Journal of Pharmacological Sciences 139 (2019) 346–351

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full Paper

Collection of biochemical samples with brain-wide electrophysiological recordings from a freely moving rodent

Daichi Konno [a, b], Ryota Nakayama [a], Makoto Tsunoda [c], Takashi Funatsu [c], Yuji Ikegaya [a, d], Takuya Sasaki [a, e, *]

[a] Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo, 113-0033, Japan
[b] Laboratory of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo, 113-0033, Japan
[c] Laboratory of Bioanalytical Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo, 113-0033, Japan
[d] Center for Information and Neural Networks, Suita City, Osaka, 565-0871, Japan
[e] Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi, Saitama, 332-0012, Japan

ARTICLE INFO

Article history:
Received 17 January 2019
Received in revised form
20 February 2019
Accepted 21 February 2019
Available online 27 February 2019

Keywords:
Brain
Local field potential
Neuromodulator
Microdialysis
Genes

ABSTRACT

Bridging accumulating insights from microscopic and macroscopic studies in neuroscience research requires monitoring of neuronal population dynamics and quantifying specific molecules or genes from the brain of identical animals. To this end, by minimizing the size and weight of an electrode array, we developed a method that records local field potential signals of multiple brain regions from one side of the hemisphere in a freely moving rodent. At the same time, extracellular cerebrospinal fluid for biochemical assays or a small part of brain tissue samples for gene expression assays are collected from the other side of the hemisphere. This method allows ongoing stable recordings and sample collections for at least two months. The methodological concept is applicable to a wide range of biological reactions at various spatiotemporal scales, allowing us to integrate an idea of physiolomics into existing omics analyses, leading to a new combination of multi-omics approaches.

Introduction

The brain is composed of multiple functional regions that must all work together to achieve precise cognitive, memory, and homeostatic functions, which have been generally studied in the field of systems neuroscience. At the microscopic level, each brain region is composed of myriad cells in which complex intracellular gene expression, signaling cascades, and intercellular transmissions continuously occur to maintain their cellular functions, which have been generally studied in the fields of molecular and cellular neuroscience. In particular, multi-omics analyses are now required in these microscopic research areas such as genomics, proteomics, and metabolomics.[1]

A large number of studies have been performed at different spatial scales to elucidate basic biological mechanisms underlying brain functions and their pathological changes. However, for further understanding of brain functions beyond the existing literature, the research fields at multiple spatial scales need to be systematically combined. In particular, activity patterns of neuronal populations, biological reactions, and pathological changes in the brain considerably vary across individuals, implying a need for collecting as much information as possible from a single living animal at different spatial scales, including whole-brain functional connection patterns, the concentration of bioactive substances, and gene expression patterns. This technical requirement has not been easily attainable for small experimental animals due to the space and weight limitations of experimental devices.

Abbreviations: LFP, local field potential; ECoG, electrocorticography; CSF, cerebrospinal fluid; EIB, electrical interface board; ACC, anterior cingulate cortex; PL, prelimbic cortex; M1, primary motor cortex; STR, striatum; S1, primary somatosensory cortex; PPC, posterior parietal cortex; HPC, hippocampus; V1, primary visual cortex; AC, auditory cortex; HPLC, high-performance liquid chromatography.

* Corresponding author. Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo, 113-0033, Japan. Fax: +81 3 5841 4786.
E-mail address: tsasaki@mol.f.u-tokyo.ac.jp (T. Sasaki).

Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2019.02.006

To address this technical issue, we developed a recording technique to monitor multiple extracellular local field potential (LFP) and electrocorticography (ECoG) signals from wide brain areas in one hemisphere while biochemical samples including genes and cerebrospinal fluid (CSF) are collected from the other hemisphere from a freely moving rodent. This new method is an improved version of our previous recording method[2–4] accomplished by minimizing the size and weight of the recording devices and optimizing the coordinates of electrode implantation. In this paper, we describe technical procedures of our novel method and representative recording examples from rats and mice. The recordings were chronically stable for at least 2 months after implantation of the device.

Materials and methods

Animals

All experiments were performed with the approval of the animal experiment ethics committee at the University of Tokyo (approval number: mouse, P29-14; rat P29-7) and according to the NIH guidelines for the care and use of animals. Male Wistar/ST rats (SLC, Shizuoka) with a preoperative weight of 300 g (8 weeks old) and ICR mice (SLC) with a preoperative weight of 30 g (8 weeks old) were used in this study. The animals were maintained on a 12-h light/12-h dark schedule with lights off at 7:00 a.m.

Preparation of an electrode array

For recordings from rats, an electrode array for brain LFP recording was assembled consisting of custom-made parts (Fig. 1D) and an electrical interface board (EIB) (Neuralynx, Bozeman, MT) (for the basic idea of the electrode array, see5). A plastic core body that contained multiple small holes with a dimeter of 0.7 mm distributed in space corresponding with the XY-coordinates of the targeted cortical areas (e.g., Fig. 1C) was created by a 3D printer (Form 2, Formlabs, MA) (Fig. 1D, left). This core body served as a template part that determined the locations of electrode implantation. An electrode assembly was created by setting metal tubes and electrodes into these holes so that the tips of the electrodes corresponded with the depth (Z-coordinate) of individual targeted brain regions (Fig. 1D, right). The other open ends of the electrodes were connected to metal holes of an EIB mounted on the top of a core body (Fig. 1D, right). An EIB consisted of 32 LFP channels and 2 ground channels, and all electrical signals from these channels were transferred to an Omnetics connector. A tetrode was used for brain LFP recording, which was constructed by bundling together four 17-μm polyimide-coated platinum–iridium (90/10%) wires (California Fine Wire, CA) plated with platinum used to adjust the electrode impedances to 150–300 kΩ. The size of the electrode assembly was width 19 mm, length 24 mm, height 41 mm, and weight 3.1 g. The open edges of all electrodes were soldered to the corresponding channels on the EIB. For recordings from mice, the assembly consisted of a 3 × 3 grid channel ECoG electrode array

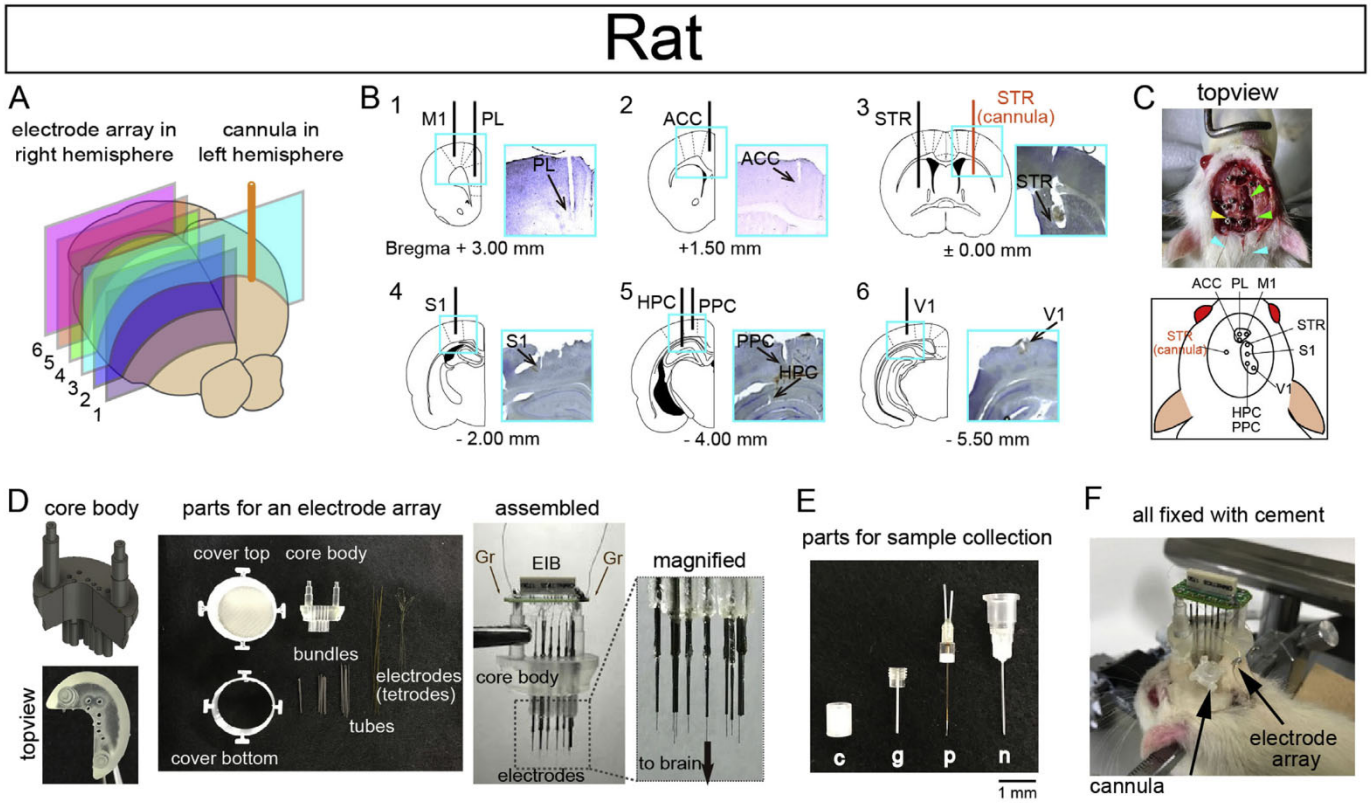

Fig. 1. Overview of the recording system for a rat. (A) Schematic illustration showing an electrode array in the right hemisphere and a cannula for sample collection in the left hemisphere for a rat. (B) Sequential coronal brain sections showing electrode locations, corresponding with the section numbers in A. Electrodes were implanted into eight brain regions, and a cannula was implanted into the contralateral STR. In each panel, the cyan regions are magnified as the right pictures with the arrows indicating the tip of the implanted cannula and electrodes. (C) An upper view of the animal's head during surgery. Two craniotomies (green arrowheads) in the right hemisphere and one craniotomy (a yellow arrowhead) in the left hemisphere were made for implanting electrodes and a cannula, respectively. (D) (From left to right) A design of the core body of an electrode array, custom-made parts for assembling an electrode array, and an assembled electrode array. The dotted box is magnified, showing electrodes protruding from the core body with a length of 1.2–4.0 mm, which were inserted into the brain for LFP recordings. (E) Plastic parts for sample collection, showing a cap (c), guide cannula (g), a microdialysis probe (p), and a collection needle (n). (F) After the implantation, all devices were secured to the skull with dental cement.

with a size of 3.5 × 3.5 mm^2 and a weight of 1.5 g, including recording sites 600 μm in diameter with 1.0 mm between sites (Unique Medial Co. Ltd., Tokyo) (Fig. 4B). Cannulas for the collection of samples from the brain were purchased from EICOM (Kyoto) (Fig. 1E).

Surgery

For surgery, animals were anesthetized with 1–2% of isoflurane gas in air. The animal was then fixed in a stereotaxic instrument with two ear bars and a nose clamp. An incision was made from the area between the eyes to the back of the head. All craniotomies were made using a high-speed drill.

For rats, two craniotomies were made as represented in Fig. 1C (green arrowheads); one covering the coordinates for the anterior cingulate cortex (ACC; 1.5 mm anterior and 0.5 mm lateral to the bregma), prelimbic cortex (PL; 3.0 mm anterior and 0.5 mm lateral to the bregma), and primary motor cortex (M1; 3.0 mm anterior and 3.0 mm lateral to the bregma), and the other covering the coordinates for the striatum (STR; 0 mm posterior and 3.0 mm lateral to the bregma), primary somatosensory cortex (S1; 2.0 mm posterior and 3.0 mm lateral to the bregma), the posterior parietal cortex (PPC) and the hippocampus (HPC; 4.0 mm posterior and 3.0 mm lateral to the bregma), and primary visual cortex (V1; 5.5 mm posterior and 4.0 mm lateral to the bregma). The electrode array was directly implanted into the cortical tissue in the right hemisphere with electrodes inserted at a depth of 1.2 mm into M1, 1.2 mm into S1, 1.2 mm into PPC, 1.2 mm into V1, 1.5 mm into ACC, 2.5 mm into PL, 2.5 mm into HPC, and 4.0 mm into STR (Fig. 1F). For the cerebellum, stainless steel screws were implanted on the skull attached to the brain surface to serve as ground/reference electrodes (Fig. 1C, cyan arrowheads). Next, a craniotomy with a dimeter of 1 mm was made at the contralateral side at a coordinate of 1.5 mm posterior and 3.0 mm left to the bregma (Fig. 1C, yellow arrowhead) and a unilateral guide cannula (12 mm length, 0.45 mm inner diameter (I.D.), 0.50 mm outer diameter (O.D.)) tilted at an angle of 15° to the posterior side and 5° to the lateral side was implanted into the contralateral STR at a depth of 3.9 mm from the dura (Fig. 1E). To prevent drying of the implanted region, a dummy cannula was inserted through the guide cannula, and they were both covered by a cap. Finally, all of the wires, the electrode array and the cannula were secured to the skull using dental cement (Fig. 1F).

For mice, a 3.0-mm square craniotomy was made at 3.0 mm posterior and 2.0 mm lateral to the bregma (Fig. 4B, green arrowheads), and an ECoG electrode array was placed on the cortical surface. The recording sites covered S1, V1, PPC, and the auditory cortex (AC). Second, a circular craniotomy was made at 0 mm posterior and 2.0 mm lateral to the bregma at the contralateral side (Fig. 4B, a yellow arrowhead), and the same cannula as was used for rats was implanted at a depth of 2.3 mm from the dura (Fig. 4D). Other procedures were similar to those for rats.

After completing all surgical procedures, the anesthesia was terminated, and the animals were spontaneously allowed to awake from the anesthesia. Following surgery, each animal was housed in a transparent Plexiglas cage with free access to water and food, with daily observation.

Collecting brain samples with ECoG/LFP recording

The dummy cannula was pulled out at least 3 h before performing microdialysis and a microdialysis probe (17 mm length, EICOM, Kyoto) was carefully inserted into the STR through the guide cannula (Fig. 1E). Before starting recording, the EIB on the animal's head was connected to a digital headstage Cereplex M (Blackrock Microsystems, Salt Lake City, UT, U.S.A.), and the digitized signals were transferred to a data acquisition system Cereplex Direct (Blackrock Microsystems, UT). Recordings commenced at a sampling rate of 2 kHz. Recordings were performed in both a familiar box (35 × 25 cm^2) with a wall height of 20 cm and a novel box (45 × 35 cm^2) with a wall height of 30 cm. The animal's moment-to-moment position was tracked at 30 Hz using a video camera. The recording was first obtained in a familiar environment for 30 min and then in a novel environment for 30 min.

Through the implanted cannula, CSF samples were repeatedly collected through the probe by perfusion of the brain with Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, and 2.2 mM $CaCl_2$) at a flow rate of 1 μl/min using a microinfusion pump (ESP-64, EICOM, Kyoto) every 15 min.

After the recording, tissue samples were collected through the cannula using a glass pipette (0.68 mm I.D., 1.20 mm O.D.) and a 5-ml syringe (Terumo, Tokyo). The glass pipette was attached to the syringe, and a tissue sample of up to 0.00005 mm^3 (~0.04 mm each side) was collected by applying a negative pressure of up to 120 hPa to the syringe.

Histology of brain tissue

After the recording and the brain tissue collection, the animals were perfused intracardially with cold 4% paraformaldehyde (PFA) in 25 mM phosphate-buffered saline (PBS) and decapitated. The electrodes were carefully removed from the brain 6–8 h after the perfusion. The brains were placed in 30% sucrose until equilibrated and coronally sectioned at a thickness of 40 μm, and the slices were stained with cresyl violet. The positions of electrodes were confirmed by identifying the corresponding electrode tracks in histological tissue.

LFP data analysis

LFP signals were convolved by a complex Morlet wavelet transformation at a frequency ranging from 1 to 250 Hz to compute the time–frequency representation of LFP power. The absolute power spectrum of the LFP during each 0.5 ms time window was calculated. All analyses were performed in MATLAB (Mathworks, MA).

High-performance liquid chromatography (HPLC)

HPLC separation was conducted on an Inertsil ODS-4 column (250 × 3.0 mm I.D., 5 μm) at 35 °C, with a flow rate of 0.5 mL/min using a mobile phase comprising 30 mmol/L sodium acetate and 200 mmol/L citrate buffer/acetonitrile (440/60, v/v) containing 1 g/L sodium 1-octanesulfonate. Dopamine was detected with an electrochemical detector.

Gene expression analysis

The tissue sample obtained through the cannula was subjected to a gene expression analysis with reverse-transcription polymerase chain reaction (RT-PCR) and agarose gel electrophoresis as previously described.[6] Briefly, collected tissue sample was homogenized with 400 μL of RLT buffer using a homogenizer and the lysate was centrifuged at 15,000 g for 3 min. Total RNA was then extracted from the lysate by removing genomic DNA using RNeasy mini kit (Qiagen, Tokyo). Then, cDNA was synthesized from the total RNA using SuperScript IV VILO Master Mix (Thermo Fisher Scientific, Tokyo). The cDNA was amplified by PCR using specific primers (eurofins, Kanagawa). The primer sequences were as follows: rat Rbfox3 (neuronal nuclei; NeuN), forward:

5′-CACCACTCTCTTGTCCGTTTGC-3′, reverse: 5′-CTCTACCATAACT GTCACTGTAGG-3′, rat Gapdh (glyceraldehyde 3-phosphate dehydrogenase; GAPDH), forward: 5′-AGACAGCCGCATCTTCTTGT-3′, reverse: 5′-CTTGCCGTGGGTAGAGTCAT-3′, mouse Rbfox3, forward: 5′-GAGGAGTGGCCCGTTCTG-3′, reverse: 5′-AGGCGGAG-GAGGGTACTG-3′, mouse Gapdh, forward: 5′-CTCCCACTCTTCCAC CTTCG-3′, reverse: 5′-GCCTCTCTTGCTCAGTGTCC-3'. Finally, the PCR products were analyzed by agarose gel electrophoresis. As positive controls, the brain and skin tissue were obtained from other animals.

Results

We developed a method to record electrophysiological signals from one side of the cerebral hemisphere and collect extracellular CSF (so-called microdialysis) and a small part of the brain tissue including RNA samples from the other side of the hemisphere from a single freely moving rodent (Fig. 2, middle). Fig. 1A–C shows the typical cortical sites where electrodes were targeted, including the M1, PL, ACC, STR, S1, HPC, PPC, and V1, in the right hemisphere. In addition, a cannula for sample collection was implanted into the contralateral STR. We applied this method to four animals and confirmed similar recording quality. We here present a representative dataset from a single rodent.

A recording dataset from a freely moving rat is shown in Fig. 2. LFP signals recorded from multiple cortical regions were converted to power spectrum by wavelet analysis, including various frequency bands, such as theta (6–10 Hz), delta (1–4 Hz), and slow-wave (0.5–2.0 Hz) bands. This rhythmic activity has been considered to link the firing of single neurons into collective neuronal ensembles and facilitate efficient information processing, including cognition, learning and memory.[7] As an example, hippocampal LFP signals were compared between familiar and novel open fields (Fig. 2, left bottom). As described previously,[8] LFP power at the theta frequency (6–10 Hz) was higher in the novel environment (familiar: 21.9; novel, 40.1 (in arbitrary unit)), confirming that our LFP recordings were successful. The same results were obtained in recordings from a mouse (familiar: 16.1; novel, 38.8 (in arbitrary unit)) and the other rats tested. From the other side of the hemisphere, CSF and tissue samples were collected (Fig. 2, right top). Microdialysis can be performed before, during, and after electrophysiological recordings. For simultaneous electrophysiological recording and microdialysis experiments, care must be taken so that the electrical cable and the microdialysis tube are not tangled.

As an example, dopamine concentration in a CSF sample collected during an electrophysiological recording was quantified by using an HPLC system. The dopamine level in a novel environment was higher than that in a familiar environment (familiar: peak amplitude = 0.43 mV; novel: peak amplitude = 0.48 mV), confirming that our CSF sample collection was sufficient for the quantification of dopamine. In addition to the microdialysis, a small tissue sample (up to 0.01 mm^3) was collected by inserting a needle though the same cannula. Based on its size, the tissue sample was considered to contain up to 200 neurons. In the tissue sample, gene expression was confirmed based on the presence of Gapdh (GAPDH) RNA, a housekeeping gene expressed in all cell types, and Rbfox3 (NeuN) RNA, a gene that is specifically expressed in neurons but not skin cells, by qPCR analysis. The results demonstrated that the collected tissue contained sufficient levels of genes for expression assays (Fig. 2, right bottom).

From the same rat shown in Fig. 2, we obtained LFP signals from the same cortical regions and collected a brain tissue sample at 2 months after the surgery (Fig. 3). Over the course of months, no significant difference in average running speed was found between these two animal groups (n = each 3 animals; naïve: 8.5 ± 0.9 cm/s; implanted 9.3 ± 0.8 cm/s; $t_5 = 1.60$, $P = 0.32$, Student's t-test). The 50-Hz LFP power represents the humming noise in electrophysiology, one of the most important electrical factor that should be minimized to obtain stable recordings with high signal-to-noise ratios. We confirmed that power spectrum analysis showed that the 50-Hz LFP power recorded 1 week and 2 months after the surgery was 23.58 and 24.47 (in arbitrary unit), respectively, demonstrating a stable LFP recording for these recording periods. These results were obtained from all the other animals tested. Similarly, Rbfox3 (NeuN) gene expression was detected from the collected tissue sample at 2 months. These results demonstrate that

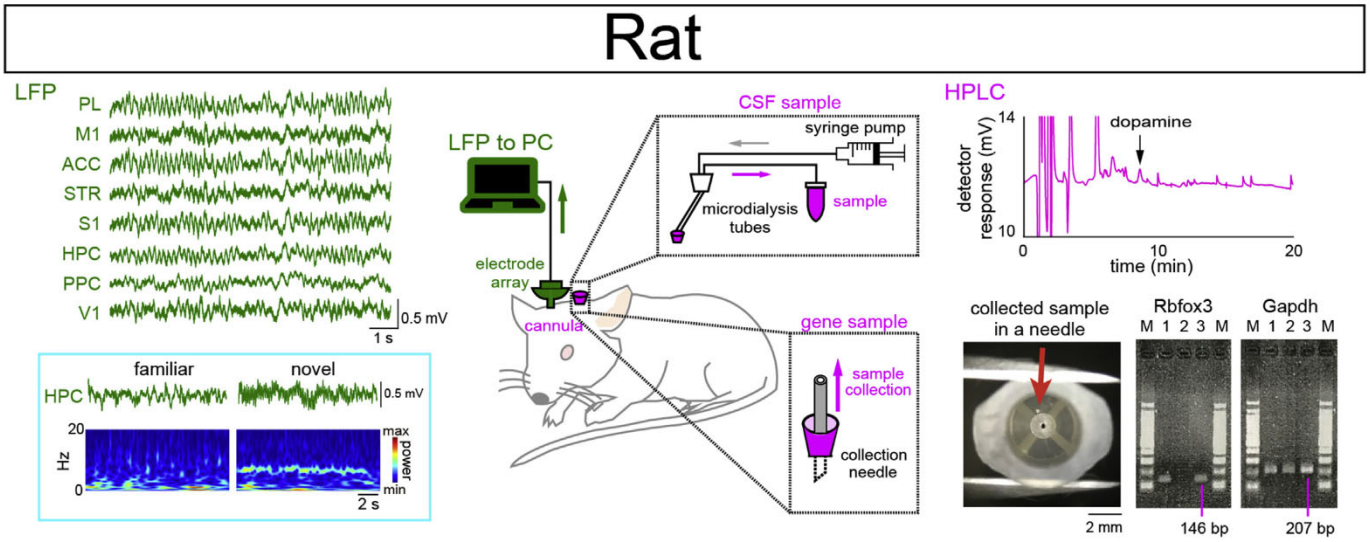

Fig. 2. Collecting CSF/gene samples with monitoring cortical wide LFP signals from a rat. (Left) Representative simultaneous recordings of extracellular LFP signals from eight cortical regions from a rat. (Left bottom cyan box) Typical hippocampal LFP signals recorded in familiar and novel open fields. The bottom panels show corresponding color-coded power spectrum of the LFP traces. Note that LFP power at theta frequency (6–10 Hz) becomes higher in the novel environment. (Middle) An experimental image (green, LFP; magenta, sample collection). (Right top) An HPLC chromatographic profile of endogenous dopamine in a collected CSF sample. (Right bottom) Collected tissue sample (indicated by the red arrow) and agarose gel electrophoresis of PCR products following amplification of Rbfox3 and Gapdh genes. Lane 1: the whole brain tissue obtained from another rat as a positive control; lane 2: the skin tissue obtained from the other rat; lane 3: the brain tissue sample collected by our method; lane M: 100 bp DNA ladder.

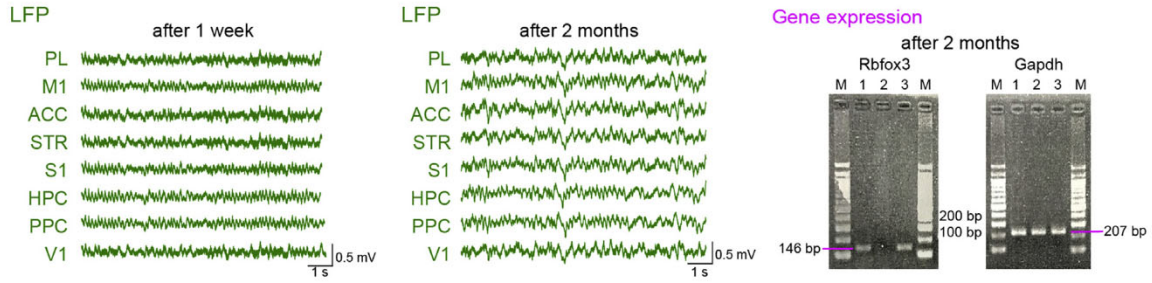

Fig. 3. Long-term chronic recordings for months. The left two panels show representative cortical LFP recordings from a rat at 1 week and 2 months after electrode implantation. Stable electrical signals were obtained during this period with no apparent changes in 50-Hz noise levels. The right panel shows a result of electrophoresis for a tissue sample obtained 2 months after the surgery.

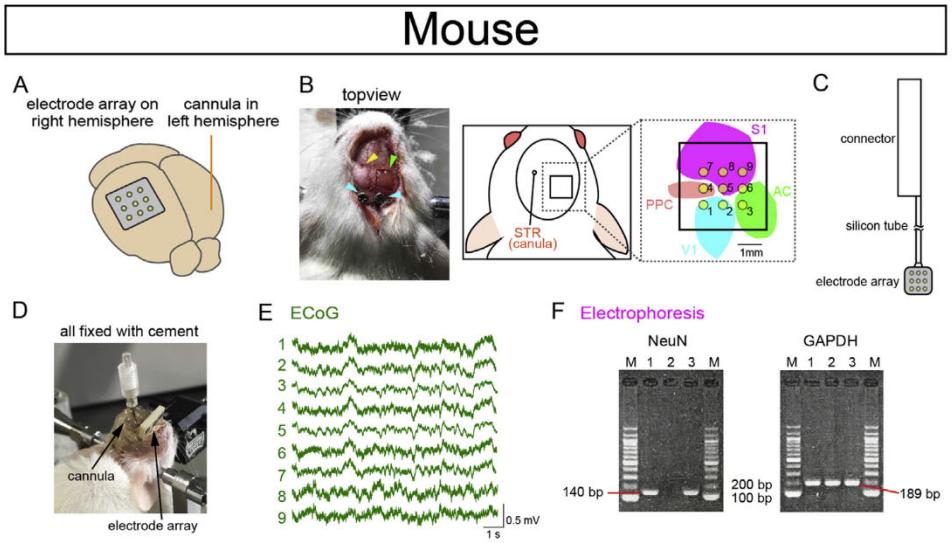

Fig. 4. Application to a mouse. (A) Schematic illustration showing an ECoG electrode array in the right hemisphere and a cannula in the left hemisphere of a mouse. (B) (Left) Same as in Fig. 1C but for a mouse. A craniotomy indicated by the green arrowhead in the right hemisphere and a craniotomy indicated by the yellow arrowhead in the left hemisphere were for implanting an ECoG electrode array and a cannula, respectively. The dotted area is magnified in the right panel, showing that the ECoG electrode array covers multiple cortical regions. Each number represents each electrode number. (C) Schematic illustration of an ECoG electrode array. (D) All devices were secured to the mouse's skull. (E) Representative recordings of cortical LFP signals from a mouse. Each number corresponds with the electrode number in B. (F) Same as in Fig. 2 right bottom, but for a mouse.

our method can be applied to obtain long-term chronic recordings for at least 2 months.

We applied a similar method to mice (Fig. 4). For mice, the electrode array was further minimized to sheet-type electrodes, containing 9 recording sites with an interelectrode interval of 1 mm (Fig. 4B and C). Fig. 4E and F demonstrate that both LFP recording and collection of the tissue sample were successful in mice as well.

Discussion

For understanding brain functions in health and disease, a large number of studies have been independently performed at molecular, genetic, and network system levels. Here, we presented a new method that enables the measurement of multiple LFP signals from multiple cortical regions from one side of the hemisphere and the collection of extracellular CSF or intracellular gene samples from the other side of the hemisphere in a freely moving rodent. The experimental configurations of both electrophysiological recordings and sample collections were stably maintained for at least two months after surgery.

Compared with a conventional electrophysiological recording method that combined extracellular electrode recordings with microdialysis,[9–11] the advantages of this method are that (1) it is applicable to both freely moving rats and mice, and (2) multiple brain regions are targeted for LFP signal recordings, and (3) it is tolerable, allowing for long-term recordings over months, leading to experiments at expanded temporal and spatial scales. In particular, by using the custom-made core body of an electrode array created by using a 3D printer, any combinations of cortical regions are targetable by adjusting the coordinates of the electrodes. The surgical technique is relatively simple, and one will be able to complete all the procedures within 2–3 h if trained on the surgical procedures several times.

The methodological concept is applicable to any research issues that require long-term tracking of biological reactions in a single rodent, such as growing, aging and long-term memory. Our method can be further combined with optical fibers for photostimulation, enabling an optogenetics study. For this application, as optical fibers and related implantation materials occupy an approximately 1-mm^2 area of the skull, we need to reduce the number of targeted brain areas. In addition, as optical fibers and related implantation materials weigh approximately 1 g, we need to minimize total weight of implantation devices especially for mice. Our method is also applicable to smaller immature animals. Recently, we established a LFP recording system for P10 mice, while only one or two brain areas are

targetable.[12] Technically, it is possible that we implant a cannula on the contralateral side of the skull for sample collection in addition to this LFP recording system for immature animals. In this case, more elaborate care must be taken for the health of small animals.

Recently, combinations of large-scale systematic analyses such as genomics, proteomics, and metabolomics has become more crucial to understand complex biological reactions. Our method enables us to combine an idea of physiolomics with these existing omics studies and help connect insights at a variety of spatiotemporal scales from microscopic to macroscopic studies.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by the Advanced Research & Development Programs for Medical Innovation (PRIME) (JP18gm6110015) from the Japan Agency for Medical Research and Development, Kaken-hi (17H05551; 17H05939), the Exploratory Research for Advanced Technology (JPMJER1801), the Takeda Science Foundation, and the Precursory Research for Embryonic Science and Technology (JPMJPR1785).

References

1. Karczewski KJ, Snyder MP. Integrative omics for health and disease. *Nat Rev Genet*. 2018;19:299–310.
2. Okada S, Igata H, Sakaguchi T, Sasaki T, Ikegaya Y. A new device for the simultaneous recording of cerebral, cardiac, and muscular electrical activity in freely moving rodents. *J Pharmacol Sci*. 2016;132:105–108.
3. Sasaki T, Nishimura Y, Ikegaya Y. Simultaneous recordings of central and peripheral bioelectrical signals in a freely moving rodent. *Biol Pharm Bull*. 2017;40:711–715.
4. Shikano Y, Sasaki T, Ikegaya Y. Simultaneous recordings of cortical local field potentials, electrocardiogram, electromyogram, and breathing rhythm from a freely moving rat. *JoVE*. 2018;(134).
5. Nguyen DP, Layton SP, Hale G, et al. Micro-drive array for chronic in vivo recording: tetrode assembly. *JoVE*. 2009;(26).
6. Nolan T, Hands RE, Bustin SA. Quantification of mRNA using real-time RT-PCR. *Nat Protoc*. 2006;1:1559–1582.
7. Buzsaki G. *Rhythms of the brain*. New York: Oxford University Press; 2006.
8. Penley SC, Hinman JR, Long LL, Markus EJ, Escabi MA, Chrobak JJ. Novel space alters theta and gamma synchrony across the longitudinal axis of the hippocampus. *Front Syst Neurosci*. 2013;7:20.
9. Ludvig N, Potter PE, Fox SE. Simultaneous single-cell recording and microdialysis within the same brain site in freely behaving rats: a novel neurobiological method. *J Neurosci Methods*. 1994;55:31–40.
10. van Duuren E, van der Plasse G, van der Blom R, et al. Pharmacological manipulation of neuronal ensemble activity by reverse microdialysis in freely moving rats: a comparative study of the effects of tetrodotoxin, lidocaine, and muscimol. *J Pharmacol Exp Therapeut*. 2007;323:61–69.
11. Dong Y, Zhang T, Li W, Doyon WM, Dani JA. Route of nicotine administration influences in vivo dopamine neuron activity: habituation, needle injection, and cannula infusion. *J Mol Neurosci*. 2010;40:164–171.
12. Kasahara Y, Igata H, Sasaki T, Ikegaya Y, Koyama R. The pharmacological assessment of GABAA receptor activation in experimental febrile seizures in mice. *eNeuro*. 2019;6.