

Video Article

Chronic Implantation of Whole-cortical Electrocorticographic Array in the Common Marmoset

Misako Komatsu¹, Takaaki Kaneko^{2,3}, Hideyuki Okano^{2,3}, Noritaka Ichinohe^{1,4}

¹Laboratory for Molecular Analysis of Higher Brain Function, RIKEN Center for Brain Science

²Laboratory for Marmoset Neural Architecture, RIKEN Center for Brain Science

³Department of Physiology, Keio University School of Medicine

⁴Department of Ultrastructural Research, National Center of Neurology and Psychiatry

Correspondence to: Misako Komatsu at mkomatsu@brain.riken.jp

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Abstract

Electrocorticography (ECoG) allows the monitoring of electrical field potentials from the cerebral cortex with high spatiotemporal resolution. Recent development of thin, flexible ECoG electrodes has enabled conduction of stable recordings of large-scale cortical activity. We have developed a whole-cortical ECoG array for the common marmoset. The array continuously covers almost the entire lateral surface of cortical hemisphere, from the occipital pole to the temporal and frontal poles, and it captures whole-cortical neural activity in one shot. This protocol describes a chronic implantation procedure of the array in the epidural space of the marmoset brain. Marmosets have two advantages regarding ECoG recordings, one being the homologous organization of anatomical structures in humans and macaques, including frontal, parietal, and temporal complexes. The other advantage is that the marmoset brain is lissencephalic and contains a large number of complexes, which are more difficult to access in macaques with ECoG, that are exposed to the brain surface. These features allow direct access to most cortical areas beneath the surface of the brain. This system provides an opportunity to investigate global cortical information processing with high resolutions at a sub-millisecond order in time and millimeter order in space.

Introduction

Cognition requires the coordination of neural ensembles across widespread brain networks, particularly the neocortex that is well-developed in humans and believed to be involved in higher cognitive behaviors. However, how the neocortex achieves this cognitive behavior is an unsolved issue in the neuroscience field. Recent development of thin, flexible electrocorticographic (ECoG) electrodes enables conduction of stable recordings from large-scale cortical activity¹. Fujii and colleagues have developed a whole-cortical ECoG array for macaque monkeys^{2,3}. The array continuously covers almost the entire lateral cortex, from the occipital pole to the temporal and frontal poles, and captures whole-cortical neural activity in one shot. We have further developed this system for application in the common marmoset^{4,5}, a small, new-world monkey with genetic manipulability^{6,7}. This animal has several advantages compared to other species. The visual, auditory, somatosensory, motor, and frontal cortical areas of this species have been previously mapped and reported to have basic homologous organization to the same areas in humans and macaques^{8,9,10,11,12,13,14,15,16}. Their brains are smooth, and most lateral cortical areas are exposed to the surface of the cortex, which is harder to access with ECoG in macaques. Based on these features, the marmoset is suitable for electrocorticographic studies. Furthermore, marmosets exhibit social behaviors and have been proposed to serve as a candidate model of human social behaviors¹⁷.

This protocol describes an epidural implantation procedure of the ECoG array on the whole lateral surface of the cortex in a common marmoset. It provides an opportunity to monitor large-scale cortical activity for primate cortical neuroscience, including sensory, motor, higher cognitive, and social domains.

Protocol

This protocol has been performed on 6 common marmosets (4 males, 2 females; body weight = 320–470 g; age = 14–53 months). All procedures were carried out in accordance with the recommendations of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The protocol was approved by the RIKEN Ethical Committee (No. H26-2-202). All surgical procedures were performed under anesthesia, and all efforts were made to minimize the number of animals used as well as their discomfort.

1. Preparation

1. Obtain a structural magnetic resonance image (MRI) of each individual brain. This will be used to identify electrode positions through registration with a marmoset brain atlas and computer tomography (CT).
2. Preparation of the ECoG array: prepare a customized multichannel ECoG array (**Figure 1A**). A 96ch ECoG array consists of two sheets with 32 and 64 electrodes. To accommodate individual differences in brain size, the ECoG array has a flexible arm. The arm can cover the

temporal pole, depending on individual brain shape. Place the reference electrodes facing opposite to the ECoG electrodes and the ground electrodes facing the same direction.

1. Assemble the ECoG array with a connector case (**Figure 1B**) and seal gaps of connector (**Figure 1C**) using acrylic glue to prevent the inflow of liquid during surgery. Sterilize the array with ethylene oxide gas.

3. Prepare and sterilize instruments.

NOTE: All instruments used are listed in the **Table of Materials**.

2. Implantation of ECoG Array

NOTE: Withdraw ingestion of food and liquids greater than 4 h prior to surgery. Perform all surgical steps with aseptic technique using sterilized gloves and instruments.

1. Pre-implant procedures

1. Induce anesthesia in the marmoset by intramuscular (i.m.) injection of ketamine (15 mg/kg) 5 min after i.m. atropine (0.08 mg/kg) injection.
2. Anesthetize and maintain anesthesia using isoflurane (1-3% diluted with oxygen) depending on the physiological state of the animal, which should be continuously monitored. Ensure that heart rate is 130-180 BPM and monitor body temperature and arterial blood oxygen saturation (SpO₂) continuously to judge the animal's condition.
3. Shave the top of the animal's head with clippers and a hair remover. Fully rinse hair-removal cream off the skin with wet gauze, or it will cause skin damage.
4. Administer an antibiotic (cefovecin; 16 mg/kg s.c.; a single injection provides up to 14 days of treatment), antihypertensive (furosemide; 2.0 mg/kg i.m.), and antihemorrhagic (carbazochrome sodium sulfonate hydrate; 0.2 mg/kg i.m.).
5. Place the animal on a stereotaxic frame. At this time, apply 2% lidocaine jelly to the ear-bars and ophthalmic ointment to the eyes to prevent dryness and postoperative pain.
6. Disinfect the surgical area with iodine solution and cover it with sterilized drapes.

2. Implantation procedures

1. Incise skin about 4 cm through the midline of the scalp with a scalpel. Detach the temporal muscle from the skull with a curette until all of the surgical area is exposed. Clean out tissues on the skull surface and stop the bleeding completely with pressure hemostasis, and with bone wax, if necessary. Wrap the edge of the skin and muscles with moistened gauze. Keep the gauze moistened during surgery.
2. Place the frontal edge of the array onto the edge of the frontal pole. Mark a planned area for the craniotomy, slits, and holes on the skull with a pencil. The craniotomy location will depend on the design of the array (**Figure 2**).
3. Drill the craniotomy along mark 1, as shown in **Figure 2**. While drilling the bone, blow air at the cutting edge to maintain a clear view for the surgeon. Next, cut the bone all the way around mark 2, as the bone piece will still be attached to dura at the center. Lift the piece up gently from one edge and peel off the dura with a spatula. This process must be conducted slowly and carefully, or it will tear the dura easily.
 1. Remove the bone tips from the bone piece and wrap the piece with moistened gauze, as this piece will be returned after implanting the array.
4. Perform craniotomy 3 and 4 as shown in **Figure 2**. These allow the insertion of electrodes into the orbitofrontal and occipital areas, respectively.
5. Drill slits on mark 5 as shown in **Figure 2**. These slits allow examination of the array to ensure that it is properly inserted.
6. The dura will now be exposed. Wash the area with saline and stop the bleeding with pressure hemostasis and a gelatin sponge, if necessary. The edge of the open craniotomy may need to be cleaned with a curette or bone rongeur.
7. Make the slits (marked 6 in **Figure 2**) into which the reference electrodes are placed. Place the reference electrodes in the epidural space at the contra-lateral sensorimotor and occipital areas. The position should be determined according to specific experimental needs.
8. Drill screw holes at four points around each stem of the connector with a 1.0 mm screw (crosses in **Figure 2**). To prevent damage to the dura matter, insert a spatula under the skull. These holes should be orthogonal against the skull. Then, install PEEK screws (1.4 x 2.5 mm) as anchors to fix the connector to the skull.
9. Insert the ECoG array into the epidural space. Use flathead forceps to hold the array.

NOTE: The array should be inserted without bending. If the array is bent, create an appropriate space by inserting a spatula between the skull and dura. If the bending was caused by the relatively small size of the brain, cut off some of the electrodes.
10. Fix the reference and ground electrodes with a dental acrylic. Place the reference electrodes in the epidural space and ground electrodes on the cranial surface. Both contacts should face the skull.
11. Put the bone piece back and fix the connector and head post to the skull with dental acrylic on the screws.
12. Suture the skin with 6-0 nylon at the forehead and rear head, and fix the skin to the sides of the connector using skin closures.

3. Post-implantation procedures

1. Remove the animal from the stereotaxic frame. Ensure that the animal is kept warm and provided with oxygen during the following steps.
2. Immediately after surgery, inject the animal with meloxicam (0.3 mg/kg i.m.) to decrease postoperative pain. Administer an anti-inflammatory corticosteroid (dexamethasone; 2.0 mg/kg i.m.) and subcutaneous infusion (lactated Ringer's solution; 5.0 mL), including famotidine (0.5 mg/kg).
3. After the animal has recovered (confirm by heart rate and SpO₂), remove vital sign monitoring and transfer the animal into the ICU for 2-3 days.

3. Postoperative Treatment

NOTE: It typically takes 5 days for animals to recover completely from the surgery.

1. To prevent brain swelling, administer the anti-inflammatory corticosteroid dexamethasone (2.0 mg/kg) twice a day on the first day after surgery. Then, reduce the dose to 1.5 mg/kg twice a day on the second and third days, and 1 mg/kg twice a day on the fourth day.
2. Administer pain relief (meloxicam; 0.1 mg/kg oral; once a day) and an antihemorrhagic (carbazochrome sodium sulfonate hydrate; 0.2 mg/kg i.m.; twice a day) for 5 days after surgery.

NOTE: In our case, 1-2 days after the surgery, some marmosets (3 out of 6) became less active and vomited. This may have been caused by increased intracranial pressure due to a blood clot. When marmosets presented these symptoms, we reopened the head and removed the clot under general anesthesia (alfaxalone). If there was no bending of the ECoG array during the implantation, the blood clot was likely in the space between the array and where the bone piece was returned. In this case, the blood clot can be washed away by running saline into the space using a catheter. This procedure usually leads to recovery in the animal.

3. Identification of electrode locations
 1. Around 1 week after surgery, perform a computer tomography (CT) scan of the animal's head.
NOTE: This is a good opportunity to check if signals can be recorded properly. Open the connector case and remove any blood clots if they are present.
 2. Align T2-weighted MRI to stereotaxic coordinates using AFNI software¹⁸ (<https://afni.nimh.nih.gov>) (**Figure 3A**). Align the CT image to T2-weighted anatomical magnetic resonance images with AFNI (**Figure 3B**). Register a marmoset brain atlas to MRI (**Figure 3C**) with AFNI and ANTS¹⁹.

Representative Results

The whole-cortical ECoG array can simultaneously capture neuronal activity from the entirety of a hemisphere. **Figure 4** shows examples of auditory evoked potentials (AEPs) from multiple auditory areas in an awake marmoset. ECoG recordings were conducted in passive listening conditions. Each marmoset was exposed to auditory stimuli, which consisted of randomized pure tones with 20 types of frequency. Then, we calculated AEPs by averaging ECoGs aligned with onsets of the tones. Different wave forms were observed from lower and higher auditory areas, which indicates that the spatial resolution of our ECoG array can capture different information processing in different cortical areas.

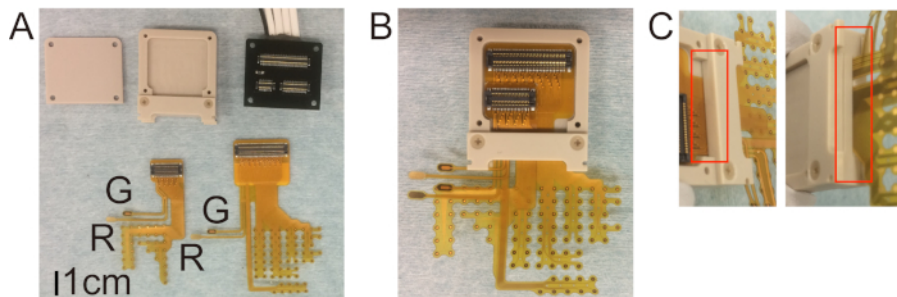


Figure 1: Preparation of an ECoG array. (A) 32 and 64 ECoG arrays (bottom left and right), a connector case (top left), and a front-end for the recording systems (top right). The "G" and "R" of each array indicate grand and reference electrodes, respectively. (B) Assembled ECoG array. (C) All gaps (red rectangles) should be sealed. [Please click here to view a larger version of this figure.](#)

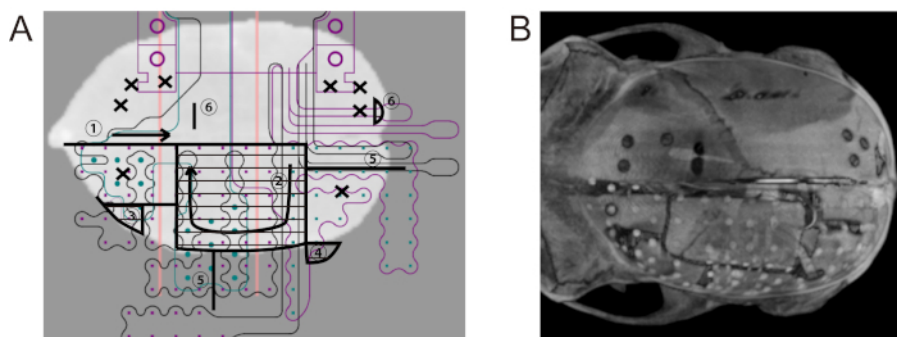


Figure 2: An example of the craniotomy. (A) The thin gray and thick black lines indicate outlines of the ECoG array and the planned area of craniotomy, respectively. The crosses correspond to anchor holes. The circled number indicates the order of drilling. (B) An example CT image of the craniotomy. [Please click here to view a larger version of this figure.](#)

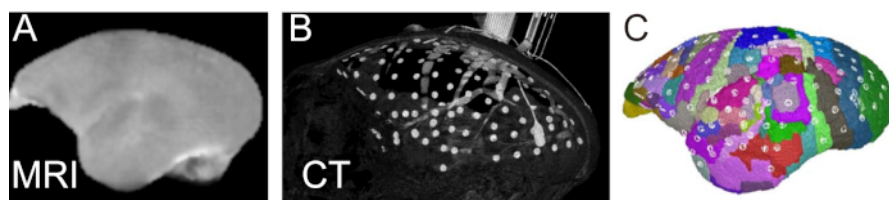


Figure 3: Localization of each electrode. (A) T2-weighted MRI, (B) CT, and (C) electrode locations on the atlas. The atlas used in this manuscript is the Woodward 3-D version based on the Hashikawa-atlas²⁰, which is an MRI-cytoarchitectural map. [Please click here to view a larger version of this figure.](#)

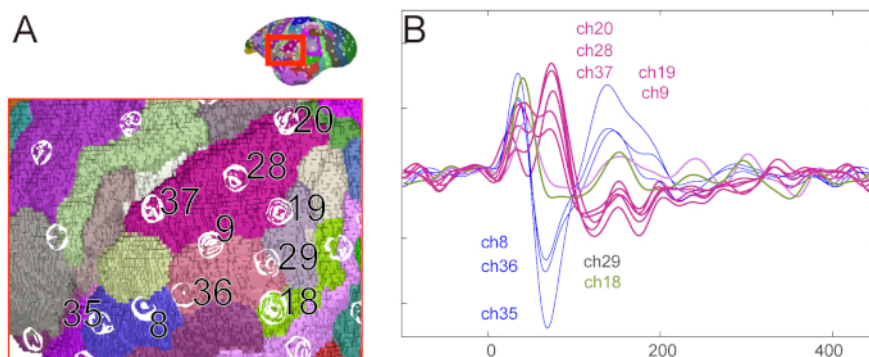


Figure 4: Examples of auditory evoked potentials. (A) Auditory area of Monkey J. (B) Examples of AEPs. Electrodes located in different auditory areas show different wave forms. [Please click here to view a larger version of this figure.](#)

9:00 a.m.	Start preparations
10:00 a.m.	Incise skin
	Exposure of skull (10 min)
	Craniotomy (30 min)
11:00 a.m.	Start to insert the array
	Insert the array (60 min)
12:30 p.m.	Close skin

Table 1: Recommended time course of the surgery.

Discussion

For successful implantation, animals should be provided with adequate nutrition before and after surgery. Short operating time is also important to optimize the animal's recovery. Preparations should be finished at least one day before surgery. To reduce operating time, previous craniotomy training with electrode array insertion in terminated animals for other experimental purposes is recommended. **Table 1** shows an example of the time course for this protocol.

We modified the anesthesia procedure and post-operative treatment on a case-by-case basis. In this video protocol, the animals were anesthetized and maintained using a mixture of isoflurane and oxygen delivered through tracheal intubation. Isoflurane can be replaced with sevoflurane, and tracheal intubation can be replaced with a mask. In other cases, we anesthetized animals with intramuscular injection of a mixture of ketamine and medetomidine. In this case, animals were initially sedated with butorphanol (0.2 mg/kg i.m.), and surgical anesthesia was achieved with a mixture of ketamine (30 mg/kg i.m.) and medetomidine (0.35 mg/kg i.m.).

Because ECoG directly records changes in electrical fields, its temporal resolution is limited by the recording system. The maximum time resolution of our recording system is 30 kHz. We usually sampled signals at a 1 kHz sampling rate and have found this to be sufficient for extraction of sensory/motor information.

Spatial resolution is dependent on electrode design. In this protocol, each electrode contact was 0.8 mm in diameter and had an inter-electrode distance of 2.5 mm. We observed different waveforms from three electrodes located in different auditory areas and separated by 2.5 mm (ch18, ch19, ch20 in **Figure 4**). Thus, the spatial resolution of our electrodes is estimated to be less than 2.5 mm. In some cases, electrode contacts were located more closely to each other. In these cases, the spatial resolution was finer.

We successfully recorded long-term, neuronal signals with good quality. In one case, the connector and dental acrylic were detached from the skull, and the electrode was broken 4 months after the surgery. This was caused by tissue growth due to blood being contained between the

dental acrylic and skull during surgery. Another marmoset was terminated due to an experimental requirement 5 months after the surgery. Four animals are still participating in experiments (1 year, 7 months, 4 months, and 4 months after the surgery, respectively).

ECoG arrays are typically implanted in the subdural space in humans and macaques. However, less invasive epidural implantations are more suited to marmosets, because they are delicate animals. The thin dura matter of marmosets allowed us to monitor high-frequency brain signals, even if the ECoG array was implanted on the dura. One of the disadvantages of epidural implantation is difficulty accessing the midline cortex and any cortex within a sulcus. Approaching these cortices requires incision of the dura matter. Furthermore, because ECoG arrays are surface electrodes, it is difficult to specify the signal source in terms of cortical depth. In order to understand precise information processing in the cortex, it is necessary to include other methods, such as depth electrodes or optical imaging. Despite these limitations, our method can provide new insight into cortical information processing. For example, sensory agency has been believed to emerge through rapid interactions between frontal and sensory areas; however, their mechanisms remain unclear since this rapid, large-scale, cortical information flow is difficult to monitor without the method presented here.

Disclosures

The authors have nothing to disclose.

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