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High-density multielectrode array with independently maneuverable electrodes and silicone oil fluid isolation system for chronic recording from macaque monkey

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HIGHLIGHTS

- ▶ Fluid drain system for reducing the postsurgical intracranial pressure.
- ▶ Heavy silicone oil system for guarding electrical circuit from CSF backflow.
- ▶ Maneuverable electrode array implant to macaque IT cortex from temporal surface.

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ABSTRACT

Chronic multielectrode recording has become a widely used technique in the past twenty years, and there are multiple standardized methods. As for recording with high-density array, the most common method in macaque monkeys is to use a subdural array with fixed electrodes. In this study, we utilized the electrode array with independently maneuverable electrodes arranged in high-density, which was originally designed for use on small animals, and redesigned it for use on macaque monkeys while maintaining the virtues of maneuverability and high-density. We successfully recorded single and multiunit activities from up to 49 channels in the V1 and inferior temporal (IT) cortex of macaque monkeys. The main change in the surgical procedure was to remove a 5 mm diameter area of dura mater. The main changes in the design were (1) to have a constricted layer of heavy silicone oil at the interface with the animal to isolate the electrical circuit from the cerebrospinal fluid, and (2) to have a fluid draining system that can shunt any potential postsurgical subcranial exudate to the extracranial space.

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1. Introduction

Recent studies have suggested that sensory information is represented in distributed manners in cortical areas using various levels of functional structures, such as neurons, columns, and other structures larger than columns (Haxby et al., 2001; Howard et al., 2009; Hung et al., 2005; Tsunoda et al., 2001; Tsao et al., 2003, 2006). One approach to investigate distributed codes of sensory information is to record activities from many neurons by penetrating an electrode repeatedly and to combine these activities together for analysis of population responses (Hung et al., 2005; Kiani et al.,

2007; Zhang et al., 2011). However, to relate population activities to functional structures, we need to map population responses in cortical space. Although fMRI and optical imaging techniques are used to map cortical activities (Haxby et al., 2001; Howard et al., 2009; Tsunoda et al., 2001), measured signals do not necessarily correspond neural activities because they are secondary hemodynamic responses induced by neural activities. Especially, we lose the information buried in temporal structure of neural activities, such as correlation and synchrony among the cells that are involved in sensory information representation (Eckhorn et al., 1988; Gray et al., 1989). Multiple electrode arrays are only the available technique so far to investigate population neural activities with high temporal and spatial resolution (Blake and Merzenich, 2002; deCharms et al., 1999; Nicolelis et al., 2003; Hochberg et al., 2006). deCharms and colleagues specially developed a densely arranged multi-electrode array to address response patterns in spatial scales equivalent to columnar representation (1999). This array led them to map

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spectrotemporal representation of sound input in primary auditory cortex (Blake and Merzenich, 2002), and to analyze plastic changes of the functional map of auditory cortex in rats (Blake and Merzenich, 2002; Blake et al., 2005). Our goal of the present study is to make this type of array feasible to map sensory information representation in macaque monkeys.

Because the fine electrodes (75 μm in diameter) adequate for above high-density recording cannot penetrate the thick dura mater of macaques, we took the approach of making an opening in the dura mater over the implant sites, as was done on some other macaque chronic recordings (Jackson and Fetz, 2007; Nicolelis et al., 2003). Leaving a dura window under the recording device will increase the risk of causing (1) biological reaction at the dural scar, (2) cerebrospinal fluid invasion into the electrical circuit, and will often limit the number of feasible electrodes and the duration of successful recording. To reduce these risks, we developed a new device which (1) has a fluid drain system at the interface between the chamber and the subcranial space over the implant site and (2) has a layer of highly viscous silicone oil to maintain isolation of the electrical circuit, in the present study.

To map spatial patterns of activity across the cortical surface, we have to take into account recent studies showing that nearby neurons generally behave very differently (DeAngelis et al., 1999; Reich et al., 2001; Sato et al., 2009; Vinje and Gallant, 2000; Yen et al., 2007). For example, Yen and colleagues revealed that even in cat V1, where columnar functional structure has been known for decades, responses of nearby cells are uncorrelated while the animal is looking at natural scenes, thus, a single cell activity cannot be treated as the representative activity in the vicinity of the recording electrode. In the present study, to make the array feasible to map spatial patterns of activity, we examined whether electrodes with large exposed tips can detect neural activity to reflect common properties across the cells within the local region. In short, the newly designed electrode array achieved higher neural activity yields in macaque monkeys. In practice, we were able to show representative spatial patterns of activity obtained from inferior temporal (IT) cortex of macaque monkeys which gave qualitatively consistent results with spatial map obtained by optical intrinsic signal imaging.

2. Materials and methods

2.1. General experimental conditions

Six rhesus monkeys (*Macaca mulatta*) were used in this study. One monkey was used for semi-acute electrophysiology experiments to search for the optimal electrode tip configuration. Five other monkeys were tested with the actual chronic multielectrode array. Chronic array was implanted on V1 cortex of two monkeys, posterior IT of another monkey and anterior IT of the other two. Electrophysiological recording experiments were conducted while the monkeys were under neuroleptoanalgesia (NLA). The experimental protocol was approved by the Experimental Animal Committee of the RIKEN Institute. All experimental procedures were performed in accordance with the guidelines of the RIKEN Institute and the National Institutes of Health.

2.2. Anesthesia

In all experiments, the animals initially received intramuscular injection of droperidol (0.25 mg/kg), atropine sulfate (0.5 mg) and ketamine (5 mg/kg). Atropine (0.5 mg) was administered every 3 h in addition to suppress saliva. On the first surgery of the animal, intravenous injection of pentobarbital (20 mg/kg initially and maintained at 5 mg/kg/h) was used to maintain the anesthesia.

Heart rate was monitored and the rectal temperature was maintained at 37.6 °C. In all cases, the animals had two stainless screws implanted over the frontal region of the skull in this first surgery. The screws penetrated the skull to touch the dura surface on the respective hemispheres and were used to monitor EEG in later anesthetized animal experiments. In the later surgeries and experiments, the animals were artificially respired with mixture of nitrous oxide (70%)/oxygen (30%). The animals were maintained in anesthesia with isoflurane (0.8–1.2%) for surgeries. In case of neurophysiology experiments, the animals were maintained in NLA with intravenous injection of fentanyl (0.91 $\mu\text{g/kg/h}$) and occasional addition of droperidol. The animals were immobilized with vecuronium bromide (73 $\mu\text{g/kg/h}$), and up to 0.3% of isoflurane was added if necessary.

2.3. Surgical procedures

In the semi-acute recording animal, a chamber typically used for cortical optical recording was attached over the IT cortex. After craniotomy and duratomy, electrodes were inserted through the dural window maintained with an artificial silicone dura (Arieli et al., 2002) on every recording session (Sato et al., 2009). Initial part of the surgery is similar to that of the chronic experiments described below, and more details on the surgery and manipulation has been described elsewhere (Tsunoda et al., 2001; Wang et al., 1996; Yamane et al., 2006).

For chronic implant experiments, animals went through two surgeries. In the first surgery, a headpost and two stainless steel screws were attached to the animals' skull. The headpost and screws were fixed with dental acrylic. The animal for acute experiment had a chamber conventionally used for optical recording attached to the temporal skull over the anterior IT cortex. Skull surface around the headpost and the region of interest was also covered with dental acrylic. The animals went through two to three weeks recovery period after the initial surgery.

The second surgery consisted of craniotomy, duratomy, chamber fixation and electrode array installation. Craniotomy was done in three steps. First, a small cranial hole was made at the center of the target site. Second, the skull over the target site was thinned so the bone thickness is approximately 2 mm, and a flat surface of at least 14 mm diameter is obtained. Third, the cranial hole is enlarged so the whole diameter is precisely 14 mm and the inner wall of the hole is as smooth as possible. A 14 mm diameter dummy metal plate was used as the template of the surface during this process. After all the bleeding from the skull was carefully stopped with bone wax, the dura was incised with 27 G needle and dura scissors to make an opening on the dura that is approximately 5 mm in diameter. Bleeding from the dura was prevented by locally coagulating the dural veins before cutting, whenever possible. The chamber was fit tightly to the cranial window and the titanium bone screws securely attached the chamber flaps on the side of the cylinder to the skull. Extra titanium bone screws were positioned around the chamber as anchors, and dental resin secured the chamber to the skull in water-tight manner. A three-way connector and two syringes filled with saline were attached to the drain silicone tube. The piston was removed from one of the syringe ('open syringe') and was fixed upright to a stable pole. The internal pressure was controlled by adjusting the height of the saline surface in the 'open syringe'. The inner space of the chamber was filled with saline and any air left in the drain path was pushed out with the other 'closed syringe'. The implant core was inserted slowly into the chamber as the proximal connection of the drain was switched to the 'open syringe'. This sequence eliminated both the air bubble and pressure elevation on the cortical surface beneath the chamber. Excess saline flowing backward into the chamber 'casing' was carefully aspirated to keep the electrical circuit dry. Once the inner core was

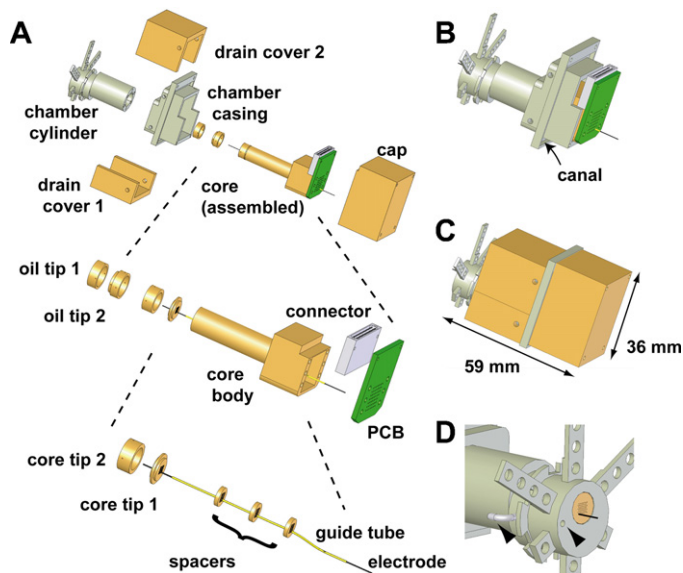


Fig. 1. The implant device in parts and assembled form. (A) The chamber and electrode array before assembly. The parts colored in gray are metal chambers attached to the skull. The 'cylinder' is made of titanium alloy for biological compatibility and the 'casing' is made of stainless steel. Parts colored in light brown are made of PEI, also known as 'Utem'. (B) The assembled chamber and the electrode array. Only one guide tube and electrode are shown, where there is 16 (4-by-4) or 49 (7-by-7) guide tubes and electrodes in the actual array. (C) The implant after the protective caps are mounted. The cap over the printed circuit board is water-tight owing to the silicone gasket mounted in the small canal surrounding the chamber and (D) Front end of the implant after assembly. Arrow heads indicate the drain outlet tube and the drain hole of the chamber.

positioned tight against the cylindrical chamber, CSF did not leak into the inner space of the chamber.

2.4. Implant design

The original system design with the outer chamber and the inner electrode array 'core' is a direct implementation from deCharms et al., at UCSF Merzenich laboratory. The difference from the conventional UCSF array is (1) use of low impedance electrode, (2) electrode arrangement for higher density with honeycomb arrangement, (3) water-tight interface to the tissue using heavy silicone oil, (4) water-tight cap, (5) fluid drain, (6) long cylinder for IT and (7) center offset. See Fig. 1 and Supplementary Figs. 1–9 for details of the device used for IT implants. The details of the V1 implants are not fully described in this article, but they are essentially the same as the IT implant, except for the length of the cylindrical part of the chamber and the inner core. See also Fig. 2 for (2) and Fig. 3 for (5). Parts were machined by commercial machinist (Nakazawa Seisakusho, Tokyo, Japan) except for the printed circuit board (PCB) manufactured by UCSF machine shop. The basic assembly procedure has been described previously (deCharms et al., 1999). The details of the difference are described briefly below.

2.4.1. Low impedance electrode

In our new design, we used low impedance electrodes (0.2–0.5 M Ω) to focus on columnar level activity as well as single cell level activity. To check whether such columnar level activity can be extracted with low impedance electrodes, we prepared electrodes having exposed tip length of 5, 20, 50, 100 and 200 μ m. These electrodes had approximate impedance of 1–2, 0.2–0.5, 0.2–0.3, 0.15, 0.15 M Ω at 1 kHz respectively. The electrodes were attached to a single electrode holder with 300 μ m separation in a fork-like

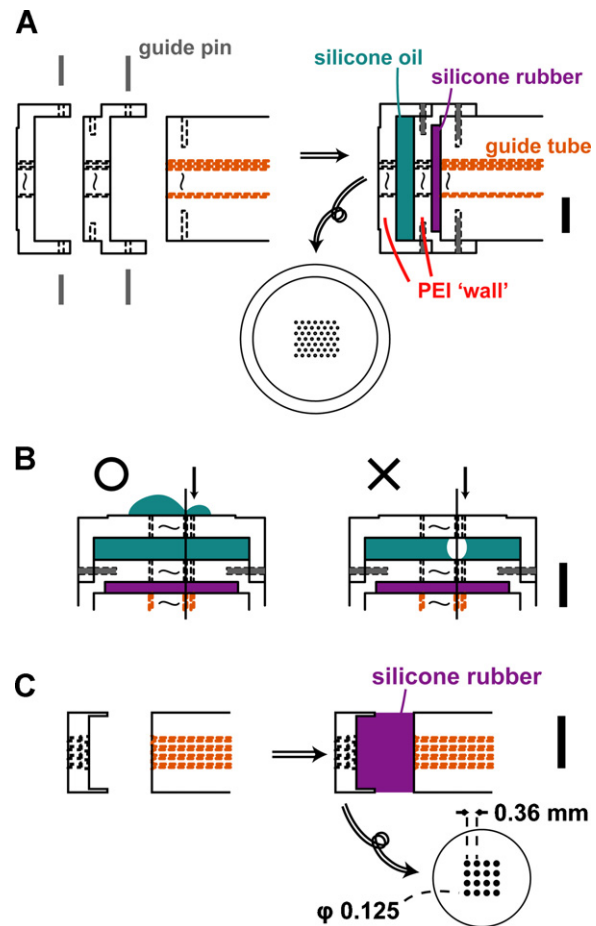


Fig. 2. The configuration of the implant front end that prevents water invasion into the electrical circuit. Note that guide tubes are parts of the electrical circuitry that passes the electrical signal measured at the electrode tips to the PCB. (A) Profile of the 'oil-type' implant. A double cap system makes two cavity spaces that are filled with heavy silicone oil and silicone rubber respectively. The silicone oil layer prevents CSF from invading into the implant, and the silicone rubber layer prevents the silicone oil from being pushed out of the oil layer into the guide tubes. (B) Schema of the electrode insertion into the implant. (Left) Electrodes are pushed in with excess amount of heavy silicone oil mounted on top of the implant. (Right) Loading electrodes without mounting the silicone oil could result in air bubble invading into the silicone oil layer and (C) Profile of the 'gasket-type' implant. The gasket is pressurized by screws so that the silicone rubber forms water-tight seal around the penetrating electrodes. Scale bars are 2 mm.

configuration and were advanced into the cortex in 250 μ m steps (Fig. 4A).

2.4.2. Electrode arrangement for higher density

In the earlier chronic arrays, electrodes were arranged in square grid configuration with the inter-electrode distance of 360 μ m (Fig. 2C), see also deCharms et al. (1999). In the later arrays, the electrodes were arranged in hexagonal grid configuration with the inter-electrode distance of 360 μ m (Fig. 2A). The density of electrodes on the cortical surface was 7.72 mm $^{-2}$ for the former and 8.91 mm $^{-2}$ for the latter.

2.4.3. Water-tight interface to the tissue using heavy silicone oil

The electrodes used for the previous (deCharms et al., 1999) and present chronic arrays were stripped off of the insulation on the back half the shank and kinked for stable electrical connection to the guide tube before being loaded into the guide tubes. Since inner surface of guide tube is not electrically insulated, invasion of cerebrospinal fluid into the guide tube causes a critical problem.

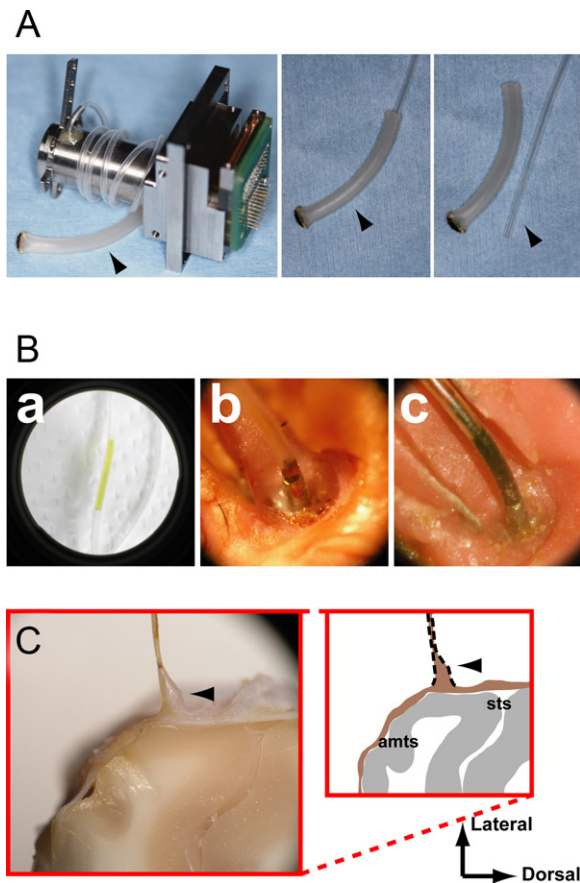


Fig. 3. Drain shunt system for depressurizing the subcranial space under the implant. (A) A silicone drain tube is connected to the assembled implant. The open end of the drain tube is capped loosely with a thicker tube with a closed end (arrowheads). (B) Drain tube at (a) 10, (b) 21 and (c) 29 days after the implant in monkey M4. When the silicone drain tube was congested for a period of time as in (B-c), or no new fluid is observed in the tube, the silicone tube was removed and the stainless tube was closed with dental resin and (C) Left is the picture of the cut surface of the cortex and the dura at the implanted site. Right is the illustration of the picture in left. When the regrown tissue (arrowhead in left, arrowhead and dotted outline in right) eventually plugs the chamber drain hole, the drain tube is removed and the drain hole is covered with dental resin.

The type of chronic array using heavy silicone oil as the main the protection against CSF invasion (the ‘oil-type’) is featured in Figs. 1 and 2A and B. In front of the guide tubes were (1) a 0.5 mm silicone rubber layer (Fig. 2A magenta), (2) a polyetherimide (PEI) layer (Fig. 2A red), (3) a 1 mm thick heavy silicone oil reservoir (Fig. 2A green) and (4) another PEI plate that interfaces against the cortical surface (Fig. 2A red). All, except the silicone oil, the reservoir had 130 μm diameter holes for the electrodes (shank diameter 102 μm) to pass through. The PEI layers were machined by commercial machinist (Nakazawa-Seisakusho, Tokyo, Japan). The silicone rubber was made prepared in the following steps.

First the dummy tungsten wires (CaliforniaFineWire, Grover Beach, USA), were placed in the guide tubes, then the ‘inner cap’ was placed on the core slowly with the freshly prepared two-solution-mixture type of silicone rubber filled in the inner cavity. Excess amount of the mixture was allowed to go out from the holes in the PEI layer, and the guide pins held the ‘core tip’ and the ‘inner cap’ to align with the holes. Dummy wires were advanced immediately to pass through the holes in the PEI layer. Dummy wires were removed after the silicone rubber layer was formed, leaving holes in the rubber layer for the electrodes to pass through.

We degassed the heavy silicone oil over-night in vacuum before use, and extra care was taken to keep air bubbles from invading

into the silicone oil reservoir. Electrode length was 89 mm, which is approximately 40 mm longer than the core length. Electrodes were stripped of the insulation on the back 50 mm of the shank by burning off the Parylene-C coating using a micro-forge (Narishige MF-77, Tokyo). The uninsulated electrode shaft was then kinked at several locations to ensure good frictional contact with the inner surface of the guide tube. Electrodes through their blunt ends were inserted into the implant ‘core tip’ part of the implant device using forceps. The blunt end came out of the implant core while the electrode held by the forceps was still the exposed portion. By pulling at this blunt end, the electrode was positioned to the final location without damaging the insulation by the forceps. The blunt end of electrodes was cut off so the final length sticking out of the guide tube on the PCB side became 5 mm (Fig. S10). Heavy silicone oil was placed on the core tip during electrode insertion (Fig. 2B left). Because of its high viscosity, the silicone oil tended to stay with the electrodes while they were loaded resulting in air bubbles invading into the oil layer (Fig. 2B right). The 0.5 mm silicone rubber layer kept the silicone oil from being drawn out of the oil reservoir and into the guide tubes. The oil on the tip assured that more oil, rather than air, was pulled into the oil reservoir while the electrodes were loaded (Fig. 2B left). (In a primitive experiment where an air bubble invaded into the oil reservoir prior to the experiment and stayed near the electrodes (data not shown), cerebrospinal fluid and the oozed exudate eventually invaded into the oil “reservoir” replacing the bubbles, and caused subsequent electrical shunting of the guide tubes that needed to be isolated to function properly as one of the routes for the electrical signal.)

The other type of chronic array (‘gasket-type’) had silicone rubber gasket at the tip of the core for protection against CSF invasion into the system (Fig. 2C), and was a direct implementation from the original design of the UCSF array (deCharms et al., 1999). Design of the ‘gasket-type’ was identical to the ‘oil-type’ from the PCB down to the guide tubes. The silicone rubber gasket was 1.5 mm thick (Fig. 2C), and heavy silicone oil (1 MPa s, Shin-etsu kagaku, Niigata, Japan) was applied at the front end of the core while the electrodes were back-filled. The core was pressurized against the chamber bottom with screws during recording so that the silicone gasket prevented cerebrospinal fluid from invading to the guide tubes. Note that in our system, signals picked up at the electrode tip were fed to the PCB through the metal guide tube.

2.4.4. Water-tight cap

A thin silicone rubber sheet was mounted in the small canal surrounding the chamber (Figs. 1B and S1). The protective cover cap was screwed onto the chamber against this silicone sheet and made a water-tight seal. Several pieces of silica-gel were put inside the cap to remove the moisture inside the sealed space. The water-tight cap and the desiccant protected the electrical circuit against the moist invasion from outside and the internal moist accumulation. Protection from the outer moist was particularly important in IT implants, because the ventro-temporal approach to the IT cortex forced the device to be positioned below the interface with the animal.

2.4.5. Fluid drain

We made a 1.2 mm drain hole on the chamber ‘cylinder’ that connects the surface of the ‘cylinder’ bottom to the surface of the ‘cylinder’ sidewall (Figs. 1D, S2 and S3). This drain hole connects the subcranial implant space to the extracranial space. A 18 G stainless steel drain outlet tube and a 20–30 cm silicone drain tube were attached to the hole on the sidewall. Approximately one-third of the silicone tube was filled with saline containing antibiotic (gentamycin, 0.25 mg/ml). The silicone tube was wound around the ‘chamber cylinder’ (Figs. 1A and 3A left), and had the open end loosely capped with another tube with a closed end (Fig. 3A center,

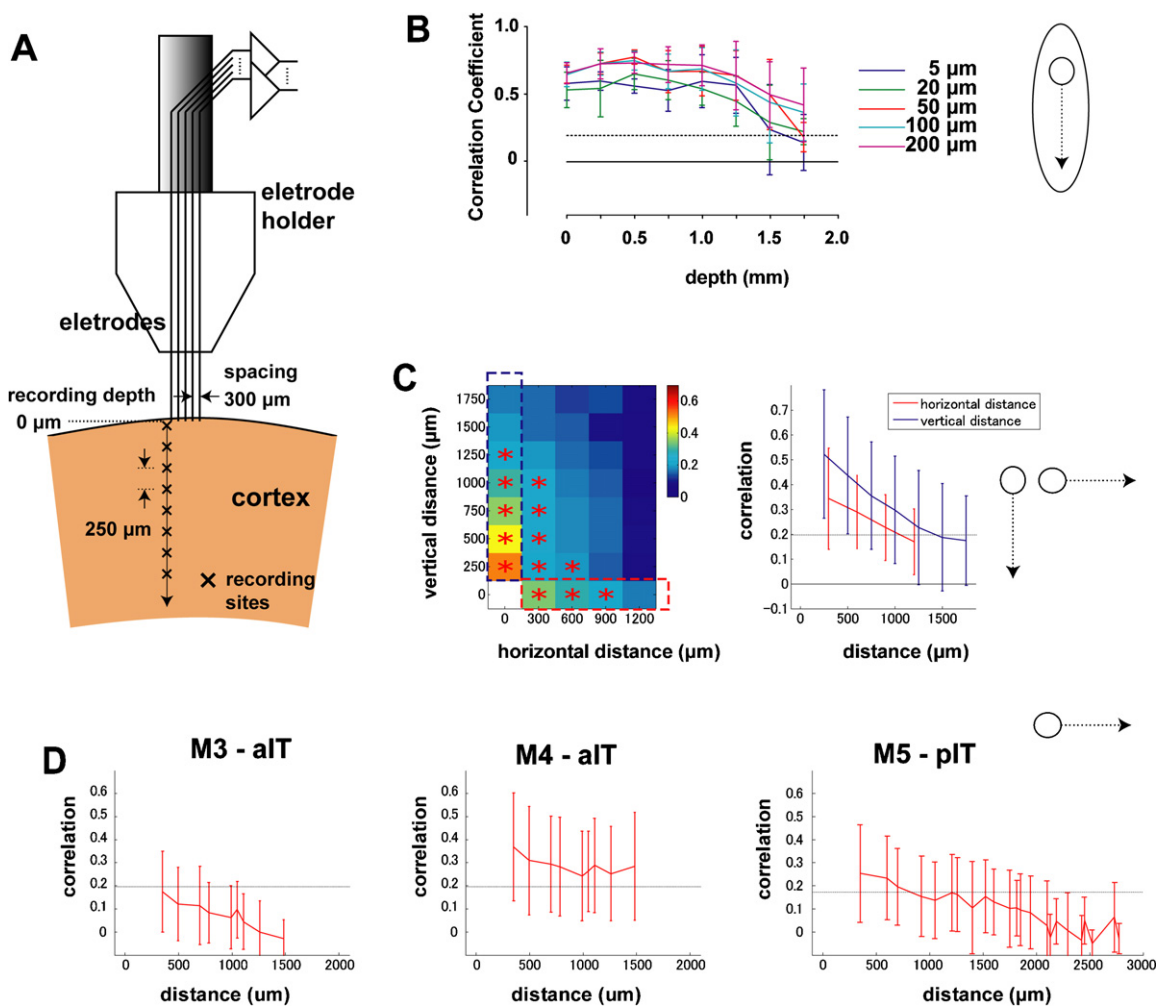


Fig. 4. Similarity of the stimulus selectivity across recording sites measured by response to complex object stimulus set. (B) and (C) are from acute data, and (D) is from implant data. (A) Schema of the acute experiment. Inter electrode distance is 300 μm and the increment of recording depths is 250 μm . (B) Correlation coefficient values calculated between depth-averaged MUs and individual MU at different depth within the same track. Responses at respective sites are excluded from the depth average when correlation coefficient at that depth is calculated. Similarity of selectivity is measured for electrodes with tip exposures 5, 20, 50, 100 and 200 μm . Dotted line, $p = 0.05$. (C) Correlation coefficient values calculated between individual MUs at different depths and tracks. Similarity of selectivity was sorted by difference of distance along axis vertical and horizontal to the cortical surface. The pseudo-color map shows the full two-dimensional plot of the correlation coefficient. The plots to the left and below of the color map shows the similarity sorted only to the vertical or horizontal axis respectively. The plot to the right of the color map shows the similarity sorted along both the vertical and the horizontal axes (blue and red dotted rectangles respectively) in overlay and (D) Correlation coefficient values calculated between MUs obtained from a 4-by-4 electrode array implanted in anterior IT and a 7-by-7 array implanted in posterior IT. Data labels, M3–M5, correspond to that in Fig. 6.

right). The drain tube was covered by a rigid cover firmly attached to the chamber with screws to avoid distraction by the animal (Fig. 1C). On daily experiments, the fluid content of the tube was examined by eye. If the content fluid was clean, the attached tube was simply changed to a new tube with sterilized saline containing antibiotics. If the fluid showed any hint of infection, the subcranial space was initially washed by gently injecting 0.5–1 ml of saline containing gentamycin back and forth through the drain hole, and then a new tube was attached. The injection did not cause significant loss of units identified before the procedure. The animal also received intravenous injection of an antibiotic (cefodizime sodium, 60 mg) in such case.

2.4.6. Elongated and thickened cylindrical chamber

The chamber 'cylinder' was increased in thickness for more mechanical rigidity (Figs. 1, S2 and S3). The device approaches the cortex from the temporal side, 20–30° rotated ventrally in the case of IT implant. It is also elongated, so the box-shaped part of the chamber does not interfere with the animals' jaw. We

introduced spacers for the guide tubes in IT implants, because of the elevated risk of interference between guide tubes (Figs. 1A bottom, S9 and S11A). The guide tubes were inserted to the 'core tip' (Figs. 1A and S5) with three spacers positioned along the length of the guide tubes (Fig. S11A). Then guide tubes and the 'core tip' were glued together with heat-tolerant epoxy (Araldite 2094, Huntsman Advanced Materials, Woodlands, USA) applied in the cavity inside the 'core tip' (Fig. S11B). Finally, the guide tubes were smeared with epoxy and inserted into the 'core'. The 'core tip' was held in strict alignment with the 'core' until epoxy hardened (Fig. S11C). The spacers gave good electrical isolation between the guide tubes which was critical for well-isolated multichannel electrophysiological recording.

2.4.7. Flexible electrode positioning within the chamber

We prepared two types of chamber 'cylinder', the 'straight cylinder' (Fig. S2) and the 'offset cylinder' (Fig. S3). When the 'straight cylinder' is used, the 7-by-7 electrode array occupies the 2.3 mm-by-1.9 mm area at the center of the cranial window. On the other

hand, with the 'offset cylinder', the electrodes occupy the area of same size, but are positioned at 2 mm offset from the center. The offset could be chosen from one of the three axial directions to the dorsal separated by 60°. The experimenter chose the position of the electrodes depending on how large veins lie on the cortical surface.

2.5. Recording device

Electrodes were advanced by pushing the back end with a small metal bar (Fig. S10) held by a hydraulic micromanipulator with pulse motor microdrive (MO-81, Narishige, Tokyo, Japan). Neural data was recorded with PLEXON MAP system (Plexon, Dallas, USA). Amplified signal was band-pass filtered between 400 Hz and 6 kHz, and was digitized at 40 kHz temporal resolution and 12 bit A-to-D resolution. Standard deviation (SD) of the signal was of the spontaneous activity measured prior to the stimulus presentation, and the signal that exceeded 3.7 SD was recorded as MU data. Wide-band data, filtered between 3 Hz and 6 kHz, was acquired simultaneously at 40 kHz temporal resolution and 16 bit digital resolution. Single unit data was sorted offline with OfflineSorter (Plexon) from the wide-band data after applying digital filter and amplitude threshold. MU data and single unit data were analyzed with in-house software made with Matlab (Mathworks, Natick, USA).

2.6. Visual stimulus

Visual stimuli were presented to the eye contralateral to the recording hemisphere. We measured the optics of the eye and focused monkey's eye on a screen of a CRT monitor placed 57 cm from the eye using a contact lens. A photograph of the fundus was taken to determine the position of the fovea. For V1 recordings, visual stimuli were whole-field moving gratings in 8 directions. The spatial frequency was 1 cycle/° and the velocity was 1°/s. For acute and chronic recordings in anterior IT cortex, we used 100 complex object images. Images were from different categories, such as fruits and vegetables, plants, tools, animals, stuffed animals, and insects. For posterior IT experiment, we used similar image set with 90 images. Stimulus images were 12–15° in size, and they were presented on a CRT monitor placed 57 cm from the eye, centered at the position of the fovea. During stimulus presentation, the images were moved in a circular path with a radius of 0.4° and at the rate of 2 cycles/s.

2.7. Analysis of neural data

Stimulus-evoked MUA response was calculated as the difference in averaged activity during the evoked period and that during the prestimulus period. The prestimulus period was the 500 ms time window before the stimulus onset, and the evoked period was the 500 ms time window with 80 ms latency from the stimulus onset. The prestimulus period started 500 ms after the offset of the previous stimulus. To compare the similarity of the stimulus response profile between MUs recorded in different locations in the IT cortex (Fig. 4C and D), Pearson's correlation was calculated between the response vectors elicited by 100 complex object stimuli for aIT implant (animal M3 and M4), and by 128 complex object stimuli for pIT (animal M5). To compare the response similarity between the depth-averaged MU response and the MU recorded at each depth, we averaged the evoked response at different depths excluding the MU of the depth to be compared, and calculated the correlation value (Fig. 4A).

When plotting the spatial pattern of the responses of pIT implant of animal M5, we converted stimulus-evoked MUA responses to z-score by the variance of the response amplitude to all the 128 stimuli and plotted them in pseudo-colored maps (Fig. 7). The number and size of column was calculated in the following

procedure. First, the local peaks that have visually evoked MU activity significantly above pre-stimulus activity level ($p > 0.05$, Kolomogorov–Smirnov test comparing mean firing rate in the pre-stimulus 500 ms time window against the post-stimulus 500 ms duration time window with 80 ms post-stimulus delay) were identified against each visual stimuli. Then for each peaks, adjacent sites above half drop of the peak value were grouped as candidate for a functional column ('candidate column'). If the extent of the 'candidate column' reached the edge of the array, it was excluded from the analysis, because we cannot know its full spatial extent. If the 'candidate column' included another local peak, giving two overlapping candidates, we excluded the larger candidate to avoid double counting. The remaining 'candidate columns' were adopted as functional columns, and calculated for average number and size within the recorded region.

2.8. Histology

To confirm the recording depth, we made electrical coagulations negative DC current of 5 μ A for 20 s from the recording electrodes at the final position of the implant recording session. One week after the lesion, we deeply anesthetized the animals, administered a lethal dose of pentobarbital sodium (70 mg/kg) intravenously, and perfused transcardially in sequence, with 0.1 M phosphate-buffered saline (pH 7.4), 4% paraformaldehyde, 10%, 20%, and 30% sucrose. Brains were processed by frozen microtomy at 50 μ m thickness. We made Nissl sections of the brain and identified the recording layer under microscope.

3. Results

The multielectrode array developed by deCharms et al. is unique in realizing high-density spatial configuration of electrodes (spacing, 350 μ m) and post-implant depth adjustment feature simultaneously. In their array, electrodes were penetrated through densely arranged metal guide tubes. Electrical signals picked up by an electrode were delivered to the head-amplifier through an electrical contact between uninsulated part of the electrode and inner surface of the metal guide tube. Since the electrodes can move within the guide tube, positions of electrode tips were adjustable. At the same time, however, the system requires the isolation of the guide tube from the cerebrospinal fluid. They used a pressurized gasket system for signal ground isolation (Fig. 2C; see Section 2 for details). The gasket was pressurized after electrode advancement to purge cerebrospinal fluid from the space in front of the guide tubes.

In preliminary test surgeries, we found that, unlike in rats, marmosets, and owl monkeys, we could not penetrate the thin electrodes (102 μ m in shank diameter) through the dura mater in macaque monkeys ($n = 2$, data not shown). Therefore, we removed the dura in the implant surgery, which sometimes resulted in strong biological reaction and subsequent implant rejection (Fig. 5A). Moreover, increased seepage of cerebrospinal fluid could not be sufficiently blocked with the pressurized gasket system especially during electrode advancement process and caused increased proportion of electrical shunting between signal and ground.

In the present study, we replaced the pressurized gasket with highly viscous silicone oil for maintaining the isolation of the electrical circuit (Fig. 2A; see Section 2 for details). This system allowed us to observe neuronal activity throughout the electrode manipulation process, as it does not require pressurizing procedure to achieve full electrical isolation. We also put a fluid drain system at the interface between the chamber and the subcranial space over the implant site (Fig. 1D, arrowhead). The drain system reduced the post-surgical rise in subcranial pressure, and the subsequent

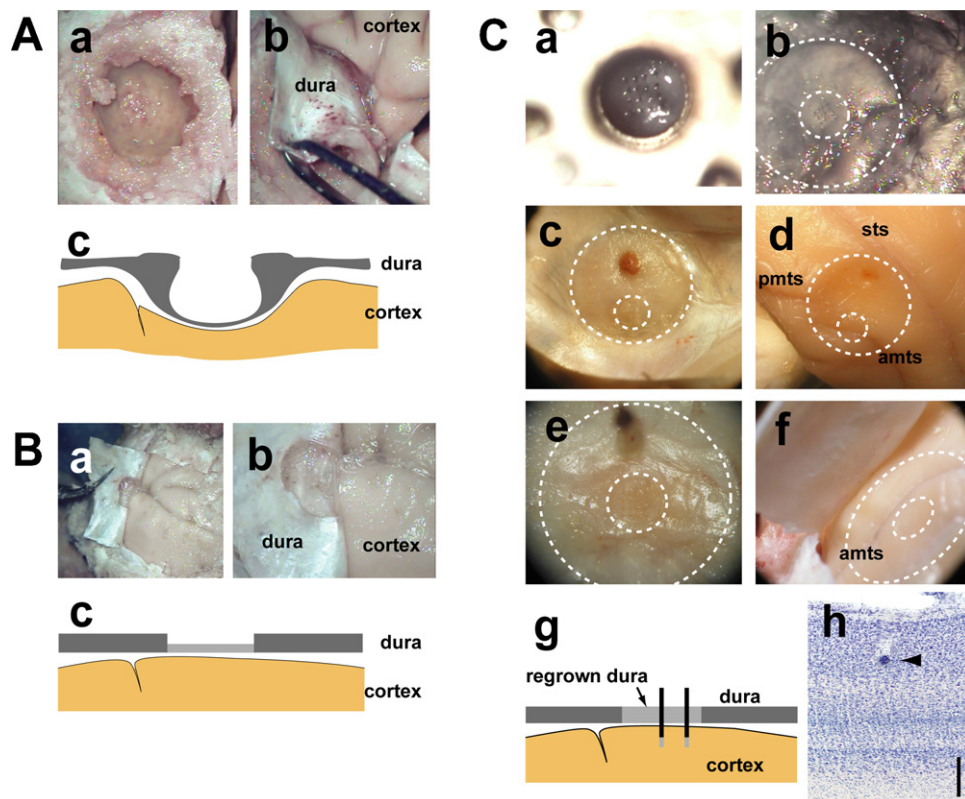


Fig. 5. Post-implant condition of the tissues. (A) and (B) are from preliminary implants not mentioned in other part of the manuscript. (A) 3 month after implanting the preliminary type of electrode array without the drain system. We observed multiunit activities from 30 to 60% of 14 electrodes up to 60 days from implant. (a) Dura mater under the implant, observed after the animal has been sacrificed and fixed. (b) The dura is lifted up to show the concave of the cortical surface underneath the dura. (c) A schematic figure of the cortex and dura after the damage. (B) 1 month after implanting the electrode array with the drain system. Data from this animal is not included in any other figure. (a) and (b) Dura mater is lifted up to show the cortical surface underneath implant site. The dura window made in the initial surgery has been closed by a thin film-like tissue. (c) A schematic figure of the cortex and dura in the process of recovery from dura incision. (C) Is from monkeys 1, 3 and 4 whose data is shown in other figures. (a) Regrown dura could be observed through the chamber implanted over V1 of monkey M1. Dura grows back tightly around the electrodes (shown in a schematic illustration in g). (b) Same implant site as that shown in (a), but with the chamber removed. Surrounding region did not show extensive regrowth or biological reaction. Note that the dark color of the dura is from the nigrosin infused intravenously prior to fixation. Large circle in white broken line shows the area covered by the chamber, and the small circle shows the location where the electrodes penetrated (see Fig. 1D). (c) and (e) The dura over the implanted sites in monkeys M3 and M4. Dura is observed from the cephalic side in (c) and the cranial side in (e). (d) and (f) The cortical surface of the implanted sites in M3 and M4. (h) An example of implanted electrode tip location shown in a Nissl stained section from monkey 1. Small DC current was delivered to the electrode for micro lesion after the implant recording period. Scale bar is 200 μm .

accumulation of tissue regrowth in the dead space formed by the internal pressure pushing down the cortex. The drain could also be used to inject topical antibiotics into the subcranial space.

3.1. Use of low-impedance electrodes for columnar activity mapping

The previous study suggested that there is common stimulus selectivity for cells in a columnar region of the anterior IT cortex (Sato et al., 2009). They also showed that each neuron has response property specific to the cell, which makes difficult to extract columnar response property from single cell activities. Similarly in primary visual cortex, neurons within a column responded almost independently to natural scenes (Vinje and Gallant, 2000; Yen et al., 2007), even though their orientation preference should be the same. In general, responses of a single cell picked up at each of the two dimensionally arranged electrodes cannot be the representative of the site of each electrode within the array. Not to map property of individual neurons that differs from nearby neuron to neuron but to map property of electrode penetration sites, we examined a possibility of using low impedance electrodes having tip exposure length of 5, 20, 50, 100 and 200 μm to detect columnar activity in separate experiments (Fig. 4A). The electrodes with a different tip exposure were attached to an electrode holder with 300 μm separation and were advanced into the cortex in 250 μm

steps. In all types of electrodes, stimulus selectivity of the MUs acquired at depths 0–1250 μm from the cortical surface, were significantly correlated to the selectivity of the depth-averaged MU activity (Fig. 4B; t -test, $p > 0.05$). There was also no significant difference between the correlation values obtained with different tip exposure lengths in these depths ($p = 0.10$, main effect of electrode tip expose in 2-way ANOVA). These results suggest that MU data obtained with all the tested low impedance electrodes can effectively extract the activities that resemble the cortical columnar activity. To further assess the feasibility of these electrodes for acquiring columnar activities, we checked the response similarities (correlation coefficients) of MU pairs with respect to their physical distance along the axis vertical and horizontal to the cortical surface and plotted as the pseudo-colored map of response similarity amplitude (Fig. 4C left). The similarity map showed a shape more elongated in the vertical direction than in the horizontal direction. The response similarities were generally higher for the pairs chosen along vertical axis than for the pairs chosen along horizontal axis and the response similarities declined in shorter distance along horizontal direction than along vertical direction (Fig. 4C right). These results indicate that multiunit activity detected by these low impedance electrodes represents activity of the functional column that elongates vertically to the cortical surface. We also plotted the relationship between the response similarities of MU pairs and the horizontal distances of the recorded sites from the implanted arrays

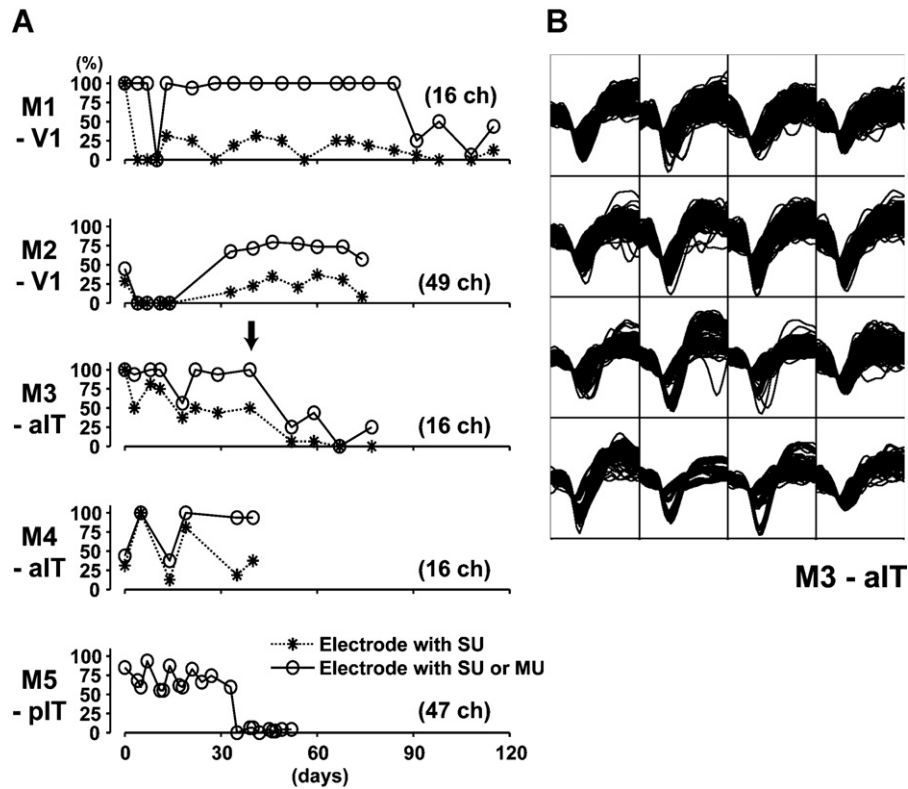


Fig. 6. Recording performance of the implant electrode arrays. (A) Time course of the recording yield from five animals. Two animals were implanted in V1, other two in anterior IT and another in posterior IT. Number of implanted electrodes also varied. (B) Example waveforms of neuronal activities recorded from the 16 channel electrode array implanted in anterior IT cortex. The data was obtained from animal M3 40 days after the implant (arrowhead in A).

used in the present study (Fig. 4D). Mean of response similarity across electrodes were higher than the statistical significance level ($p < 0.05$) in distances 900 μm or smaller along the direction vertical to the cortical surface, whereas the similarity was higher than the significance level in distance shorter than 1250 μm or smaller

along the direction horizontal to the cortex. The tendency of higher similarity for shorter horizontal distance persisted in 2 out of 3 animals with implant (Fig. 4D, left and right). One animal (M4) was an exception showing no drop of similarity for distant pairs (Fig. 4D, center). The recording site of M4 was atypical in that MUs from all

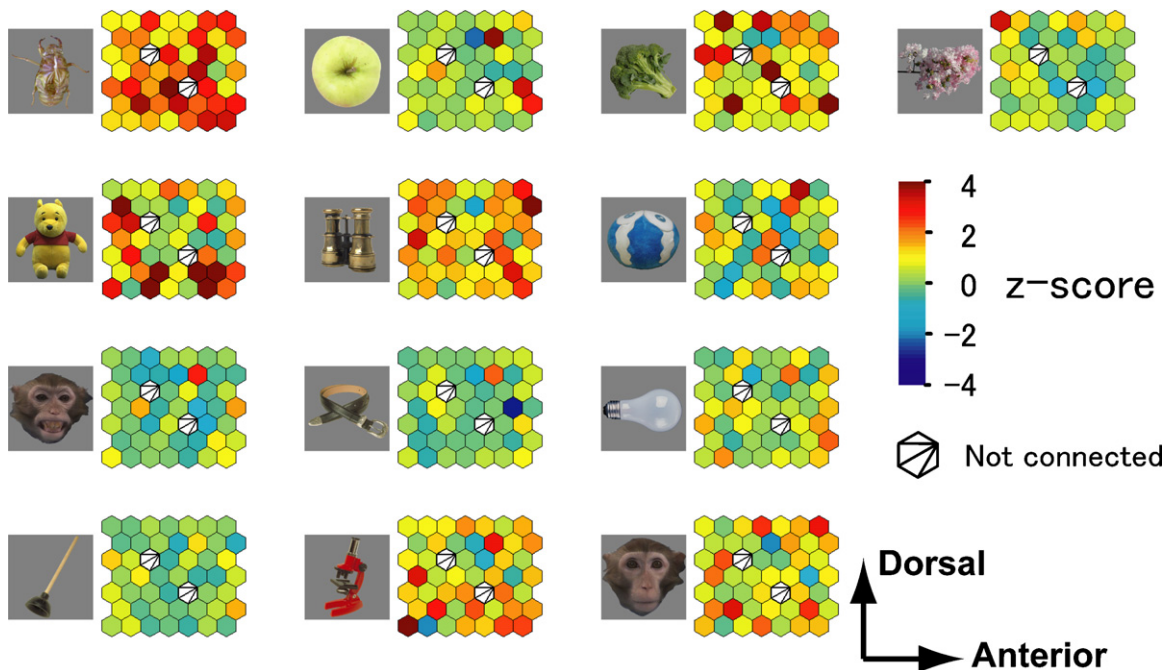


Fig. 7. Object image stimuli elicit different response patterns over the IT cortical surface with densely arranged multiple electrode array. Stimulus-evoked MUA response is converted to z-score by the variance of the response amplitude to all the 128 stimuli. Object image stimuli elicited response over the pIT cortex in different spatial patterns.

Table 1
Surgical and experimental processes applied for each animal.

	Implant	Advance electrode	Duratomy	Drain	Oil system
M acute	—	+	+	—	—
M1	+	—	+	+	—
M2	+	+	+	+	—
M3	+	+	+	+	—
M4	+	+	+	+	+
M5	+	+	+	+	+

the electrodes showed high selectivity to faces, and we assume that the whole array was in one of the face patches described by Tsao et al. (2003), most likely the anterior lateral patch speculated from the anatomical location (Moeller et al., 2008).

3.2. Performance of the chronic MEA

The two animals with V1 implant recorded MU activity up to 80 and 70 days after implant. The implant on the first animal (M1) achieved 100% yield from 16 electrodes, and the implant on the second animal (M2) had up to 75% yield from 49 channels (Fig. 6A). The site of the duratomy recovered and formed a smooth dura-like membrane tightly forming around the electrode shanks (Fig. 5C-a, c and e. See Fig. 5B-a and b for the intermediate stage of the optimal dura recovery). In the animal M1 implant, electrodes were advanced into the cortex only on the day of the implant and they were not removed till 80 days after the implant. In the animal M2 implant, electrodes were advanced on the day of implant, left untouched for the first 30 days, and then readjusted after 30 days. Both of these implants had the fluid drain system. The electrode tips were confirmed to be in the cortex at the end of the implant period by DC current injection and Nissl staining (Fig. 5C-h). For the other two animals with anterior IT implant (M3 and M4), the stable recording persisted for 40 days and with nearly 100% yield in both cases (Fig. 6A). However, where the recording quality gradually dropped after the 40-day stable recording period in M3, recording period with clear visually responsive multiunits (the ‘intact recording’ period) was interrupted by an accidental damage to the device in M4. Thus the similarity of the intact recording period was coincidental for these two animals. For the animal with the posterior IT implant, intact recording could be acquired for as long as 30 days with about 90% of the 47 electrodes (Fig. 6A).

Data with the quality for single unit recording could be acquired with 70 to nearly 100% of the electrodes during the recording in the initial 1–2 weeks. It soon leveled at around 30–50%, and dropped further down shortly before all the multiunit activity disappeared into the background noise. Fig. 6B shows a typical example of the single and multi units observed during the implant period. The example comes from anterior IT (M3), 38 days post implant (Fig. 6A, middle row, arrow). In this case, total of 11 single units could be isolated from 8 channels, and 8 more multi units could be detected in the other 8 channels. In M1 where the electrodes were positioned at fixed depth (Table 1) signal-to-noise ratio (S/N) of the single and multi units was 14.0 ± 8.5 SD of the noise standard deviation (SD) on the day of the implant, and it dropped to 5.1 ± 1.5 noise SD 41 days after the implant. In M3 where the electrodes were adjusted for depth (Table 1), S/N was 13.2 ± 4.6 noise SD on the day of the implant and dropped to 7.5 ± 4.5 noise SD 38 days after the implant. There was a tendency for better S/N when the electrodes were readjusted for position.

3.3. Observations on the drain system

Typically, fluid was observed in the drain tube in the first one to two weeks after the implant surgery. We distinguished four

different types of fluid, (1) colorless and transparent, likely to be CSF (data not shown), (2) pale yellow, likely to be exudate from damaged tissue (Fig. 3B-a), (3) red, likely to be exudate containing blood (Fig. 3B-c) and (4) white-yellow and cloudy (data not shown), fibrous tissue growing from the exudate or possible infection. In case (4), we retrieved a sample of the fluid to check for bacterial types, if any, and sensitivities to different types of antibiotics. The subcranial space was initially washed by gently injecting 0.5–1 ml of saline containing gentamycin (0.25 mg/ml) back and forth through the drain hole, and then a new tube was attached. The injection did not cause a significant loss of units identified before the procedure. The animal was also treated with intravenous injection of either cefodizime sodium (60 mg, TAIHO Pharmaceuticals, Tokyo) or panipenem/betamipron mixture (125 mg, Daiichi-Sankyo Pharmaceuticals, Tokyo) depending on the drug sensitivity of the bacteria. After the first week, fluid was less frequently observed within the drain tube. After the second week, the drain tube typically dried up and became clogged (Fig. 3B-c). Then the soft silicone tube was removed and the outlet tube was closed with dental resin. The remaining hole in the cylindrical chamber was finally closed with soft tissue grown from the dura (Fig. 3C, arrowheads).

Comparison of implant with and without drain tube was carried out by evaluating the biological reaction of the tissue under the implant. In an animal implanted WITHOUT the drain tube, we initially detected MU signals from 30 to 60% of the electrodes by adjusting the electrode positions each day (data not shown). However, after advancing eight weeks, we suddenly lost good recording. We perfused the animal one month later to find a large concave of the dura (Fig. 5A). In contrast, the duratomy site of another animal WITH the drain tube went through optimal recovery. The animal was sacrificed 4 weeks after the implant surgery to check for tissue reaction. There was no apparent deformation of the cortex, and thin sheet-like tissue formed back over the cortex (Fig. 5B). MU signal could be detected only from 3 out of 14 electrodes with this animal, which was most likely due to improper electrical connection within the device judging from large background noise observed on the inactive electrodes. Of the other 5 animals implanted WITH the drain tube and had 5–12 weeks of successful chronic recording with 75–100% yield (Fig. 6A), 3 animals (M1, M3 and M4) were sacrificed 7–18 weeks after the implant surgery to check for tissue reaction. The duratomy sites were closed with regrown dura of normal thickness (Fig. 5C-a and C-b from M1, C-c from M3 and C-e from M4). There was no apparent biological reaction in the dura and the cortical surface in the implanted region, except for some tissue that grew into the drain hole in 2 of 3 animals (Fig. 3C arrow head, Fig. 5C-c and e). Other two animals were not sacrificed, because they were used for other experiments.

3.4. Spatial pattern

High yield of active electrodes made us possible to map spatial patterns of activity across the cortical surface. We recorded response patterns of visually evoked object responses in inferior temporal cortex (Fig. 7). The previous studies with intrinsic signal imaging revealed that object stimuli activate multiple columns in IT cortex (diameter of each column, about 0.5 mm) and the patterns of columnar activation were different from object to object (Tsunoda et al., 2001; Yamane et al., 2006). Although spatial resolution of our densely arranged electrode array was still not as high as that of intrinsic signal imaging, high yield of active electrodes enabled us to visualize spatial patterns of object responses similar to those obtained by intrinsic signal imaging: object image stimuli elicited responses in multiple locations (columns) over IT cortex and in different spatial patterns. Consistent with the previous study (Tsunoda et al., 2001), the density of local activities observed in M5

with the 47 electrode array in the pIT region was $0.3 \pm 0.1 \text{ mm}^{-2}$ and the size of local activities was below 0.67 mm, the Nyquist limit of the present electrode array.

4. Discussion

4.1. Summary

In the present study, we introduced an implant recording system with densely arranged and independently maneuverable electrodes applicable to macaque cortices and achieved high yield of active electrodes. High yield of active electrodes enable us to map cortical activities across cortical surface.

To make implantation feasible for exposed cortices in macaques, we made multiple critical modifications. The oil system achieved (1) great reduction of the electrical shunting problem, (2) maintaining signal-to-noise ratio of the recording even during the electrode advancement process, and (3) maintaining the electrode tip position along the depth after the fine adjustment. It was critical to give a higher recording quality and stability immediately after electrode advancement, and to allow the experimenter to immediately continue with high-quality recording session. Another critical factor is to apply the drain mechanism for reducing the electrical shunting problem. The drain system also helped reduce the local damage to the subcranial tissue by preventing rise in the local pressure from oozing exudates.

4.2. Pitfalls of the chronic array

The chronic electrode array introduced in the current study is in contrast with other types of arrays that have fixed electrodes. It provides better means of post-operative adjusting of electrode depth, giving experimenters the chance to search for more neurons after the implant surgery. However, there are other pitfalls with this movable array. First is the risk of clogging the guide tubes with debris during assembly. Through the assembly procedure, we placed dummy tungsten wires in the guide tubes to block invasion of debris, but in some cases were unsuccessful and ended with (animal M5, see Figs. 6A and 7). Second is the difficulty of aligning the 'oil tip' (Figs. 1A and S6) parts during assembly. When there was any misalignment of the two 'oil tip' parts, the loaded electrode had difficulty entering the PEI 'wall' in front of the silicone rubber layer, and also the guide tube to a lesser extent (because the guide tube has larger inner diameter than the holes on the PEI 'wall'). The "blunt" back end of the guide wires and electrodes are actually slightly "sharpened" by filing with motor, to assist smooth back loading. The slightest bending of the electrode (guide wire) on its back end had to be carefully readjusted for smooth insertion into the guide tube.

4.3. Future application

The chronic array used in this study has $360 \mu\text{m}$ inter-electrode distance. This distance is shorter than size of the functional structures such as face patches and other category specific structures (Tsao et al., 2003, 2006; Bell et al., 2011; Op de Beeck et al., 2008). This distance is even shorter than the size of the cortical columns known both in macaque V1 and IT cortices (Hubel and Wiesel, 1968; Fujita et al., 1992). Density ($0.3 \pm 0.1 \text{ mm}^{-2}$) and size ($<0.67 \text{ mm}$) of local activities recorded from IT with the present electrode array were in good accordance with those suggested by the previous study with intrinsic signal imaging, where the density of functional column is 0.26 mm^{-2} , and its mean ($\pm \text{SD}$) size is $0.50 \pm 0.13 \text{ mm}$ in the longer axis and $0.35 \pm 0.09 \text{ mm}$ in the shorter axis (Tsunoda et al., 2001).

Intrinsic signal imaging has been a powerful technique to explore fine detailed functional structures such as the pin-wheel structure in V1 (Ts'o et al., 1990), segregation of color and orientation coding areas in V4 (Tanigawa et al., 2012), and continuous mapping of rotating face in IT (Wang et al., 1996). However, intrinsic signal imaging measures the neural activities in an indirect manner through light reflectance change following deoxygenation of hemoglobin. The present high-density, high-yield chronic electrode array is a great candidate to replace the intrinsic signal imaging for the better by directly measuring the electrical activity of the neuron.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jneumeth.2012.08.019>.

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