Supporting information

S1 Pressure and flow in the µPPPS probe

Operation of the µPPPS probe requires careful tuning of the inlet flow rates and outlet pressure in order to achieve a decent recovery rate without disturbing the brain environment with significant convection. The various microchannels and connecting capillaries are collected in an equivalent electrical circuit. From this, a transfer function is determined which can be plotted as a function of various input flow rates and pressures.

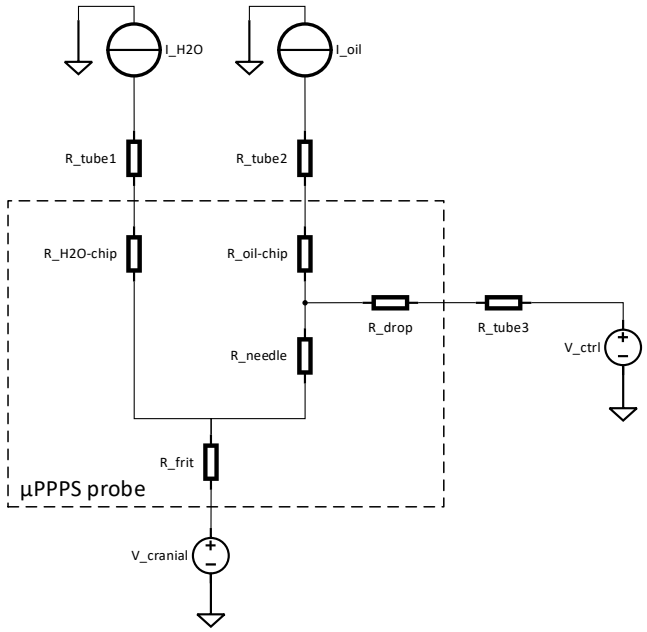

Figure S1A. Electrical equivalent circuit of the hydraulic resistance and pressure of the microfluidic channel network of the µPPPS probe.

With the component values as given in table S1B.
Now, the outlet flow rate and the sampling flow through the sampling channels can be calculated:

$$I_{out} = \frac{V_{cranial} - V_{ctrl} + I_{H2O}R_{frit} + I_{oil}(R_{frit} + R_{needle})}{R_{frit} + R_{needle} + R_{drop} + R_{tube3}}$$

$$I_{sample}$$

$$= \frac{V_{cranial} - V_{ctrl} - I_{H2O}(R_{needle} + R_{drop} + R_{tube3}) - I_{oil}(R_{drop} + R_{tube3})}{R_{frit} + R_{needle} + R_{drop} + R_{tube3}}$$

The component values are listed in table S1B. Using the equations and data from table S1.1, the inward flow rate through the probe tip (Isample) and total outflow (Iout) can be calculated (see figure S1C). From this, an estimation can be made for the desired outlet pressure.

Table S1B. Component values related to the electrical equivalent circuit of the µPPPS probe.

Component	Value	Explanation
I_H_2O	$8.3 \cdot 10^{-13}$ m³/s	Flow rate of water (*in vitro*) or perfusion fluid (*in vivo*)
I_oil	$3.3 \cdot 10^{-12}$ m³/s	Flow rate of decane
V_ctrl	$-8 \cdot 10^{4}$ Pa	Pressure at the outlet
V_cranial	0 Pa	Cranial pressure (*in vivo*, set to 0 Pa for *in vitro*)
R_tube1	$1.1 \cdot 10^{15}$ Pa·s/m³	Total hydraulic resistance of inlet tubing for water
R_tube2	$1.2 \cdot 10^{15}$ Pa·s/m³	Total hydraulic resistance of inlet tubing for *n*-decane
R_tube3	$1.2 \cdot 10^{15}$ Pa·s/m³	Total hydraulic resistance of outlet tubing for water droplets in *n*-decane, assuming a 50%/50% volume ratio
R_H_2O-chip	$1.8 \cdot 10^{15}$ Pa·s/m³	Hydraulic resistance of microchannel for water infusion
R_oil-chip	$8.1 \cdot 10^{15}$ Pa·s/m³	Hydraulic resistance of microchannel for oil until droplet generator
R_needle	$23 \cdot 10^{15}$ Pa·s/m³	Hydraulic resistance of microchannel for sampling until droplet generator
R_frit	$25 \cdot 10^{12}$ Pa·s/m³	Hydraulic resistance of sampling channel array
R_drop	$7.2 \cdot 10^{14}$ Pa·s/m³	Hydraulic resistance of microchannel for droplet transport to outlet

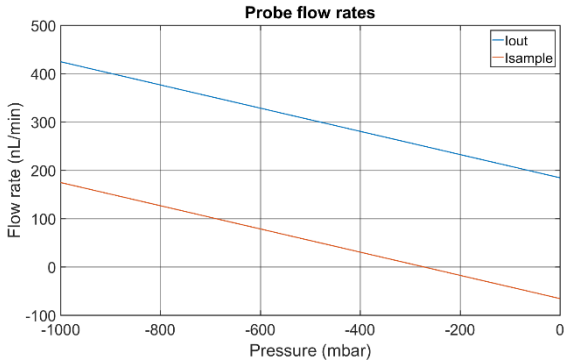

Figure S1C. Calculated flow rates through the outlet capillary (I_{out}) and through the sampling channels in the probe tip (I_{sample}). Set flow rates are I_H_2O=50 nL/min and I_oil=200 nL/min.

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S2 Optical setup

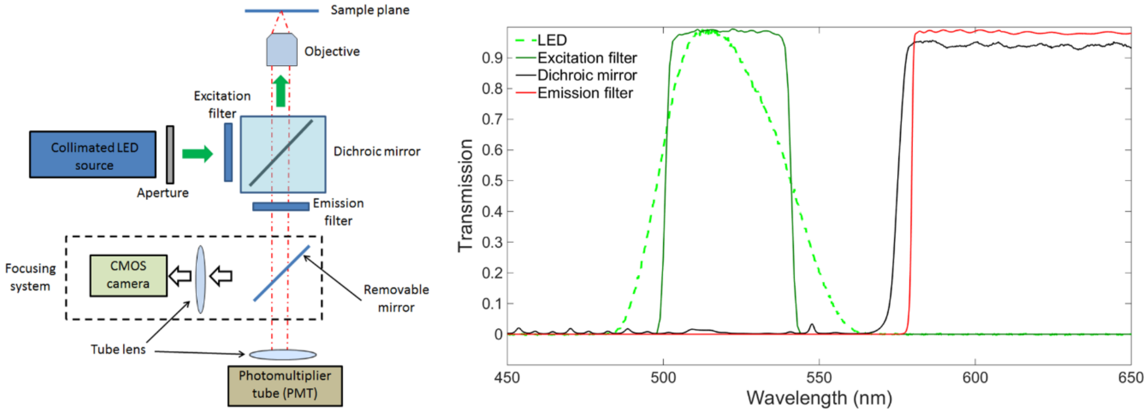

Figure S2A. Schematic representation of the optical setup designed for the fluorescence measurements. Resorufin has excitation and emission maxima of 571 and 585 nm, respectively.

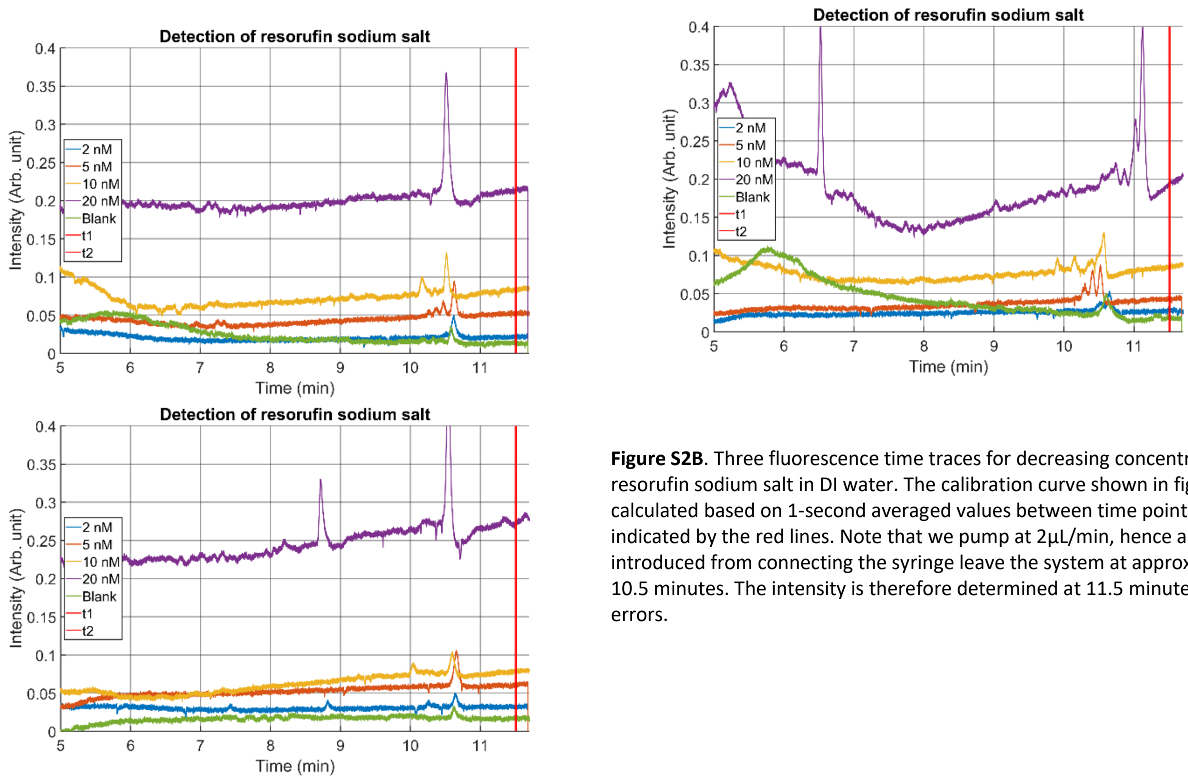

Figure S2B. Three fluorescence time traces for decreasing concentrations of resorufin sodium salt in DI water. The calibration curve shown in figure 6 is calculated based on 1-second averaged values between time points t1 and t2 indicated by the red lines. Note that we pump at 2µL/min, hence air bubbles introduced from connecting the syringe leave the system at approximately 10.5 minutes. The intensity is therefore determined at 11.5 minutes to avoid errors.

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S3 *In vitro* fluorescein sensing experiments

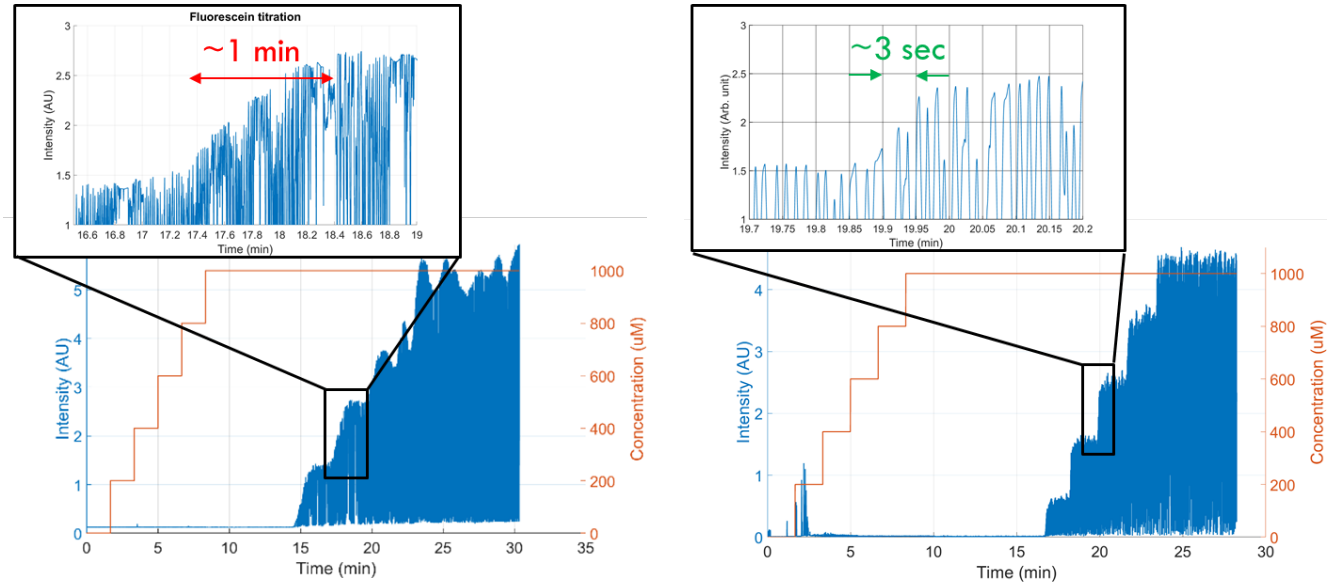


Figure S3A. Comparison of the obtained temporal resolution of probe type 1, using fluorescein titration with (right) and without (left) droplet merging. Due to dispersion of the small droplets, the temporal resolution reduces down to 1 minute, while merging the droplets enhances the temporal resolution to approximately 3 seconds.

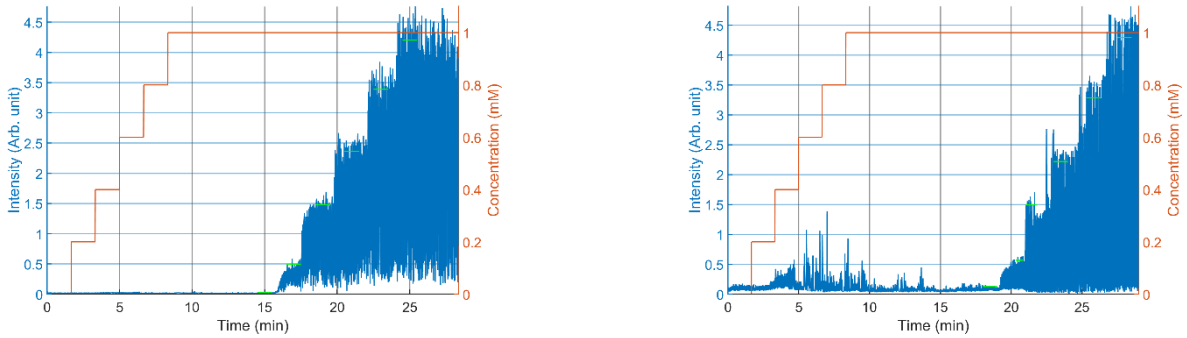


Figure S3B. Additional repeats of the Fluorescein titration experiments.

S4 Additional *In vitro* glutamate sensing experiments

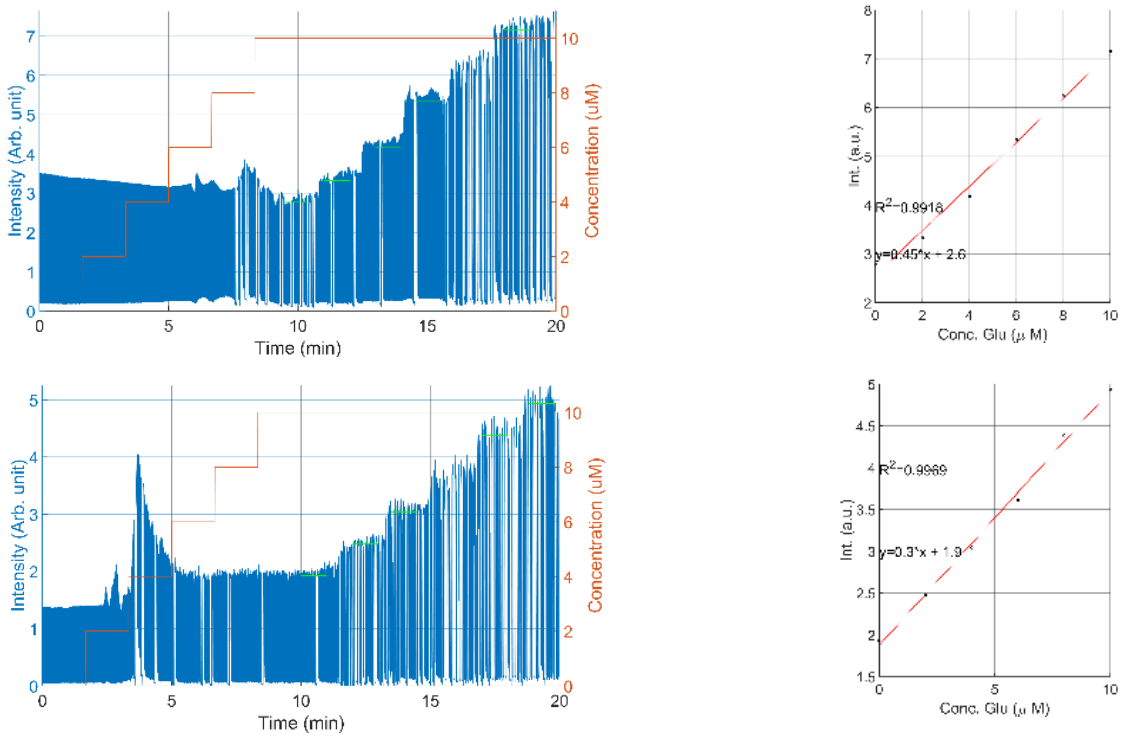


Figure S4. Additional repeats of the Glutamate titration experiments, indicating reproducible behaviour with different sensitivity (0.45 and 0.3 a.u/µM, respectively).

S5 *In vivo* glutamate sensing experiments

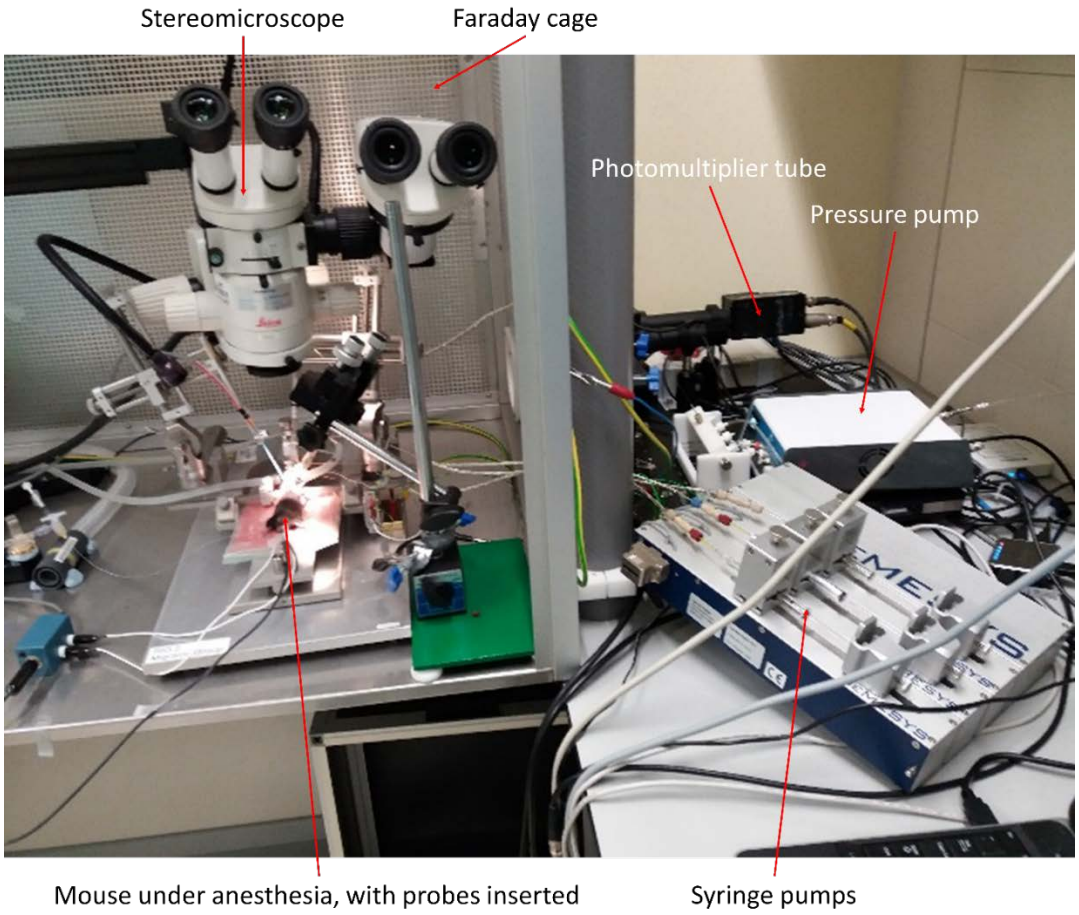


Figure S5A. Setup for *in vivo* glutamate measurements. Various pieces of equipment used are indicated. See figure S5B for details on the placement or the µPPPS probe and other electrodes in the mouse brain.

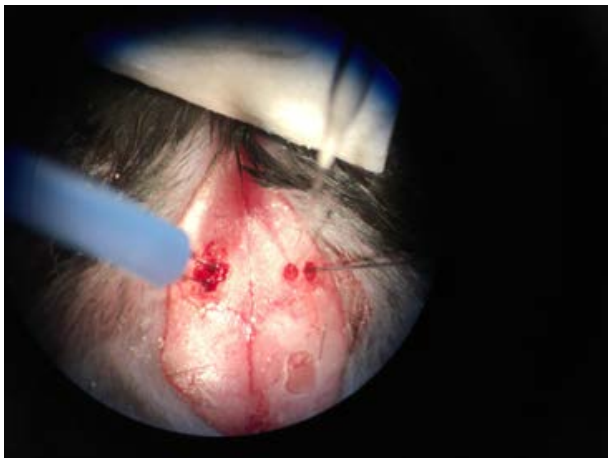
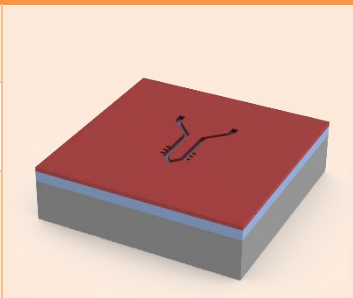
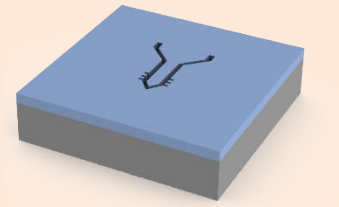
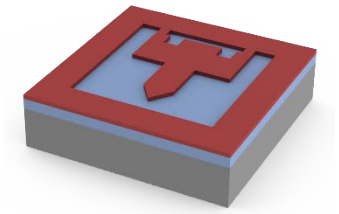
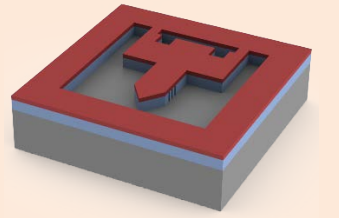
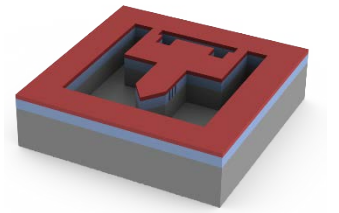
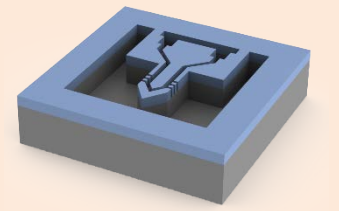
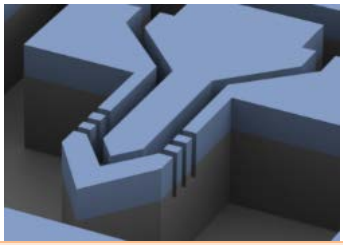
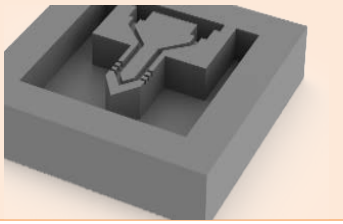
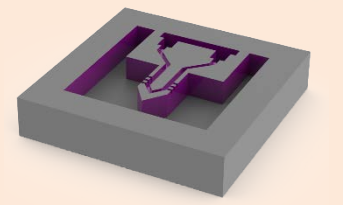
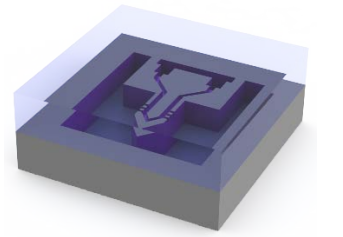
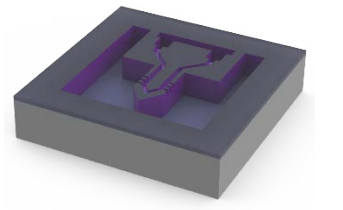
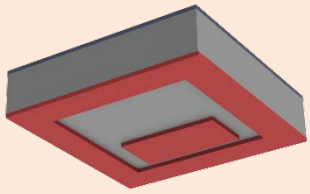
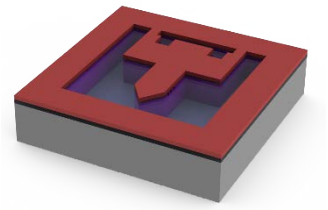
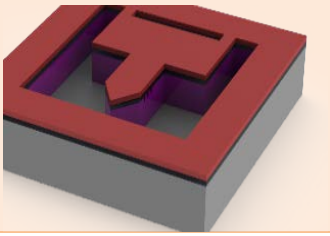


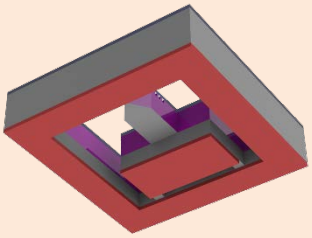
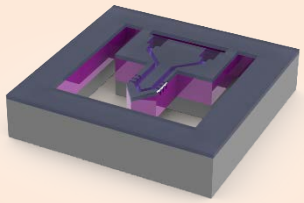
Figure S5B. Image obtained through the stereomicroscope showing the surgical set-up used for the *in vivo* uPPPS measurements in an anesthetized mouse. Shown is the mouse skull overlaying the cortex, with the craniotomies for (from left to right) electrical stimulation, DC glass recording, and µPPPS probe. More details can be found in figure 7 and the corresponding text.

S6 Process flow of the probe fabrication

Step	Process	Parameters	
1	Wafer selection & cleaning	p-type <1-0-0> 5-10 Ω*cm OneSide Polished, 525 µm thickness.	
2	Thermal oxidation	oven B2 – wet 1150; 20 min 1150 °C wet oxidation – ~450 nm	
3	Photolithography Mask 1 IW – fluidic channels	WB21 - st. OiR907-12 with HMDS, 4 s. exposure <check sampling channels, optically>	
4	SiO2 buried mask etching	AdixenDE – SiO_2 STD; etchrate is load dependent : ~400 nm/min. @ 80% load; 1:30 min.	
5	Resist strip	Tepla 360M – recipe 011; load dependent 20 min.; check carrier masking, repeat if necessary	
6	Photolithography Mask 2 IB – Needles & inlets	WB21 – 3000 rpm OiR907-17 with HMDS, 6 s. exposure <check alignment, after development>	
7	Remove SiO_2	AdixenDE – SiO2 STD; etchrate is load dependent : ~400 nm/min. @ 80% load; 1:30 min.	
8	DRIE – 120 µm Needles & inlets	SPTS Pegasus; Si etch st. etchrate is load dependent: ~20 µm/min.; 6 min., check depth, add extra time	
9	Resist strip	Tepla 360M – recipe 035; load dependent 20 min.; repeat if necessary	

10	DRIE – 20 µm Fluidic channels	SPTS Pegasus; HARS Etch rate is load dependent: ~10 µm/min; 2 min., check depth, add extra time	
11a	FC removal I oxygen plasma	TEtske: 100 mT / 25 W, 50 sccm O_2 / 10 sccm N_2; ~1 min.	
11b	FC removal II Piranha	H_2SO_4/H_2O_2 2:1 op 110 ºC; ~10 min.	
12	Buried mask strip	WB6 – BHF; ~7 min., until full hydrophobicity	
13	Pre-furnace cleaning	standard cleaning WB14 + 1 min 1% HF dip	
14	Oxidation 450 nm	furnace B2: 20 min. program Wet 1150B	
15	Anodic bonding	MEMpax® glass wafer, 500 µm thickness. Anodic Bonder EV-501 – st. program-**no vacuum** (Elizaveta-no-vac) 3 min. 400 V, 3 min. 600 V, 3 min. 800 V, 10 min. 1000 V	
16	Back Etching	WB10 – 25% HF, etch rate ~1 µm/min; temperature and freshness dependent Wafer thickness measurement: Heidehain etch/measure/etch; ~ 480 min. After 240 min. turn wafers 180º to reduce non-uniformity.	
17	Polishing (CMP)	Cemapol; 240 s. standard chemical mechanical polishing	

15	Lithography Mask 3 IW - electrodes	WB21 - st. OiR907-17 with vapour phase HMDS <check alignment, after development>	**Please note that these steps have not been carried out in practice, but indicate that it would be relatively easy to add electrodes to the glass cover of the shaft of the probe. A similar process has been demonstrated previously as described in [23]**
16	Recess etch	WB10 – 6 min. BHF @ RT	
17	Sputtering 10/125/10nm Ta/Pt/Ta	Sputterke – 110 sccm Ar, 200W 45 s. 150 W Ta, 5 min 20 s. 200 W Pt, 45 s. 150 W Ta	
18	Lift-off	WB11 – beaker acetone 20 s. ultrasonic, soak until complete delamination (few hours), ultrasonic in new acetone for 2 min., rinse with acetone resp. IPA (spray bottle). Do not let dry in between.	
19	Cleaning	WB6 – fuming HNO_3 remove residues of the lift-off; 5 min.	
20	PECVD ONO	Oxford 80 – Bios JB ONO Min.	
21	Lithography Mask 4 IW – electrode pads	WB21 - st. OiR907-17 with HMDS <check alignment, after development>	
22	ONO + Ta etch		
23	Resist strip	Tepla 360M – recipe 012; load dependent 30 min.; repeat if necessary	
24	Lithography Mask 5 IB mirror - Needle body	WB21 – 2000 rpm OiR907-17 with HMDS	
25	Lithography Mask 6 IW – Top cover clearance	WB21 – 3 times 2000 rpm OiR907-17 with HMDS bottom alignment, crosshair 3 times 15 s. interval exposure, 10 s. relax	
26	DRIE glass Top Cover Clearance	AdixenDE program BFloatexp9 2 times 20 min.	
27	Resist strip frontside	TEtske: 40mT/60W 50/20 sccm O_2/N_2	

28	DRIE 385 µm - Needle body	SPTS Pegasus; 14 min., until He leakage. Too long will ruin the sampling channels and needle!	
29	RIE Needle Body	TEtske: 100 mT/ 80W , 35/30/25 sccm $SF_6/O_2/CHF_3$ Until all needles are cleared.	
30	Resist strip	Tepla 360M – recipe 012; load dependent 30 min.; repeat if necessary	

Note: Machine names mentioned in the parameter column refer to specific equipment present in the MESA+ Nanolab cleanroom facility. A full list of equipment can be found here: https://mesaplusnanolab.ewi.utwente.nl/mis/