



Concave-shaped transparent electrode to simultaneously monitor electrical activity from multiple sites within the optical sampling area of the intact rat cerebral cortex

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Abstract

We have developed a concave-shaped transparent electrode unit that enables the placement of several electrodes within the optical sampling area on the spherical surface of the rat brain. This concave-shaped transparent electrode unit consists of an insulator base (a plano-concave lens) and a gallium-doped zinc oxide film that is a transparent conductor coating the base. Most of the unit is wrapped in an insulator film made of silicon dioxide, and the few areas left unwrapped act as electrodes. In the study reported here this newly developed transparent electrode unit worked well within the optical detection area without affecting optical recording. We applied this unit to our multiple-site optical recording system for membrane potential in order to eliminate pulsation artifacts and succeeded in optically recording spontaneous neural activity, including small changes in membrane potential, in the cerebral cortex in a single-sweep recording.

Keywords Transparent electrode · Spontaneous neural activity · Single-sweep optical recording · Epifluorescence optics · Cerebral cortex · Voltage-sensitive dye

Introduction

Optical methods are powerful tools in physiological research, particularly when used to simultaneously detect signals from many sites in living tissue that are associated with brain functional activities, such as the membrane potential of neurons, intracellular ionic concentration and metabolic activity [1–4]. However, optical signals are very small and often intermingled with large artifacts that are mainly caused by mechanical perturbations associated with the heartbeat and respiration. Consequently, it is frequently difficult to distinguish small optical signals from noise and

to analyze these due to the low signal-to-noise (S/N) ratio. Since signals obtained by an electrophysiological method have much higher S/N ratios, the combination of optical and simultaneously recorded electrophysiological data taken from the optical recording field may contribute to advances in physiological research. One of the difficulties associated with combining these two methods can be attributed to the properties of conventionally used electrodes and their lead wires, which are generally opaque; it is extremely difficult to place the electrodes and lead wires within the optical sampling area without interfering with the optical measurements. Transparent conductors, which are optically transparent and made of electrically conductive materials, are widely used in a number of electronic devices, including liquid-crystal displays, touchscreens and photovoltaics, which led us to attempt to utilize these materials to construct transparent electrodes. A transparent electrode unit composed of a transparent conductor and electrode base, if realized, may be of great value for the simultaneous recording of electrical events within the optical sampling area. Since the mammalian cortex has a spherical surface, the shape of the contact surface of the electrode unit must have a similar curvature to prevent damage to the cortex. We have invented transparent electrodes on a plano-concave lens that have been shown to

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improve signal quality by reducing peripheral defocusing [5].

We developed a multiple-site optical recording system for the *in vivo* opaque mammalian brain using fluorescent voltage-sensitive dyes. This optical recording system records neural activity continuously for practically an unlimited time period [6, 7]. With improvements in optics and the use of newly developed software, the S/N ratio of optical recordings has become an adequate measure to analyze the evoked responses of small changes in the membrane potential, namely, changes in the subthreshold membrane potential, even in single-sweep fluorescence measurements [8]. Our software precisely estimates pulsation artifacts based on signals during a ‘neural activity-free period’ and removes these from raw optical signals, even when heart beat intervals periodically change due to spontaneous breathing [8]. However, the identification of the ‘neural activity-free period’ may be difficult. We previously reported that some neural activity did not expand to the whole optical recording area [9]. Also, the electrical signal recorded by the electrode placed on the cortical surface may reflect activity within approximately 0.5 to 1 mm [10, 11]. Thus, it is impossible to precisely confirm whether neural activity occurs within a recording period using an electrode placed at the edge of or outside the optical recording area.

In the study reported here for the precise detection of the ‘neural activity free period’, we adopt a transparent electrode to record neural activity within the optical recording area.

Materials and methods

Fabrication of the transparent electrode

The transparent electrode unit described herein essentially consists of an insulator base covered by a thin conductor layer, with most of the unit wrapped in insulating film, but with a few spots left unwrapped to act as electrodes.

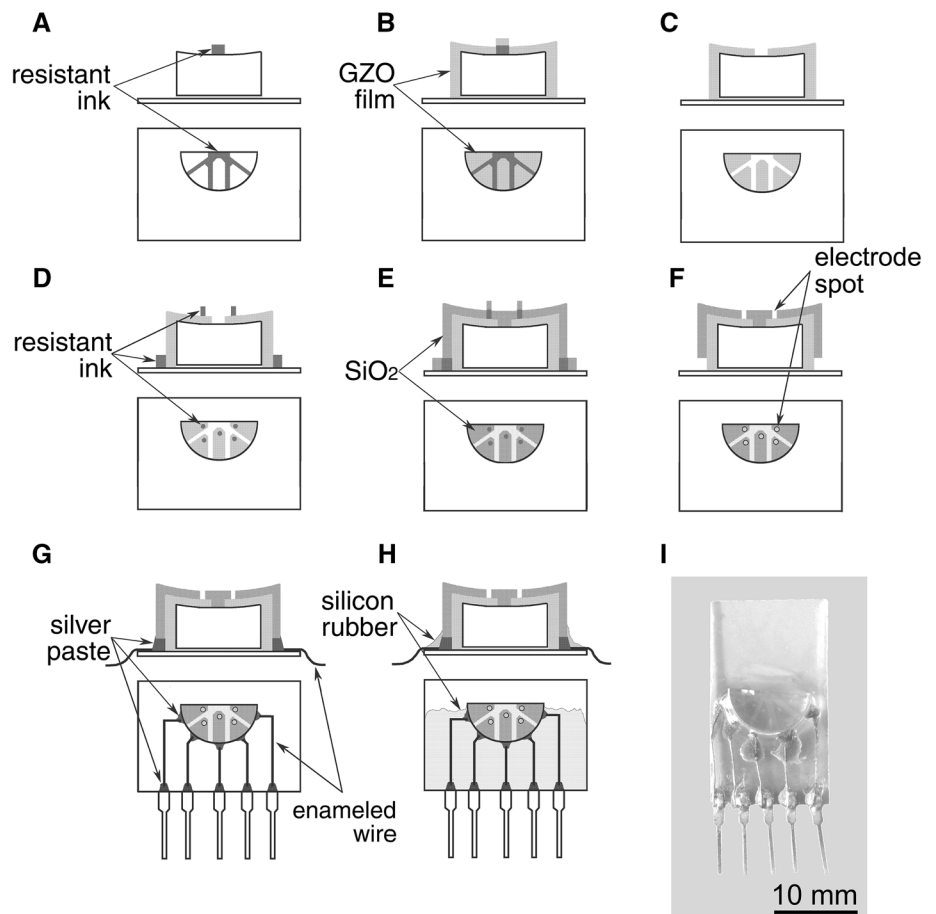
We initially searched for a transparent insulation material suitable for the electrode base. Since the mammalian cerebral cortex is extremely sensitive to mechanical pressure, the shape of the contact surface of the electrode base needs to conform to the round shape of the cerebral cortex. The plano-concave lens, which we had been using to improve the focus in our experiment at that time [5], appeared to be one of the most suitable materials for this purpose. The shape of our optical recording area, including the primary sensory area of the adult rat cortex, is globe-shaped with a radius of approximately 20 mm [12]. Therefore, a glass plano-concave lens with a radius of curvature equal to 20 mm was the closest shape to the surface of the cortex. When the depth from the skull surface to the dura was taken into consideration, the lens needed to be thicker than the surrounding skull in order

to be held in the appropriate position; therefore, the edge needed to have a thickness of at least 3 mm. However, since a thick plano-concave lens was not commercially available, we converted a concave mirror (type TS-0880R; Sugitoh Co., Ltd., Tokyo, Japan) into a plano-concave lens to meet these requirements. The mirror had a diameter of 10 mm, a focal length of 10 mm and a central thickness of 3 mm. The aluminum plating on the concave surface that formed the mirror was removed with acid, and the planar surface on the opposite side was mirror-polished. We produced the electrode base from this lens. We cut the lens into a half circle and polished the edge so that it fit into the elliptical opening in the skull that had been prepared to optically record neural activity. We also rounded off the corner between the concave and lateral surfaces to prevent the thin film coatings from crazing. We then glued the planar surface of the lens to a flat glass board using transparent silicone rubber (Sylgard® 184 Silicone Elastomer; Dow Corning Corp., Midland, MI, USA). This glass board provided a platform for connectors as well as for holding the electrode base.

The next step was to cover this insulator base with five blocks of thin transparent conductor film; this film was used to electrically isolate the electrode spot and its lead from each other. The conductor films were separated into five blocks in this electrode unit, and five electrode spots were created independently on the electrode unit. This set-up and the following processes are summarized in Fig. 1. In the first step, the separator pattern was hand-drawn with an etch-resistant ink using a 0.3-mm-wide printed circuit-board pen (Type RP-3; Sunhayato Corp., Tokyo, Japan) on both the concave and lateral surfaces of the lens (Fig. 1A). In the second step, gallium-doped zinc oxide (GZO) film [13], which is a transparent conductor, was deposited onto the concave and lateral surfaces of the lens by radio-frequency magnetron sputtering (Fig. 1B) with a thickness of approximately 500 nm. In the third step, the resistant ink was removed with organic solvent, and five separate blocks of GZO emerged (Fig. 1C). Within each block, the concave and lateral surfaces of the lens were covered in a continuous layer of the GZO film.

The next key step was to wrap the electrode unit, consisting of the insulator base covered with five thin transparent conductor films, with insulator film, leaving five unwrapped spots and output terminal portions. Prior to wrapping, we plotted five dots (with a diameter of approx. 300 μm) onto each block of GZO on the concave surface using a resistant pen; these dots were designated to become the electrode spots. We also drew masking patterns on each block of GZO at the lateral surface near the supporting glass, which were to become the output terminals (Fig. 1D). Silicon dioxide (SiO_2) film, a transparent insulator, was then deposited onto the concave and lateral surfaces by sputtering (Fig. 1E), to a

Fig. 1 Schematic drawing of the process used to make the transparent electrode unit. See section [Fabrication of the transparent electrode](#) for explanation of each step



thickness of approximately 3 nm. After the resistant ink was dissolved, the five electrode spots and output terminals remained unwrapped by the thin SiO_2 film, and the lower layer of the GZO conductor film was exposed on the electrode unit (Fig. 1F). We attached five metal connector pins to the edge of the flat glass board, then connected their roots to the output terminal of the transparent electrode body with an enameled wire using silver paste (EPO-TEK® H20E; Epoxy Technology Inc., Billerica, MA, USA (Fig. 1G). All exposed conductive materials around the output terminals were then shielded with silicone rubber (Fig. 1H). This completed the construction of the transparent electrode. The five spots (diameter of approx. 300 μm each) on the concave surface, which were the only exposed conductors made of GZO film, served as the independent electrodes. A photograph of the actual electrode unit is shown in Fig 1I.

Animals and optical recording

Following the application of this electrode to strictly eliminate pulsation artifacts in our multi-site optical recording system, we performed the following experiment. Female Sprague–Dawley rats (weight 180–240 g; age 9–12 weeks) were used, and the methods for optical recording and animal treatment were essentially the same as those described previously [5, 7–9]. Briefly, the exposed cortex of a rat anesthetized with a mixture of α -chloralose (80 mg/kg) and urethane (800 mg/kg) was stained with the voltage-sensitive dye RH414 (0.4 mg/mL; Molecular Probes, Eugene, OR, USA), with the dura left intact. The stained cortex was illuminated by stable excitation light, and the intensity of fluorescent light was detected by the 1020-element photodiode array. Since the active element was 1.35×1.35 mm in size and separated by an insulating area with a width of 0.15 mm, each photodiode received fluorescence from a 225×225 - μm region, and the pixel interval corresponded to 250 μm on the preparation.

Electrical recording and stimulation

We placed the transparent electrode unit on the stained cortex covered with the dura to monitor electrocorticogram (ECoG) signals. The electrode unit was held by a micro-manipulator to prevent it from physically pressing against the cortex. A thin, silver plate coated with Ag/AgCl was inserted underneath the neck skin to function as an indifferent electrode. The electrical signal from the transparent electrode was amplified ($\times 5000$) and band-pass filtered (0.5–150 Hz) with a conventional amplifier (DEN-751S; Dia Medical System Co., Tokyo, Japan), the input impedance of which was 500 M Ω . The amplified signal was digitized (1024 Hz) and fed into a FA computer as an ECoG signal together with the optical signals [7]. Somatic evoked cortical responses were induced by an electrical pulse (intensity 1 mA, duration 0.5 ms) applied through the electrode inserted under the skin of the contralateral hindlimb. Lead I of the ECG was recorded simultaneously with the optical measurement and provided to software designed to reduce pulsation artifacts mixed in optical signals. All animal experiments were performed in compliance with the Guidelines for Animal Experimentation of the Center for Integrated Research in Science, Shimane University.

Results

Basal measurement of neural activity

Figure 2A is an actual top view image of the transparent electrode unit placed over the rat somatosensory cortex covered with dura. The entire concave surface of the electrode base fit well over the cortical surface. As expected, neither the electrode spots nor the lead conductors on the electrode base were visible. We drew the approximate positions of the five sets of leads and electrode spots on the concave surface of the electrode base for clarification (Fig. 2B). Figure 2C shows the ECoG traces recorded by these electrodes. These five traces were recorded sequentially as #1 through #5, not simultaneously. All five electrodes successfully detected both evoked and spontaneous neural activities in the cortex.

We performed multi-site optical measurements of the membrane potential of the rat somatosensory cortex using the voltage-sensitive dye together with this transparent electrode unit. The plano-concave lens not only improved optical signal quality by enhancing peripheral defocusing [5], it also served as the base of the transparent electrode unit, covering the entire optical monitoring area. The ECoG trace, detected by one of the five transparent electrodes placed within the optical sampling area, was recorded simultaneously with the optical traces. As expected, the optical traces obtained from

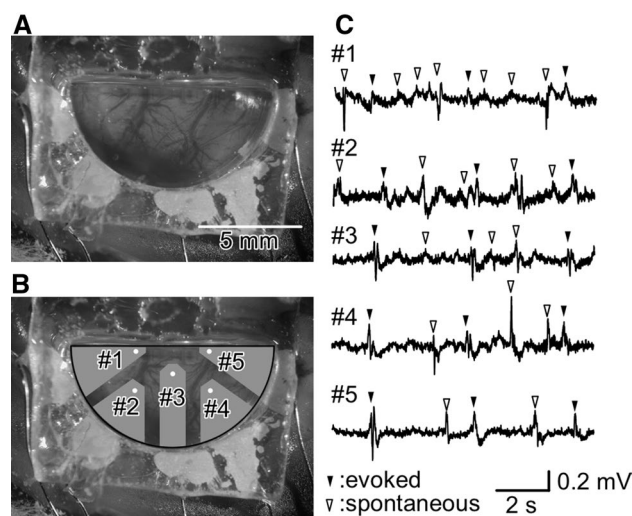


Fig. 2 **A** An actual top view image of the transparent electrode unit positioned on the rat cortex, **B** the five estimated sets of leads and electrode spots (#1–#5) on the glass electrode base, **C** electrocorticogram (ECoG) traces recorded by the five electrodes. The neural response in the somatosensory cortex (filled arrowhead) was evoked by an electrical pulse. The open arrowhead indicates spontaneous activity in the somatosensory cortex

the area beneath the lead and electrode were not optically affected.

Application to the removal of pulsation artifacts in optical recordings of spontaneous neural activity

Although we successfully performed quantitative analyses of the optical signal corresponding to an evoked response [9, 14], we frequently encountered difficulties with quantitatively analyzing a spontaneous event. This difficulty appeared to be attributable to the procedure used to reduce pulsation artifacts. As described in a previous study [8], our procedure requires the detection of ‘neural activity-free periods’ for estimating a pulsation artifact signal; however, this detection process is not always sufficiently accurate. Nevertheless, this inaccuracy was not a serious issue in the evoked response analysis as the distortion of the signal due to inaccuracies in the pulsation artifact signal was negligible because data were averaged to eliminate trial-to-trial variability [9, 14]. However, in the analysis of spontaneous events, for which averaging was essentially unavailable, errors due to the inaccuracy may persist and cause distortion. To select the correct ‘neural activity-free period’, the ECoG signal needs to be recorded directly within the optical recording area—not from the edge of or outside the optical recording area. Therefore, we applied the transparent electrode to this area.

An example of the detection process of the ‘neural activity-free period’ from raw optical recording signals is shown

in Fig. 3. Raw signal traces (upper three traces in Fig. 3A) contained large pulsation artifacts. A close inspection detected two upward deflections superposing the pulsation artifact (white arrowheads pixel #1 in Fig. 3A). Based on raw optical signals, the period between these two deflections appeared to be a ‘neural activity-free period’, but this

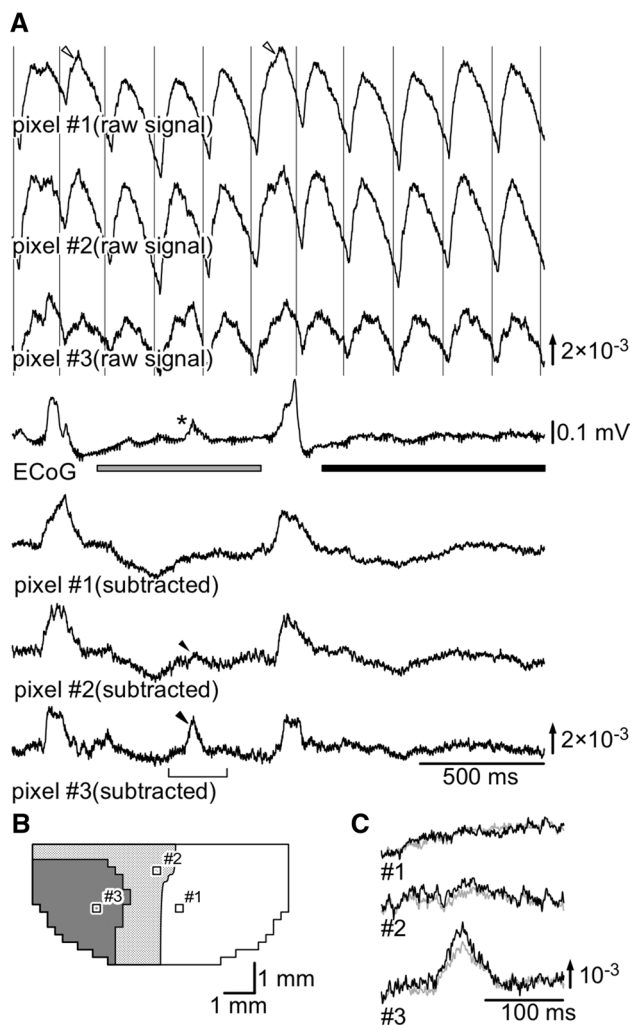


Fig. 3 **A** Example of reductions in pulsation artifact. Raw (top 3 traces, indicated as detectors #1–#3) and pulsation artifact-removed optical signals (bottom 3 traces, indicated as detectors #1–#3) obtained at three different pixels are shown with the ECoG signal. Pixel #1 was located underneath the electrode spot (corresponding to electrode #3 in Fig. 2) where the electrocorticogram signal was recorded; the two other pixels were 1 mm away from #1. **B** Distribution of neural activity, as indicated by the asterisk or arrowhead in A. Dark-gray and light-gray areas indicate the areas in which this neural activity was clearly (similar to the arrowhead in pixel #3) or poorly (similar to the arrowhead in pixel #2) detected, respectively. Within the white area, no neural activity was detected at this time period [similar to pixel #1 (subtracted)]. **C** Comparison of artifact compensation based on two different neural activity-free periods, real (thick black lines) and pseudo (thick gray lines). The black and gray traces were based on the periods indicated by the thick black and gray bar in Fig. 3A, respectively. See the text for details

assumption was incorrect when the transparent electrode-recorded ECoG signal was taken into consideration. Two large upward deflections were observed in ECoG traces, consistent with the deflections in the raw optical signals. However, weak additional neural activity (asterisk in ECoG trace in Fig. 3A) was also detected in between, clearly indicating that this was not a ‘neural activity-free period’. Although the raw optical recordings may have contained a signal corresponding to this weak activity, it was almost impossible to recognize the deflection, if there at all. Thus, the use of the ECoG signal taken within the optical sampling area enabled a ‘real’ neural activity-free period (thick black bar under ECoG trace in Fig. 3A) to be distinguished from pseudo ones, including spontaneous activity (thick gray bar under ECoG trace in Fig. 3A). Therefore, we were able to construct more accurate pulsation artifact signals.

After the removal of pulsation artifacts, a small upward deflection emerged in pixels #2 (subtracted; Fig. 3A) and #3 (subtracted; Fig. 3A) in the optical recording, but not pixel #1 (subtracted; Fig. 3A); the observed deflection was quite distinct on pixel #3 but only slight in pixel #2. Figure 3B shows the distribution of this weak neural activity. The spatial extent and amplitude of this weak spontaneous activity appeared to be reduced by other preceding spontaneous activities (the left-hand white arrowhead in Fig. 3A) (compare to [14, 15]) and, consequently, this signal was hardly recognized in the raw traces. If we considered this period (thick gray bar under ECoG in Fig. 3A) as a ‘neural activity-free period’, the pulsation artifact was estimated inaccurately. We actually examined this and observed the outcome of these spontaneous activity-related optical signals (Fig. 3C). This inaccurate estimation resulted in a decrement in the signal corresponding to the weak spontaneous activity (pixel #3), and the signal in some traces was buried in noise (pixel #2). Therefore, using the ECoG signal within the optical sampling area contributed to a more accurate estimation of the ‘neural activity-free period’.

Figure 4A shows an example of a multi-site optical recording of neural activity without a stimulation after the elimination of the pulsation artifact with our software. An ECoG trace, detected by an electrode spot (the asterisk in Fig. 4A) within the optical sampling area, was used to assume the ‘neural activity-free period’. In this example, spontaneous optical signals were broadly distributed in the somatosensory cortex. Six traces (shown circled in Fig. 4A) are illustrated at a higher sweep speed in Fig. 4B. Although nearly synchronized, the time course of each signal slightly varied among the pixels. In this example, the onset of the optical signal initially occurred in trace “a” and finally in “e” (51 ms after “a”) among the six traces. We measured time differences in the onset of this spontaneous activity among all pixels and constructed an isochrone map of this spontaneous activity (Fig. 4C). The isochrone map formed a nearly

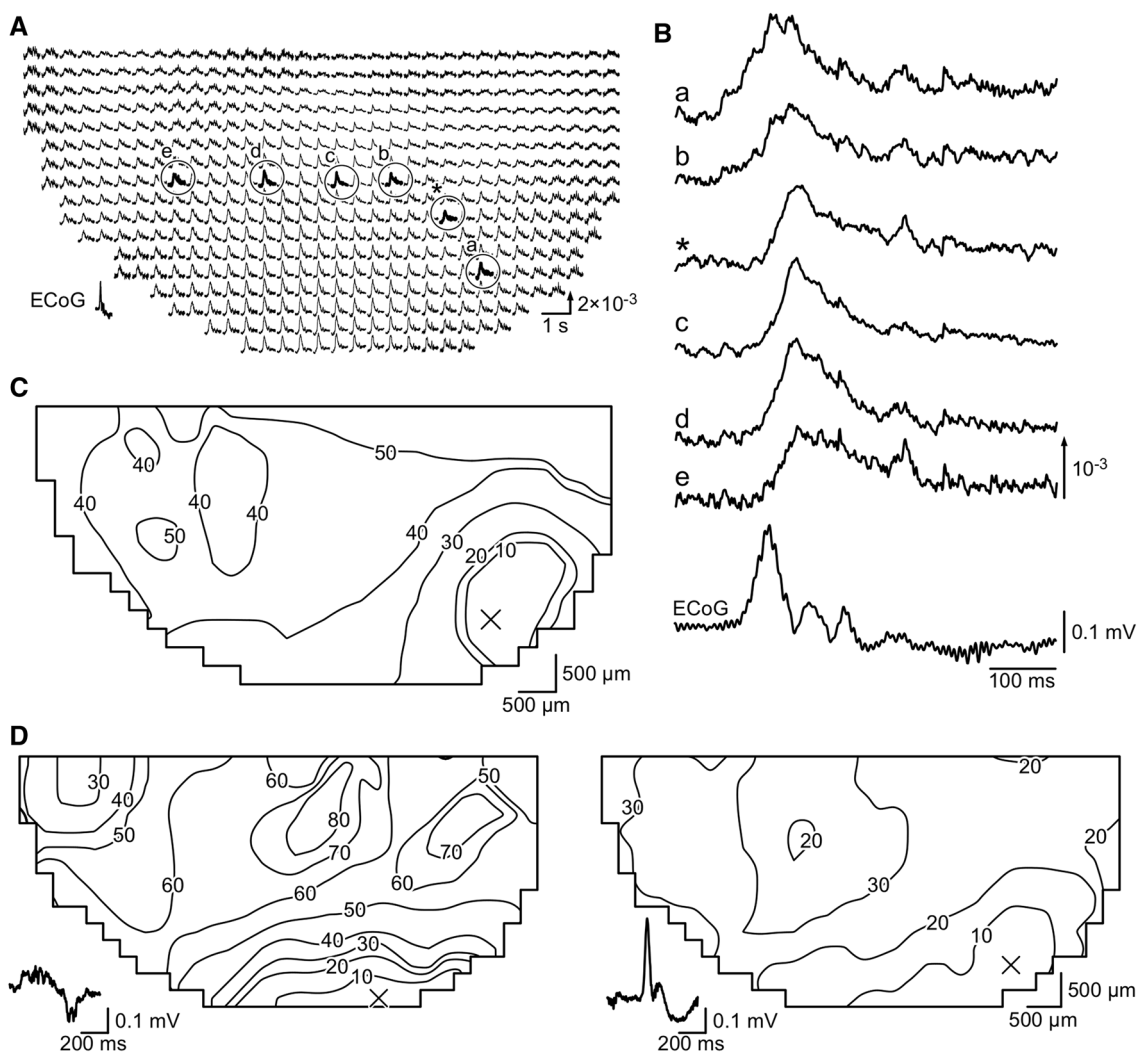


Fig. 4 Examples of multi-site optical recordings of spontaneous neural activity. **A** An example of a single-sweep simultaneous multi-site optical recording after reduction of the pulsation artifact. The six traces surrounded with a circle (labeled *a–e* or with an asterisk) are shown in **B**. **B** Magnified traces obtained from the six sites (*a–e* and asterisk) in **A**. The ECoG signal was recorded using the transparent electrode spot near the asterisk. **C** Isochrone map for the onset time

constructed from the data in **A**. The X indicates the initiation site of spontaneous neural activity and corresponds to trace “a” in **A**. Each number represents the time lag (in milliseconds) relative to this site. **D** Two further examples of the isochrone map of spontaneous neural activity, the initiation sites of which were located closely to that shown in **A**. The ECoG trace is shown at the left bottom corner in each panel

concentric circle pattern, indicating that spontaneous activity was initiated within a small area and expanded radially.

After analyzing several spontaneous signals, we noted that the initiation site of each spontaneous neural activity was not fixed, but varied across the cortex. In addition, propagation patterns differed among spontaneous activities, even when the initiation site was close. A typical example of this phenomenon is shown in Fig. 4D. These two isochrone maps were made from two different neural activities, recorded from the same preparation as that shown in Fig. 4A. The two activities were initiated in very close locations (X in Fig. 4D) to each other, which were also close to the initiation site in Fig. 4A–C (X). Nevertheless, propagation patterns

differed among these three activities. Thus, we concluded that the placement of the transparent electrode within the optical recording area was a powerful tool not only to establish the ‘neural activity-free period’ in order to create the ‘pulsation artifact template’ for removing pulsation artifacts, but also to obtain the timing of the occurrence of the spontaneous signal via the ECoG trace.

In summary, the advantages of the transparent electrode in our study were as follows: (1) it provided better estimations of the ‘neural activity-free period’ for creating the ‘pulsation artifact template’ in order to remove pulsation artifacts, and (2) it contributed to the recognition of small and spatially restricted spontaneous signals via the ECoG trace.

Discussion

In this study, we demonstrate the use of a transparent electrode unit which we developed to monitor neural activity with both electrical and optical techniques simultaneously. Additional electrophysiological information obtained by this electrode makes it possible to determine more accurately whether a small optical signal is noise or a physiologically significant signal. This combination of electrical and optical recording methods may advance the analysis of brain function.

Necessity of the transparent electrode

In this section we discuss the application of this electrode for the elimination of pulsation artifacts in optical recordings as one of the examples of the potential uses of this electrode. Although a neural activity-free period is essential for our software to accurately eliminate pulsation artifacts [8], we encountered difficulties associated with selecting the ‘neural activity-free period’ based solely on raw optical signals (Fig. 3). Therefore, simultaneous electrophysiological monitoring is very important for the optical detection of neural activity using our system. Furthermore, although all evoked neural activities expanded to the whole optical detection area, some spontaneous ones did not (see Fig. 4B). These localized and small neural activities may be overlooked if the electrode is placed at the edge of or outside the optical recording area because surface electrodes record the activity of neurons within a distance of up to 1 mm [10, 11]. The results shown in Fig. 3 indicate that the ECoG trace taken within the optical sampling area was required to accurately construct the pulsation artifact signal and analyze spontaneous events. The introduction of the transparent electrode markedly improved the accuracy of removing pulsation artifacts from raw optical signals.

One key feature of the transparent electrode is its ability to record from several electrode spots on a semicircular surface using one electrode base (Patent pending “MULTI-CHANNEL ELECTRODE”: publication number JP, 2009-172265). As reported in our previous study [5], the curved surface of our plano-concave lens (electrode base) fit well to the shape of the rat cerebral hemisphere at almost every position. This compatibility allows for the placement of several electrode spots at preferred locations on the curved surface of the electrode base or plano-concave lens. The spatial error of the hand-drawn electrode spots in this trial product was estimated to be up to several hundred micrometers on the cortex. The shape and size of the ECoG trace detected by neighboring electrode spots on the

same electrode unit, at a distance of approximately 2 mm, were similar. Therefore, the spatial error was negligibly small in practice. Furthermore, the conduction velocity of the excitation wave on the cortex was at least several tens of millimeters per second, and any temporal error due to positional differences was also negligibly small as it pertained to current usage.

Using GZO for animal experiments as a recording electrode

The contact between a semiconductor and a metal often forms non-ohmic contact, and at this contact, current and voltage are not directly proportional. Since GZO is a semiconductor and the tip of the probe of an amperometric device is made of metal, then it is possible that the contact between these two materials is a non-ohmic contact. In this case, the contact resistance is not considered to be negligibly small. Nevertheless, while it is practically impossible to accurately measure the resistance of the electrode, it was $< 1 \text{ M}\Omega$, including this contact resistance, based on our rough measurements. This value is markedly smaller than the input impedance of the amplifier ($500 \text{ M}\Omega$); therefore, the voltage decrease caused by electrode impedance is negligibly small. Furthermore, the capacitance contribution of our electrode to the biological signal is also negligible because no marked differences were observed between the shapes of the ECoG signals recorded by our electrode and those obtained by a generally used metal electrode.

During the experiment, the GZO transparent electrode spot was placed in close proximity to nervous tissue; the maximum distance of the gap between the cortex and the concave surface of the electrode base was $100 \mu\text{m}$, as estimated from the difference in curvature between the dura and the concave surface of the electrode base. The GZO material may have toxic effects on the cortical neurons due to the close proximity of the electrode surface to the dura mater. In earlier studies, we used a plano-concave lens without a GZO film to reduce peripheral defocusing and detected cortical neural activity without any deterioration over a 1-h recording period [5, 9]. In the present study, we used a lens of the same shape with a GZO film, and no significant differences were noted in signals obtained with and without the GZO film. From these results, we conclude that the toxic effects of the GZO film were negligible in the acute experiments, although we did not quantitatively evaluate the toxicity of GZO.

Possibility to adopt our transparent electrode to other optical recording methods and long-term experiments

There are many types of optical recording methods, and we expect our transparent electrode to be useful in optical

experiments. For example, two-photon microscopy has been used in the three-dimensional functional imaging of brain structures. Images obtained with two-photon microscopy are dark and the S/N ratio of the signals is low; therefore, averaging (200–400 times) is unavoidable when data are analyzed quantitatively [16]. The use of our transparent electrode for two-photon microscopy will decrease the number of averaging times needed.

Optical recording methods are applicable to long-term experiments and, thus, the use of a transparent electrode is required. Implantable transparent electrode arrays are fabricated by depositing a transparent conductive film on a flexible film [17–20]. The total size of these electrodes, including lead wires, is markedly smaller than our electrode unit, while their density is markedly higher. In contrast, our electrode is too large to implant; it was originally intended to be applied to wider areas of the cerebral cortex. Its size (5×10 mm) was sufficiently large to record from the somatosensory area of the rat cortex. To date, our transparent electrode has been useful in acute experiments. If our electrode is to be applied to long-term experiments, its shape needs to be modified in order for it to be implantable. While short-term toxicity is negligible, as discussed above (section [Using GZO for animal experiments as a recording electrode](#) section), its long-term effects currently remain unknown. Detailed assessments, including histological examinations, may also be required.

Prospects for a transparent stimulating electrode

Throughout a functional analysis of the cerebral cortex, it is important to achieve the electrical stimulation of the cortical surface at specific points within the optically detected field during optical recording. Based on the evoked neural activity generated at an electrode spot, it is possible to more accurately identify the position of the electrode spot. As described above, in our study the range of spatial error regarding the position of the electrode spot was negligibly small under current usage; however, more accurate positioning may be needed in the future, particularly in cases in which higher magnification of the image plane is needed than the currently used sixfold magnification. This accurate positioning will be accomplished by introducing the technique used for drawing the electronic circuit pattern on a printed circuit board.

We made several attempts to flow a current through this electrode and found that the strength was several-fold greater than the quantity needed for a minimum electrical stimulation, with the longest duration being 10 ms. Therefore, the current damaged the insulating SiO_2 coating and/or cracked the GZO film itself. These issues may have been due to the heat produced in the lead due to high resistance. Some of these issues may be resolved by thickening the transparent

lead film to lower resistance; however, the effect of the improvement is limited.

In addition to the limitations associated with high electrical resistance, there is another issue related to flowing current through this electrode. The materials used as transparent conductors, such as indium tin oxide or zinc oxide doped with a metal (such as gallium, indium and aluminum), may dissolve in electrolytic solution, and mammalian body fluid in particular is considered to be a strong electrolytic solution. Therefore, current flow between these materials and the electrolyte may produce an electrochemical reaction that promotes the dissolution of the transparent conductor film at the electrode spot, possibly releasing metal ions. Moreover, the use of the electrode as an anode may also release a large amount of metal ions. Since divalent or trivalent metal ions may modify calcium channels, the use of these substances in neural preparations needs to be avoided.

We consider it important to develop a transparent electrode made from alternative materials in order to enable current flow. We are currently searching for various alternative materials, including newly developed materials, that are both suitable as a transparent electrode for applying a stimulus current to the cerebral cortex and insoluble in a strong electrolytic solution.

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