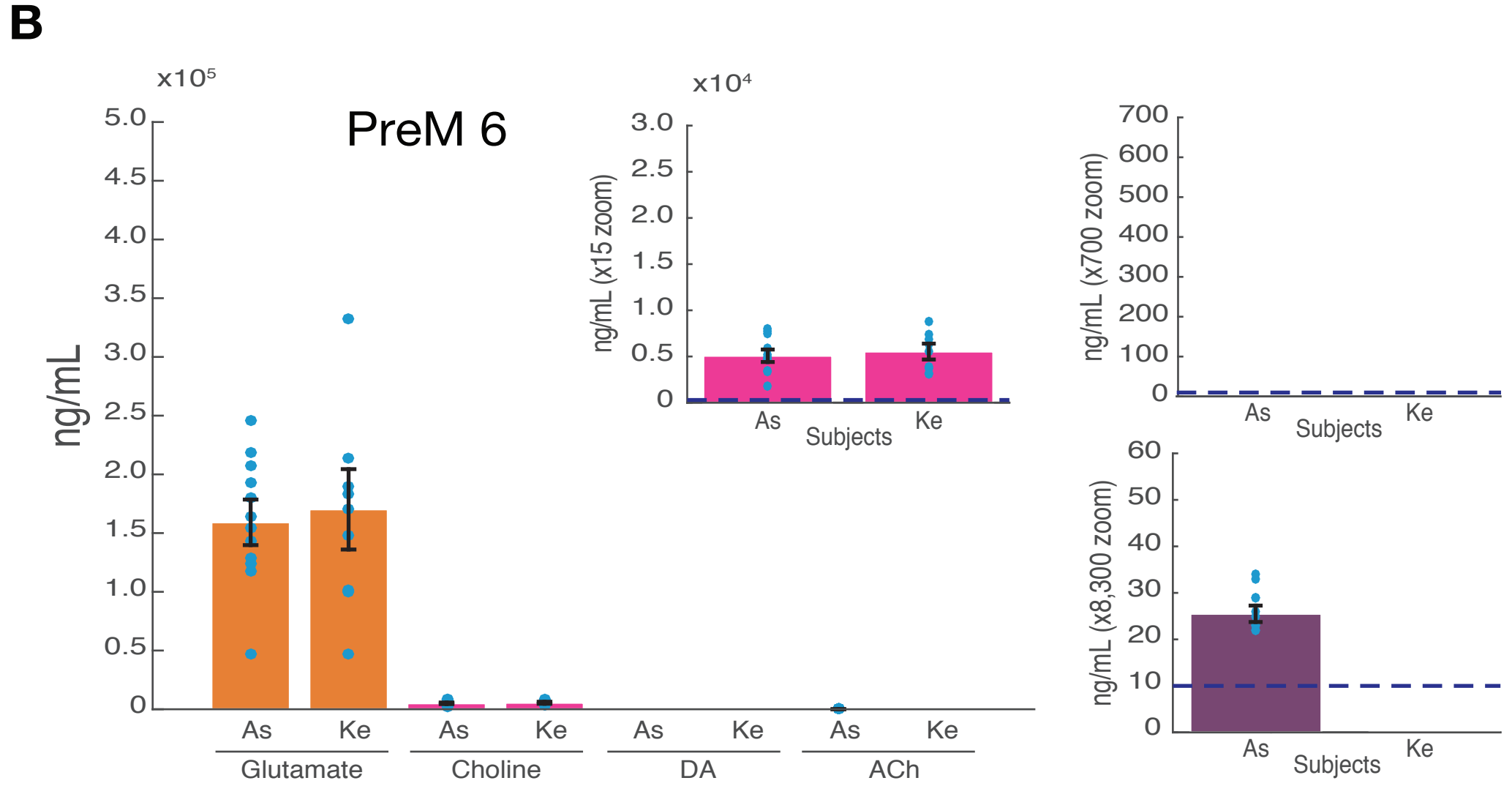


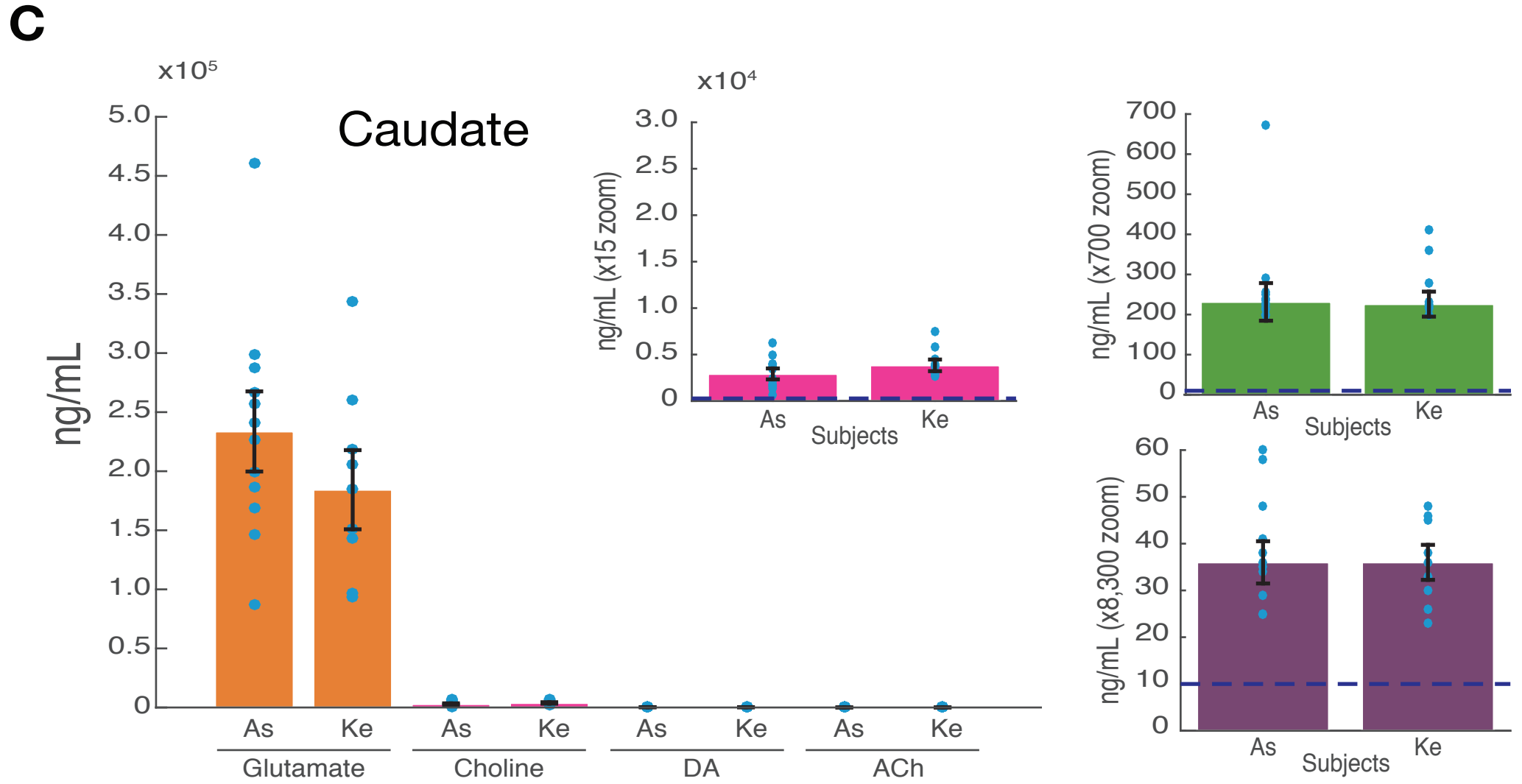





Glutamate
Acetylcholine
Choline
Dopamine
change in normalized concentration (%)
250
200
150
100
50
0
sample 1
sample 2
sample 3
PFC8a
PreM6
Caudate


A
PFC 8
x10⁵
ng/mL
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
As
Ke
Glutamate
Choline
DA
ACh
x10⁴
ng/mL (x15 zoom)
Subjects
LOD
Acetylcholine
Choline
Dopamine
Glutamate
ng/mL (x700 zoom)
ng/mL (x 8,300 zoom)


B
PreM 6
x10⁵
ng/mL
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
As
Ke
Glutamate
Choline
DA
ACh
x10⁴
ng/mL (x15 zoom)
Subjects
ng/mL (x700 zoom)
ng/mL (x8,300 zoom)


C
Caudate
x10⁵
ng/mL
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
As
Ke
Glutamate
Choline
DA
ACh
x10⁴
ng/mL (x15 zoom)
Subjects
ng/mL (x700 zoom)
ng/mL (x8,300 zoom)

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

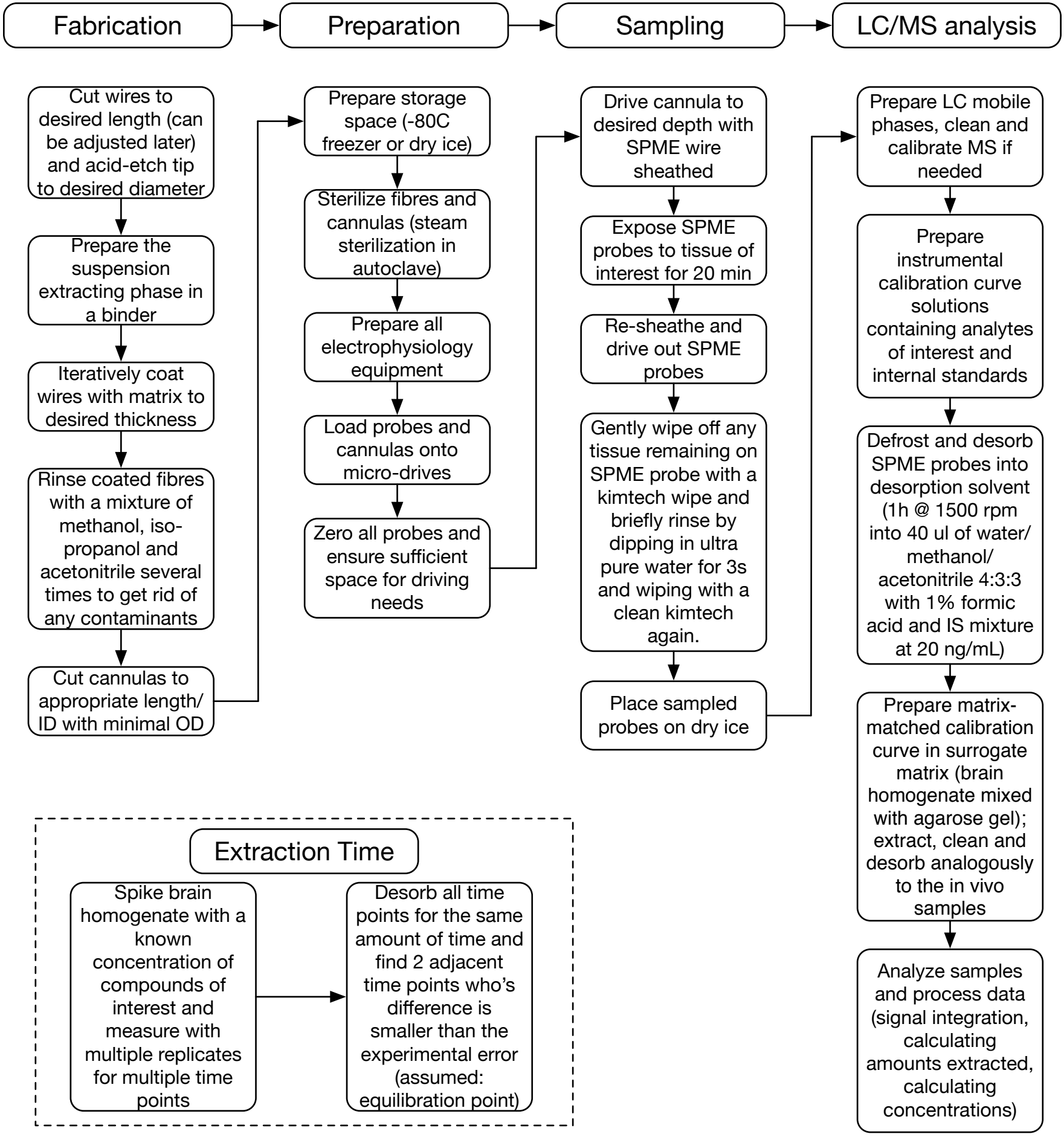
Fig S1. Experimental procedure from SPME probe fabrication to quantitation. Fabrication described the in-house procedure to prepare SPME probes. Preparation describes experimental set-up. Sampling describes the actual data collection process. LC/MS analysis describes the chemical quantitation of collected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