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Stereotaxic Surgery for Implantation of Microelectrode Arrays in the Common Marmoset (Callithrix jacchus)

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Dr. Moshe Pritsker, Ph.D.
CEO, Co-Founder, Editor-in-Chief
JoVE

Dr. Ronald Myers, Ph.D.
Senior Science Editor
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Dear Dr. Pritsker and Dr. Myers,

Please find enclosed a revised version of the previously submitted manuscript # 60240_R1, titled "Stereotaxic surgery for implantation of microelectrode arrays in the common marmoset (*Callithrix jacchus*)." Thank you for giving us the opportunity to revise and submit this manuscript after expressing an interest in our work published earlier this year. We would particularly like to thank the reviewers for their careful and constructive reviews. We appreciate the time and detailed comments provided by each reviewer, and are very encouraged that the reviewers' each agreed our work addresses an important need. We would like to reaffirm that, as highlighted by your community's interest, disseminating surgical knowledge related to the common marmoset will greatly help the Neurosciences community.

We believe we have addressed the major and minor concerns raised by each reviewer and the editorial team. In so doing we are happy to say that by incorporating the suggested changes, wherever possible, we have greatly improved our manuscript. The editors asked for us to describe the results of the experiment we conducted and the conclusion that we were able to draw from it. In our detailed response we have highlighted the types of data and results that will be obtained using all of these relevant techniques together, and we have provided exemplary references throughout the manuscript.

Reviewer 1 indicated concern regarding our postoperative anesthetic protocol used when attaching the connectors. We completely agree that frequent anesthesia should be avoided and have taken great care in minimizing the use of such procedures; in line with the NIH's Guide for the use and Care of laboratory animals, and our institution's policies. In addition to respecting these internationally recognized animal welfare guidelines, we follow

strict experimental procedures to reduce confounding effects. We provide a more detailed description of the analgesic procedures we employ, further helping our manuscript conform with the NC3Rs ARRIVE guidelines, while demonstrating our full compliance with our institution's rules and the NIH's Guide for the use and Care of laboratory animals.

Reviewers 2 and 3 expressed interest in our electrode design and fabrication methodology. We have provided more details on the specific design of the electrode arrays that we have implanted. Also, we made reference to published work that have previously documented the methodology to manufacture this kind of electrode array. Appended to this letter is a point-by-point response to the comments raised by the reviewers and editors.

To date, there are few accounts of how to perform neurosurgery in the marmoset. This is because >80% of NHPs used in research involving surgeries are macaques. However, with the growing use of the common marmosets in recent years it is important for more researchers to be educated on the nuances of working with this species. We hope our revisions will allow us to share what we have learned with the scientific community.

We confirm that the manuscript describes original work and is not under consideration for publication elsewhere. We have no conflicts of interest, and all authors approved the final version of the manuscript and agreed with its submission to *JoVE*.

I look forward to hearing from you, as the corresponding author, in due time regarding our submission and to respond to any further questions and comments.

Kind regards,

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TITLE:

Stereotaxic Surgery for Implantation of Microelectrode Arrays in the Common Marmoset (*Callithrix jacchus*)

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KEYWORDS:

marmoset, stereotaxic surgery, electrophysiology, neurosurgery, non-human primates, microelectrode array

SUMMARY:

This work presents a protocol to perform a stereotaxic, neurosurgical implantation of microelectrode arrays in the common marmoset. This method specifically enables electrophysiological recordings in freely behaving animals but can be easily adapted to any other similar neurosurgical intervention in this species (e.g., cannula for drug administration or electrodes for brain stimulation).

ABSTRACT:

Marmosets (*Callithrix jacchus*) are small non-human primates that are gaining popularity in biomedical and preclinical research, including the neurosciences. Phylogenetically, these animals are much closer to humans than rodents. They also display complex behaviors, including a wide range of vocalizations and social interactions. Here, an effective stereotaxic neurosurgical procedure for implantation of recording electrode arrays in the common marmoset is described. This protocol also details the pre- and postoperative steps of animal care that are required to

successfully perform such a surgery. Finally, this protocol shows an example of local field potential and spike activity recordings in a freely behaving marmoset 1 week after the surgical procedure. Overall, this method provides an opportunity to study the brain function in awake and freely behaving marmosets. The same protocol can be readily used by researchers working with other small primates. In addition, it can be easily modified to allow other studies requiring implants, such as stimulating electrodes, microinjections, implantation of optrodes or guide cannulas, or ablation of discrete tissue regions.

INTRODUCTION:

Common marmosets (*Callithrix jacchus*) are gaining recognition as an important model organism in many fields of research, including neuroscience. These new-world primates represent an important complementary animal model to both rodents and other non-human primates (NHPs), such as the rhesus macaque. Like rodents, these animals are small, easy to manipulate, and relatively economical to care for and breed[1-4], as compared with larger NHPs. Furthermore, these animals have a propensity for twinning and high fecundity relative to other NHPs[1-3]. Another advantage the marmoset has over many other primates is that modern molecular biology tools[3-7] and a sequenced genome[2-5,8] have been used to genetically modify them. Both knock-in animals using lentivirus[5], and knock-out animals using zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENS)[7], have yielded viable founder animals.

An advantage in relation to rodents is that marmosets, as primates, are phylogenetically closer to humans[3,5-6,9-11]. Like humans, marmosets are diurnal animals that depend on a highly developed visual system to guide much of their behavior[10]. Further, marmosets exhibit behavioral complexity, including a wide range of social behaviors such as the use of different vocalizations[3], allowing researchers to address questions not possible in other species. From a neuroscientific perspective, marmosets have lissencephaly brains, unlike the more commonly used rhesus macaque[9]. Furthermore, marmosets have a central nervous system similar to humans, including a more highly developed prefrontal cortex[9]. Together, all these characteristics position marmosets as a valuable model to study brain function in health and disease.

A common method for studying brain function involves implanting electrodes in anatomically specific locations by means of stereotaxic neurosurgery. This allows for chronical recording of the neural activity in different target areas in awake and freely behaving animals[12-13]. Stereotaxic neurosurgery is an indispensable technique used in many lines of research, as it allows precise targeting of neuroanatomical regions. Compared to macaque and rodent literature, there are fewer published studies describing the stereotaxic neurosurgery specific to the marmoset, and they tend to provide sparse detail of the steps involved in the surgery. Moreover, those with greater detail mainly focus on procedures for electrophysiology recording in head-restrained animals[14-17].

In order to facilitate wider adoption of marmosets as a model organism in neuroscience research, the present method defines specific steps necessary for a successful stereotaxic neurosurgery in this species. In addition to implantation of recording arrays, as detailed in the present method, the same technique can be adapted for many other experimental ends, including implantation of

stimulating electrodes for the treatment of diseases[18] or causally driving circuit behavior[19]; implantation of guide cannulas for extraction and quantification of neurotransmitters[20], injections of reagents, including those for inducing disease models[12] or for circuit tracing studies[15]; ablation of discrete tissue regions[21]; implantation of optrodes for optogenetic studies[22]; implantation of optical windows for cortical microscopic analysis[23]; and implantation of electrocorticographic (ECoG) arrays[24]. Thus, the overall goal of this procedure is to outline the surgical steps involved in the implantation of microelectrode arrays for chronic electrophysiological recordings in freely behaving marmosets.

PROTOCOL:

Animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Santos Dumont Institute Ethics Committee (protocol 02/2015AAS).

1. Surgery preparation

1.1. Attach each electrode array to an electrode holder compatible with the stereotaxic frame to be used.

1.2. Connect one electrode holder to the stereotaxic micromanipulator and set one microwire to the interaural coordinates. Repeat this for the additional electrode arrays and holders, if necessary.

NOTE: The interaural coordinate of any microwire can be used to calculate the implantation coordinates for the entire array, because the relative distance between the microwires is constant. When the array has bundles with different lengths, the interaural coordinate of the longest wire is the most convenient to use for setting the interaural zero.

1.3. Detach the electrode holders from the stereotaxic micromanipulator and sterilize the assemblies (electrode attached to the electrode holder) in an ultraviolet light (UV) cabinet for at least 2 h.

1.4. Attach a 24 G needle to a stereotaxic probe holder, connect it to the micromanipulator and set the interaural coordinates for the tip of the needle.

NOTE: Prior to the surgery, the coordinates of all craniotomies must be predefined as a perimeter 200 μm^2 larger than the anteroposterior (AP) and mediolateral (ML) position of the array's target implantation site. Use the 24 G needle-probe holder assembly to determine the position of the craniotomies in the skull based on the zero interaural coordinates.

1.5. Detach the probe holder from the micromanipulator and sterilize the assembly in a UV cabinet for at least 2 h.

1.6. Gather 6−8 titanium or stainless-steel screws. Solder a ground wire to half of them.

1.7. Organize and sterilize all remaining instruments, equipment, and disposables required for the surgery.

2. Preoperative procedures

NOTE: Two adult male marmosets (*Callithrix jacchus*) weighing 320–370 g were used in this study. Ensure that the animal has not eaten for 6 h prior to the induction of anesthesia.

2.1. Anesthetize the animal with an intramuscular injection of atropine (0.05 mg/kg) to reduce salivation and bronchial secretions. Check for the lack of pedal responses.

2.2. After 5 min apply ketamine (10−20 mg/kg) intramuscularly.

2.3. Shave the animal's head using an electric barber clipper.

2.4. Administer tramadol (2 mg/kg) intramuscularly as a general analgesic.

2.5. Intubate the animal.

2.5.1. Using a mask, expose the marmoset to isoflurane in 1−2% oxygen with a flow rate of 1−5 L/min to induce deep anesthesia. When the animal is deeply anesthetized, reduce and maintain isoflurane to 1−3 L/min.

2.5.2. Attach an elastic band to the surgical table with tape.

2.5.3. Position the marmoset in a supine position with the head toward the technician and place the elastic band in the marmoset's mouth behind its canines.

NOTE: It is best to position the head such that the dorsal surface is pointed toward the floor and its face is toward the technician.

2.5.4. Using a cotton-tipped probe, swab dry the marmoset's tongue, and grasp it in one hand to keep the mouth open.

2.5.5. Spray 10% lidocaine on the tip of the endotracheal tube.

2.5.6. Insert the uncuffed, 2.0 mm diameter endotracheal tube into the trachea until the 4.0 cm mark is at the entrance of the trachea.

2.5.7. Attach the tube to the anesthesia assembly with the artificial ventilator set to 40 breaths/min and confirm proper expansion and contraction of the chest.

NOTE: At this time the isoflurane and oxygen should be delivered via the endotracheal tube, not the mask.

2.5.8. Remove the elastic band from the marmoset's mouth so the endotracheal tube can be taped to the jaw.

2.6. Position the marmoset in a prone position in line with the stereotaxic frame and fix the animal's head into the stereotaxic frame.

2.6.1. First, insert the tip of the right ear bar into the animal's right auditory canal.

2.6.2. Next, insert the tip of the left ear bar into the left auditory canal.

2.6.3. Center the animal's head at the center of the stereotaxic frame and fix the ear bars in place.

2.6.4. Insert the mouthpiece into the animal's mouth and adjust its height so that it touches the animal's palate. At the same time, position the orbital bars at the lower surface of the orbital bone.

2.6.5. Make sure that the lower surface of the orbital bone is horizontally aligned with the center of the ear bars.

2.7. Connect a portable pulse oximeter to the marmoset's hand. Ensure that the heart rate is within 154−180 beats/min (bpm) for the duration of the surgery; often a heart rate above 200 bpm implies the animal is waking up. Ensure that the oxygen saturation is above 95%. It may occasionally drop to 90% without harm.

NOTE: Should the heart rate drop below 154 bpm, decrease the isoflurane.

2.8. Position the rectal temperature probe connected to a homeothermic heating pad into the anus, with the desired temperature set for 37 °C. Tape this sensor to the tail to keep it fixed in place.

2.9. Apply sterile ophthalmic lubricant to the eyes.

2.10. Clean and disinfect the animal's head with chlorhexidine and povidone iodine before covering the animal with a surgical field.

NOTE: Carry out all the following surgical procedures under aseptic conditions.

3. Surgery procedures

3.1. Apply local analgesic subcutaneously (e.g., lidocaine 20 mg/mL, 0.1 mL) at the site of the intended incision. Make an incision in the midline of the scalp.

3.2. Expose and prepare the surface of the skull.

3.2.1. Carefully detach the temporal muscle from the cranium. First, use a scalpel to cut the fascia at its insertion into the skull. Then, gently separate the temporal muscle from the cranium using a periosteal raspatory.

3.2.2. Remove the periosteum from all exposed cranium using a periosteal raspatory.

3.2.3. Control the bleeding with a sterile cotton swab, if necessary.

3.2.4. Clean the bone surface with hydrogen peroxide.

3.3. Delineate the location of the craniotomy by marking its corners with shallow burr holes into the bone surface. Then, drill out the perimeter of the craniotomy using a dental drill at maximum speed (i.e., 350,000 rpm). Add a few drops of sterile saline over the skull while drilling to prevent overheating. Measure the position of the craniotomy and the coordinates of the electrode implant with respect to the interaural coordinates.

3.4. Implant screws into the skull.

3.4.1. Drill 6−8 screw holes into the cranium.

3.4.2. Implant the screws such that each ground wire fused screw is adjacent to and in the proximity of an unaltered screw (i.e., without a ground wire attached to it).

3.4.3. Wind each ground wire around the adjacent, unaltered screw.

3.4.4. Add a drop of silver paint between the ground wire and each screw.

3.5. Remove the bone at the center of the craniotomy using forceps with a curved tip (e.g., McPherson forceps). Keep the dura mater hydrated with sterile saline.

3.6. Remove the dura mater. Use a sterile hypodermic needle (25 or 26 G) with the bevel bent at approximately 90˚ to puncture and lift the surface of the dura mater away from the brain surface. Then, cut the dura mater with microscissors. Keep the exposed brain hydrated with saline.

NOTE: If significant dural bleeding is observed, use cautery or sterile absorbent gelatin sponges soaked in thrombine[25].

3.7. Implant microelectrode arrays.

3.7.1. Attach the sterilized electrode holder and electrode array to the stereotaxic micromanipulator.

3.7.2. Position the micromanipulator such that the electrode is at the desired anteroposterior and mediolateral coordinates.

3.7.3. Lower the electrode array until the tip of the longest bundle touches the surface of the brain.

3.7.4. Slowly insert the array into the brain tissue until it reaches the dorsoventral coordinates.

3.7.5. Cover the exposed cortex with small pieces of sterile, absorbent gelatin sponges.

3.7.6. Secure the electrode to the skull by applying dental acrylic to the exposed skull, one screw, and the electrode.

3.7.7. Detach the electrode holder and remove it from the micromanipulator.

3.8. Repeat the implantation procedure from step 3.7 with the additional arrays, if necessary.

3.9. Wind together and weld the ground wires of the separate arrays and screws. Use silver paint to form a bridge around the weld to ensure an electrical connection has been achieved.

3.10. Using dental acrylic, make a sturdy headcap around the lateral extent of the arrays, and completely encase the ground wires and any exposed skull and screws.

3.11. If necessary, insert a support bar into the headcap. This can be a sturdy plastic cylinder like those from a cotton swab. Seal it into place with the dental acrylic.

NOTE: This can be helpful in securing the electrophysiology cable connectors in place but may be unnecessary depending on the equipment used. In the present method, a similar support rod is affixed to the head stage such that an elastic band can robustly hold the head stages in place on the connectors.

3.12. Suture the skin around the headcap.

4. Postoperative recovery

4.1. Apply antiseptic solution (e.g., chlorhexidine) around the wound.

4.2. Turn off the isoflurane supply but not the oxygen and remove the animal from the stereotaxic frame.

4.3. Place the animal onto the heating pad with the oxygen maintained through the endotracheal tube.

4.4. Remove the endotracheal tube when the first signs of neurogenic reflexes, such as laryngospasms, are observed.

4.5. Keep supplying the oxygen with a mask until the animal presents clear signs of anesthetic recovery, such as protective reflexes, postural tone, and attempts to ambulate.

4.6. Place the animal inside a clean cage in a recovery room for 24−48 h before moving the animal to its home cage. House each implanted animal individually.

NOTE: Because marmosets tend to climb the cage walls, use a cage with smooth walls or cover the cage walls with a smooth surface to prevent the animal from falling.

4.7. In the first hour following surgery, observe the animal to watch for signs of distress or uncoordinated head contact against the side of the cage.

4.8. Administer antibiotics (e.g., enrofloxacin 5 mg/kg, subcutaneously, once a day for 5−7 days), analgesics (e.g., oral tramadol 1 mg/kg, every 8 h for 3−5 days) and anti-inflammatory drugs (e.g., dexamethasone 0.5−1.5 mg/kg, subcutaneously, once a day for 1−3 days).

NOTE: After a successful surgery, animals will be fully recovered within 3−5 days.

5. Chronic electrophysiological recordings in freely behaving marmosets

5.1. Start the electrophysiological recording sessions at least 1 week after the surgery.

NOTE: Habituate the animals to the researcher and experimental environments before starting all experimental procedures for at least 1 month.

5.2. At the beginning of each session, lightly anesthetize the animal using isoflurane (1−5 L/min, 1% O_2).

NOTE: Follow the relevant institution's guidelines regarding the sedation of small primates. If recording sessions are very frequent, habituate the animals to be handled so that cables can be connected without anesthesia.

5.3. Connect the electrode arrays to a commercial neural recording system.

5.4. Place the animal inside the experimental chamber.

NOTE: The experimental chamber used here is a cubic acrylic box (0.45 m x 0.45 m x 0.45 m) designed to evaluate the amount and pattern of spontaneous motor activity[26,27].

5.5. Wait for 30 min before starting the recordings to ensure the animal is fully recovered from anesthesia.

NOTE: Isoflurane has a rapid onset and offset action which allows for rapid sedation and awakening[28]. Once the isoflurane supply is turned off, the animal will start to wake up. The animal is awake when it stays in the upright position and can ambulate freely in the experimental chamber without falling. This takes less than 15 min. To ensure the absence of any sedative effects, begin the recordings 30 min after the isoflurane is discontinued.

5.6. Confirm the position of the microelectrode array implants postmortem by NISSL staining after fixing and sectioning the tissue[29].

REPRESENTATIVE RESULTS:

The purpose of this study was to describe a stereotaxic neurosurgical procedure for implantation of microelectrode arrays for electrophysiological recordings in the common marmoset. A typical surgery (from anesthesia induction to anesthesia recovery) will last for approximately 5−7 h, depending on the number of arrays implanted. Here, two arrays were symmetrically implanted, one in each brain hemisphere. Each array contained 32 stainless steel microwires arranged in seven bundles targeting several structures of the basal ganglia-corticothalamic circuit (**Figure 1**), but the electrode design and targeted brain regions may vary depending on the experiment. After successful surgery and postoperative procedures, the animal should be fully recovered within 3−5 days. If the array has been grounded and implanted properly it will be possible to record spikes (**Figure 2A**) and local field potentials (**Figure 2B**) in freely behaving animals over several weeks or months, before a mature gliotic scar is established[13,30]. As an example, the electrophysiological data collected in the experimental paradigm described here has been effectively used to study the concurrent activity of different regions of the basal ganglia-corticothalamic circuit during spontaneous, ground-based locomotion in a model of Parkinson's disease[26].

Finally, a successful surgery also involves implanting the arrays into the targeted structures. Non-invasive imaging methodologies, such as MRI or tomography may be performed following the surgery and prior to beginning experimental recordings. Use of such methodology will be possible only if the specific implants used are manufactured to be compatible with such techniques, and if the researcher has access to appropriate small animal equipment. Ultimate confirmation can also be performed postmortem. Nissl stained sections containing electrode tracks can be used to precisely determine the position of each implanted microwire (**Figure 3**). Notice that electrode tracks in coronal sections appear as tears in the tissue. Thus extreme care must be used when sectioning is performed to reduce the chance of creating artifacts that will confound interpretation.

FIGURE AND TABLE LEGENDS:

Figure 1: Microelectrode array for implantation in small primates. The array was composed of 32 stainless steel microwires. The wires were 50 µm in diameter and were organized in seven bundles aimed to reach the following areas: primary motor cortex (M1), putamen (Put), caudate (Cd), globus pallidus (GPe), ventrolateral and ventroposterior lateral thalamic nucleus (VPL), and subthalamic nucleus (STN). The interelectrode spacing in each bundle was 300 µm. The

interbundle spacing depends on the target coordinates for each brain region. More detailed information about microelectrode array design and manufacturing can be found in Nicolelis[31], Lehew and Nicolelis[32], and Dizirasa et al.[33]. Scale bar = 5 mm.

Figure 2: Representative electrophysiological result following a successful surgery. The left panel shows spike activity of two neurons (yellow and green waveforms) recorded from one electrode. The right panel shows local field potential oscillations recorded from 14 electrodes.

Figure 3: Nissl stained tissue section demonstrating an electrode track. This section (anteroposterior coordinate, relative to interaural line: +8.0, according to the atlas by Paxinos and Watson[34]) depicts an electrode track with the tip at the Putamen, as indicated by the black triangle. Scale bar = 1 mm.

DISCUSSION:

This work provides a detailed description of the procedures involved in the implantation of microelectrode recording arrays in the marmoset brain. This same protocol can be readily used when implanting electrodes, whether homemade or commercially available, in other small primates. Additionally, it can be easily adapted for other experimental ends that require precise targeting of brain structures. Therefore, this protocol is purposefully vague regarding stereotaxic coordinates and cranial drilling techniques, because those are the aspects that may vary the most. For instance, to implant the arrays used in this surgery, craniotomies were performed to open two appropriately sized windows in each hemisphere. However, when implanting sturdy, individual structures, such as guide cannulas, neither this nor the durectomy is necessary. Rather, a simple burr hole to the level of the dura will suffice. Similarly, when nonelectrical implants are involved it is not necessary for the screws to be grounded. Thus, step 3.9 in the surgical protocol can be omitted. Instead, dental acrylic can be used to simply cover the exposed skull, implant, and screws.

Regardless of the specific experimental goal of the stereotaxic neurosurgery, successful implementation of the given procedure largely revolves around good surgical practices. This means that rigorous protocols must be followed to perform the surgery under aseptic conditions in order to prevent postoperative infections[35]. Some of the most critical moments are inducing and removing anesthesia. It is therefore essential that the vital signs of the animal (heart rate, blood oxygen saturation, and body temperature) be monitored throughout the entire surgical procedure[36]. If a decrease in the heart rate with a concurrent drop in oxygen saturation occurs, confirm that the chest is inflating and deflating normally, otherwise the connection to the breathing machine may be at fault. The first thing that can be done to attempt to recover the heart rate and oxygen saturation is to decrease the isoflurane concentration. If this does not resolve the issue, atropine may be administered intramuscularly to increase the heart rate and attempt to stabilize the animal. This must be done extremely cautiously, because previous experience shows that a heart rate above 200 bpm without sufficient isoflurane will awaken the animal.

Unlike rodents, in primates all coordinates are usually measured relative to the interaural coordinate, not the bregma and lambda[34]. Therefore, it is important to measure the interaural zero coordinates of the electrode arrays and other probes before fixing the animal's head in the stereotaxic apparatus. Moreover, in marmosets the horizontal plane is defined as the plane passing through the lower margin of the orbital bone and the center of the external auditory meatus. Thus, it is important to align the lower surface of the orbital bone with the center of the ear bars before fixing the head in the stereotaxic frame. Furthermore, the temporal muscles of the marmoset cover a wide area of the cranium. Thus, many neural targets require craniotomies to be performed under or in very close proximity to this musculature. Because these muscles are important for marmoset communication[38], the surgeon must slowly and carefully detach this musculature from the cranium to minimize damage.

Researchers familiar with behavioral work involving either rodents or marmosets should be aware of several limitations when performing electrophysiology in freely behaving NHPs. First, in the present arrangement and others involving high density arrays or multiple arrays, it is likely that inducing light anesthesia will be required to attach the cable connectors, even after appropriate habituation. This procedure, while within the scope of NIH's and other countries' regulatory guidelines, should be performed sparingly to reduce the mental and physical stress on the marmoset. Furthermore, it is critical that the researcher ensure the animal is fully recovered from anesthesia before beginning data acquisition, otherwise the anesthesia may confound the data[39]. Another related limitation is the physical presence of the cable itself. While wireless recording solutions are becoming available[40], the more common wired options impose a physical restriction on the animal. Finally, the experimental chamber being used will also restrict the range of behaviors available to the marmoset. Unlike rodents, marmosets display unique behaviors (e.g., climbing) that will not be possible depending on the experimental chamber being used.

Advances in material science and engineering are leading to the novel neural interfaces[41]. Effective neurosurgical procedures, such as the one described in this manuscript, will allow researchers to implement these new and forthcoming tools in marmosets. Combined with the concurrent developments in molecular biology[3-5], marmosets have the potential to allow investigations of important basic and clinical questions in neuroscience.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

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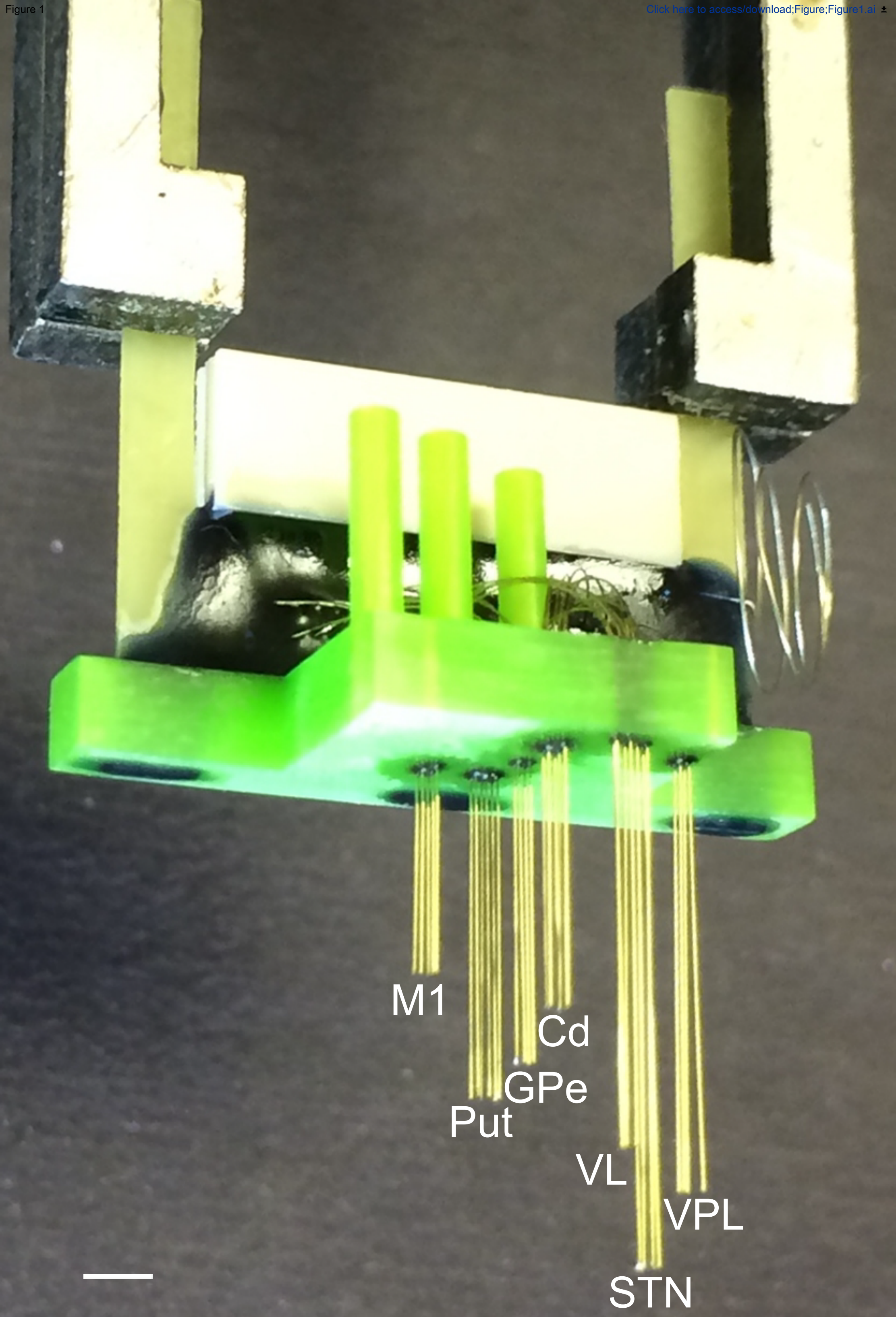
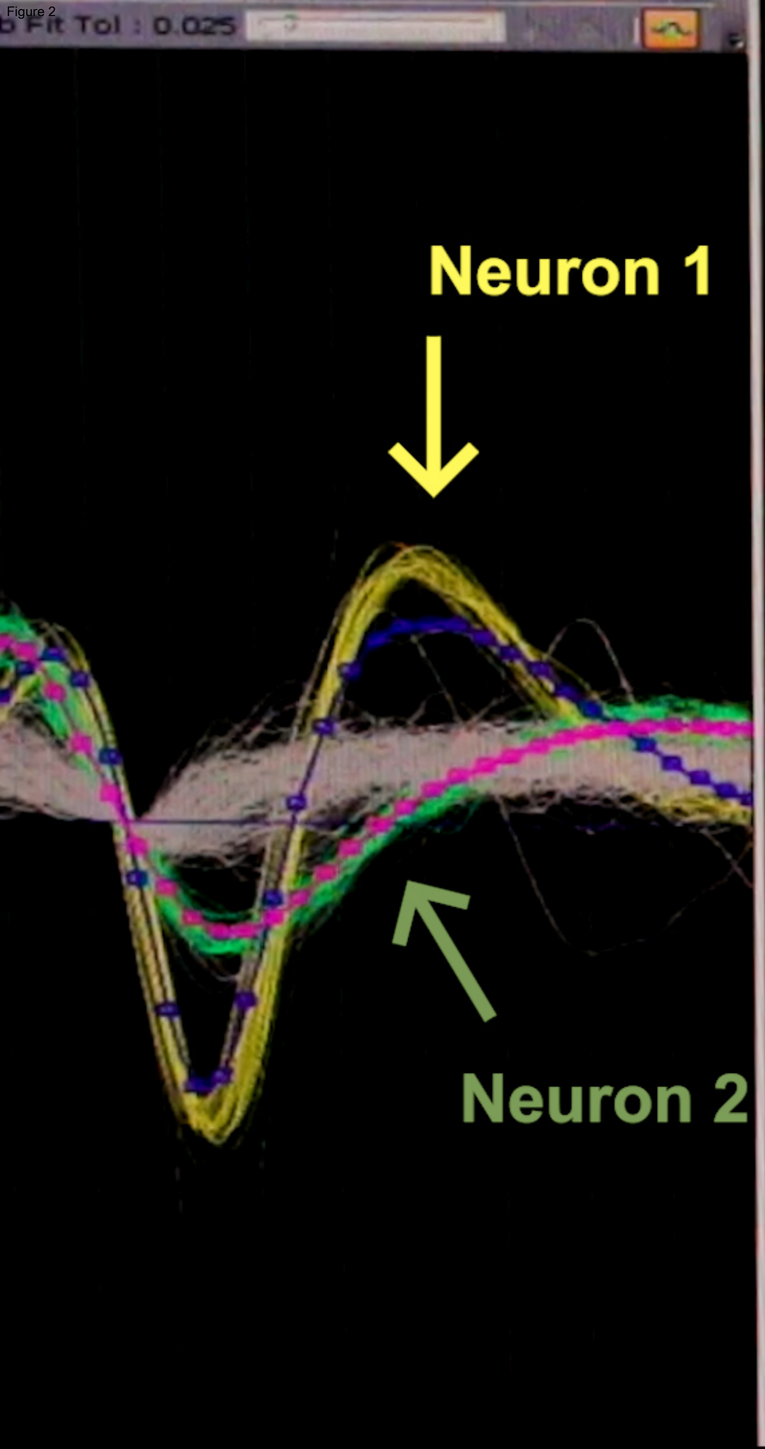


Figure 2



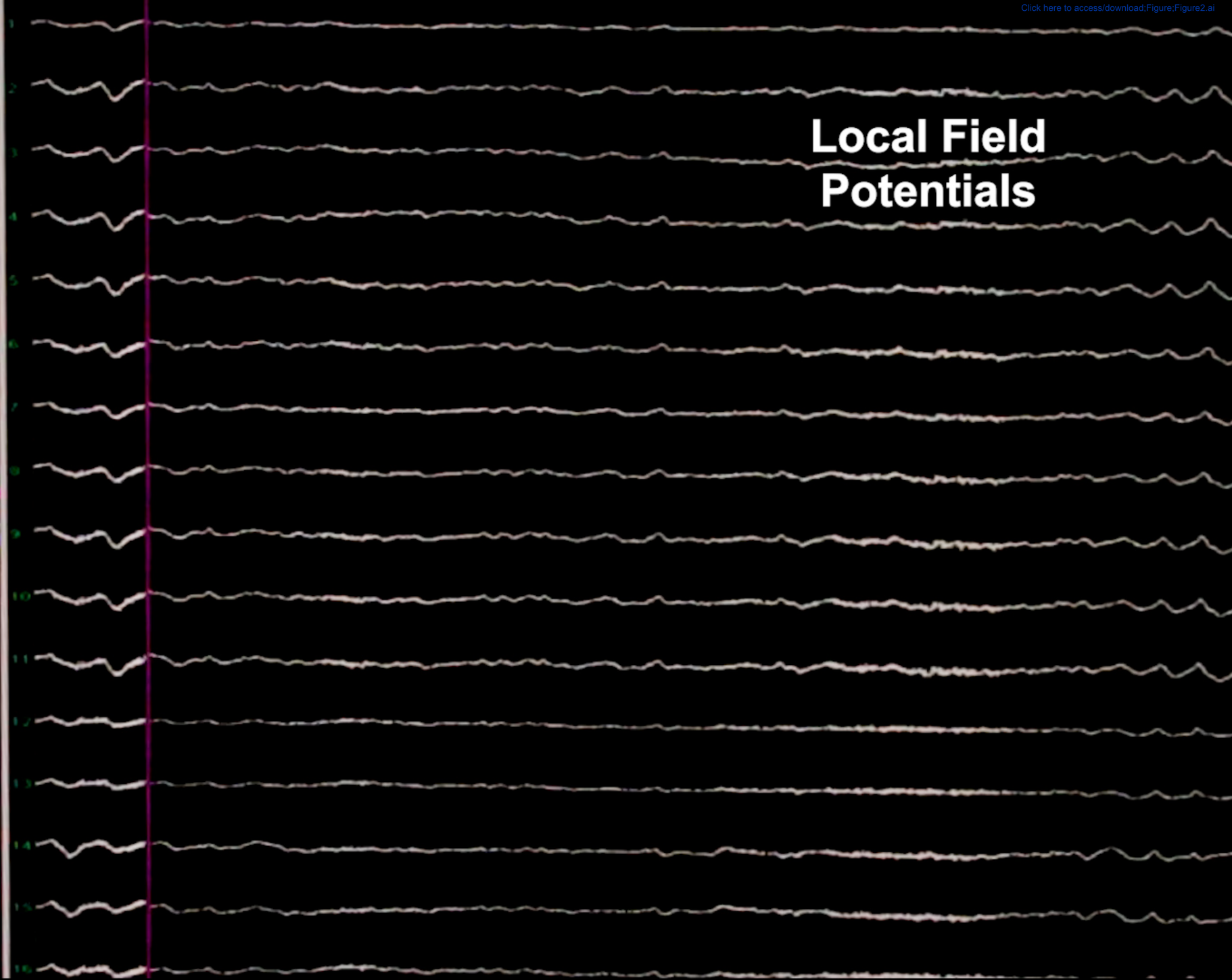
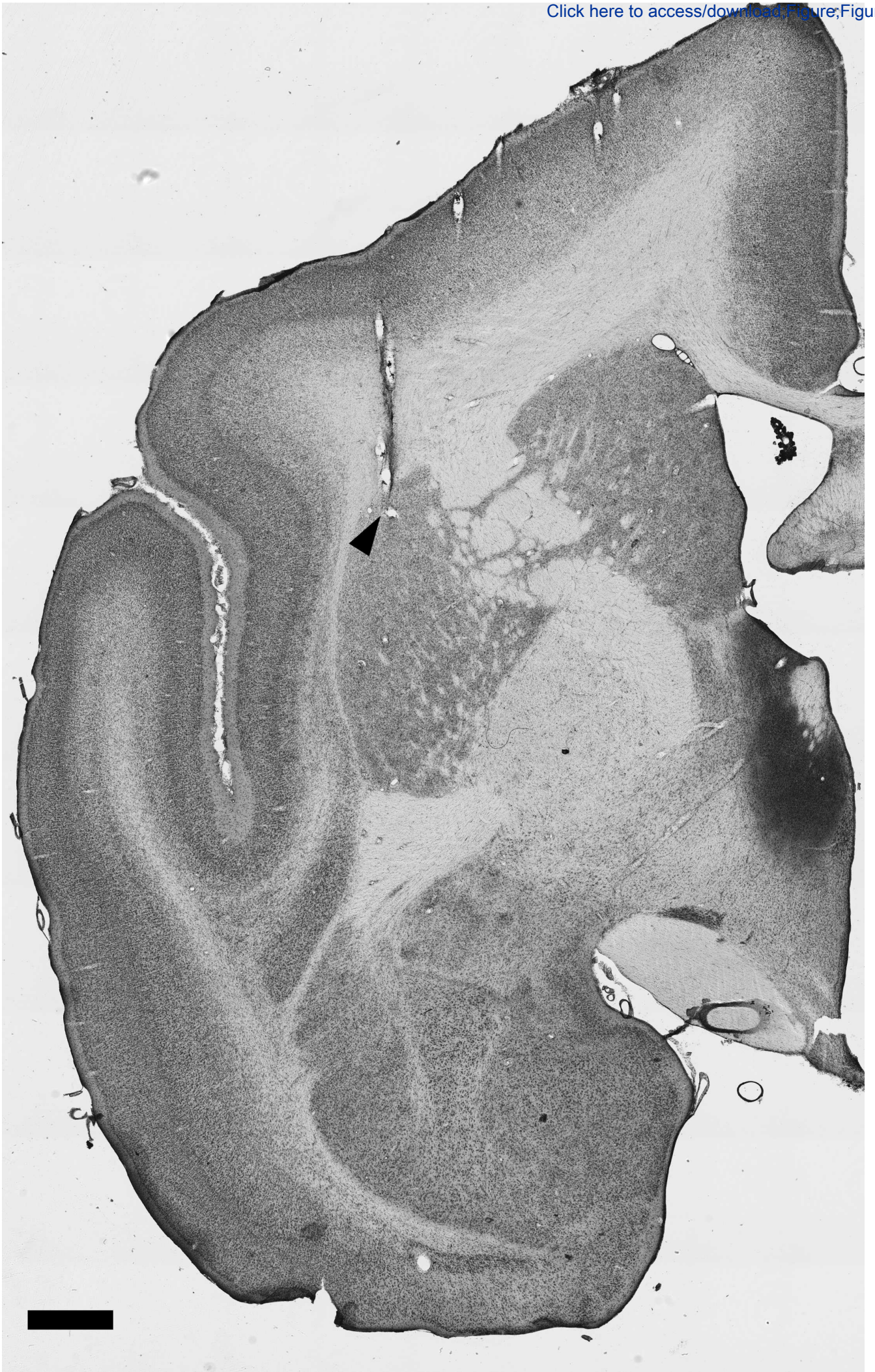


Figure 3



Name of Material/ Equipment	Company
Equipments	
683 Small Animal Ventilator	Harvard Apparatus, Inc.
Anesthesia Assembly	BRASMED
Barber Clippers	Mundial
Dental Drill	Norgen
Homeothermic Heating Pad and Monitor	Harvard Apparatus, Inc.
Marmoset Stereotaxic Frame	Narishige Scientific
Patient Monitor and Pulse Oximeter	Bionet Co., Ltd
Stereotaxic Micromanipulator	Narishige Scientific
Surgical Microscope	Opto
Instruments	
Allis tissue forceps	Sklar
Alm Retractor, rounded point, 4x4 teeth	Rhosse
Angled McPherson Forceps	Oftalmologiabr
Curved Surgial Scissors	Harvard Apparatus, Inc.
Curved Tissue Forceps	Sklar
Delicate Dissection forceps	WPI
Dental Drill Bit	Microdont
Essring Tissue Forceps	Sklar
FG 1/4 Dental Drill Bit	Microdont
Halsey Needle Holder	WPI
Halstead Mosquito forceps	WPI
Hemostatic Forceps, Straight	Sklar
Jewler Forceps	Sklar
McPherson-Vannas Optathalmic microscissor, 3 mm point	Argos Instrumental
Pereosteal Raspatory	Golgran
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Screwdrivers	Eurotool
Sodering Iron	Hikari
Surgical Scissor	Harvard Apparatus, Inc.
Toothed forceps	WPI
Disposables/Single Use	
1 ml sterile syringe with 26 G needle	Descarpack
130 cm x 140 cm surgical field, presterilized	ProtDesc
24G Needle, presterilized	Descarpack
50 cm x 50 cm surgical field, presterilized	Esterili-med
Cotton Tipped Probes, Presterilized	Jiangsu Suyun Medical
Cotton tipped Qutips	Higie Topp
Electrode Array	
Endotracheal tube without cuff, internal diameter 2.0 mm,	Solidor
Tinned copper wire, 0.15 mm diameter	
M1.4x3 Stainless steel screws	USMICROSCREW

Medical Tape	Missner
Nylon surgical sutures	Shalon
Scalpal Blade, presterilized	AdvantiVe
solder	Kester
Sterile Saline 0.9%	Isofarma
Sterile Surgical Gloves	Maxitex
Sterile Surgical Gown	ProtDesc
Surgical Gauze, 15 cm x 26 cm presterilized	Héika
Gelfoam	Pfizer

Drugs/Chemicals

0.25mg/ml Atropine	Isofarma
10% Lidocaine Spray	Produtos Químicos
2.5% Enrofloxacino veterinary antibiotic	Chemitec
Dexametasona Veterinary Anti inflammatory	MSD
Hydrogen Peroxide	Farmax
Isoflourane	BioChimico
Jet Acrylic polymerization solution	Artigos Odontológicos
Jet Auto Polymerizing Acrylic	Artigos Odontológicos
Ketamine 10%	Syntec
Lidocaine and Phenylephrine 1.8 ml local anesthetic	SS White
Povidone-Iodine solutiom	Farmax
Riohex 2% surgical Soap	Rioquímica
Silver Paint	SPI Supplies
Tramadol chloride 50 mg/ml	União Química
Refresh gel (polyacrylic acid)	Allergan

Catalog Number	Comments/Description
55-0000	
COLIBRI	
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B07-201-M1KG	
50-7212	
SR-6C-HT	
BM3	
SM-15R	
SM PLUS IBZ	
36-2275	
RH11078	
11301A	
72-8422	
47-1186	
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ISO.806.314.001.524.010	
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ISO.700.314.001.006.005	
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Title:	Stereotaxic Surgery for Implantation of Microelectrode Arrays in the Common Marmoset (Callithrix jacchus)
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Answer: The protocol was thoroughly revised and all steps contain only 2-3 actions.

9. Please describe the result with respect to your experiment, you performed an experiment, how did it help you to conclude what you wanted to and how is it in line with the title. Please include some results to show how electrophysiological results obtained via this method helped you answer your scientific question.
Answer: The goal of this work is to describe an effective stereotaxic neurosurgical procedure for implantation of recording electrode arrays in the common marmoset that may be used to investigate a variety of scientific questions. If the protocol is followed successfully, it is expected that after 3-5 days of recovery it will be possible to record spikes and local field potentials (LFPs) in freely behaving animals. In our representative results, we focused on assessing whether the surgical procedure was successful through physiological parameters, animal behavior, electrophysiological signal detection, and histologic analysis.

The spike dataset obtained from this experiment is part of a larger study and will help to understand neuronal variability and coding in the basal ganglia - corticothalamic circuit during free behavior. At the same time, the local field potentials are being used to study the dynamic of communication between the structures of this circuit in healthy and parkinsonian marmosets.

To demonstrate what a full experiment can produce, we have now highlighted in the manuscript (page 9, lines 403-407) previous work by our research group using this surgical and a very similar electrophysiologic protocol. Santana et al. (2014) recorded the brain activity of multiple structures of the basal ganglia corticothalamic loop in parkinsonian marmosets and demonstrated that spinal cord stimulation disrupted abnormal synchronized activity in each of the recorded structures. It is important to note that this surgical protocol can be easily adapted to include other brain regions and answer different questions. Thus, we believe that the present manuscript will help to disseminate the knowledge needed to establish more electrophysiologic studies of marmosets in the literature.

10. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."
Answer: None of the figures depicted in the manuscript were reused from a previous publication.

11. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol.
Answer: All figure legends include a title and a short description of the data presented.

12. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
Answer: The discussion was revised and modified to include all the items listed above (page 11, lines 487-500).

Video:
Please ensure that the protocol subheadings are the same in the text and in the video.
Answer: Protocol subheadings were revised and now they are the same in the text and in the video.

Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.
Answer: Modifications were made both in the written protocol and the narration to increase the homogeneity between them.

Please ensure all the result figures are present in the video as well. Presently figure 3 is not shown.
Answer: We have added Figure 3 to the video.

Vet Review:

The vet review did not require any modifications in the video. The following comments were included in the Vet Review document: ***"Procedures were approved by facility's ethic committee. Anesthesia and intraoperative monitoring were acceptable. Analgesia is described post-operatively, would prefer to see analgesia started pre-surgery as a suggested improvement This could be addressed in the text. Otherwise no concerns with video or article. Surgery was performed well, instructions are thorough, video quality is good. Appreciated that post-op recovery including 1 week post recording session info was included."***
We thank the reviewer for the comments and highlight that we have included the pre-surgery analgesia description (step 2.4, page 3, line 166), that was missing in the original submission.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
This article describes general procedures about the stereotaxic implantation of microelectrode arrays in the marmoset brain. This is not new method but provides useful information for readers, who intend to start primate neuroscience with marmosets. Thus, I believe that the content is appropriate for the journal. However I have a major concern which should be solved.

Major Concerns:
Additional to surgical procedures, they mentioned about a protocol of an electrophysiological recording. In this recording protocol, for attaching the connector,

they anaesthetized animals to immobilize. This is inacceptable as an experimental protocol with non-human primates at least with following two reasons.
1. Frequent anesthesia should be avoided.
2. To reduce animals' physical and mental stress, it is important to acclimate animals to the experimenter and experimental environments. In our experience, one month of acclimatization enables us to handle marmosets' connector without neither anesthesia nor head fixing.
Authors' protocol may give a wrong understanding of chronic experiments with non-human primates for readers. Chronic measurement methods would be better removed from the protocol.
Answer: We agree with the reviewer that frequent anesthesia should be avoided. In this protocol, deep anesthesia was utilized only during the surgical procedure. To connect the recording cable, we briefly induced light sedation using isoflurane administered in 1% oxygen. This procedure was performed for 2-3 minutes, only the time necessary to attach the connector. Isoflurane has a rapid onset and offset effect which allows for momentary sedation and rapid re-awakening (Misra and Koshy, 2012). According to the NIH's Guide for the use and Care of laboratory animals, "Loss of consciousness occurs at a light plane of anesthesia, before antinociception, and is sufficient for purposes of restraint or minor, less invasive procedures" (page 122 of the Guide). These same guidelines call for the overseeing veterinarian (VA) to exercise their judgement in determining anesthesia induction on a per experimental basis, and in this case the determination was made that this light anesthesia is safe.

Regarding the concern that this procedure may impact our results, we waited 30 minutes following the end of isoflurane administration to begin electrophysiological recordings, ensuring the absence of any sedative effect. We chose this method because we used a small connector attached to each headstage with 32 channels. The connector's high pin density coupled with its small size makes it quite hard to attach the connector in awake marmosets without breaking the pins; even after long periods of handling. In addition, since we performed the recordings once a week, this method was considered acceptable by our institution's Ethics Committee and guidelines.

We added a note stating that frequent anesthesia should be avoided and that sedation protocols must follow their countries' and institutions' guidelines regarding sedation of non-human primates. We also highlighted that when experimental design involves frequent recordings, the experimenters must habituate the animals to be handled to connect the cables without anesthesia (step 5.2, page 8, lines 367-369). Finally, we highlighted in the discussion that anesthesia, as part of the recording procedure, is a limitation of the method we are describing (page 11, line 487-500).

We also agree that it is essential that the marmosets are acclimated with the experimenter and experimental environments. All marmosets that are used in our experiments are acclimated with the experimenter and to the room and chamber used in the experiments for at least 1 month before the surgery. We use a positive reinforcement training regiment so that the animals learn to enter the transport cage and the experimental chamber by themselves. Accordingly, we have added a note stating the utmost importance of performing animal acclimation before the surgery and recordings (step 5.1, page 8, lines 361-362).

Minor Concerns:
Step 2.7. Are there administrations of a local analgesic to auditory canals and to eyelids under the eyes?

Answer: In our protocol we only fix the animal's head in the stereotaxic frame after it is deeply anesthetized. We neither administer local analgesic to the auditory canals nor to the eyelids. Instead, immediately before the surgery, we apply tramadol (2 mg/Kg, i.m.). This information was added to step 2.4, page 3, line 166.

Before step 2.8. Did you apply ophthalmic ointment to the eyes to prevent dryness?
Answer: After anesthesia, refresh gel (allergan) was applied to the eyes. This information was added as step 2.9, page 5, line 234.

Before step 3. Is there any administration of analgesic to decrease operative pain?
Answer: We applied tramadol (2 mg/Kg, i.m.) immediately before the surgery. This information was added to step 2.4 (page 3, line 166). In addition, we also apply tramadol orally (1 mg/Kg, i.m) for 3-5 days after the surgery, as described on step 4.8 (page 7. Lines 350-352).

Reviewer #2:
Manuscript Summary:
This manuscript summarizes a stereotaxical neurosurgery method to implant microelectrode arrays for chronic electrophysiological recordings in the common marmoset. As the common marmoset is becoming more popular as a non-human primate animal model, this video/manuscript will be useful to increasing number of researchers using the animal.

Major Concerns:
Line 196 (Video 6:45): The authors should clarify how they handled the interface between the exposed cortex and the dental acrylic. Was any ointment, vaseline and/or medical-grade silicone applied on top of the exposed cortex before covering with the dental acrylic?
Answer: Exposed cortex was covered with small pieces of sterile gelfoam to prevent direct contact between the dental acrylic and the cortex. This information was added on step 3.7.5 (page 6, line 299-300).

Minor Concerns:
Line 93: Make a brief note on the homemade electrode array here. Clarify if it come from a conventional design? Appropriate reference should be inserted if it has been used in a prior publication/presentation.
Answer: The microelectrode array utilized in this manuscript was previously developed by Lehew and Nicolelis (2008). The array is composed of 32 stainless steel microwires. The wires are 50 µm in diameter and are organized in 7 bundles aimed to reach the following areas: primary motor cortex (M1), putamen (Put), caudate (Cd), globus pallidus (GPe), ventrolateral and ventroposterior lateral thalamic nucleus (VPL), and subthalamic nucleus (STN). The interelectrode spacing in each bundle is 300 µm. The interbundle spacing depends on the target coordinates for each brain region. More detailed information about microelectrode array design and manufacturing can be found in Lehew and Nicolelis (2008) and Dizirasa et al. (2011). Others works have utilized a large variety of microwire array configurations for targeting different cortical and subcortical brain areas (Nicolelis et al., 2003; Santana et al., 2014; Barz et al., 2017). These references have been added to figure 1 (page 9, line 429-432).

Line 187: Describe source(s) of critical bleeding points when dissecting the dura (e.g., sinus and meningeal arteries). Also comment on how to stop dural bleeding when it occurs (e.g., if they used bipolar coagulators or not).
Answer: We rarely experience significant bleeding when dissecting the dura. However, bleeding can be stopped with cautery or placement of gelfoam soaked in thrombin. This information was added as a note on step 3.6 (page 6, line 283).

Line 224: During the recovery, marmosets may prefer to climb to high places in the cage and end up falling down, making unwanted head banging. Please comment within the manuscript if the authors take care to prevent this.
Answer: During the recovery the animals are housed in a padded cage (all bars are covered with absorbent mats) to minimize the animal's ability to climb and therefore the chances of an animal falling down. This information was added as a note on the protocol on step 4.6 (page 7, line 344-345).

Line 233: Describe how long the authors wait for the "lightly anesthetized" animal to recover before they start the recording sessions.
Answer: Isoflurane has a rapid onset and offset action which allows for rapid sedation and awakening (Misra and Koshy, 2012). Therefore, once the isoflurane supply is turned off, the animal starts to wake up. We waited until the animal was awake and in an upright position, while able to ambulate freely in the experimental chamber without falling down, which takes less than 15 minutes. To ensure the absence of any sedative effects, however, we only begin the recordings 30 minutes after the offset of isoflurane. This information was added as a note on step 5.5 (page 8, line 382-386).

Reviewer #3:
This study by Budoff and colleagues describes a methodology for the stereotaxic surgical implantation of chronic recording electrodes in marmoset. It provides a detailed account of surgical materials, procedural steps during the surgery, and pre- and post-operative care. While there are several existing descriptions of stereotaxic methods in the marmoset neuroscientific literature (e.g. Johnston et al. 2018, Wakabayashi et al 2018), previous papers have focused primarily on preparations for the restrained paradigm. The present study emphasizes a generalizable approach for chronic implants, which can be used in studies of the freely moving animal. It provides a new level of detail in both visual and written format, which may prove helpful to those who are developing research lines in the marmoset. The manuscript and video could be improved further by addressing both general and specific points, as described below.

General comments
(1) In addition to providing a visual guide to basic surgical techniques, the present study stands to offer a new perspective on the methodology of implanting chronic electrode arrays, which permit recording in a less restrained setting. In its present form, however, this potential benefit of the study is hampered by two factors. First, there is a lack of clarity and detail about the nature of the arrays, their holding devices, and the manner in which they are implanted. The utility of the study would benefit from additional descriptions of these components, and from a clearer exposition of the steps

required for accurate localization (see 'Comments for video and narration' for specific suggestions). Second, the authors should acknowledge the potential limitations to the 'freely behaving' paradigm in the current set-up, due to recovery from brief anesthesia and due to the recording tether.

Answer: The reviewer is correct in stating that there is a lack of detail in the nature of the arrays. The electrode arrays used in the surgeries were custom-made and the specifics on how to manufacture them have been previously described (Lehew and Nicolelis 2008; Dzirasa et al., 2011). We have changed the legend of Figure 1 to include this information (page 9, line 429-432). In addition, the surgical protocol described in the manuscript can also be used by experimenters intending to implant other types of electrodes, including commercially available ones, in small primates. This information was also added in the discussion (page 10, line 449).

There was also a lack of detail in the manner in which the electrodes were implanted, especially regarding zeroing the electrodes and determining the appropriate size for the craniotomies. Prior to surgery, we predetermine the coordinates of the craniotomies and of the array implants based on the interaural coordinates, since the bregma is highly variable in primates. Our electrode arrays usually have different bundles designed to target specific brain areas. The position of each microwire is determined based on a target stereotaxic coordinate. The distance between the microwires of the same bundle is 300 µm and the distance between the bundles vary depending on the brain areas that are being targeted. When the array attached to the micromanipulator is moved, all microwires are moved together, meaning that the relative distance between the microwires are constant. Therefore, we can use the interaural coordinate of any microwire of the array to calculate the implantation coordinates of the entire array. When the bundles of the array have different lengths (different dorsoventral coordinates), we prefer to use the interaural coordinate of one microwire belonging to the longest bundle, since this is the bundle that first touches the surface of the brain. This information has been added as a note under 1.2 (page 2, line 128-131) to prevent confusing other readers.

We use a 24G needle attached to the stereotaxic probe holder to outline the desired craniotomies at the surface of the skull. The coordinates of the craniotomies depend on the target coordinates of the microwires of the array and are calculated based on the interaural coordinates of the 24G needle - probe holder assembly. The size of the craniotomies is based on size and relative distance of the bundles of the array. They are 200 μm^2 wider than the AP and ML distances between the microwires at the borders of the craniotomy. This information was added as a note under step 1.4 (page 3, line 140-143). Please see also the answers to the points raised at the topic “related to general comment #1” for further explanations.

We also agree with the reviewer that we must acknowledge the potential limitations to freely-behaving paradigm in the current set-up, and these limitations were included in the discussion (page 11, lines 487-500).

(2) While it is clear to experienced researchers that the animals have been carefully and conscientiously handled in accordance with guidelines, I would encourage the authors to be mindful of descriptions and footage that may be misinterpreted by audiences less familiar with animal surgeries. Again see 'Comments for video and narration' for suggestions.

Answer: According to the reviewer suggestions we have changed some marmoset footages (please, see the answer for comments for video and narration related to general comment #2).

Specific comments

Line 52 - currently states "These new-world primates represent an important compromise between rodents and historically popular non-human primates (NHPs)." The word 'popular' can give the mistaken impression that rhesus are a frequently used model in animal research overall (when in fact they constitute <1% of animals used in biomedical research). Consider rephrasing simply as 'represent an important complementary animal model to both rodent and rhesus macaque'; or specify that among non-human primate models, old-world monkeys the historical standard.
Answer: We originally used this terminology as rhesus macaques have constituted more than 80% of NHPs used in research (Bertrand et al, 2018). However, the reviewer is correct, and we made the changes according to the suggestion above (page 1, line 59-60).

Line 65 - replace 'like' with 'unlike' so it reads 'lissencephalic, unlike the macaque' and explain why this is advantageous
Answer: The words were replaced accordingly.

Line 115 Place the NOTE before the first preoperative procedure, i.e. move up to line 113
Answer: The note was moved to the suggested position.

Lines 140-144 The suggested ranges for heart rate are very pragmatic; are there citations for these values as well?
Answer: Once the animal is awake a normal heart rate is 240-350 BPM (Kindlovits & Kindlovits, 2009). The range for heart rate suggested here is based on our experience in marmoset surgeries over more than 10 years. We use several physiological parameters to measure anesthetic depth and choose the optimal anesthetic dose, such as heart rate, breathing pattern, motor activity and eyelid reflexes. All these signals have been indicative of deep anesthesia when heart rate is between 154-180 bpm. We have added the information that those ranges are based on our experience to the discussion (page 10, line 472).

Lines 173, 177 - if possible, add details on drilling (type of drill used, optimal rpm, methods to prevent overheating, and stopper if relevant to prevent drilling too far in the case of screws
Answer: We used a high-speed air-driven dental drill with a maximum speed of 350.000 rpm. To prevent overheating, we may add a few drops of sterile saline over the skull during drilling. This information was added on step 3.3, page 5, line 259-261.

Line 179 - what is meant by the 'unaltered' screw?
Answer: Unaltered screw, in the present context, is the screw without a ground wire soldered to it. This explanation was added to the text on step 3.4.2 (page 6, line 269).

Line 194 - Can the authors describe their method for estimating the depths of each of the bundles? Are any checks done on using the dorsoventral values expected on the skull at the location of these arrays/bundles?
Answer: All the bundles of each array are inserted at the same time, as they are manufactured as a single artifact. The coordinates for the implant are calculated based on the dorsoventral interaural zero coordinate of one microwire (located on the longest bundle, if the bundles have different lengths). Since all of the microwires are at a relative distance from each other, when we set the dorsoventral coordinate for one microwire all of the other microwires are also

positioned at their expected coordinates. Therefore, the depths of each bundle are determined during the design and manufacture of the array (electrode array manufacturing was previously described in Lehew and Nicolelis (2008) and Dzirasa et al. (2011)). This information has been clarified as a note under step 1.2 (page 2, line 128-131), and references to the relevant literature have been highlighted in the legend for figure 1 (page 9, line 429-432).

Line 206 - 'support bar' - it is hard to understand this description from text alone; please elaborate further on the purpose of this bar and materials used
Answer: The 'support bar,' in this context, is a plastic rod made from a Q-tip and embedded in the dental acrylic of the head cap. During our particular experiment, we are using two headstages connected to the electrode connectors and recording unit. We have found that by wrapping elastic bands around the support bars and a similar support bar annealed to the headstage we are able to maintain a secure connection during free motion of the animal. Elaborations were added to the text on step 3.11 (page 7, lines 318-321).

Line 224 - use 'movement' or 'contact' instead of 'battering'
Answer: The word 'battering' was replaced by 'contact'.

Line 232 - how is this light anesthesia achieved (mask or chamber?), approximately how long is recovery time, and how frequently and repeatedly can this be used as the strategy for restraint?
Answer: We used a chamber (48 cm x 29 cm x 27 cm) to induce anesthesia with gaseous isoflurane. The chamber should not be precharged. Isoflurane is administered at concentrations of 1% in oxygen. Once the animal is lightly sedated, we use a custom mask to keep isoflurane administration until the connectors are attached. Isoflurane has a rapid onset and offset action which allows for rapid sedation and awakening (Misra and Koshy, 2012). Therefore, the animal starts to awake from anesthesia as soon as the anesthetic supply is turned off. We waited 30 minutes following offset of the gas to begin electrophysiological recordings as this ensures the absence of any sedative effect.

The frequency od light anesthesia induction must follow each countries' and institutions' guidelines regarding sedation of non-human primates. In our experiments, we performed recordings once a week, and it was considered acceptable by our institution's Ethics Committee and guidelines. We added a note in the manuscript stating that frequent anesthesia should be avoided and that sedation protocols must follow their countries' and institutions' guidelines regarding sedation of non-human primates (step 5.2, page 8, lines 367-369).

Line 236 - provide a brief description of the experimental chamber & its general purpose
Answer: The experimental chamber is a cubic acrylic box (0.45 x 0.45 x 0.45 m^3) that is used to assess the amount and the pattern of spontaneous motor activity. A brief description of the experimental chamber and the additional 2 references (Santana et al., 2014; Santana et al. 2015) with more details on its design and experimental purpose were added as a note to step 5.5 (page 8, line 382-386).

Line 242 - does the typical surgery duration include only steps in Section 3 (ie. not intubation and other presurgical steps?) This seems surprisingly long, so it would be instructive to know the stages of the procedure that are most time-consuming and why

Answer: Typical surgery duration includes all steps from anesthesia induction to the time the animal is placed inside a cage after anesthetic recovery (steps described in sections 2, 3 and 4). No step is particularly more time-consuming, should no adverse events be encountered. The sentence (page 9, line 395) was rephrased to clarify that issue.

Line 250 - instead of recommending only a post-mortem confirmation, the authors should point out that in-vivo confirmation of electrode location may also be feasible if MR-compatible materials are used
Answer: The reviewer is correct, and we added this information (page 9, line 409-414).

Figure 1 - the description of the microwire bundles is not clear in the text. Each array has 32 microwires arranged in 7 bundles? How many wires per bundle? What's the inter-bundle distance? I gather each is determined to target and encompass the brain areas of interest, but it would be helpful to have details here
Answer: Each electrode array has 32 microwires arranged in 7 bundles (4-6 wires per bundle). The distance between the wires in each bundle is 300 µm. As the reviewer pointed out, each bundle is designed to target a different brain structure on the cortico-basal ganglia-thalamic circuit. Therefore the inter-bundle distance varies amongst the bundles, and it depends on the target coordinates of each structure. More detailed information regarding arrays manufacturing can be found on Lehew and Nicolelis, 2008. This information was added to the representative results (page 9, line 398-400), and to the legend of Figure 1 (page 9, line 429-432).

Line 292 - use 'critical' instead of 'perilous' (can replace the next 'critical' in 293 with essential)
Answer: The changes were made accordingly.

Comments from video and narration
The narration states that "anesthesia is administered" using atropine and ketamine. The appropriate terminology is that atropine and ketamine are used to induce anesthesia
Answer: We have changed the terminology in the narration accordingly.

Was ophthalmic ointment used to protect the eyes?
Answer: After anesthesia, refresh gel (allergan) was applied to the eyes. This information was added on step 2.9 (page 5, line 234).

Related to general comment #1:
The terminology became somewhat unclear regarding the implanted electrodes. In the video, it looks as if the entire set of arrays went down all at once. Was this the case, following multiple craniotomies for each array? Or is this considered a single array with multiple bundles?
Answer: Each microelectrode array (containing 32 microwires arranged in 7 bundles) is gently inserted in the brain at once following the craniotomy. As clarified above, each array is a single artifact manufactured as previously described in Lehew and Nicolelis (2008) and Dzirasa et

al. (2011). In this particular surgery we made 2 adjacent craniotomies and implanted 1 array into each hemisphere.

Can the authors say more about the device and method for simultaneously advancing each of the bundles/arrays and ensuring that they reach the target areas and with sufficiently (but not excessively) large craniotomy openings? It's also not clear how this step relates to the earlier description of zeroing a single electrode for each of the bundles.

Answer: The electrode arrays that we use in both rodent and primate research are custom-made according to the aims of each specific experiment. Detailed description of the steps involved in the manufacture of these arrays has already been detailed in previous publications (Lehew and Nicolelis, 2008; Dzirasa et al., 2011).

The arrays used in the video have 7 bundles designed to reach 7 different brain areas of the cortical - basal ganglia - thalamic circuit. The spacing between the electrode bundles is fixed and is based on the targeted anteroposterior (AP) and mediolateral (ML) coordinates of each microwire. The length of each bundle is based on the targeted dorsoventral (DV) coordinate for each brain region. Therefore, the position of each microwire is fixed relative to the other microwires in the same bundle and other bundles. All coordinates are relative to the interaural line. Before the surgery, we fix the array into an electrode holder (Lehew and Nicolelis, 2008) that can be attached to the micromanipulator. The electrode attached to its holder is depicted in Figure 1. We then position one microwire in the array (usually belonging to the longest bundle) at the interaural zero of the stereotaxic frame. These AP, ML and DV coordinates are then used to calculate the microwire implantation coordinates. Since the coordinates of this microwire is relative to the coordinates of all other microwires, when we position the array at the calculated coordinates all wires are positioned into their predicted target coordinates.

The size and position of the craniotomies are outlined based on the relative coordinates of the microwires and bundles. If the bundles are sufficiently far apart from each other, separate craniotomies may be drilled. If the bundles are close to each other, one craniotomy large enough to encompass all microwires is drilled for each array. The craniotomies are 200 μm^2 larger than the AP and ML distances between the microwires that are at the borders of the intended craniotomy. This information was added as a note on step 1.4 (page 3, line 140-143).

Related to general comment #2: If there is footage of the animal looking more alert and responsive in (1) post-operative stages and (2) during recording, that would be preferable.

Answer: According to the reviewer's suggestion, we have changed the footage in postoperative stages and during recording.

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