

Multifunctional Fibers for Simultaneous Optical, Electrical and Chemical Interrogation of Neural Circuits *in Vivo*

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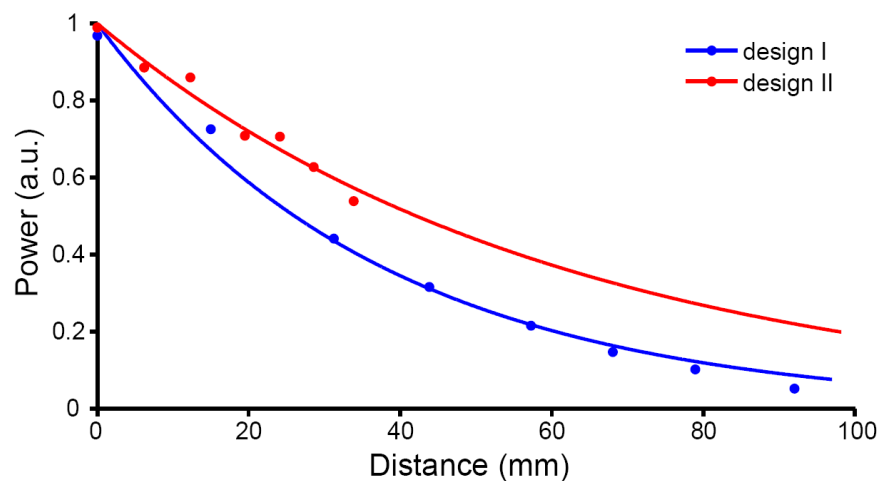
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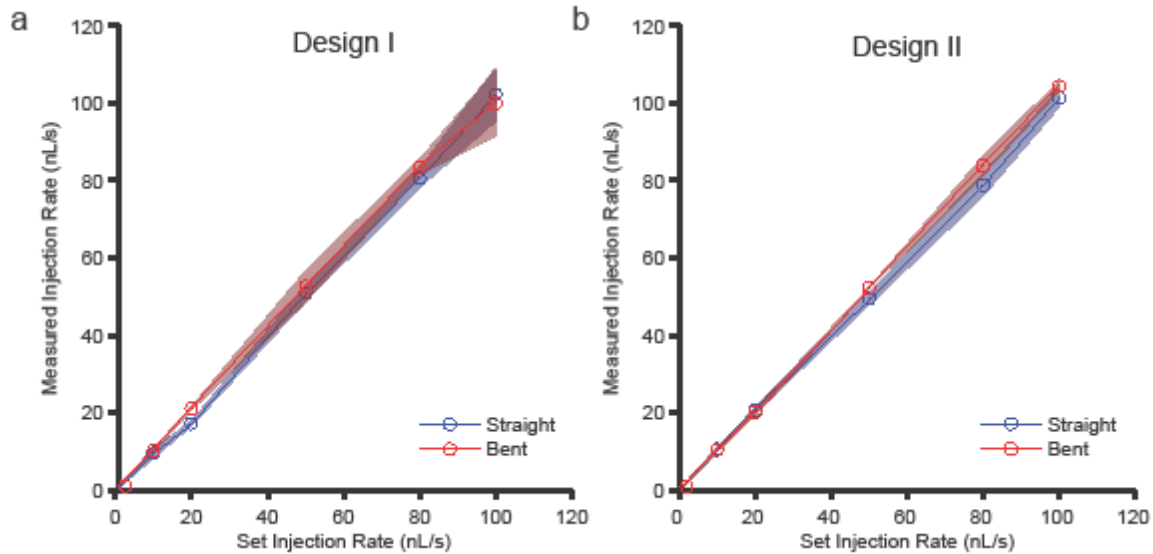
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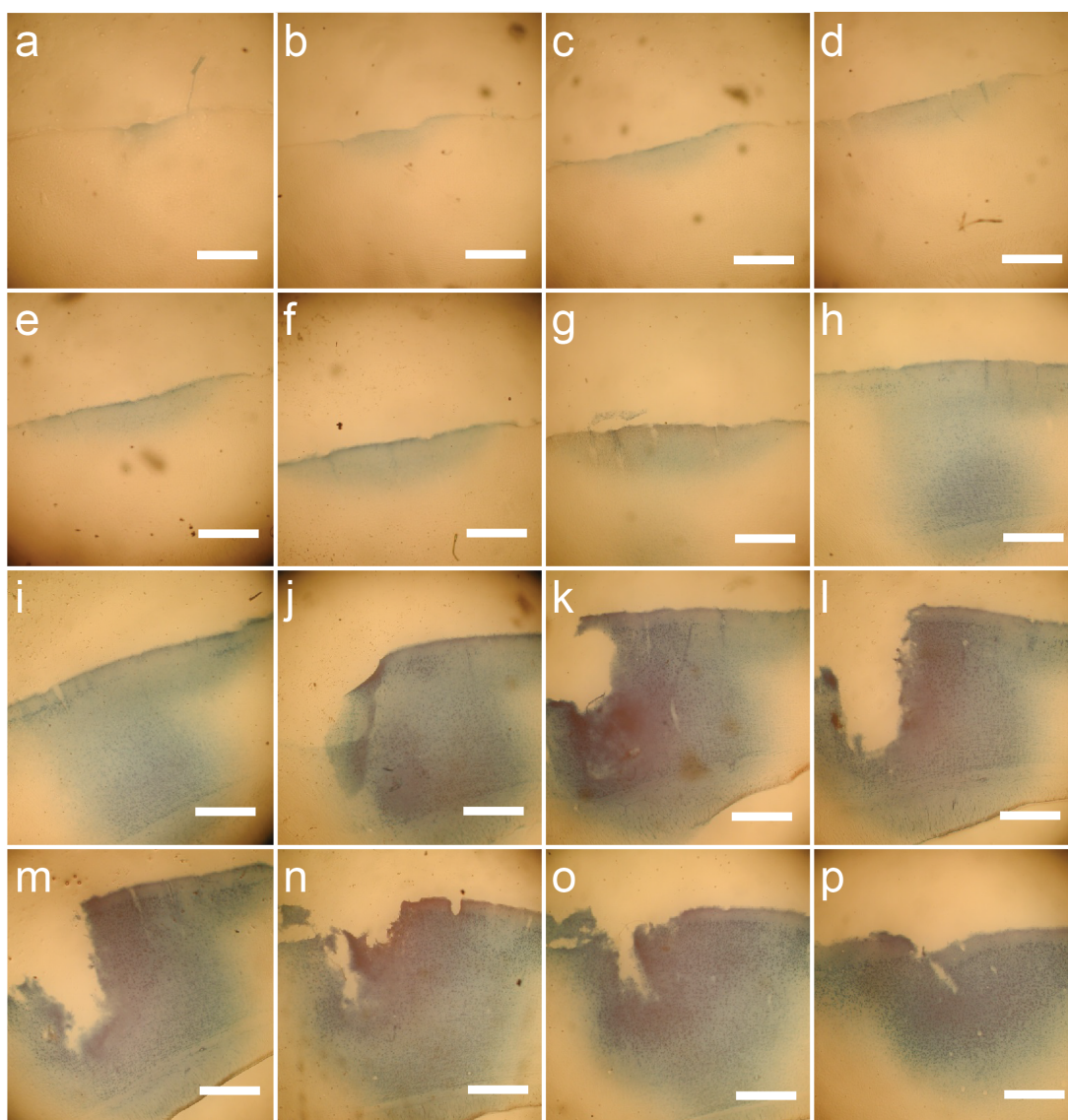
Supplementary Information



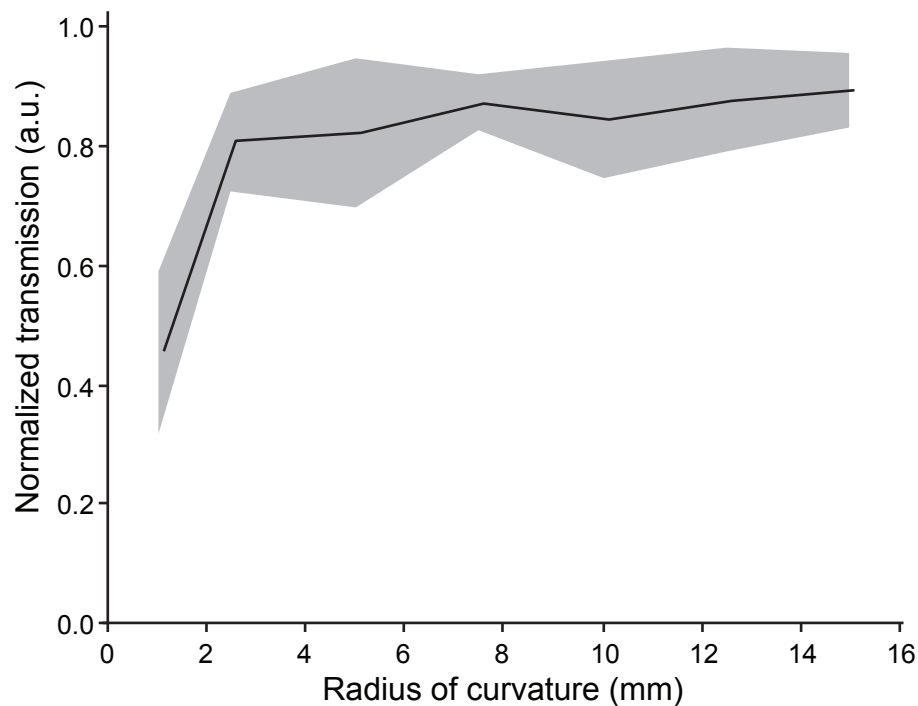
Supplementary Figure 1 | Characterization of optical transmission through multimodality probes. Optical power measured for different probe lengths. The decibel losses derived from these measurements are 2.7 dB/cm and 1.6 dB/cm for multimodality probe designs I and II respectively.



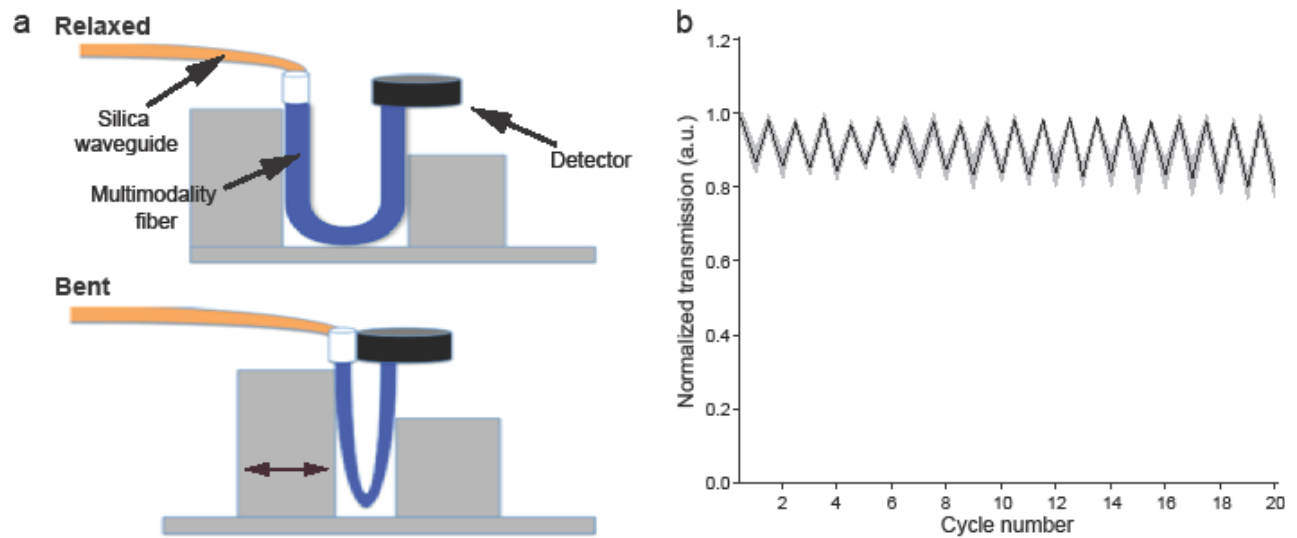
Supplementary Figure 2 | Characterization of microfluidic channels of the multimodality probes. We confirmed reliable injection at rates [nL/s]: 1, 10, 20, 50, 80, 100, even when the multimodality probe designs **(a)** I and **(b)** II were bent (90° , 4 mm radius of curvature, $n = 3$).



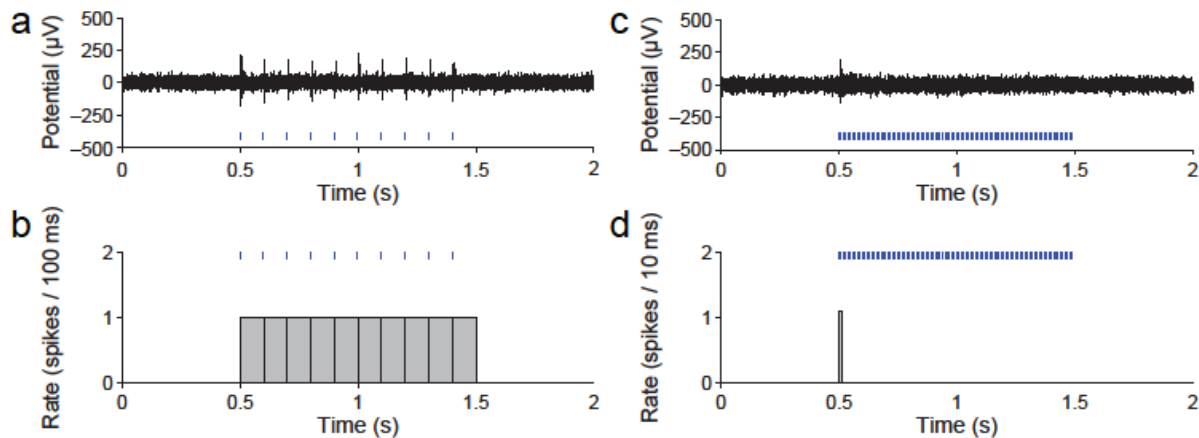
Supplementary Figure 3 | Evans Blue injection. To confirm functionality of the fluidic channel in design I multimodality probes we injected Evans Blue (1 μL , 50 μM) into the mouse mPFC, immediately perfused the mouse and sectioned the brain (sagittal plane, 50 μm thick sections). Sections were imaged using optical microscopy (scale bars = 500 μm).



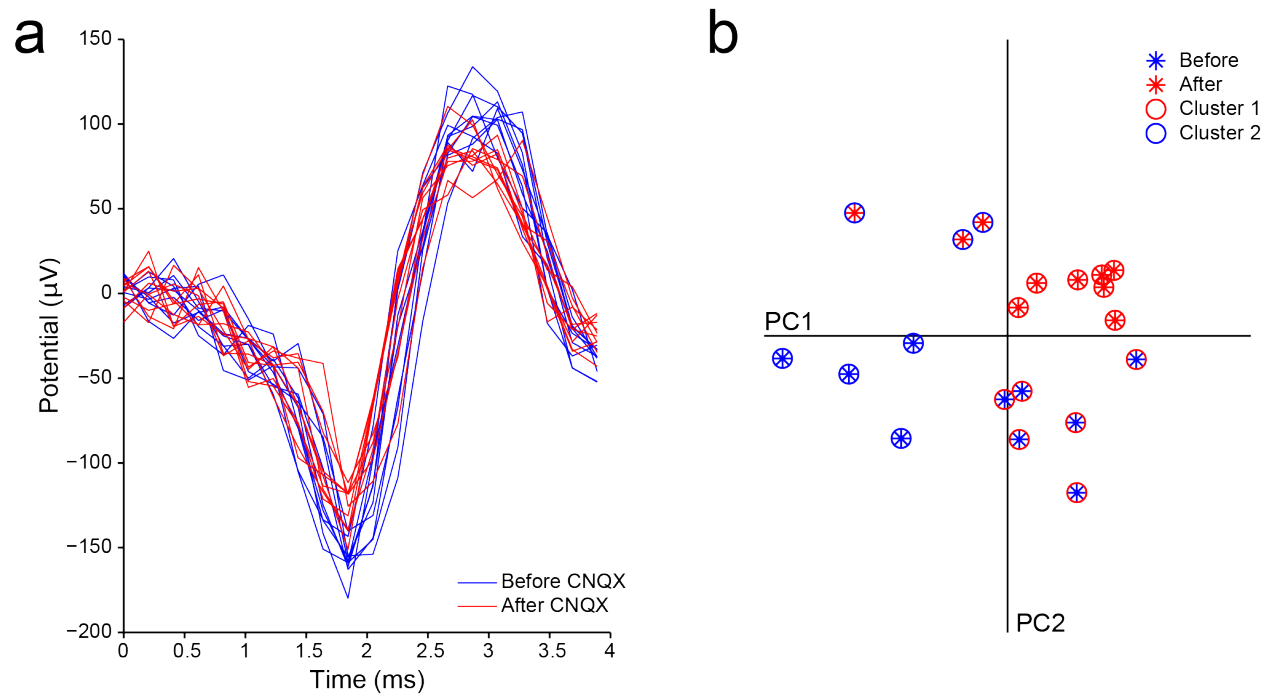
Supplementary Figure 4 | Optical transmission of bent multimodality probes. To characterize the optical transmission as a function of radius of curvature we bent design I probes ($n = 3$, 90° bending angle) at different radii [mm]: 1, 2.5, 5, 7.5, 10, 12.5, 15. The data show a plateau at larger radii of curvature followed by a steep decrease in transmission at 1 mm radius, where the devices no longer satisfy the total internal reflection condition. Transmission values were normalized to the values obtained from straight probes.



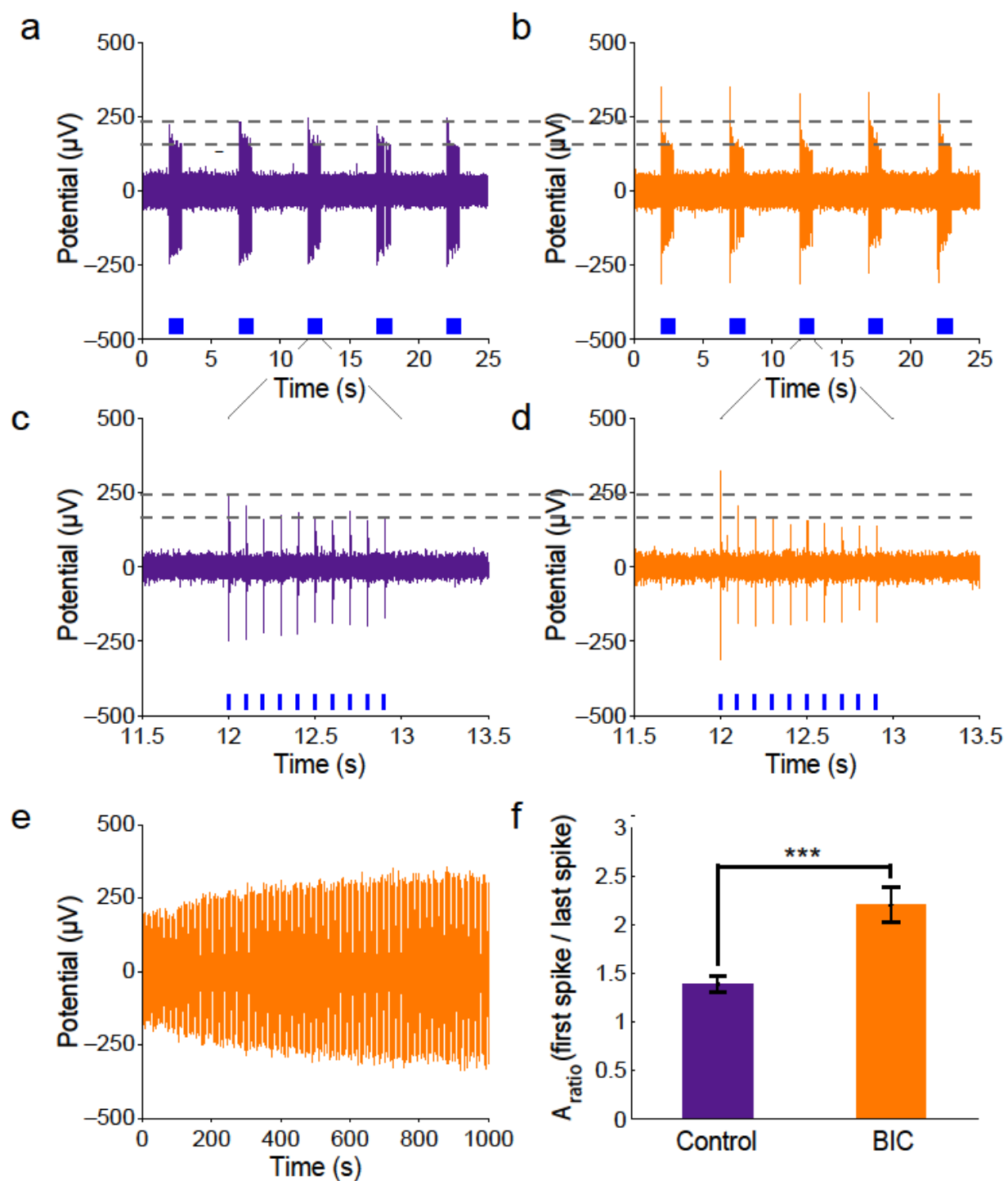
Supplementary Figure 5 | Optical transmission of multimodality probes during repeated deformation. **(a)** Schematic of the measurement setup comparing optical transmission under relaxed (top) and deformed (bottom) conditions. **(b)** Transmission was measured in the relaxed and deformed positions (n=20 repetitions). No decrease in performance was found. Transmission values were normalized to values obtained from a straight fiber.



Supplementary Figure 6 | Electrophysiological recording and optogenetic interrogation using a multimodality probe under acute conditions. **(a)** Simultaneous optogenetic stimulation and electrophysiology in the mPFC of anesthetized Thy1-ChR2-YFP mice using a design I multimodality probe (5 ms pulse width, 10 Hz, 1 s, cyan background, blue lines indicate laser onsets). **(b)** PSTH over 20 stimulation trials confirming stability of the neural response in (a). **(c)** Since, on average, groups of neurons are unable to maintain synchronized activity under ChR2-facilitated optical excitation at frequencies exceeding 50 Hz, we increased the stimulation frequency to 100 Hz. The observed decay in cortical activity is consistent with previous reports¹ ensuring physiological origin of the activity measured. We confirmed stability of this finding by calculating a PSTH across 20 trials **(d)**.

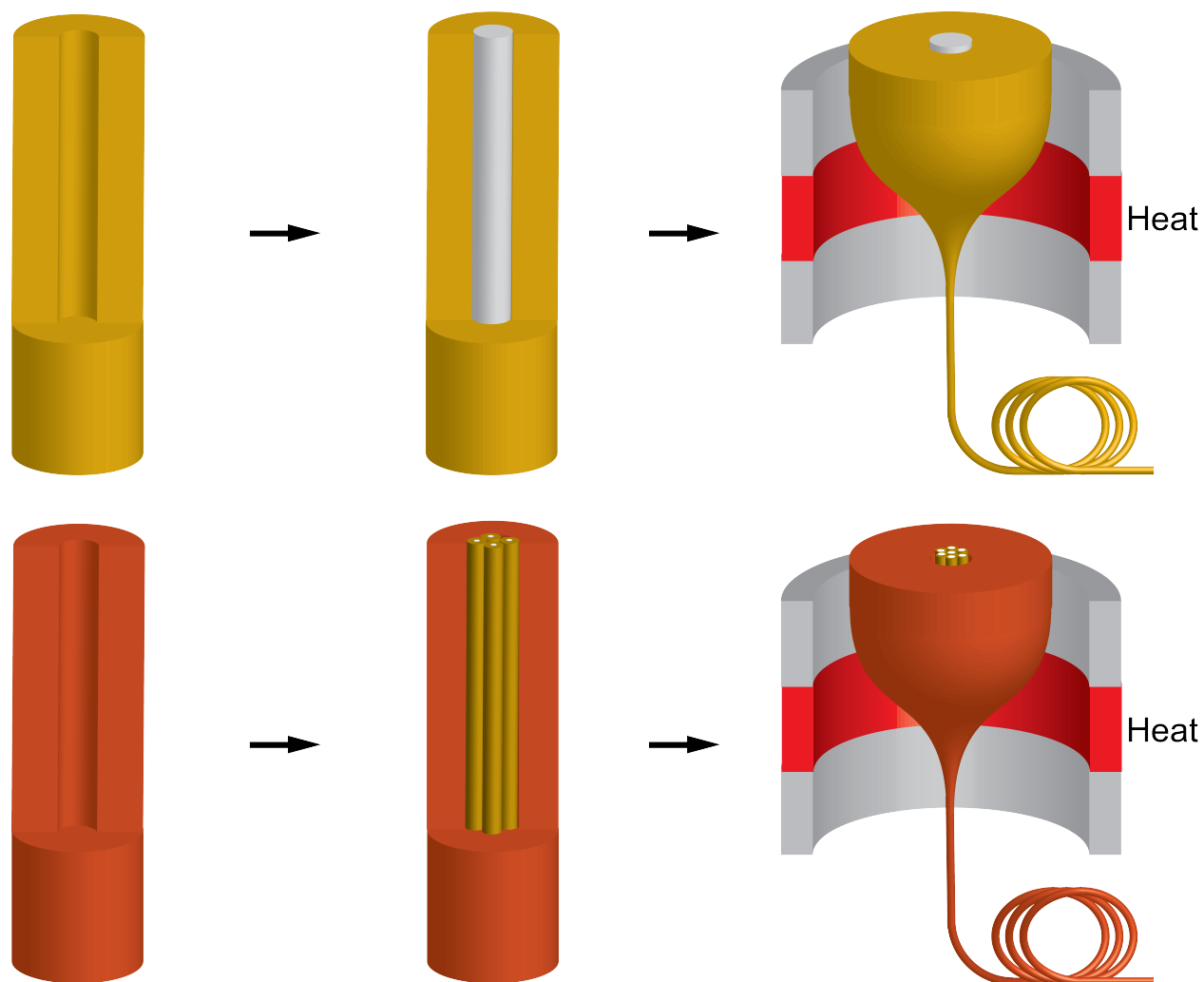


Supplementary Figure 7 | Comparison of neural response before and after CNQX injection. (a) Neural response waveforms following laser stimulation prior (blue) and following (red) injection of CNQX via a hollow channel of multimodality fiber probes are similar, indicating that the evoked responses stem from the same neural population (cf. main document **Fig. 2i-n**). (b) To further confirm that the waveforms before and after injection are not distinguishable, we performed principle component analysis (PCA) analog to **Fig. 2g**. We found very poor separation (Isolation distance: 1.86, L-ratio: 0.71) between the waveforms recorded before and after CNQX injection.

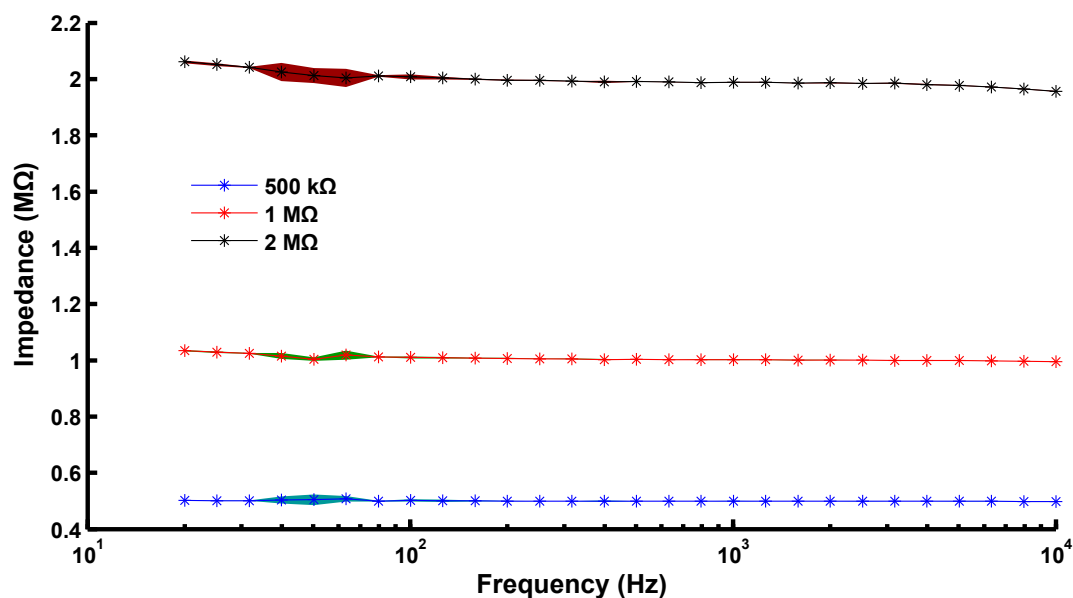


Supplementary Figure 8 | Delivery of the γ -aminobutyric acid receptor (GABA_A) antagonist bicuculline methiodide (BIC, ~50 μ l, 2 mM)² through a microfluidic channel of multimodality probe reshapes the neural response to optogenetic stimulation. (a) Example

trace showing five trials of 10 Hz optical stimulation under control conditions. **(b)** Same as (a) but 15 minutes after administration of 2 mM BIC. The amplitude of neural activity in response to the first optical pulse of a given trial increased, while the amplitudes of the subsequent responses decreased. **(c)** and **(d)** Expanded views of single trials in (a) and (b) respectively. The dashed lines indicate the amplitudes following the first and last stimulation pulse under control conditions to allow for comparison with the corresponding BIC amplitudes. **(e)** Full recording trace started ~ 5 seconds after BIC injection. The traces shown in b and d were obtained at 910 – 935 s. Following injection of BIC into the mPFC using a multimodality probe design II, the amplitudes of the first response of each optical stimulation trial increased constantly over time. **(f)** We quantified this observation by calculating the amplitude ratios (A_{ratio}) between the first and the last spike for ten control stimulation trials ($A_{\text{ratio}} = 1.39 \pm 0.09$, mean $\pm$ SD) and ten BIC stimulation trials 15 minutes after the injection ($A_{\text{ratio}} = 2.20 \pm 0.18$, mean $\pm$ SD)). A_{ratio} was significantly different between control and BIC conditions ($p = 2.64 \times 10^{-11}$, Student's t-test, $n = 10$). Since BIC blocks inhibition, this change in activity can be attributed to the recruitment of a larger excitatory neuronal population during the first trial pulse followed by neurotransmitter depletion and decreased neuronal response to the following pulses in each stimulation trial.

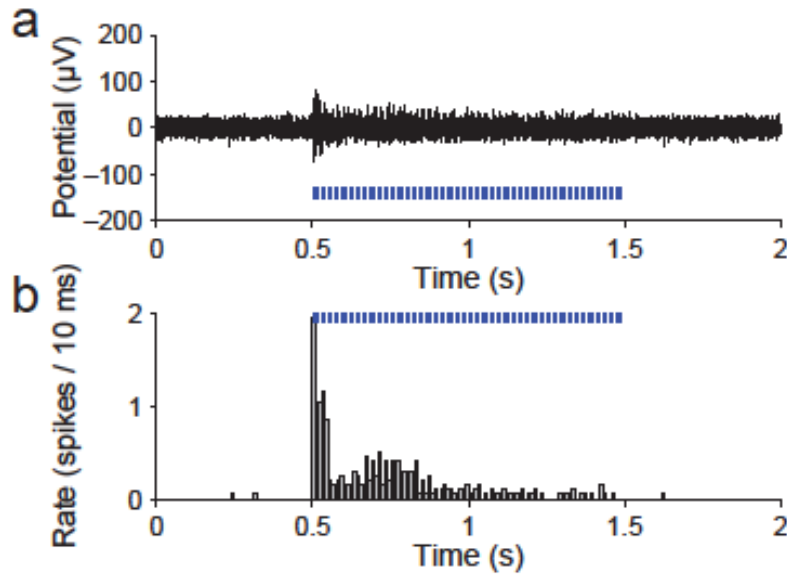


Supplementary Figure 9 | Two-step thermal drawing process. To achieve a high electrode count in a multi-electrode probe and to decrease electrode diameter, we use a two-step TDP depicted in this schematic. During the first step, we draw a fiber with a single tin core. During the second step, a bundle of sections of the fibers obtained from the first step is drawn together, which further reduces the diameter of individual electrodes.

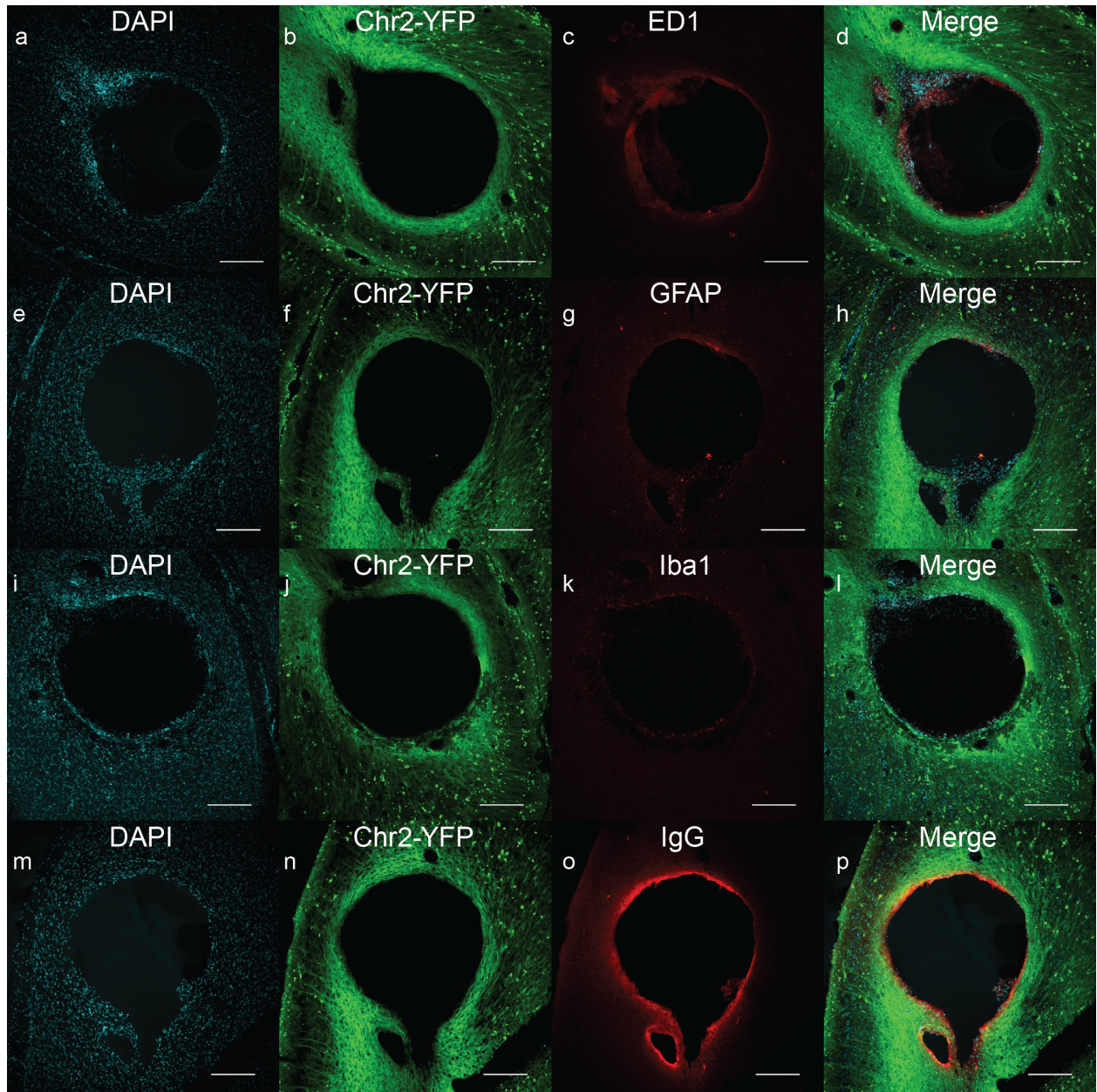


Supplementary Figure 10 | Calibration curves of the impedance measurement system.

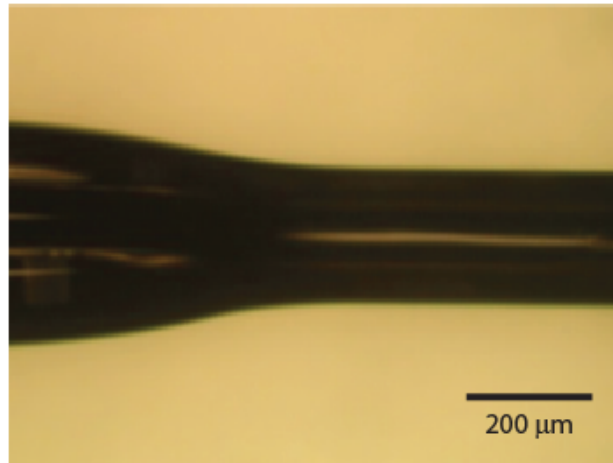
Impedance spectra of carbon film resistors (0.5, 1 and 2 MΩ, Stackpole Electronics) are similar to the spectra obtained from the multielectrode probes (**Fig. 3f**). This indicates that the frequency-dependent impedance of the multielectrode probe electrodes is dominated by resistive rather than capacitive behavior.



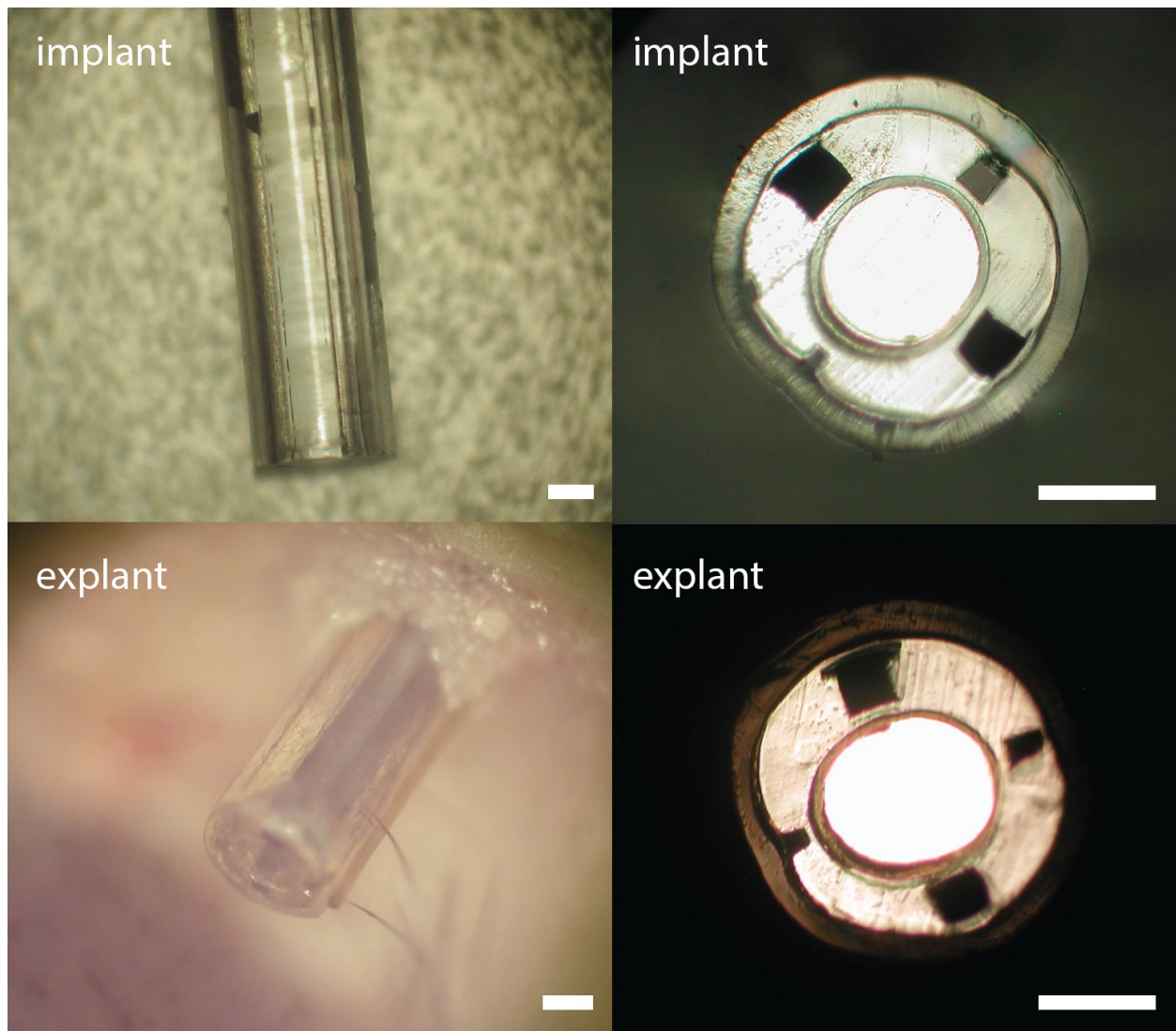
Supplementary Figure 11 | Multielectrode probe recording in the presence of high frequency optical stimulation. (a) Increasing the stimulation frequency to 100 Hz led to a rapid decay of response amplitudes after initial stimulation pulses, which verifies that the activity measured using a multielectrode probe (cf. **Fig. 3m,n**) reflected a neural response to the stimulation. **(b)** Stability of the finding was confirmed by PSTH analysis of 20 repetitions of (a).



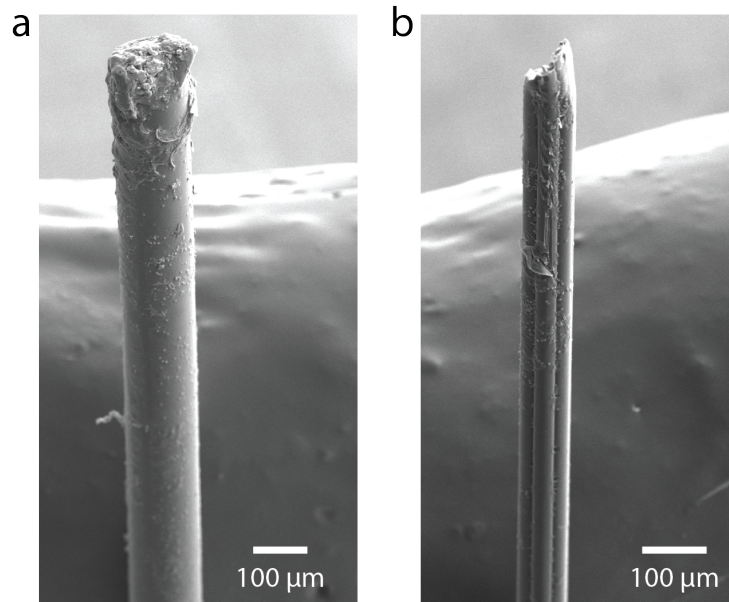
Supplementary Figure 12 | Tissue response to implanted multimodality probes. Representative fluorescent confocal images show tissue response 3 months after implantation of multimodality probes in the mPFC of a Thy1-ChR2-YFP mouse (scale bars = 250 μ m).



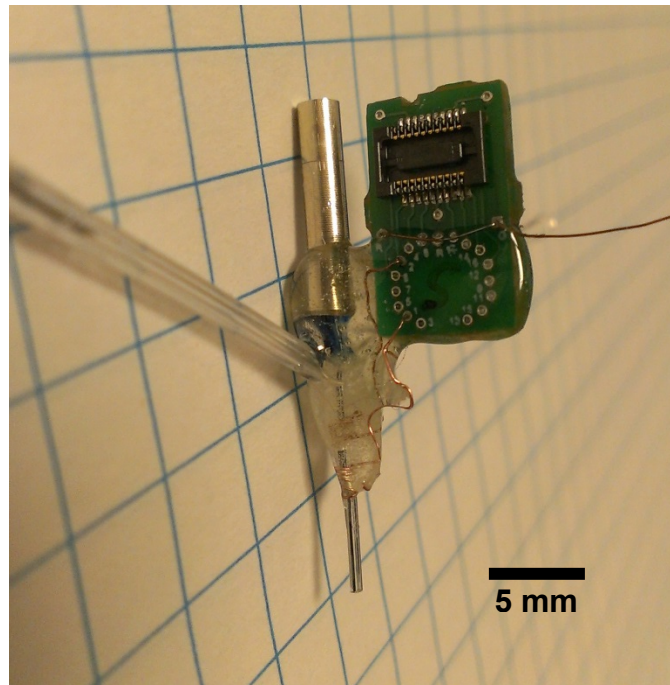
Supplementary Figure 13 | Selective etching of the outer cladding of a multimodality probe (design II). To reduce the overall diameter, the outer PC cladding of multimodality probes can be selectively etched using dichloromethane. In this case the diameter was reduced from ~ 350 μm to ~ 210 μm .



Supplementary Figure 14 | Comparison of multimodality probes before and after implantation. There is no visible difference between the multimodality probes before and after implantation in the mouse mPFC (3 months after implantation, scale bars = 200 μm).



Supplementary Figure 15 | Comparison between a steel microwire and a multielectrode probe after implantation. SEM images of explanted microwire (**a**) and multielectrode probe (**b**) two weeks after implantation.



Supplementary Figure 16 | Interfacing with electrical, optical and microfluidic functionalities of the multimodality fiber probes. To make use of all functionalities of a multimodality probe (design I) simultaneously, the electrodes were exposed by removing the cladding from a section of drawn fiber, and electrically connected to copper wires using conductive silver paint. The wires were in turn connected to a printed circuit board (PCB). The fluidic channels were connected by exposing the channel in a way similar to the electrodes, inserting the fiber perpendicularly through a piece of EVA tubing and gluing it in place. The tip of the fiber was glued into a stainless steel ferrule (710 μm ID, Thorlabs), followed by mechanical polishing of the tip to reduce light losses at the optical interface. The photograph shows a fully assembled design I multimodality probe.

Supplementary Table 1: Materials selection for probe fabrication

Material	T _g /T _m (°C)	Refractive index	Young's modulus (GPa)
PC ³	150	1.586	2.38
CPE**	123	—	—
COC ⁴	158	1.53	3
PEI	215 ³	1.66 ⁵	3 ³
PPSU ⁶	220	1.67	2.3
Tin	232	—	50 ⁷
Silicon	1414 ⁸	3.42 ⁸	130–188 ⁹
Silica ¹⁰	1667	1.458	69

** CPE data is from Michael Rein (personal communication).

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