

Supporting Information for:

Microfabricated Sampling Probes for *In Vivo* Monitoring of Neurotransmitters

Woong Hee Lee, Thomas R. Slaney and Robert T. Kennedy*

Contents:

Experimental

- materials and reagents
- microfabrication process
- microfabrication of probe holder
- surgical details
- sample analysis details

Figure S1. Silicon probe assembly on a holder.

Figure S2. Determination of response time with Si probe.

Figure S3. Histology indicating probe placement and minimal tissue displacement.

Table S1. Comparison Basal Extracellular Concentration by Silicon Microneedle Push-pull Probe with previous reports using microdialysis.

Experimental

Reagents and Materials

All chemicals were purchased from Sigma Aldrich (St. Louis, MO). Buffers were prepared in 18.2 M Ω -cm resistivity water purified by Milli-Q system (Millipore, MA). Fused silica capillary tubing was purchased from Molex (Phoenix, AZ). Unions for 360 μ m outer diameter (OD) capillaries were purchased from IDEX Health and Science (P-772, Oak Harbor, WA). Epoxy resin was purchased from ITW Devcon (Danvers, MA). Crystalbond Adhesive was purchased from Structure Probe (West Chester, PA). Artificial cerebrospinal fluid (aCSF) contained 145 mM NaCl, 2.68 mM KCl, 1.01 mM MgSO₄, 1.22 mM CaCl₂, 1.55 mM Na₂HPO₄, and 0.45 mM NaH₂PO₄, pH 7.4. All solutions used for sampling were filtered with a 0.2 μ m filter.

Microfabrication of Sampling Probes

Sampling probes were designed in L-EDIT software (Tanner EDA). Probes were fabricated on 4 inch silicon-on-insulator (SOI) wafers (University Wafer, MA) which had a 70 μ m thick Si layer over a 1 μ m thick insulator layer over a 500 μ m thick Si wafer. A 1 μ m silicon dioxide layer was grown on a 4 inch SOI wafer by wet oxidation (Tempress TS 6604 S3). The desired pattern of fluidic channel was created by lithography in 3 μ m of photoresist, SPR220 (Dow, MA). Deep reactive ion-etching (DRIE) was performed using the Bosch process (which uses C₄F₈ as a passivation material) to create 30 μ m deep and 3 μ m wide trenches in the pattern of the fluidic lines. Lithographic patterning and isotropic etching (Xactix XeF₂ Etcher) were used to form microchannels with 20 μ m diameter at the bottom of the trench. Channels were sealed by chemical vapor deposition of 3 μ m of polysilicon (Tempress TS 6604 S3). A 1 μ m thick additional silicon dioxide layer was grown on SOI wafer. The outer probe shape and orifices were patterned by lithography and etched with DRIE. The resulting structure was bonded to a

carrier wafer with Crystalbond 555 (SPI Supplies / Structure Probe, PA) for backside etching by DRIE. Backside etching was stopped when it reached to the buried insulator layer in SOI and probes were released from carrier wafer in hot water. Probes were imaged by scanning electron microscopy (SEM) (Hitachi SU8000 Ultra-High Resolution SEM).

Microfabrication of probe holder

Probe holders were fabricated from 4 inch Si wafers into the shape shown in Figure S1. After growth of a 1 μm silicon dioxide layer, lithography was performed to create the 2 mm wide rectangle pattern on a wafer for the probe installation. Using DRIE, the SiO_2 layer on the wide rectangle pattern and trenches for capillary tubes were patterned with SPR 220. SiO_2 was removed and 100 μm deep trenches were etched by DRIE. Photoresist on the wafer was removed by positive resist stripper (PRS 2000; Avantor Performance Materials, PA) and DRIE used to etch 70 μm deep features as in Figure S1 (d).

Assembly of Push-pull Probes

To connect probes to capillaries the probe holder in Figure S1 was used. A 1 cm length of 150 μm inner diameter (ID) and 360 μm OD capillary was fixed on each trench of a probe holder with epoxy gel resin. Probe ports were inserted into capillaries using the holder as a guide and the probe then glued to the holder with epoxy gel resin. A 10 cm length of 20 μm ID and 90 μm OD capillary was joined to each probe port through this connection as shown in Figure S1.

Surgical Procedures and in Vivo Sampling.

All procedures were performed according to a protocol approved by the University Committee for the Use and Care of Animals. Male Sprague-Dawley rats between 250 and 350 g were anesthetized using 65 mg/kg ketamine and 0.25 mg/kg dexmedetomidine and placed in a stereotaxic frame (963 model, David Kopf, Tujunga, CA). The probe holder containing the

silicon probe was fixed to the stereotax. The skull was exposed and a burr hole drilled at 1 mm anterior and 2.3 mm lateral to bregma. The dura was carefully incised with a 27 gauge hypodermic needle and the surface of the cortex to allow easy penetration of the brain tissue.

Probes were inserted stereotaxically over a period of 2 min. During insertion, both channels of the probe were flushed with aCSF at 200 nL/min using a syringe pump (Fusion 400, Chemyx, Stafford, TX) so that a total of 800 nL was infused along the insertion track. After reaching final position, push-pull flow at 50 nL/min for each channel was initiated. Flow on the push channel was controlled using the syringe pump. Pull flow was initiated by applying vacuum to the outlet of a 13 cm length of 100 μ m ID by 360 μ m OD capillary connected to the probe. Flow rate was monitored by measuring linear velocity of fluid filling the capillary. Vacuum was adjusted as necessary to maintain 50 nL/min. After a capillary was filled (corresponding to 1 μ L), it was removed and sample deposited into an autosampler vial using gas pressure. Fractions collected within the first hour were discarded.

Analysis of Fractions.

Samples were derivatized with benzoyl chloride for analysis. For sample derivatization, the following were added to samples immediately after collection: 1.5 μ L of 100 mM sodium tetraborate, 1.5 μ L of 2% (v/v) benzoyl chloride in acetonitrile, 1.5 μ L of 13 C-labeled internal standards in dimethylsulfoxide containing 1% (v/v) acetic acid, and 1 μ L of 100 nM d₄-acetylcholine (CDN Isotopes, Pointe-Claire, Quebec, CA). Internal standards were prepared by labeling standards with 13 C-benzoyl chloride. Analysis was conducted using Waters nanoACQUITY UPLC equipped with a Waters Acquity T3 column (1.8 μ m, 1 \times 50 mm) interfaced with electrospray ionization to an Agilent 6410 triple quadrupole MS. The MS was operated in multiple reaction monitoring mode for MS-MS detection of all analytes as reported

previously. Calibration standards of 5 different concentrations that bracketed the in vivo concentrations were prepared for all measured chemicals. Internal standards were added to be within 2-fold of the in vivo concentrations.

Histology

After sampling, 100 nL of filtered saturated FastGreen FCF was infused at 50 nL/min through the probe. Brains were placed in 10% paraformaldehyde in 100 mM phosphate buffered saline and fixed at $\sim 4^{\circ}\text{C}$ for at least 24 h. Brains were frozen and sliced coronally using a cryostat. Slices (50 μm thick) were placed on microscope slides (SuperFrost, Fisher, Fairlawn, NJ) and imaged on an optical microscope to verify probe location.

Figure S1. Silicon probe assembly on a holder. (a) A union capillary was mounted into the holder and the probe ports then slid into the capillary using the holder as a guide. (b) A fluidic or connection capillary was inserted into the union capillary from the opposite end. The union capillary aligned the internal channels of the probe and fluidic capillaries as seen in the inset. (c) SEM image of microfabricated holder. (d) Photograph of assembled holder, probe, and fluidic capillaries. (e) For operation, the fluidic capillaries were connected to a syringe pump (for push flow) and a vacuum (for pull flow). The capillary on the pull arm was disconnected to remove samples for analysis. For complete description of operation see Experimental section.

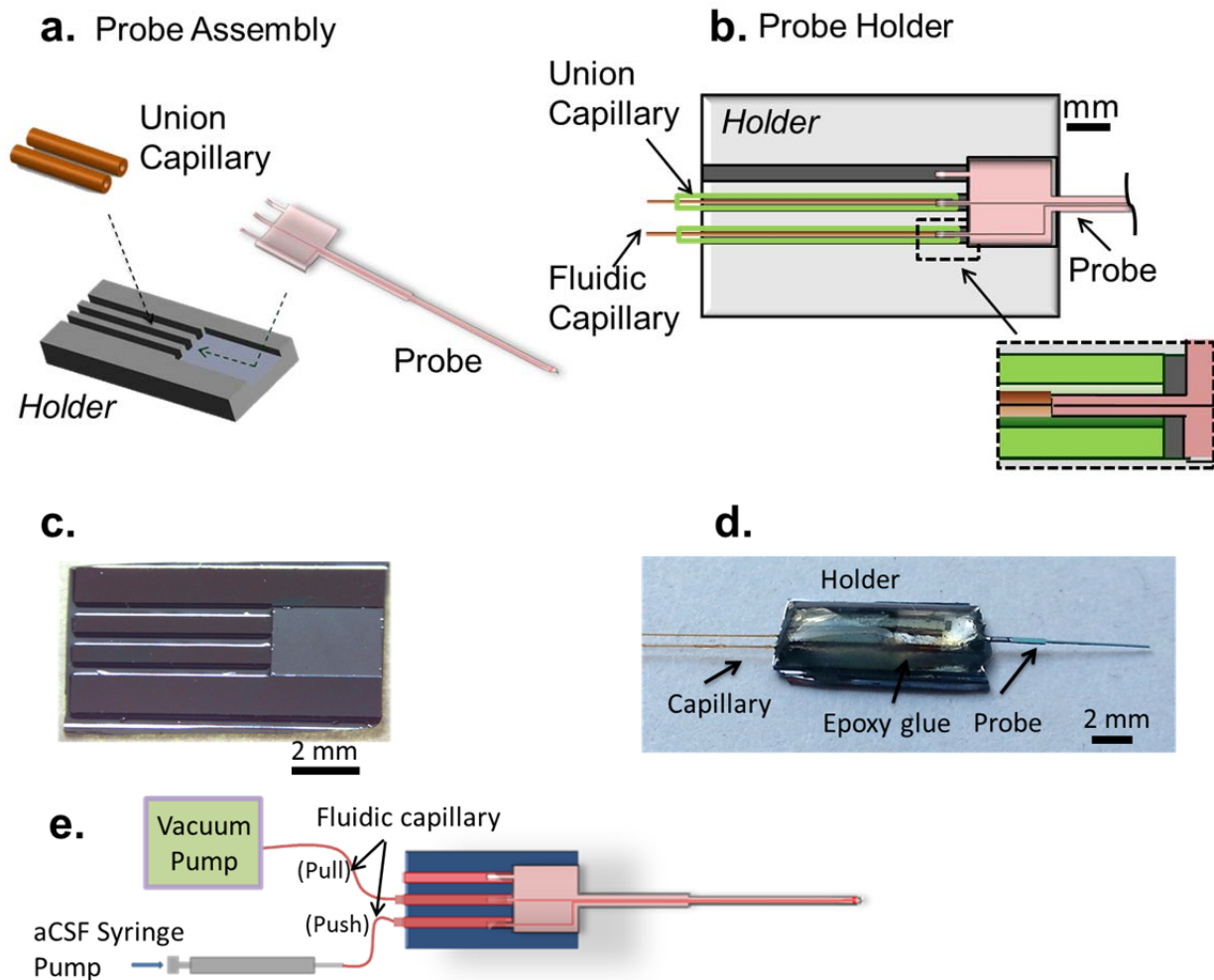


Figure S2. Determination of the response time with Si probe. Probe was connected to pump and collection capillaries as described in Experimental Section. Probe was dipped into a 2 μM fluorescein solution and fluorescent signal recorded by laser-induced fluorescence at a point 30 cm from the tip inside the fused silica collection capillary. After a stable signal was recorded for 15 min, the vacuum for sampling was removed, the solution changed to 4 μM fluorescein, and vacuum reapplied to resume flow. Trace shows representative example for period of detection fluorescent change. The 10% to 90% response time is 26 s. Dip in signal was associated with the stoppage of flow.

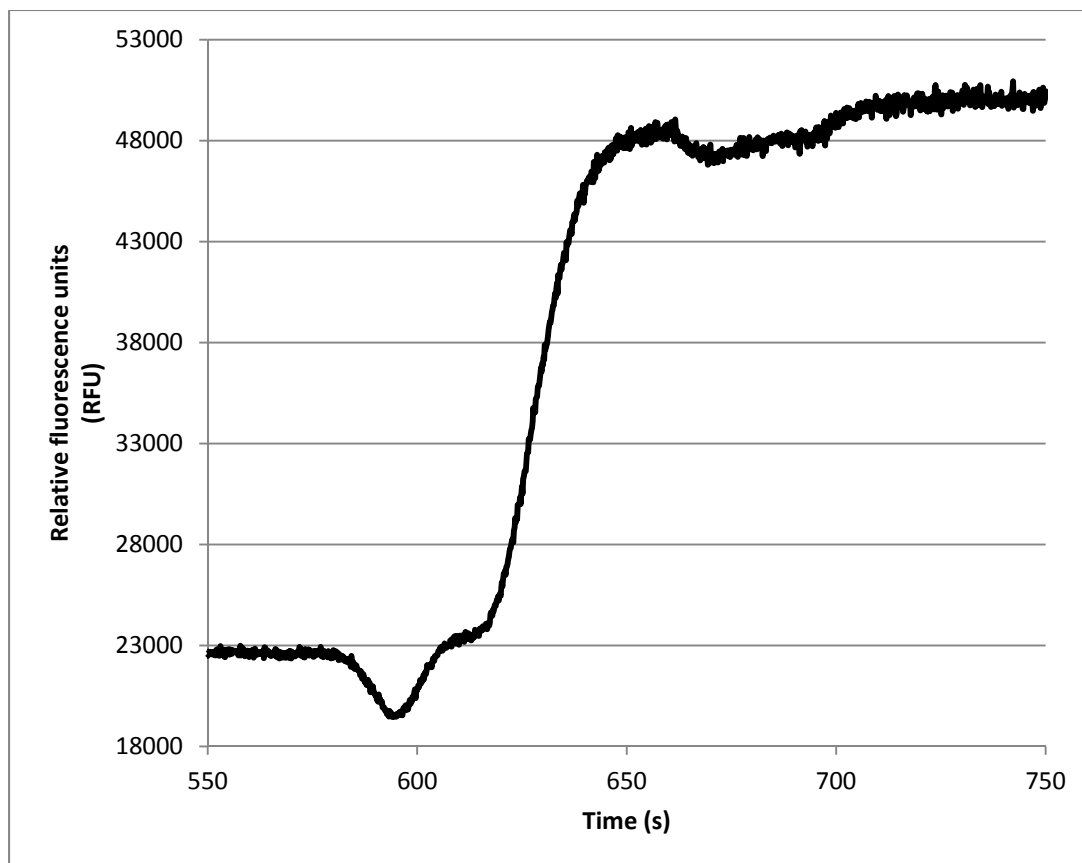


Figure S3. Histology indicating probe placement and minimal tissue displacement. (A) After sampling, FastGreen FCF was infused through the probe, which labeled the sampling site (arrow) and probe track blue-green. (B) Dashed line indicates track overlaid on a rat brain atlas diagram. Sampling occurred at the tip of the probe (dashed line) within the striatum. (See experimental section for details.)

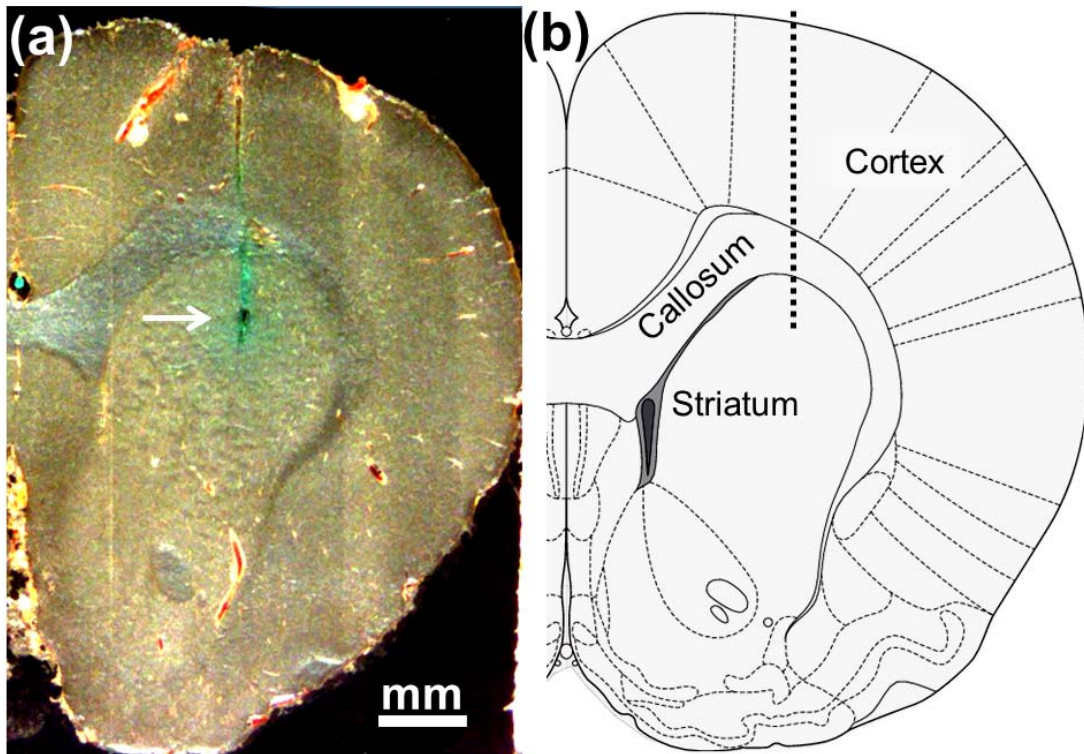


Table S1. Comparison Basal Extracellular Concentration by Silicon Microneedle Push-pull Probes with previous reports using microdialysis. All measurements are from the striatum of rats. Unless noted otherwise, values are reported as recovered concentration with no correction for recovery. Values given as mean \pm SEM. (references are in main text list).

References	Basal Concentrations (nM)	36	37	38	39	40	41	42	43	44
Anesthesia	Ketamine	Urethane*	Ketamine	Halothane	Halothane	Urethane	Awake	Awake	Halothane	Halothane
Sampling Method	Push-pull perfusion	Microdialysis	Microdialysis	Microdialysis	Microdialysis	Microdialysis	Microdialysis	Microdialysis	Microdialysis	Microdialysis
Flow Rate	50 nL/min	1.2 μ L/min	300 nL/min	3.0 μ L/min	2 μ L/min	1.0 μ L/min	1.2 μ L/min	3.0 μ L/min	2 μ L/min	2 μ L/min
Acetylcholine	26 \pm 12						2.9 \pm 0.38			
Taurine	1500 \pm 700									12000 \pm 3000
Histamine	5.9 \pm 1.5									
Serine	6600 \pm 1800		34100 \pm 4400							9500 \pm 1200
Aspartate	530 \pm 110		700 \pm 300		90 \pm 10	75.7 \pm 9.3			95 \pm 23	810 \pm 100
Glycine	3000 \pm 600		6000 \pm 1100							5500 \pm 1000
Glutamate	1100 \pm 500		1800 \pm 400	340 \pm 30	1320 \pm 230	4400 \pm 900			861 \pm 128	2500 \pm 800
γ -aminobutyric acid	78 \pm 30			11 \pm 0.91	24.04 \pm 2.34				24 \pm 8	130 \pm 20
Adenosine	39 \pm 21							17 \pm 2		
5-Hydroxyindoleacetic acid	140 \pm 80	1134 \pm 53			140 \pm 10				720 \pm 55	
Homovanillic acid	380 \pm 240	2160 \pm 276			730 \pm 150				485 \pm 40	
Normetanephrine	0.1 \pm 0.0									
3,4-dihydroxyphenylacetic acid	750 \pm 450	1347 \pm 145			750 \pm 420				554 \pm 30	
Serotonin	0.36 \pm 0.16									
3-methoxytyramine	1.3 \pm 0.7									
Norepinephrine	1.5 \pm 1.3									
Dopamine	19 \pm 7	16 \pm 1		3.5 \pm 0.2	4.2 \pm 0.05	8.9 \pm 1.5			5 \pm 0.6	

*concentrations corrected for in vitro recovery