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Chronic implantation of a whole-cortical electrocorticographic array in a common marmoset

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TITLE:

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

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KEYWORDS:

ECoG, non-human primate, surgical operation, electrophysiology, local field potential, awake recording

SUMMARY:

We have developed a whole-cortical electrocorticographic array for the common marmoset that continuously covers almost the entire lateral surface of cortex, from the occipital pole to the temporal and frontal poles. This protocol describes a chronic implantation procedure of the array in the epidural space of the marmoset brain.

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 activity[1]. 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[8-16]. 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 behaviors[17].

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

1.1. 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).

1.2. 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.

1.2.1. 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.

1.3. 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.

2.1. Pre-implant procedures

2.1.1. 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.

2.1.2. 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.

2.1.3. 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.

2.1.4. 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.).

2.1.5. 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.

2.1.6. Disinfect the surgical area with isodine solution and cover it with sterilized drapes.

2.2. Implantation procedures

2.2.1. 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.

2.2.2. 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**).

2.2.3. 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.

2.2.3.1. 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.

2.2.4. Perform craniotomy 3 and 4 as shown in **Figure 2**. These allow the insertion of electrodes into the orbitofrontal and occipital areas, respectively.

2.2.5. Drill slits on mark 5 as shown in **Figure 2**. These slits allow examination of the array to ensure that it is properly inserted.

2.2.6. 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.

2.2.7. 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.

2.2.8. 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.

2.2.9. 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.

2.2.10. 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.

2.2.11. Put the bone piece back and fix the connector and head post to the skull with dental acrylic on the screws.

2.2.12. 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.

2.3. Post-implantation procedures

2.3.1. Remove the animal from the stereotaxic frame. Ensure that the animal is kept warm and provided with oxygen during the following steps.

2.3.2. 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).

2.3.3. 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.

3.1. 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.

3.2. 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.

3.3. Identification of electrode locations

3.3.1. 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.

3.3.2. Align T2-weighted MRI to stereotaxic coordinates using AFNI software[18] (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 ANTS[19].

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 AND TABLE LEGENDS:

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.

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.

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-atlas[20], which is an MRI-cytoarchitectual map.

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.

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.

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DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1 Fukushima, M., Chao, Z. C., Fujii, N. Studying brain functions with mesoscopic measurements: Advances in electrocorticography for non-human primates. *Current Opinion in Neurobiology.* **32,** 124-131, doi:10.1016/j.conb.2015.03.015, (2015).

2 Nagasaka, Y., Shimoda, K., Fujii, N. Multidimensional recording (MDR) and data sharing: an ecological open research and educational platform for neuroscience. *PLoS One.* **6** (7), e22561, doi:10.1371/journal.pone.0022561, (2011).

3 Fukushima, M., *et al.* An electrocorticographic electrode array for simultaneous recording from medial, lateral, and intrasulcal surface of the cortex in macaque monkeys. *Journal of Neuroscience Methods.* **233,** 155-165, doi:10.1016/j.jneumeth.2014.06.022, (2014).

4 Komatsu, M., Sugano, E., Tomita, H., Fujii, N. A Chronically Implantable Bidirectional Neural Interface for Non-human Primates. *Frontiers in Neuroscience.* **11,** 514, doi:10.3389/fnins.2017.00514, (2017).

5 Komatsu, M., Takaura, K., Fujii, N. Mismatch negativity in common marmosets: Whole-cortical recordings with multi-channel electrocorticograms. *Scientific Reports.* **5,** 15006, doi:10.1038/srep15006, (2015).

6 Sasaki, E., *et al.* Generation of transgenic non-human primates with germline transmission. *Nature.* **459** (7246), 523-527, doi:10.1038/nature08090, (2009).

7 Okano, H., *et al.* Brain/MINDS: A Japanese National Brain Project for Marmoset Neuroscience. *Neuron.* **92** (3), 582-590, doi:10.1016/j.neuron.2016.10.018, (2016).

8 de la Mothe, L. A., Blumell, S., Kajikawa, Y., Hackett, T. A. Cortical connections of auditory cortex in marmoset monkeys: lateral belt and parabelt regions. *Anatomical Record.* **295** (5), 800-821, doi:10.1002/ar.22451, (2012).

9 Kaas, J. H., Hackett, T. A. Subdivisions of auditory cortex and processing streams in primates. *Proceedings of National Academy of Sciences of the United States of America.* **97** (22), 11793-11799, doi:10.1073/pnas.97.22.11793, (2000).

10 Ghahremani, M., Hutchison, R. M., Menon, R. S., Everling, S. Frontoparietal Functional Connectivity in the Common Marmoset. *Cerebral Cortex.* doi:10.1093/cercor/bhw198, (2016).

11 Belcher, A. M., *et al.* Functional Connectivity Hubs and Networks in the Awake Marmoset Brain. *Frontiers in Integrative Neuroscience.* **10,** 9, doi:10.3389/fnint.2016.00009, (2016).

12 Mitchell, J. F., Leopold, D. A. The marmoset monkey as a model for visual neuroscience. *Neuroscience Research.* **93,** 20-46, doi:10.1016/j.neures.2015.01.008, (2015).

13 Solomon, S. G., Rosa, M. G. A simpler primate brain: the visual system of the marmoset monkey. *Frontiers in Neural Circuits.* **8,** 96, doi:10.3389/fncir.2014.00096, (2014).
14 Burman, K. J., Palmer, S. M., Gamberini, M., Rosa, M. G. Cytoarchitectonic subdivisions of the dorsolateral frontal cortex of the marmoset monkey (Callithrix jacchus), and their projections to dorsal visual areas. *Journals of Comparative Neurology.* **495** (2), 149-172, doi:10.1002/cne.20837, (2006).
15 Bakola, S., Burman, K. J., Rosa, M. G. The cortical motor system of the marmoset monkey (Callithrix jacchus). *Neuroscience Research.* **93,** 72-81, doi:10.1016/j.neures.2014.11.003, (2015).
16 Krubitzer, L. A., Kaas, J. H. The organization and connections of somatosensory cortex in marmosets. *Journal of Neuroscience.* **10** (3), 952-974 (1990).
17 Miller, C. T., *et al.* Marmosets: A Neuroscientific Model of Human Social Behavior. *Neuron.* **90** (2), 219-233, doi:10.1016/j.neuron.2016.03.018, (2016).
18 Cox, R. W. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research.* **29** (3), 162-173 (1996).
19 Avants, B. B., *et al.* A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage.* **54** (3), 2033-2044, doi:10.1016/j.neuroimage.2010.09.025, (2011).
20 Hashikawa, T., Nakatomi, R., Iriki, A. Current models of the marmoset brain. *Neuroscience Research.* **93,** 116-127, doi:10.1016/j.neures.2015.01.009, (2015).

Figure1

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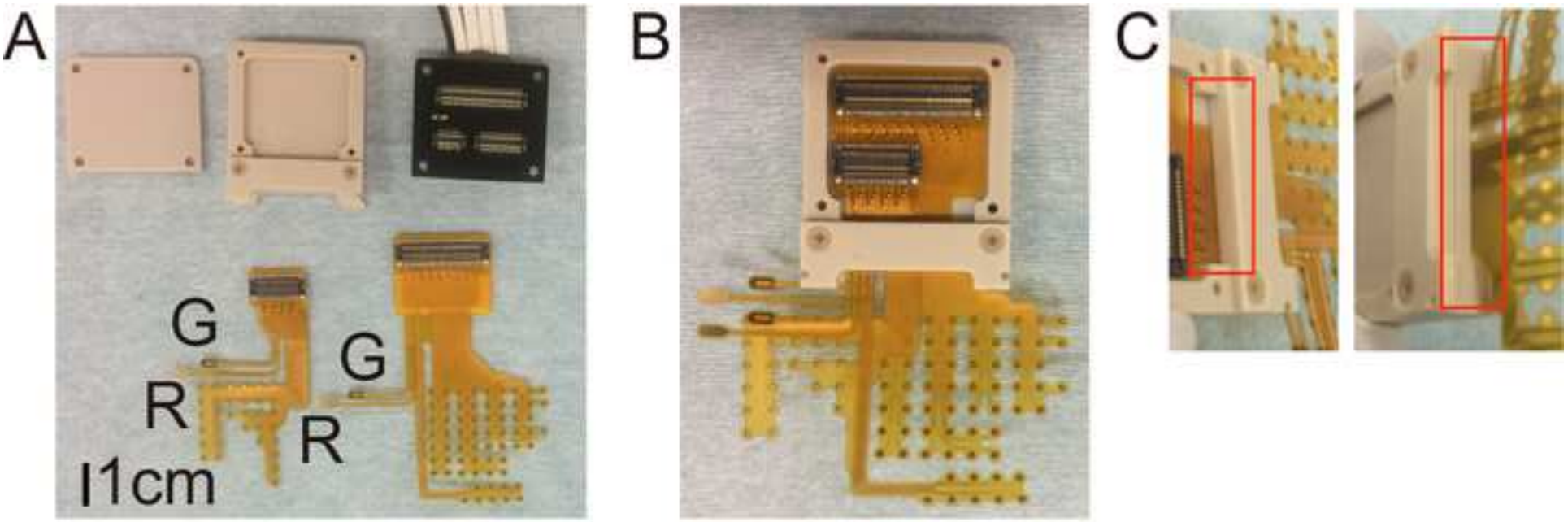


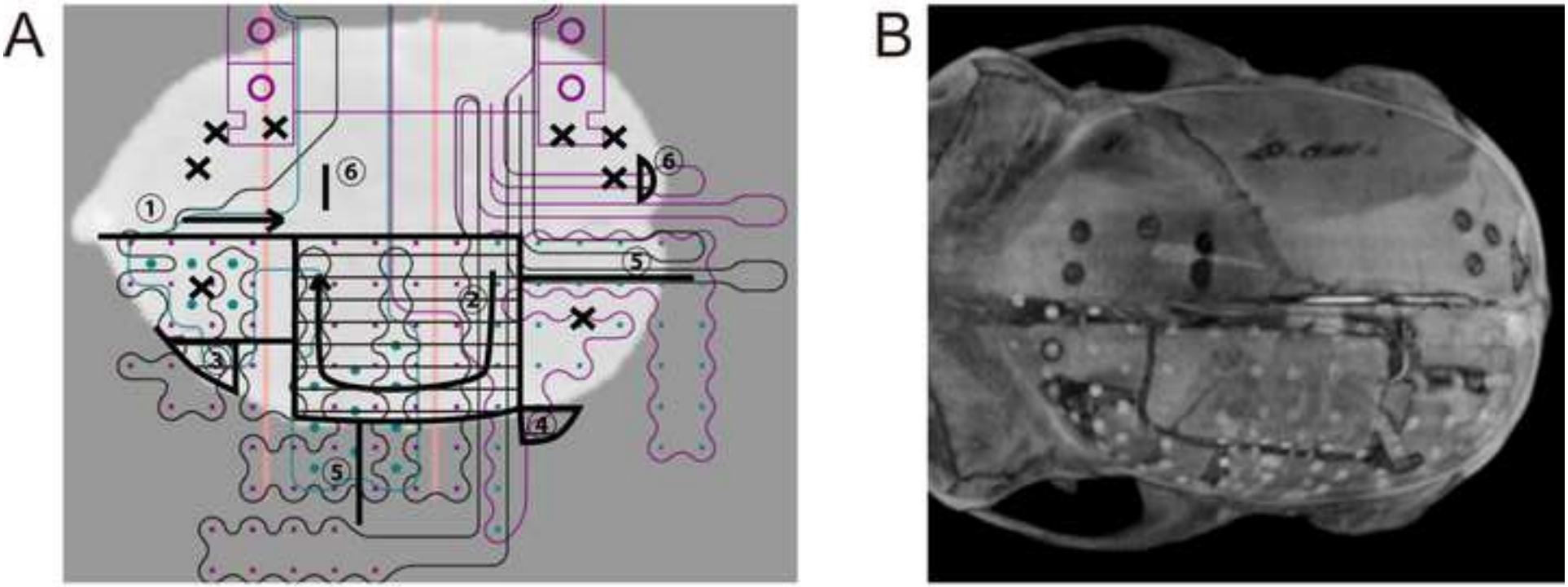
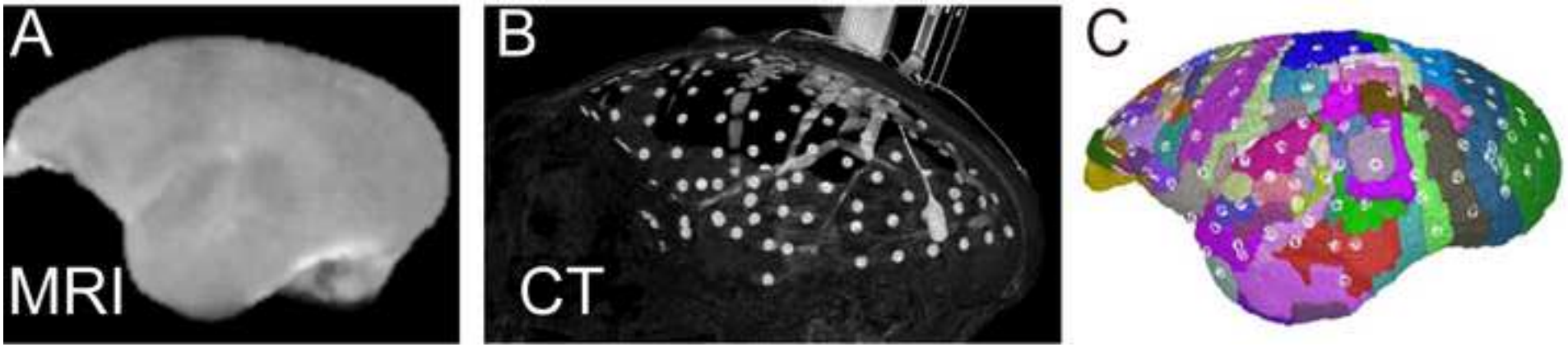
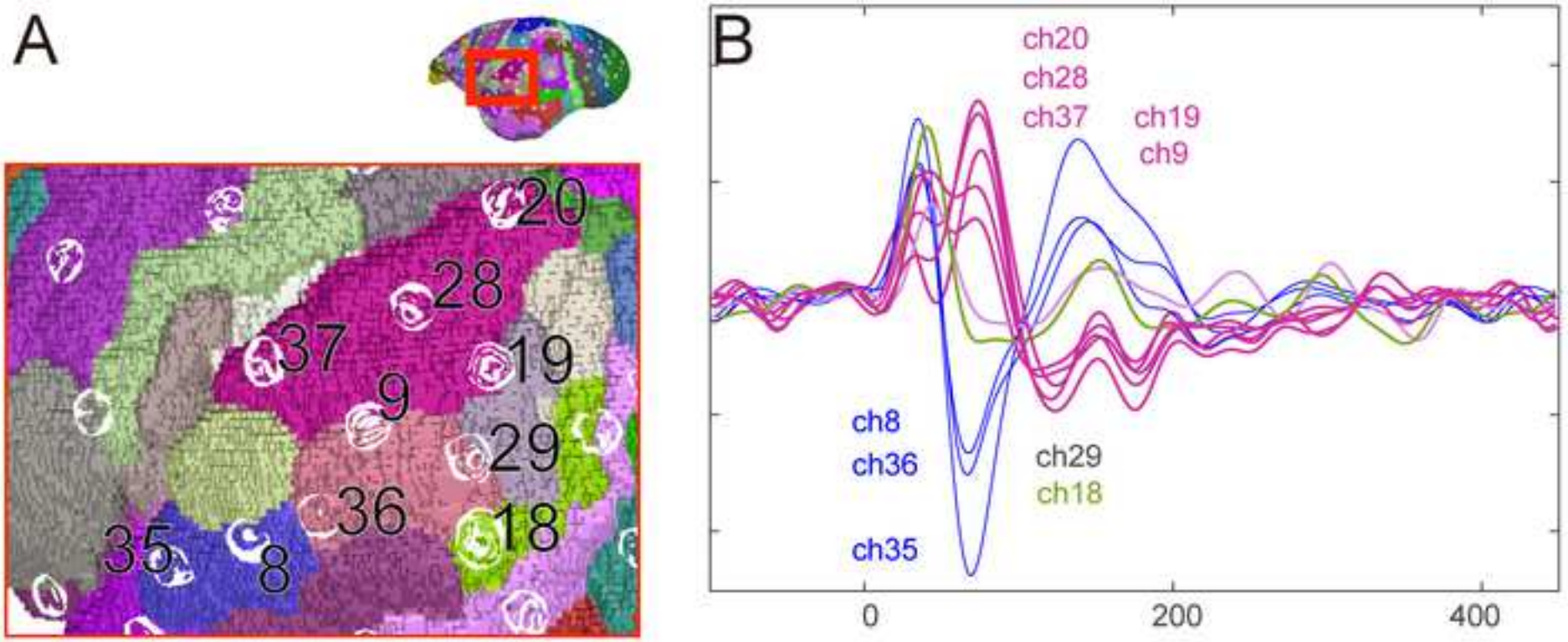


Figure3





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 of Materials

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Name of Material/ Equipment	Company
Beaker (100 cc)	
Cotton ball	
Absorption triangles	Fine Science Tools Inc.
Cotton swab with fine tip	Clean Cross Co., Ltd.
Gauze	
Towel forceps	
Scalpel handle	
Needle Holder	
Iris Scissor	
Micro-Mosquito Forceps	
Adson, 1x2 teeth	
Bone Curette	
Micro spatura	Fine Science Tools Inc.
Needle Holders, 12.5cm, Curved, Smooth Jaws	World Precision Instruments
Vessel Dilator, 12cm, 0.1mm tip	Fine Science Tools Inc.
Vessel Dilator, 12cm, 0.2 mm tip	Fine Science Tools Inc.
Fine-tipped rongeur	Fine Science Tools Inc.
Manipurator of a stereotaxic frame	
Wrench for the manipurator	
Hand-made fixture for the connector	
Silicon cup for dental acril	

Silicon cup hlder

Paintbrush

Pencil

Micro screw, 1.4 mm x 2.0 mm	Nippon Chemical Screw Co., Ltd.

Screw driver for the micro screw

Micromotor handpiece of a drill

Stainless steel burr, 1.4 mm

Stainless steel burr, 1.0 mm

Drill bit, 1.2 mm

Rubber air blower

Catalog Number	Comments/Description
	Outocrave
	Outocrave
18105-03	Outocrave
HUBY340 BB-013	Outocrave
	Outocrave
	Outocrave
	Outocrave
	Outocrave
	Outocrave
	Outocrave
	Outocrave
	Outocrave
10091-12	Outocrave
14132	Outocrave
18131-12	Outocrave
18132-12	Outocrave
16221-14	Outocrave
	Gas sterilization
	Gas sterilization
	Gas sterilization
	Gas sterilization

	Gas sterilization
	Gas sterilization
	Gas sterilization
PEEK/MPH-M1.4-L2	Gas sterilization
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Response to reviewers:

We greatly appreciate the reviewers' helpful comments and suggestions. Below, please find our point-by-point responses to these comments and queries, with the editorial and reviewers' comments appearing in italic typeface and our responses set in Times New Roman typeface.

Editorial comments:
Changes to be made by the author(s) regarding the written manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We used a professional English editorial service.

2. Please revise lines 114-117 and 125-127 to avoid previously published text.

We have revised these lines.

3. Please provide an email address for each author.

An email address for each author has been added.

4. Please expand your Introduction to include the following: A clear statement of the overall goal of this method; Information that can help readers to determine if the method is appropriate for their application.

We have revised the introduction.

5. JoVE cannot publish manuscripts containing commercial language… Examples of commercial sounding language in your manuscript are: Cir-Tech Inc., Shizuoka,

We have revised the relevant text.

6. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
7. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.)…

In accordance with comments 6, 7, and 11, we have entirely revised the protocol text.

8. Please add more details to your protocol step. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the

protocol. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below:

1.1: Obtain MRI on what animals and how? Please specify.

We have specified what animals were used, but further details are beyond the scope of our protocol.

1.2.3: Which gas is used to sterilize and how? Please specify.

We specified which gas was used (Line 92), but further details are beyond the scope of our protocol.

1.3: Please specify how to sterilize instruments. Please reference Table of Materials for all instrument used.

We have referred to the Table of Materials and have now specified sterilization methods for each instrument.

2.1.1: How is ketamine given to the marmoset? Also please specify the age and gender of marmoset used.

Ketamine was administered by an intramuscular injection. This information has been added. We have also specified the age, sex, and body weight of marmosets used (Line 70-71).

2.1.2: Please mention how proper anesthetization is confirmed.

Proper anesthetization is confirmed using body temperature, heart rate, and arterial blood oxygen saturation (SpO2). We have added this information.

2.1.3: What is used to rinse/remove the cream?

We have now specified what we used.

2.1.6: How to disinfect the surgical area?

We have now specified how to disinfect the surgical area.

2.2.1: How large is the incision? Please specify the surgical instrument used in this step. Please write all text in the imperative tense.

We have specified the size of the incision and have revised our description of this step.

2.2.6: What is used to wash?

This has now been specified.

2.3.2: How to keep the animal warm, using a blanket?

We used a heat pad; however, a blanket is also acceptable. We do not believe that an apparatus needs to be specified in this step.

2.3.4: What is SPO2?

SpO2 is arterial blood oxygen saturation (Line 111).

9. 3.4.2-3.4.4: If the authors plan to film these software steps, they must be more explicitly explained (e.g. button clicks for software actions, numerical values for settings, etc.).

We do not plan to film these steps.

10. What happens to the animal after the procedure? Do the electrodes stay in their head?

Yes, "chronic implantation" means the electrodes stay in their head.

11. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

In accordance with comments 6, 7, and 11, we have revised the protocol text entirely.

12. Please include single-line spaces between all paragraphs, headings, steps, etc.

We have corrected this.

13. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

14. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia.

15. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

In accordance with comments 13–15, we have highlighted the protocol for this video.

16. Table 1: Please change "mins" to "min" for the time unit.

We have revised "mins" to "min."

17. Figure 2: Please avoid handwriting. The labels may be difficult to read for some readers.

We avoided handwriting labels in Figure 2.

18. References: Please do not abbreviate journal titles.

This has been corrected.

Reviewers' comments:
Reviewer#1:
Major Concerns:
The paper is really short, and needs to be improved in terms of outlining a motivation for establishing a protocol for epidural EcoG in the marmoset. It lacks statistics (I have listed a few suggestions in my detailed comments below)

We revised the introduction and discussion sections to explain our motivation for establishing a protocol for epidural ECoG in the marmoset. We have also added some statistics.

Minor Concerns:
Needs a careful English revision

We have used a professional English editorial service.

Specific points:
1.2.2 Is "acryl" acrylic? I would like to see some expansion of the explanation of why there is a risk of blood inflow, where, and in which circumstances. The sentence as it stands is unclear (in terms of someone "visualising" what to do, and why this is important).

We changed the description in Line 90-91 to "Seal gaps of connector by acrylic glue to prevent the inflow of liquid during surgery."

1.2.3 How do you gas sterilise (how long, using what, etc.)

We used ethylene oxide gas. We have added this information (Line 92).

2. NOTE: The animals are not "required to abstain", the food is withdrawn.

This has been corrected.

2.1.1 ketamine is not a muscle relaxant, but an anaesthetic. Perhaps "anesthetize" or "heavily sedate" (this depends on the species and dose), but not "immobilize".

We changed "immobilize" to "induce anesthesia."

2.2.1 Temporal muscle (note: muscles) are removed from skull. Do you mean removed from skull (i.e. do you cut them off) or "detached from the skull" (i.e. just separated, but left intact). In general, this paragraph needs some English revisions.

We changed “removed from skull” to “detached from the skull.”

2.2.3 is very hard to visualise without a video, or a series of photos. This seems to be an important step but I can't comment.

2.2.4 do you mean "orbitofrontal"?

Yes, this has been corrected.

2.2.6 I am not sure what is meant by "remove bone tips". This seems like an English error, but I can't suggest an alternative without seeing the video.

We meant make the edge of the craniotomy area smooth by “removing bone tips.” However, we realize the same things is mentioned later, thus, we have removed the phrase “remove bone tips.”

2.2.9 unclear what this sentence means: "If a cause of the bending is small size of the brain compared with ECoG array, cut some of electrodes". Consider re-phrasing for clarity.

We re-phrased this to read: “If the bending was caused by the relatively small size of the brain, cut off some of the electrodes.”

2.2.11 "episkull" is not a word (mixes Greek and English). Epicranial, or simply "on the cranial surface".

The term “episkull” has been revised to “on the cranial surface.” (Line 170)

3.3 It would be useful to know some statistics. Out of how many experiments done, how many animals experience complications requiring further surgery.

We have implanted the array into 6 animals, and 3 of the animals required further surgery. We have now included these statistics.

Discussion:

- The table with surgery time would benefit from some statistics. For n surgeries, what is the average time to reach these steps, and the SD. Maybe consider time zero being "start preparations".

We added the number of surgeries (lines 70-71). We did not change the table because this is just an example plan of a surgery. We have now stated that the table is a recommended time course.

- "We successfully recorded neuronal signals with good quality at least for 4 months" - again, some statistics required.

We have added details.

- Has some comparison been made between epidural and subdural array implantations, and if not what are the advantages and disadvantages? The second approach seems to be required in order for arrays to cover the midline (cingulate) cortex, or cortex within a sulcus.

We have added the following description. "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 animal. 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 the difficulty of accessing the midline cortex and the cortex within a sulcus. Approach of these cortices require incision of the dura matter."

General points:

- This paper is a bit slim in terms of references. We are shown brain maps in the figures but the legends are minimal. The cortical map shown in the figures seems to be the Woodward 3-d version of the Paxinos cytoarchitectural map (the areas seem to follow the Paxinos scheme). This should be stated.

The atlas used in the manuscript is the Woodward 3D version based on the Hashikawa atlas (Hashikawa et al. 2015), which is a MRI-cytoarchitectural map. The annotation of this atlas indeed follows that of the Paxinos cytoarchitectural map, as the reviewer noted. We have added these statements and the reference in the legend of Figure 3.

- An introduction covering more of the motivation for studying the marmoset would make this paper more appealing to a wider audience. For example, it is unclear form the text that the visual, somatosensory, dorsolateral prefrontal, orbitofrontal, etc. cortical areas of this species have been mapped, and what is known about them that makes this species useful. What we get is a blanket statement and 4 references, which seem to be selected randomly. For example, 2 of the 4 references are to auditory papers, including one (number 7), which hardly talks about marmosets at all.

We have revised the statement about advantages of marmosets in the introduction section and references (Line 56-63).

- The discussion could also make a stronger case for the relevance of this procedure by mentioning specific issues in the literature, which could be solved by application of this method.

We have added the relevance of this procedure in the discussion section (line 299-302).

There are minor English errors throughout, which should be attended to before publication. For example (among others)

- "array consists of two sheet with 32…" (two sheets) This has been corrected.

- "monitored by vital signs" (for vital signs) This has been corrected.

- "should be totally rinsed on a skin" (off the skin) This has been corrected.

- "The both contacts face to skull" (maybe? Both contacts face the skull?) This has been corrected.

- "Resister marmoset brain atlas" (register) This has been corrected.

- "To success implantations, critical steps in our protocol is the same of the basics of surgical…" (not sure what is meant here) This has been removed.

- "Outocrave" is not a word - autoclave. Other spelling errors: spatura, manipurator. This has been corrected.

Reviewer#2:

Major Concerns:

In implantation procedures, authors made a plan for craniotomy and for design of the electrode array as shown in figure 2. However, this information is needed to scan the animal's head.

An MRI scan is needed before the surgery. We described this in 1.1.

Minor Concerns:

The authors used animal and monkey. The reviewer recommend to use one word for the same subject.

We have changed the term “monkey” to “animal.”

Reviewer#3:

Line 22 and 30 "... entire lateral surface ..." should be "entire dorsal surface."

Our array covers not only the dorsal surface but also the ventral surface. Thus, we used the term “lateral surface” rather than “dorsal surface.”

Line 71 to 73, the layout of the ECoG array is not described. To successfully cover a full hemisphere of a marmoset cortex, is it necessary to consider individual differences in brain size? Are the reference and grounding electrodes from the array facing the same side as the ECoG electrodes or not? In my opinion, these information about the design is as important as the detailed procedure for implantation in order to lead the readers having their own success if they try to use this manuscript as a guideline.

We have added the following explanation regarding the layout of the ECoG array. "To accommodate individual difference of brain size, the ECoG array has a flexible arm. The arm can cover the temporal pole, depending on individual brain shape. Place reference electrodes facing the opposite side to the ECoG electrodes, and the ground electrodes facing the same side." (Line 85-88).

Line 77, is the sealing acryl dental acrylic?

We changed "acryl" to "acrylic glue".

Line 128, they mentioned the bone piece will be returned after implanting the array. But until the end of the procedure, I didn't find any other sentence mentioning when and how they keep the bone during the surgery and put the bone piece back.

We have added the instruction "Put the bone piece back" (Line 173).

Line 141, they mentioned where the reference electrodes are placed in the other hemisphere, however, in Line 157, the reference electrodes were then facing the skull according to their protocol. It does not make sense. Thus, there is a small contradiction. Please clarify.

The reference electrodes are placed on the other hemisphere and their contacts face toward the skull. To clarify this, we changed "placed at the contra-lateral…" to "placed in the epidural space at the contra-lateral…" (Line 155).

Line 156, are the grounding electrodes facing skull with or without being fixed? If with, how did they fix the electrodes onto the skull. I believe they should be fixed, otherwise if there is any loose of contact to grounding, one might get artifact in the recorded data.

As you have mentioned, the ground electrodes were fixed onto the skull with dental acrylic. We have added this information (Line 169).

Line 188 to 191, how to remove the blood clot when there is already an ECoG implanted

needs to be further illustrated or at least described more in detail. Marmosets are delicate animal. The post-op treatments are especially important. To further illustrate this procedure will help readers to perform better in their own experiments when using the same technique.

We have added an explanation as to how to remove the blood clot (Line 208-210).

Line 225 "... and thick block lines indicate ...." Base on their figure and also the texts, I believe it should be "black lines."

We have corrected “block” to “black.”

In the Discussion section, the limitation of using ECoG array should be mentioned briefly. Because these are surface electrodes, it is difficult to pin down the specific signal source in cortical depth. Also, the current ECoG array, although covering as large as the technic allows, there are still several cortical areas in the medial and ventral sides that may be important in coordinating cognitive behavior but cannot be accessed with the current method.

We have added a section regarding the limitations of using an ECoG array, and the current method, to the discussion (Line 293-298).

Figure 2, 3, and 4, the resolution of the figures seems to be low. Please replace with higher quality images, if possible.

We have replaced the figures with higher resolution versions.

Thank you again for all your comments and suggestions. We hope we have adequately addressed all the points and question raised by the reviewers.

Response to the editor:

We greatly appreciate the editor's helpful comments and suggestions. Below, please find our point-by-point responses to these comments and queries, with the editorial comments appearing in italic typeface and our responses set in Times New Roman typeface.

Editorial comments:
Changes to be made by the author(s) regarding the written manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We used a professional English editorial service.

2. Please do not abbreviate journal titles for all references.

This has been corrected.

3. The short abstract is over 50 word limit.

We have revised the length of the short abstract.

4. Please use h, min, s for time units.

This has been corrected.

5. Please mention how proper anesthetization is confirmed.

We have added more details about how proper anesthetization is confirmed in Step 2.1.2.

6. Step 3.1: Please write this step in imperative tense.

We have revised this line as a notation.

Thank you again for all your comments and suggestions. We hope we have adequately addressed all the points and question raised by the reviewers.