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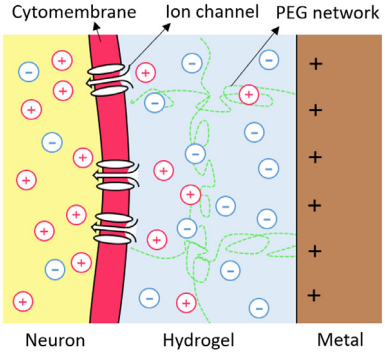
Neural interfaces by hydrogels

Hao Sheng [a,1], Xiaomeng Wang [b,1], Ning Kong [c,1], Wang Xi [d], Hang Yang [e], Xiaotong Wu [b], Kangling Wu [e], Chenghai Li [e], Jian Hu [e], Jingda Tang [e], Jinxiong Zhou [e], Shumin Duan [b], Hao Wang [b,*], Zhigang Suo [a,*]

[a] John A. Paulson School of Engineering and Applied Sciences, Harvard University, 29 Oxford St. Cambridge, MA 02138, USA
[b] Department of Neurobiology and Department of Neurosurgery of Second Affiliated Hospital, Key Laboratory of Medical Neurobiology of the Ministry of Health of China, Key Laboratory of Neurobiology, School of Medicine, Zhejiang University, 866 Yuhangtang St. Hangzhou, Zhejiang 310058, China
[c] Department of Orthopaedics, Second Affiliated Hospital of Health Science Center, Xi'an Jiaotong University, 157 Xiwu St. Xi'an, Shanxi 710004, China
[d] Interdisciplinary Institute of Neuroscience and Technology, Zhejiang University, 268 KaiXuan Road, Hangzhou 310020, China
[e] State Key Laboratory for Strength and Vibration of Mechanical Structures and School of Aerospace, Xi'an Jiaotong University, 28 Nianning St. Xi'an, Shanxi 710049, China

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrogel neural interface mimics the living environment of neurons mechanically, chemically, and electrically, incurs much less immunochemical rejection than commonly used metallic electrodes (platinum and silver). It readily records local field potentials and individual spikes *in vivo*. In optogenetic experiments, a single fiber of the hydrogel can function as both an optic fiber and an ionic conductor, co-locating the site of laser stimulation and electrical recording.

Contents

* Corresponding authors.
E-mail addresses: haowang@zju.edu.cn (H. Wang), suo@seas.harvard.edu (Z. Suo).
[1] Co-first author.

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1. Introduction

Implanted neural interfaces are fundamental to biology and medicine, but existing interfaces struggle to be compatible with neurons. We report a hydrogel neural interface that mimics the living environment of neurons chemically, mechanically, and electrically, incurs much less immunochemical rejection than existing interfaces, and records high-quality signals from brains *in vitro* and *in vivo*. In an optogenetic experiment, a single fiber of the hydrogel enables simultaneous optical stimulation and electrical recording. We further make an elastomer-sealed hydrogel cable, freeze the hydrogel, and insert the stiff cable into the brain of a mouse. Inside the brain, the hydrogel melts and collects neural signals. The hydrogel neural interfaces can be broadly useful in biology and medicine.

Implanted neural interfaces are central to the analysis of neural circuits, treatment of neurological diseases, and control of artificial limbs [1]. Traditional interfaces are made of metals, which cause glial cells to wrap around the interfaces and interdict the signals [2,3]. Efforts to mitigate the problem include using sheet metals [4], or further embedding sheet metals in soft substrates [5]. It remains challenging, however, to fully mask the fundamental dissimilarity between neurons and existing interfaces [6,7].

By contrast, many hydrogels are compatible with living tissues chemically, mechanically, and electrically. In particular, polyethylene glycol (PEG) hydrogels [8] have been used to culture neurons [9]. Hydrogels are stretchable, transparent, ionic conductors, and have enabled the recent development of hydrogel ionotronics [10,11] and soft optical fibers [12,13]. Also note that artificial cerebrospinal fluid (ACSF) has long been used in extracellular electrophysiological recording, but the flow of the fluid has limited its applications as implanted interfaces [14].

2. Results and discussion

Here we demonstrate hydrogels as highly neuro-compatible interfaces. To mimic the living environment of neurons, we synthesize a PEG hydrogel using ACSF as solvent. The PEG-ACSF hydrogel readily records neural signals *in vitro* and *in vivo*. We show that the hydrogel causes much less immunochemical rejection than platinum. In an optogenetic experiment, a single hydrogel fiber can enable simultaneous optical stimulation and electrical recording of a free-moving mouse. We further describe a method to insert a soft neural interface into a brain.

To illustrate the basic physics and chemistry of a hydrogel neuroelectronic interface, consider how the hydrogel transforms an action potential of a neuron to an electronic current in a detector (Fig. 1A and Movie S1). The hydrogel contacts the cytomembrane of the neuron and a metallic electrode of the detector. The hydrogel and the neuron exchange matter (i.e., ions, water, and possibly other molecules). This exchange mimics that between the extracellular fluid and the neuron, and is effective with or without extracellular fluid between the hydrogel and the cytomembrane. Thus, the PEG-ACSF hydrogel mimics the extracellular fluid chemically and electrically, but is a soft solid mechanically that can maintain designed shape *in vivo*. The hydrogel and the metallic electrode do not exchange matter, but form an electrical double layer (EDL) [11]. The voltage associated with neural signals is small, so that no electrochemical reaction occurs at the EDL. Rather, the EDL is under the non-Faradaic condition and functions as a capacitor. The hydrogel is an ionic conductor, and the metal is an electronic conductor. When the neuron fires an action potential, ion channels in the cytomembrane allow ions (mostly positive) move from the hydrogel into the neuron. To regain electrical neutrality, in the hydrogel, some negative ions migrate towards the EDL, and some positive ions migrate away from the EDL. In the metal, some electrons flow from the EDL to the detector. This cascade of events enables the detector to register the action potential.

We tested the hydrogel as a neuroelectronic interface in a loose patch-clamp experiment (Fig. 1B). We synthesized a PEG-ACSF hydrogel (Figure S1) in a glass pipette of a 2.5 μm-diameter tip, and placed the tip gently on a Purkinje cell of a brain slice of a mouse (C57BL/6J) (Fig. 1C). The other end of the hydrogel was connected, via a silver wire, to a detector (Axopatch 700B). For comparison, we used both a hydrogel-filled pipette and an ACSF-filled pipette to record the spontaneous firing of Purkinje cells (Fig. 1D). The records by the hydrogel and by the ACSF had similar average waveforms (Fig. 1E) and similar frequency distributions (Fig. 1F).

We next used the hydrogel to record local field potentials of a free-moving mouse *in vivo* (Fig. 2A). For convenience of the surgery, we designed the neural interface as a cap (Fig. 2B, Figure S2). The cap hosted a hydrogel-filled glass tube, which was implanted into the hippocampus of a free-moving mouse (C57BL/6J) (Fig. 2C). The recorded signals clearly differentiated the two states of the mouse, wake and sleep (Movie S2). When the mouse fell asleep, local field potentials showed waveforms of low frequencies and large amplitudes, suggesting synchronized activities of a large number of neurons (Fig. 2D). The recorded signals by the hydrogel were comparable to those by platinum. The spectrogram showed that low-frequency waves increased significantly when the mouse transitioned from wake to sleep (Fig. 2E). We repeated the experiment in six mice, and all of them recorded high-quality signals two weeks after implantation.

We also recorded spikes of individual neurons of an anesthetic cat *in vivo* to prove the capability of our interface. We used a micromanipulator (Narishige MO-10) to insert into the brain of the cat a hydrogel-filled glass tube of 5 μm-diameter tip (Fig. 2F). We slowly adjusted the depth of insertion by the micromanipulator and simultaneously monitored the output of the detector. The tip of the hydrogel was taken to be close enough to a neuron when the detector recorded clear spikes (Fig. 2G).

We next confirm that the PEG-ACSF hydrogel incurs much less immunological rejection than metals. In a brain, glial cells (microglia and astrocytes) tend to migrate to a foreign body [15]. It is known that implanted materials of lower moduli incur less immunological rejection [2,6]. Young's modus of a metal is on the order of 100 GPa, while Young's modulus of the brain is in the range 100 Pa-100 kPa [6]. Hydrogels have tunable modulus and can be softer than brains. We measured the modulus of the PEG-ACSF hydrogel by a rheometer (Anton Paar, MCR 302) and found that its modulus was 45 Pa (Figure S3). We placed the hydrogel, silver and platinum in glass tubes (Polymicro TECHNOLOGIES,

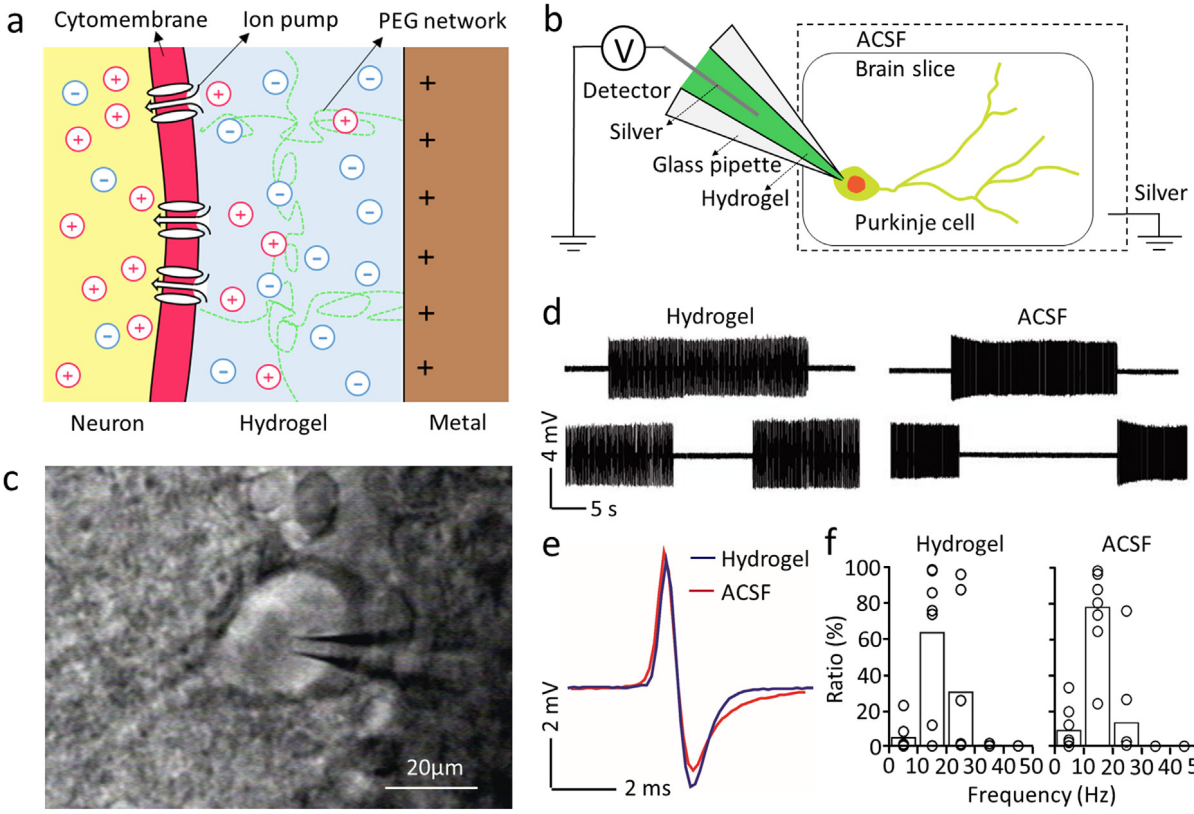

Fig. 1. Hydrogel as a neuroelectronic interface. (A) The working principle. (B) A hydrogel in a glass pipette connects a Purkinje cell in a brain slice and a silver electrode of a detector. The brain slice is immersed in the artificial cerebrospinal fluid (ACSF), which is grounded via a silver wire. (C) An optical image of the tip of the pipette on a Purkinje cell. (D) Two representative sweeps detected by the hydrogel-filled pipette and by the ACSF-filled pipette. Each episode contains roughly 100 spikes. (E) The averages of spikes. (F) The frequency distribution of the signals detected by the hydrogel-filled pipette and ACSF-filled pipette. Each circle represents a sample. Seven samples per group were tested.

TSP320450). One end of each tube was sealed, and the other end was open (Fig. 3A). We implanted each tube into the lateral hypothalamus (LH) of the brain of a mouse. After one week, we sliced the brain beneath the open end of the tube, immunochemically treated the slices, and observed them in a confocal microscope (Olympus FV1200). As expected from the existing literature [16], platinum incurred less immunochemical reaction than silver (Fig. 3B). Our experiment showed that the hydrogel incurred far less immunochemical reaction than platinum. For each type of the neural interface (hydrogel, platinum, or silver), we repeated the experiment using six mice. We counted glia cells within a square area (300 μm × 300 μm) at three depths. The numbers of glial cells in the slices tested using the hydrogel were significantly less than those using the metals (Fig. 3C). The fluorescence intensity of slices tested using the hydrogel was also much less than that using platinum (Fig. 3D).

Optogenetics enables neural stimulation with high resolution in time and space [17]. The existing practice is to use glass fibers to transmit light to neurons and use metal wires to record electrical signals. Both glass fibers and metal wires are stiff materials, susceptible to immunological rejection. Furthermore, using different materials for the two tasks pose challenge to co-locate the site of stimulation and recording. A polyacrylamide hydrogel have been used as optical fibers in an optogenetic experiment [13]. Here we show that the PEG-ACSF hydrogel can simultaneously stimulate and record neurons.

We measured the transmittance of the PEG-ACSF hydrogel by a spectrometer (PerkinElmer Lambda 950), where a 5 mm-thick (approximately the thickness of the brain of a mouse) hydrogel showed an average transmittance of 98.4% in the visible range (Figure S4). We next confirmed that the hydrogel could transmit laser in an optogenetic experiment. We inserted a 0.2 mm-diameter optical fiber into a 0.32 mm-inner diameter, 0.45 mm-outer diameter PEG-ACSF hydrogel-filled tube (Polymicro TECHNOLOGIES, TSP320450) and made sure that they are on the same axis. We implanted them by a cap (Figure S5) in the laterodorsal tegmentum (LDT) of a mouse (Fig. 4C). The LDT controls olfactory-cue induced innate fear. When GABAergic cells in this brain area are activated, the mouse expresses the behavior of fear, such as freeze [18]. We tested two groups of mice, optogentically modified (VGAT-ChR2(H134R)-eYFP, abbreviated to VGAT-ChR2), and unmodified (C57BL/6J). We stimulated the mice by 470 nm, 5 mW, 20 Hz blue lasers (NEWDOON, Aurora-220-473) with each pulse of 20 ms for 20 s. When the laser was off, both groups of mice did not freeze. When the laser was on, the unmodified mice did not freeze, but the optogenetic mice did (Fig. 4D and Movie S3).

We then demonstrate that the hydrogel can simultaneously stimulate and record neurons. We inserted both a 0.2 mm-diameter optical fiber and a 60 μm-diameter silver wire into the hydrogel from the top end of the tube (Fig. 4A, B). We then fixed the tube in a cap (Figure S6) and implanted the bottom end of the tube into the brain of a VGAT-ChR2 mouse. Then we stimulated the mouse by 470 nm, 5 mW blue lasers of 1, 2, 5 Hz with each pulse of 20 ms and detected neural signals at the same time. Signals showed that neurons were activated following the frequency of the laser pulses (Fig. 4E). Moreover, we implanted the tube into the LH (Fig. 4F), the brain area involved in sleep regulation [19] of the VGAT-ChR2 mouse. We selectively

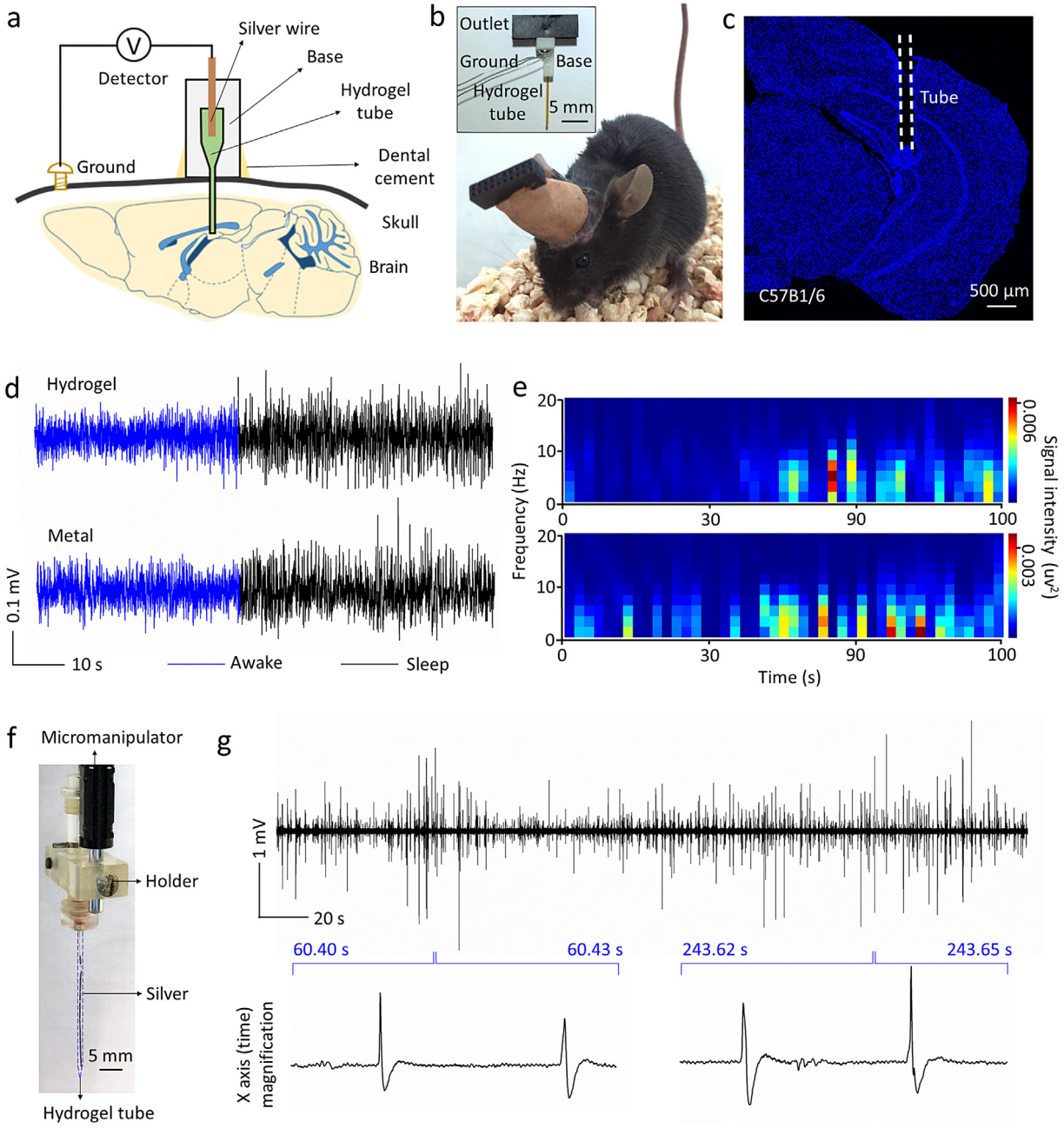

Fig. 2. Detecting neural signals *in vivo*. (A) A schematic of the experimental setup to detect local field potentials of mouse *in vivo*. (B) Photos of a cap and an implanted mouse. (C) A micrograph of a hydrogel-filled glass tube implanted in the brain of a mouse, using DAPI as nuclear fluorescent stain. (D) Local field potentials detected by hydrogel and by metal of a mouse *in vivo*. (E) The spectrograms of the signals in figure D. (F) The device for recording spikes of cat *in vivo*. (G) Spikes of a cat recorded *in vivo*: a 300 s signal and two 3 ms segments.

stimulated GABAergic cells when the mouse fell asleep. They woke up quickly after the light stimulation (Fig. 4G and Movie S4). The sleep–wake transition could be further identified by the power density (Fig. 4H). The spectrogram of the recorded signal clearly showed the changes in frequency during the process (Fig. 4I). The experiment was repeated six times and all of them worked well.

In the *in vivo* experiments above, the glass tube served two functions: a dielectric that electrically insulates the hydrogel from the surrounding tissues, and a stiff material that can be inserted into the brain. Ideal neural interfaces should be stiff to be inserted into a brain, but soft afterwards to match the brain. The conflicting requirements cannot be fulfilled by the hydrogel-filled glass tube. We hypothesize that the conflicting requirements can be fulfilled by the phase transition of water. The hydrogel contains ∼90 wt% water and freezes at ∼ zero centigrade. To make a soft neural interface, a hydrogel can be insulated in a tube of dielectric elastomer. The frozen hydrogel-filled elastomer tube is stiff and can be inserted into the brain. After the insertion, the water in the hydrogel melts and the tube becomes soft. Neurons in the brain can survive zero centigrade for a short time, as routinely demonstrated in the preparation of brain slices at zero centigrade.

We designed an experiment to ascertain this hypothesis. For the dielectric tube we used a soft silicone tube (DOW CORNING, NO. 508–001) with an inner diameter of 0.8 mm and an outer diameter of 1.2 mm. This hydrogel-elastomer coaxial cable

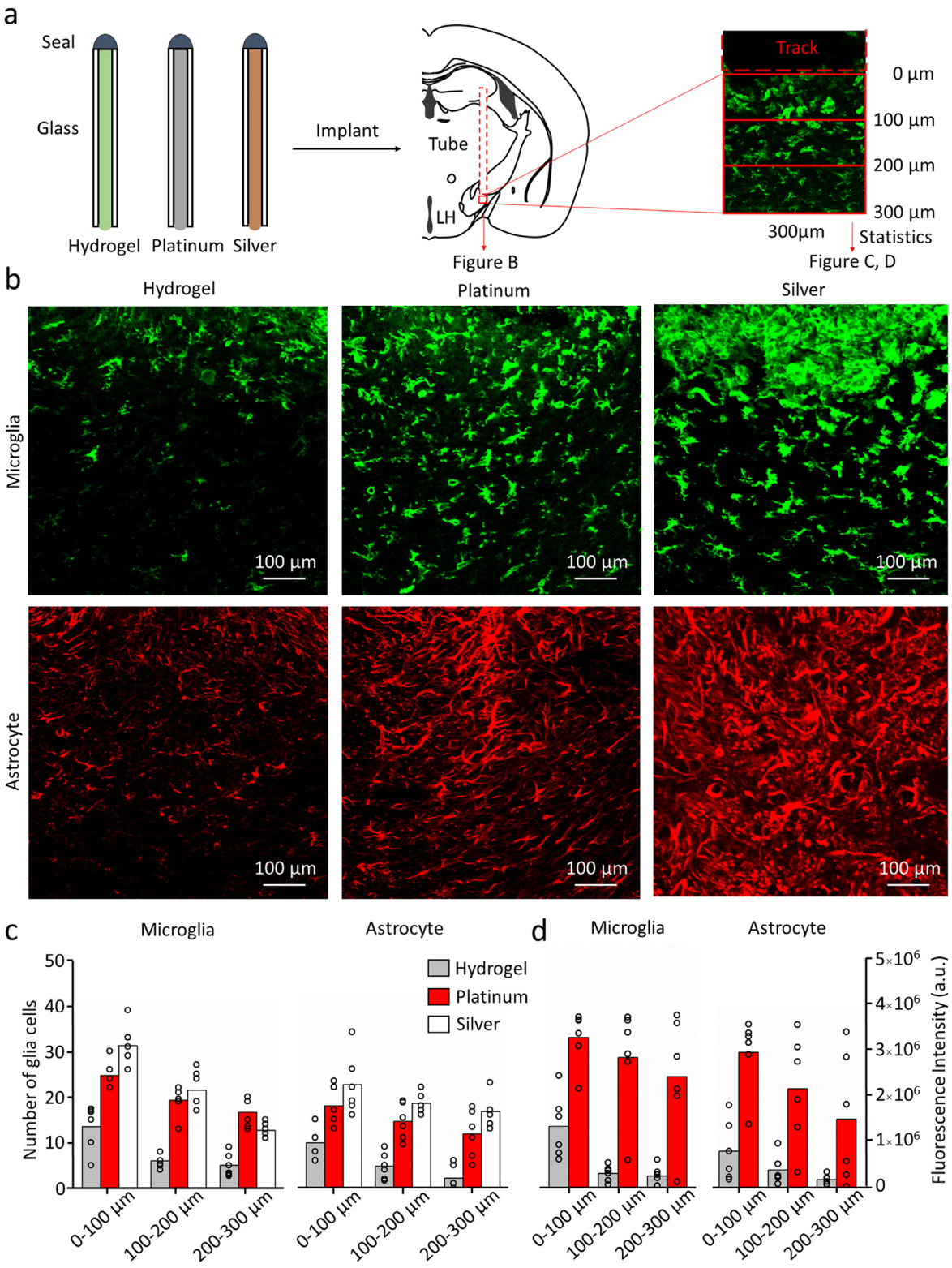

Fig. 3. Biocompatibility of PEG-ACSF hydrogel. (A) PEG-ACSF hydrogels and metal wires were placed in glass tubes and implanted in brains of mice. After one week, the brains beneath the open end of the tubes were sliced and immunochemically treated. (B) Confocal micrographs of the brain slices showed microglia cells (green) and astrocyte cells (red). More cells represent worse immuno response, and imply worse biocompatibility of material. (C) The numbers of microglia and astrocyte cells. (D) The fluorescence intensity of confocal images. Circles represent each sample. The height of each bar represents the mean of the group . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mimics the myelinated axon [11,20], and can transmit signals up to 10^5 Hz (Figure S7). By comparison, the time scale for an individual action potential is ms. Thus, this artificial axon is much faster than needed to transport neural signals. Young's modulus of the silicones was 16.56 kPa (Figure S8), which was much lower than that of metals. Dielectric elastomers of even lower moduli

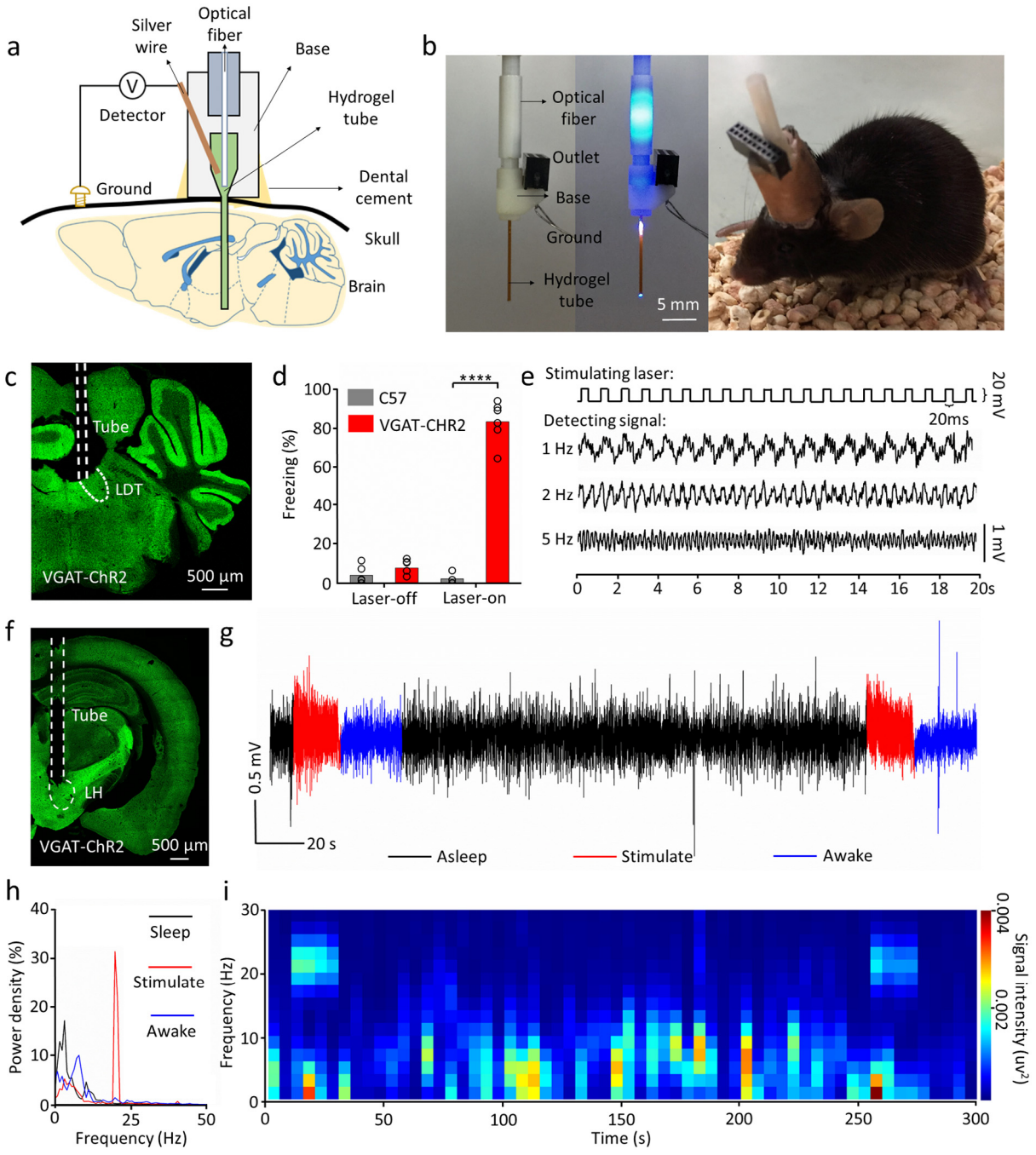

Fig. 4. Hydrogel as both a neuroelectronic and a neuro-optic interface in an optogenetic experiment. (A) The device of applying laser stimulations and detecting neural signals in a hydrogel tube. (B) Photos of the hydrogel interface when the laser was off and on. A photo of a mouse implanted with the interface. (C) A confocal image of the interface placement in the LDT. (D) Percentage freezing time during 20 s with or without laser stimulation of C57BL/6J and VGAT-ChR2 mice. Circles represent each sample. ****One-way ANOVA, n = 6 per group, F = 327.889, P = 0 < 0.05. (E) We used 5 mW blue lasers of 1, 2, 5 Hz with each pulse of 20 ms to stimulate neurons through the hydrogel interface. Detected signals are shown corresponding to its stimulus frequency. Neurons are activated at the same frequency as stimulating lasers. (F) An example of the interface placement in the LH. (G) Optical stimulations and electrical recordings by the hydrogel interface. We used lasers to stimulate sleeping mice at the LH, where neurons are activated and mice turn awake immediately. Here we show recording signals during this process. (H) The power density corresponding to figure G. The frequency of the sleep state concentrated under 5 Hz, while the frequency of the stimulation state gathered sharply in 20 Hz. (I) The spectrogram analysis corresponding to figure G. Low frequency waves increase at the sleep state and 20 Hz-waves appear at the stimulation state.

can be made [21], but are not pursued in this paper. We used the hydrogel-elastomer interface (Fig. 5A) to repeat the sleep–wake experiment of free-moving mice (C57BL/6J). The interface was first kept at −20 centigrade for 24 h and then in an ice–water mixture for 10 min. Immediately afterwards we inserted the frozen interface into the hippocampus (Fig. 5B). In three repeated experiments, recorded signals of the interface clearly showed changes between awake and asleep states (Fig. 5C).

3. Conclusion

In summary, we have demonstrated that the PEG-ACSF hydrogel incurs much less immunochemical reaction than metals,

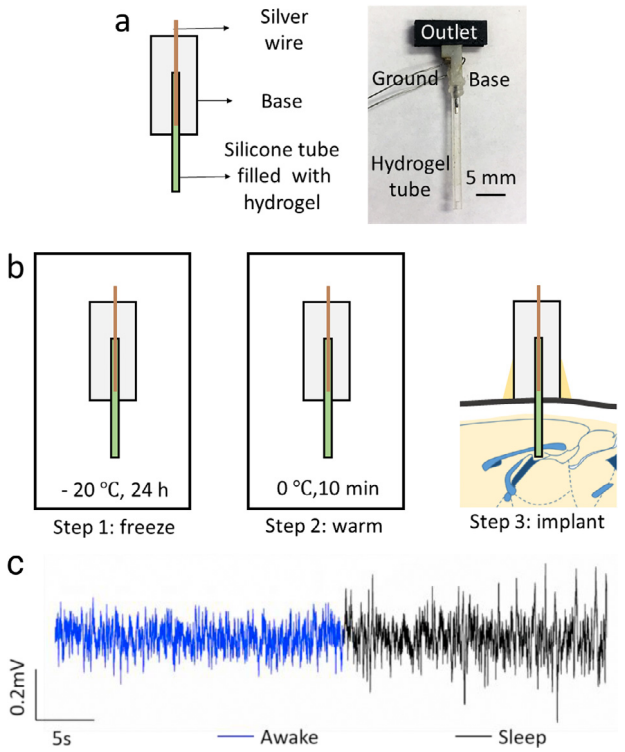

Fig. 5. Frozen-hydrogel interface. (A) The Design and a photo of the interface. (B) Steps of implantation. Step 1. Freeze the interface at −20 centigrade for 24 h. Step 2. Warm it in ice–water mixture for 10 mins. Step 3. Implant it in the brain. (C) Signals recorded by the frozen-hydrogel interface.

and enables long-time use *in vivo*. A single fiber of hydrogel effects simultaneous optical stimulation and electrical recording. A frozen hydrogel is stiff enough to be inserted into a brain, but is soft enough after melting to match the brain. Hydrogels are inexpensive, and can greatly reduce the cost of neurobiological experiments. Hydrogels can readily host drugs and neuromimicking molecules. The stretchability of elastomer-sealed hydrogel interfaces can enable applications in peripheral nervous systems. The neurocompatible hydrogels have enormous potentials to enable discoveries and innovations in biology and medicine.

4. Experimental section

Animals

In this study, we used VGAT-ChR2(H134R)-eYFP male mice at the age of 2–4 months, C57BL/6J male mice at the age of 15–25 days and 2–4 months, cats. During experiments, all animals were fed with food and water ad libitum, under 22 ± 1 °C and 55 ± 5% humidity. All animal experiments performed were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Zhejiang University.

Artificial cerebrospinal fluid (ACSF)

The ACSF used in our experiments contained (in mM): 125 NaCl (Sigma, S5886), 2.5 KCl (Sigma, P5405), 2 $CaCl_2 \cdot H_2O$ (Sigma, C3881), 1.3 $MgCl_2 \cdot 6H_2O$ (Sigma, M0250), 1.3 NaH_2PO_4 (Sigma, 71505), 25 $NaHCO_3$ (Sigma, 792519), 10 glucose (Sigma, G6152). All solutes were fully dissolved in deionized water.

Hydrogel

The hydrogel used in our experiments consisted of a polyethylene glycol (PEG) network and the ACSF solvent. The hydrogel was synthesized as follows. We first dissolved 0.1 g/ml PEGDA (20000Da) [8], 0.004 g/ml ammonium persulfate (Sigma, A3678) in ACSF and stirred the solution for 30 min. Then we dissolved 0.8 μl/ml tetramethylethylenediamine (Sigma, T9281) in the solution and stirred it for 30 s. Finally, we kept the precursor at 37 centigrade for 1 h to crosslink the polymer network.

Brain slices for the patch-clamp experiment

C57BL/6J mice (15–25 days old) were anesthetized with chloral hydrate and perfused with ice-cold oxygenated slicing solution. The solution contained (in mM): 110 choline chloride (Sigma, C7527), 7 $MgCl_2 \cdot 6H2O$ (Sigma, M0250), 2.5 KCl (Sigma, P5405), 0.5 $CaCl_2 \cdot H2O$ (Sigma, C3881), 1.3 NaH_2PO_4 (Sigma, 71505), 25 $NaHCO_3$ (Sigma, 792519), 20 glucose (Sigma, G6152), saturated with 95% O_2 and 5% CO_2. The brain was removed rapidly and immersed in the ice-cold solution. 300 μm-thick sagittal slices of the cerebellar cortex were cut using a vibratome (Leica VT1200s). The slices were subsequently incubated in the oxygenated ACSF at room temperature.

Electrophysiological recordings in the patch-clamp experiment

The recording chamber was continuously perfused with the ACSF for recording. The glass pipette was pulled using a P-97 micropipette puller (SUTTER) and had a resistance of 2–4 MΩ when filled with the ACSF. The Purkinje cell layer and the molecular layer of the cerebellar cortex were visualized using an upright microscope (BX51WI, Olympus) with infrared optics. Loose patch recordings were made from the soma of Purkinje cells with an Axopatch 700B amplifier and Digidata 1440A with PCLAMP 10.4 software (Molecular Devices). Signals were filtered at 2 kHz and digitized at 10 kHz. GraphPad Prism 6 (La Jolla, CA) software was used for all statistical analyses.

Recording system of recording local field potentials

The detective channel was connected to a preamplifier headstage (Plexon). All signals were amplified, filtered between 0.1 Hz and 10 kHz, and spike waveforms were digitized at 40 kHz. Spikes were sorted using the software Offline sorter (Plexon), units were accepted only if a distinct cluster was visible in a two-dimensional plot of the largest two principal components. NeuroExplorer software was used for signal processing.

Hydrogel interfaces for recording local field potentials

We used an outlet (Risym, 2*10 contacts, 1.27 mm contact space, 4.3 mm high). The outlet had ten external contacts, but we only used four middle contacts. We first welded one of the middle contacts with a 7 cm-long, 0.2 mm-diameter silver wire. Then we inserted the outlet into the base of a long hole and three short holes (Figure S2). The contact welded with the silver wire was the signal channel, and was inserted into the long hole of the base. The three other contacts of the outlet were ground channels, and were inserted into the three short holes of the base. The silver wire and three ground contacts were all longer than their holes and exposed small pieces. After that, we welded a 0.5 mm-diameter silver wire with each of the three ground-channel contacts as the ground wire. Then we prepared the PEG-ACSF hydrogel in a 2 cm-long glass tube (Polymicro TECHNOLOGIES, TSP320450). We inserted the tube into the base, with the signal silver wire inserted into the hydrogel.

Implanting hydrogel interfaces for recording local field potentials

Mice were deeply anesthetized with sodium pentobarbital (1% wt/vol) and then placed in stereotactic apparatus (KOPF). After exposing the skull, we located the right field hippocampus (AP: −2.9 mm, ML: −3.2 mm, DV: −3.2 mm from bregma) of the brain and drilled an implant hole on the skull. We then inserted the glass tube into the implant hole.

Recording local field potentials of free-moving mice

The mouse rested 48 h after the surgery for the recovering and circadian regulation. The experimental time was afternoon, when the mouse was asleep. We connected the interface on its head with the detecting system. Then we put the mouse inside an electromagnetic shielding case with artificial lightings and started detecting. The mouse moved around to get familiar with the new surroundings and gradually fell asleep.

Recording spikes of cats in vivo

We fabricated the PEG-ACSF hydrogel in a glass tube with a 5 μm-diameter tip. The cat was deeply anesthetized by ketamine (25 mg/kg, i.m.), intubated, artificially ventilated with a 70:30 mixture of N_2O and O_2, and fixed on a stereotaxic apparatus (Narishige). Isoflurane (1.0–2.5%) was used to maintain anesthesia, and the depth of anesthesia was assessed continuously by monitoring end-tidal CO_2, saturation of peripheral oxygen, electro-cardiogram, blood pressure, withdrawal, and corneal reflexes. Temperature was also monitored, and the animal was wrapped in a water blanket. To expose the recording area V2 and V1, a large (20*20 mm) craniotomy and a durotomy were performed. On completion of the surgery, the craniotomy was filled with 3% agar. At this point isofluorane was turned off, anesthesia maintained with propofol (2–5 mg/kg/h), the glass pipette electrode was controlled by the micromanipulator (Narishige MO-10), and the electrode recording of the spike was using the cerebus DAQ system (Blackrock, USA).

Hydrogel, platinum and silver interfaces for the immunohistochemical experiment

We prepared PEG-ACSF hydrogels in 3 cm-long compound glass tubes (Polymicro TECHNOLOGIES, TSP320450). We inserted 0.3 mm-diameter platinum or sliver wires in the same kind of glass tubes. Then we sealed one end of each tube using a biomedical glue (3M Vetbond, No. 1469SB) and put them under UV light for 72 h for sterilization.

Immunohistochemistry and imaging

We drilled a hole in the skull of each mouse, and implanted a glass tube (containing hydrogel, platinum or silver) in the lateral hypothalamus (AP: −1.4 mm, ML: −1.1 mm, DV: −4.6 mm from bregma). We avoided touching the implanted part during implanting. After waiting for 7 days, the mouse was perfused for the immunochemical experiment. Firstly, the mouse was deeply anesthetized with sodium pentobarbital (1% wt/vol) and was transcardially perfused with saline buffer followed by 4% paraformaldehyde (wt/vol) in 0.1 M phosphate buffer. Then the brain was removed into 4% paraformaldehyde buffer for 6–8 h fixation at 4 centigrade. Next, the brain was dehydrated with 25% sucrose (wt/vol) overnight. Finally, coronal cryo-sections were cut at 40 μm on a freezing microtome (Leica CM1900) for confocal imaging and immunostaining. After being gently rinsed within 0.1 M phosphate buffer (3 × 10 min) and ice-cold methanol (1 × 10 min) and blocked with 5% (wt/vol) normal bovine serum albumin (BSA) for 1 h at room temperature (RT), the brain slices were incubated with one of the following primary antibodies: anti-GFAP (mouse 1:400, Millipore), anti-Iba1 (rabbit, 1:400, Wako) at 4 °C overnight. Next day, the sections were rocked and washed 3 × 10 min in 0.1 M phosphate buffer and then incubated with fluorophore-conjugated secondary antibody for 2 h at RT (1:800: Millipore). Antibodies were diluted in phosphate-buffered saline containing 5% BSA. Finally, all images were acquired with 20× or 40× objectives using an Olympus FV1200 confocal microscope.

Counting the number of glial cells and calculating fluorescence radio

Three people counted glial cells on each confocal image, and the median of the three counts was used to represent the image. To compare fluorescence intensity, confocal images were acquired under a same microscope parameter (Channel488 : HV614 laser 8% power, background off 20%, Channel cy3: HV615 laser 25% power, background off 20%). Then we use Image J to calculate fluorescence radio a same parameter (MinThr 2.41, MaxThr 0.09).

Hydrogel interfaces for laser stimulations

We first prepared the PEG-ACSF hydrogel in a 2 cm-long glass tube (Polymicro TECHNOLOGIES, TSP320450). We inserted the tube, then a 0.2 mm-diameter optical fiber into the base (Figure S5).

Implanting hydrogel interfaces for laser stimulations

Stereotaxic surgeries were as described above. We implanted the interface in the right LDT (AP: −5.2 mm, ML: −0.4 mm, DV: −3.7 mm from bregma).

Behavioral tests by laser stimulations via hydrogel interfaces

We followed the optogenetic manipulation as previously described [18]. After surgery, the mouse was allowed 48 h for recovery. The experimental time was morning, when the mouse was awake. We connected the interface on the mouse's head with a laser source (NEWDOON, Aurora-220-473) through an optic fiber sleeve. Then we put the mouse inside a case with artificial lightings. After 10mins habituation, the mouse performed behavior test. For each cycle, we stimulated the mouse by 470 nm, 5 mW, 20 Hz blue lasers (NEWDOON, Aurora-220-473) with each pulse of 20 ms for 20 s and then wait for 40 s until next cycle. The freezing level was calculated as the percentage freezing time during 20 s of direct light stimulation in the LDT.

Hydrogel interfaces for simultaneous optical stimulation and electrical recording

We prepared the PEG-ACSF hydrogel in a 2 cm-long glass tube (Polymicro TECHNOLOGIES, TSP320450). We first assembled the outlet and the ground with the base (Figure S6) by the same method shown in assembling hydrogel interfaces of recording local field potentials of free-moving mice. The only difference was that the signal silver wire used in the multi-functional hydrogel interfaces had a diameter of 0.1 mm. Then we inserted the hydrogel tube into the base, and made sure that the signal silver wire was inserted into the hydrogel tube. We then inserted a 0.2 mm-diameter optical fiber into the base. The optical fiber was automatically inserted into the hydrogel tube since they had the same axis. In multi-functional hydrogel interfaces, the ending of the optical fiber and the signal silver wire were both inserted in the hydrogel tube.

Implanting hydrogel interfaces of applying laser stimulations and detecting neural signals

We implanted the multi-functional hydrogel interfaces by the same method shown in implanting hydrogel interfaces of recording local field potentials of free-moving mice. The only difference was that the implanted area was the lateral hypothalamus (AP: −1.4 mm, ML: −1.1 mm, DV: −4.8 mm from bregma).

Optogenetics by hydrogel interfaces for simultaneous laser stimulation and electrical recording

The mouse rested 48 h after the surgery for the recovering and circadian regulation. The experimental time was afternoon, when the mouse was asleep. We put the mouse inside an electromagnetic shielding case with artificial lightings and started detecting. We connected the interface on the mouse's head with a laser source (NEWDOON, Aurora-220-473) through an optic fiber sleeve. The mouse moved around to get familiar with the new surroundings and gradually fell asleep. Then we stimulated the mouse by 470 nm, 5 mW, 20 Hz blue lasers with each pulse of 20 ms for 20 s. The mouse woke up immediately.

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Hao Sheng, Xiaomeng W and Ning Kong contributed equally to this work. Detecting spikes by the patch clamp was achieved by Xiaotong W and H.S. Detecting spikes signal *in vivo* was achieved by W.X, H.S. and Xiaomeng W Detecting local field potentials, immunohistochemical experiments and frozen implantations were achieved by Xiaomeng W and H.S. Results of immunohistochemical experiments were calculated by H.S and N.K. Hydrogel interfaces were designed and fabricated by H.S and H.Y. The modulus of hydrogel was measured by K.W. The frequency range of hydrogel interfaces was checked by H.S and C.L. The paper was drafted by H.S., Xiaotong W and Xiaomeng W, and revised by Z.S. and H.W. Z.S., H.W., J.H., J.T., S.D. and J.Z. guided the work.

Declaration of competing interest

The authors (H.S., X.W., H.W. and Z.S.) have filed patent applications relating to neural interfaces by hydrogels and the frozen implantation.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eml.2019.100510.Supporting Information is available from the Wiley Online Library or from the author.

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