

Assessment of Corticospinal Excitability in Awake Rodents Using EMG-Controlled Intracortical Stimulation

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[Abstract] Assessment of corticospinal excitability (CSE) is an essential component of experiments designed to induce or study neuronal plasticity in the motor system. Common examples are paired associative stimulation (PAS), theta-burst stimulation (TBS), intensive motor training, or any methods aimed at potentiating the corticomotor system in the hope of promoting better recovery after neurological insult. To date, rodent models of CSE assessment have mostly been completed under anaesthesia, which greatly affects the level of CSE, as well as the mechanisms of plasticity. Experiments in awake animals are difficult because the ongoing state of behavior affects the excitability of the motor system and complicates the assessment of CSE. To address this issue, we have designed a novel approach for CSE assessment in awake behaving rodents, enabling a reliable measure of evoked motor responses obtained from cortical microstimulation in repeatable conditions of ongoing motor activity. The system relies on chronically implanted intracortical and intramuscular electrodes and a custom-made software control system, enabling the user to require that precise parameters of EMG activity be met before cortical stimulation probes are delivered. This approach could be used for further studies of PAS, TBS or other interventions requiring the assessment of CSE under repeatable conditions. We provide fabrication schematics and a list of materials for the implant, as well as instructions for running a custom-made MATLAB codebase, customizing the PAS protocol, and performing the complete analysis of experimental data. We hope these tools can further facilitate animal research in the field of neuroplasticity and neurorehabilitation.

Keywords: Corticospinal excitability, Neuronal plasticity, Motor cortex, Rodent models, Spinal cord, Paired associative stimulation

[Background]

A. Corticospinal Excitability

Neuroplasticity and sensorimotor network reorganization are determining factors for functional recovery after stroke (Dancause and Nudo, 2011; Zeiler and Krakauer, 2013) and spinal cord injury (Serradj *et al.*, 2017; Brown and Martinez, 2019). The corticospinal system is the major neural pathway facilitating fine voluntary motor control in non-human primates and humans, connecting the major output neurons in layer Vb of the motor cortex with the spinal circuits controlling arm and hand muscles (Lemon, 2008). A deeper understanding of how beneficial plasticity can be induced in this pathway will be instrumental to designing better therapeutic interventions after stroke. Research in humans and non-

human primates can directly investigate the fine motor function of hands and digits. Despite the fact that rats lack monosynaptic corticomotoneuronal cells (Alstermark *et al.*, 2004) and opposable thumbs or individuated finger movement, they have a good level of distal forelimb dexterity and constitute a useful model of forelimb motor function and recovery from lesion (Kleim *et al.*, 2007). They also present significant advantages for research over non-human primates in terms of ethical justification, cost, and the growing availability of transgenic strains. A potential difficulty in using rodent models to investigate corticospinal plasticity resides in obtaining accurate and consistent measures of corticomotor pathway excitability during behaviour, without the influence of anaesthesia. We have approached that goal by implementing a novel EMG-controlled intracortical micro-stimulation approach using chronically implanted electrodes in the rat. The purpose of this paper is to further document and make available the blueprints for our system as a companion to the main scientific paper (Ting *et al.*, 2020), so that the interested parties may use and adapt them to suit their needs.

B. Paired Associative Stimulation (PAS) to Modulate Corticospinal Excitability

One demonstrated method to modulate corticospinal excitability (CSE) is paired associative stimulation (PAS). In humans, transcranial magnetic stimulation and transcutaneous neuromuscular stimulation are non-invasive stimulation methods used to target the motor cortex and a contralateral peripheral nerve. The concept, based on Hebbian spike-timing-dependent plasticity (Dan and Poo, 2004; Feldman, 2012), is that repetitive forced coincidence of pre- and post-synaptic action potentials can lead to a LTP-like conditioning of the intervening synapses, and result in a beneficial strengthening of corticomotor connections. The first PAS studies aimed to induce plasticity in the sensorimotor cortex (Stefan *et al.*, 2000), but subsequent investigations confirmed the possibility for spinal plasticity as well (Taylor and Martin, 2009). In the latter case, antidromic potentials in motoneurons were made to coincide with orthodromic descending corticospinal volleys to transiently modulate CSE.

PAS has been shown to increase corticomotor excitability in healthy human participants (Suppa *et al.*, 2017) and promote recovery after human neurological insult (Rothwell, 2016; Urbin *et al.*, 2017; Christiansen and Perez, 2018). However, PAS effects are highly variable across subjects (Sale *et al.*, 2007; McGie *et al.*, 2014; Tarri *et al.*, 2018), and failure at inducing plasticity with PAS has also been reported (McGie *et al.*, 2014). Therefore, the promise of neuromodulation therapies such as paired stimulation warrant further studies in animal models (Mishra *et al.*, 2017), to improve our understanding of the factors leading to effective control of neuronal plasticity for functional benefits.

There are sparse animal investigations in the PAS literature. Most published studies are completed under anesthesia, or require some degree of restraint (Zhang *et al.*, 2018). Hence there continues to be an unaddressed need to develop a readily extensible chronic model of CSE in awake, ecologically behaving rats. Such a system should provide a way of assessing the CSE during normal behavior, while ensuring that the CSE assessments are made at similar levels of corticomotor activity. It should also permit an effective delivery of therapeutic stimulation protocols. Here, we present a closed-loop CSE assessment model, which provides a reliable means to investigate the effectiveness of interventions aimed at driving corticospinal plasticity such as PAS. This closed-loop, EMG-controlled CSE

assessment protocol contains a list of equipment and fabrication instructions. We also include detailed instructions on a functional MATLAB codebase that we developed specifically to acquire and analyze data obtained from PAS experiments using this model. In this way, we demonstrate a full prototyping ecosystem for conducting closed-loop CSE assessment in rodents.

We first discuss the specific goals of our system along with the roles of each major component, and follow with the main protocol describing the implant surgery. Standalone sub-protocols are also included, detailing step-by-step fabrication of (1) the cortical array to be inserted in the brain; (2) the cortical connector to which the cortical array attaches, including the EMG wires to be inserted in the muscle; and (3) the connector cable which tethers the rat to (4) the hardware and software platform which permits closed-loop EMG recording and control. Explanatory pictures and schematics are provided at key steps to aid understanding and help facilitate assembly of the necessary components.

C. General Approach

In designing this system, we had several considerations in mind:

1. A chronic preparation to preclude the need for anesthesia. Ketamine-xylazine and other associated agents have an effect on NMDA-dependent plasticity, due to their purported mechanism as NMDA-receptor antagonists (Zanos and Gould, 2018). We wanted to streamline the process so that only one recovery surgery is required to perform the full implant, for ethical concerns as well as for efficiency.
2. The system should permit repeated assessment of CSE under relatively consistent conditions of EMG activity. We selected the trapezius, biceps and extensor carpi radialis (ECR) as three potential plasticity targets and for EMG readout, because electrical and excitatory optogenetic stimulation of the caudal forelimb area (CFA) in the primary motor cortex of the rat is most likely to induce movements in one or more of these muscles. The ECR was the primary target because it is involved in fine motor control of the wrists, forepaws and fingers of the rat, and one goal of this model is to facilitate the study of dexterity and neurostimulation after motor cortex lesioning.
3. CSE assessment or any interventions should not require fixed head or limb restraint, but perhaps a temporary tether permitting a high degree of movement flexibility.
4. We wanted to have some degree of redundancy in the cortical and muscle stimulation. In case one or more electrodes failed, we could still continue with a PAS intervention and assessment of CSE with a different target muscle. This could reduce the total amount of rats required for a particular study.

Our overall design is shown in Figure 1, with details in Figure 2. The implant itself consists of three main components: (1) a 2×2 array of electrodes in the primary motor cortex (Figure 3), which is used to stimulate the descending corticomotor tract, thus inducing a motor evoked potential (MEP) which could be measured by the (2) EMG electrodes embedded in the wrist extensor, shoulder and biceps muscles, and (3) a Samtec® head-mounted connector with clipping latches, which permitted detachable

and reliable interfacing with a nearby computer.

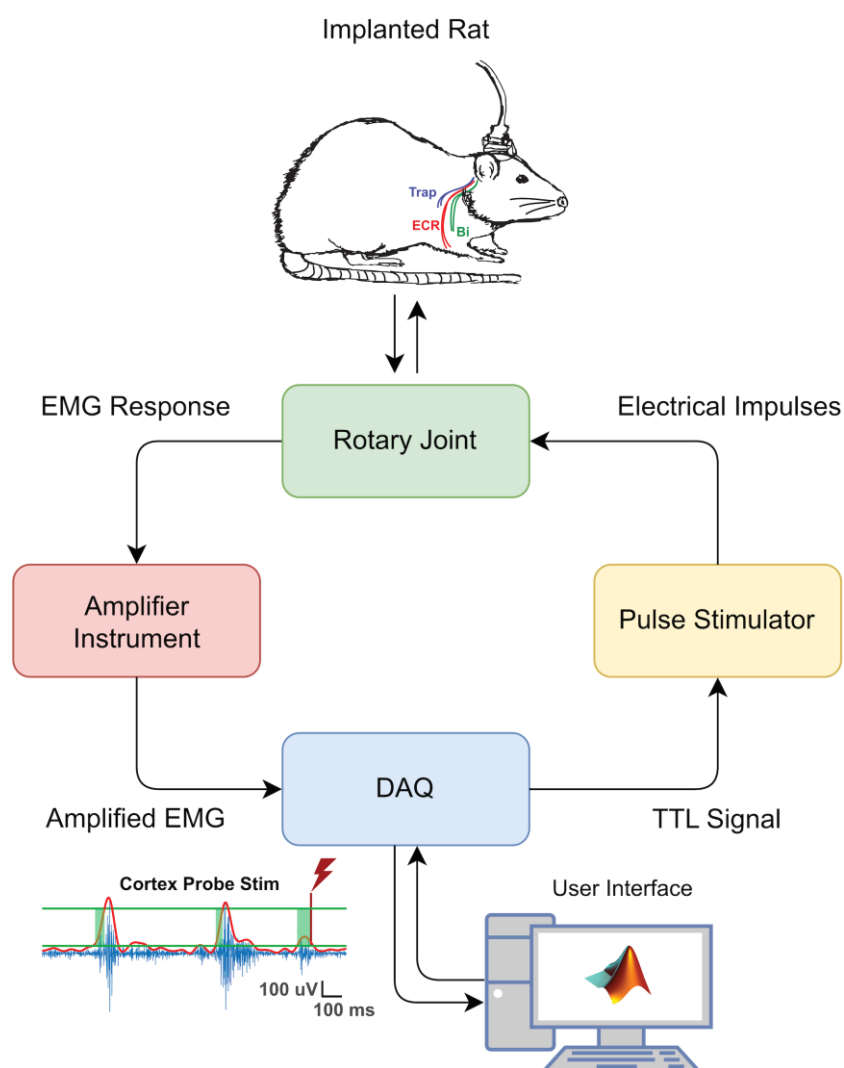


Figure 1. CSE Assessment Hardware Schematic.

Stimulation protocols and EMG data capture are facilitated by a Data Acquisition (DAQ) interface on a desktop computer. The implanted rat (top) has both cortical and EMG electrodes, and both sets are connected to an access port on the rat's head. A custom-made cable connects this access port to a fixed frame with a rotary joint commutator, permitting relatively free movement and preventing significant torsion on the cable. The raw EMG signal obtained from muscle activity is amplified, processed through a DAQ hardware system, and stimulation is triggered in an EMG-dependent manner. Electrical impulses are routed via a stimulator to the same access port, which delivers current to the cortical electrodes. Abbreviations: ECR = extensor carpi radialis; Bi = biceps; Trap = trapezius; TTL = transistor-transistor logic; DAQ = data acquisition; EMG = electromyogram.

D. Key Component: Chronic Implant

The chronic implant is made of two components which are soldered together peri-operatively: a Samtec® connector interface and the stimulation array. The Samtec® connector should be made beforehand, consisting of the connector itself, soldered to 2 cm 32 AWG gauge wires for soldering to the cortical array, and EMG wires soldered directly to the appropriate Samtec® pins. The stimulation array consists of four electrode leads made of platinum-iridium microwire, apposed together to a central cylindrical core, made of layers of heat shrink wrapped around a male electrical pin. For our experiments, the implant itself is made such that each electrode is equidistant from one another laterally by 1 mm. This ensures that the square area of the array itself encompasses a relatively large portion of the caudal forelimb area (CFA) of the motor cortex. The entire square array is encased in epoxy, to fix and insulate the electrodes. We recommend making the connector a few days prior to the implantation surgery, to leave time for quality control of all the connections and to perform two applications of epoxy to secure the wires. We soak both components in 70% alcohol the morning of the surgery for asepsis, but other sterilization methods are possible if stainless metal wire is used, including autoclaving.

E. Key Component: Connector Cable

The connecting cable from the rat implant to the commutator setup is, in our experience, often the weakest link in the entire hardware setup. This is unsurprising because the greatest torsion and other mechanical forces are placed on the wires within the cable. Rats will often try to bite the connecting cable once they are connected, to try to release themselves from the tether. Initially, we experienced poor EMG recording quality and permanent cable damage because one or more fine wires, including the plastic insulation within the connector cable were bitten off by the rat. We found that metal spring wire shielding provided sufficient protection of the cable from physical damage but also retained enough flexibility to permit the rat a high freedom of movement while tethered to the commutator.

EXAMPLE PINOUT

C1= Red Wire on Samtec = Anterior Lateral Cortical Electrode
C2= Yellow Wire on Samtec = Posterior Lateral Cortical Electrode
C3= Green Wire on Samtec = Anterior Medial Cortical Electrode
C4= Black Wire on Samtec = Posterior Medial Cortical Electrode
G = Ground Wire = Skull Screw
R = EMG Reference
M1₁-M1₂ = Right Trapezius EMG Pair
M2₁-M2₂ = Right Biceps EMG Pair
M3₁-M3₂ = Right Extensor Carpi Radialis EMG Pair

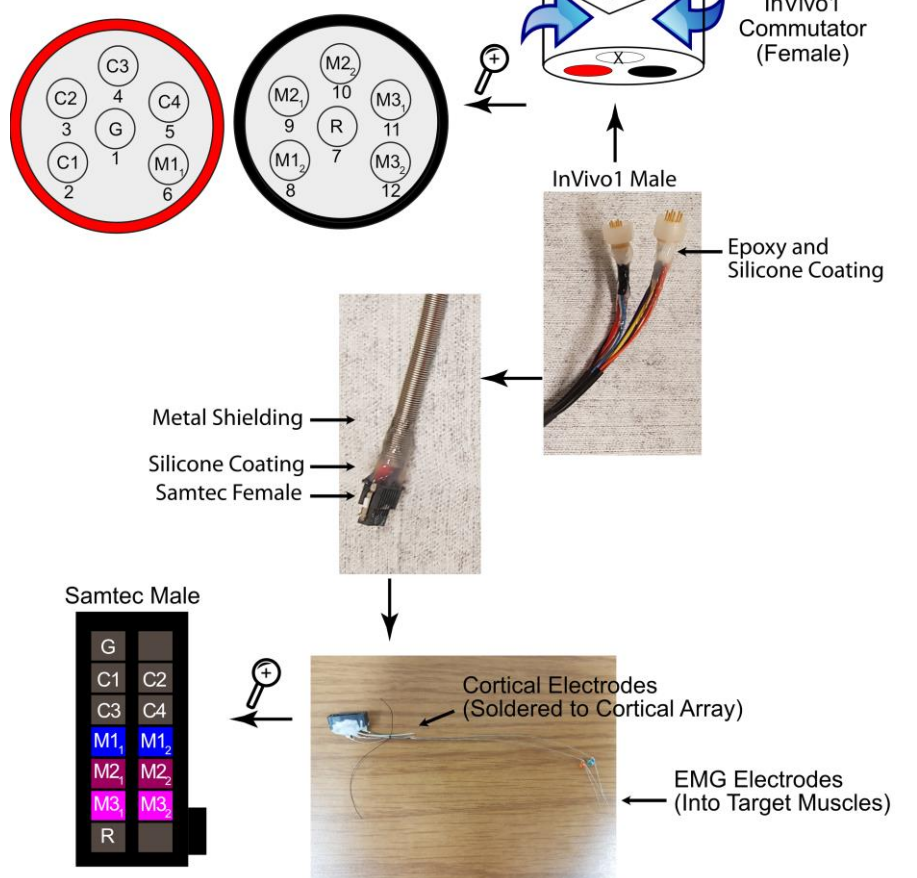


Figure 2. Commutator to Rat Hybrid Schematic.

From top to bottom: A breakout board is used to connect the commutator to the DAQ system and the aforementioned computer system. The commutator is mounted on a rigid stand next to the rat's cage and releases torsion on the connector cable, as the rat may rotate during locomotion and normal behavior. This maintains a secure electrical connection. Two out of the three sockets in the commutator are occupied according to the pinout provided. The metal shielding on the cable prevents physical damage to the wires within, and is a robust solution. The connection between the cable and the rat's head is facilitated by one Samtec® female plugging into a Samtec® male connector port on the rat's head, continuing the pinout described above. The Samtec® male connector's designated muscle wires are implanted into the muscles during the first part of the surgery, and the designated cortex wires are soldered onto the

corresponding wires on the cortical array during the latter part of the surgery.

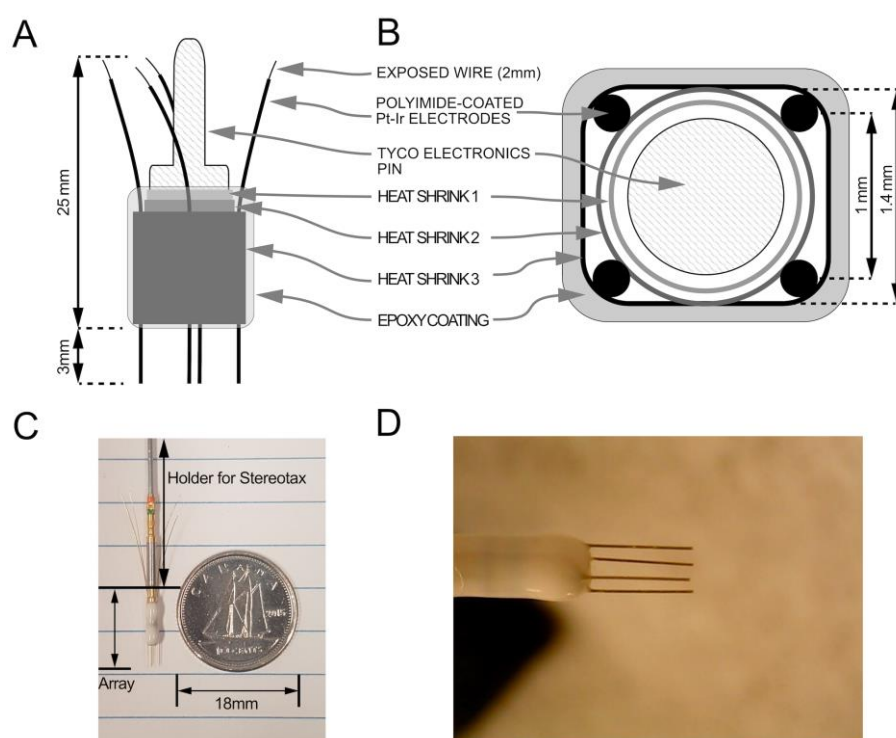


Figure 3. Intracortical electrode array blueprint (not to scale).

A. Condensed array side view with measurements. B. Top view. C. Photograph of finished array and scale for reference. Array holder with female pin facilitates attachment to stereotaxic manipulator. D. Magnified view of finished array.

Equipment

To facilitate assembly, an electronic workbench with tools such as a soldering setup, low-magnification microscope, helping hands, crimpers, and flexible light source are required. Basic surgical tools, like scissors, blunt tweezers and scalpel (with flat Teflon® surface for removing insulation on EMG wires) are also necessary to manipulate the small components. A multimeter is helpful for verifying electrical contacts and the absence of shorts, as well as a heat gun.

A. Part A: Intracortical Stimulation Electrode Array (Peri-Operatively Soldered to Part B)

Chronic Implant

1. 80/20 Platinum-Iridium Wire (California Fine Wire Company, catalog number: 100168), 75 μm \times 1 or equivalent
2. 101 Epoxy adhesive (Omegabond, catalog number: OB-101-2) (with Part A and Part B mixable components)
3. "Iridium" Clear Polyolefin Heat Shrink Tubing, Min Expanded ID 0.066" (Cobalt Polymers, catalog number: X2-070-CLR), and 0.050" (Cobalt Polymers, catalog number: U2-055-CLR)

4. Tyco Electronics Series 90 Amplimate Connectors Pin (Tyco Electronics, catalog number: M39029/58-360)

For mount

1. Tyco Electronics Series 90 Amplimate Connectors Socket (Tyco Electronics, catalog number: M39029/57-354)
2. 21 Gauge Stainless Steel Hypodermic Tubing, 0.032" OD (A-M Systems, catalog number: 843600)

B. Part B: Head Connector and EMG Electrodes (Port on Rat Head, and Electrodes in Muscles)

1. 2 × 7 Male Connector (Samtec, catalog number: TFM-107-01-L-D-WT) × 1
2. Stainless Steel 7 Strand. 0.001" Bare, 0.0055" Coated, variable length × desired number of EMG electrodes (the number must be even to calculate a differential) and 5 cm reference electrode × 1 (A-M Systems, catalog number: 793200)
3. Generic Small Gauge (32 American Wire Gauge) Single Cored Electrical Wires, about 2 cm (maximum 4, or fewer desired cortical electrodes)
4. 101 Epoxy adhesive (Omegabond, catalog number: OB-101-2) (with Part A and Part B mixable components).

C. Connector Cable (Rat to Commutator)

1. 2 × 7 Female Connector and wire assembly with retention latches (Samtec, catalog number: SFSD-07-30C-G-12.00-SR) × 1
2. Silbione Silicone Medical Adhesive A-4300
3. Metal Spring Shielding (InVivo1, catalog number: 120 X .156)
4. 12-Channel Component Cable with Open Ends (InVivo1, catalog number: 363/2-000)
5. Suitable Size Heat Shrink for Insulating Soldered Connections
6. 101 Epoxy adhesive (Omegabond, catalog number: OB-101-2) (with Part A and Part B mixable components)

D. Stimulation and Recording Hardware (Commutator to Computer):

1. Laboratory Computer with MATLAB installation
2. A-M Systems 2,100 Stimulator × 2 or equivalent
3. Brownlee Precision Model 400 Amplifier or equivalent
4. Labchart Hardware System
5. Digital to Analog (DAC) SCB-68A Converter Digital Acquisition System
6. Invivo1 SL12C Double Brush Commutator
7. Associated Support Frames and BNC Connecting Cables

Software

1. PAS Analyzer Code at: <https://github.com/ethierlab>. Download repositories: PAS_interface_stim, PAS, Plotting, and Data_processing
2. MATLAB for Windows, Version 2018 or greater
3. LabChart Software Version 7 or higher
4. Latest drivers for stimulation and recording hardware, as well as DAQ System

Procedure

A. Making Part A: Cortical Stimulation Array (Considerations in Brackets)

1. Start with obtaining the necessary components (see list of equipment).
2. First, make the mount for the array: pick up the rigid straight piece of stainless steel tubing and cut about a 5 cm length (this part will be used to mount the array onto the stereotaxic frame during the implant).
3. Take the female socket using tweezers and use a crimper tool to crimp the shorter end of the socket onto the piece of wire, ensuring that both components are firmly attached. Set the mount aside for now (Figure 4A).
4. Pick up the male pin and observe its construction. There is one end with a smaller outer diameter (OD) which fits into the corresponding female socket. This side will be left untouched for the time being. Our attention will be focused on the other end (demarcated by the asterisk in Figure 4B). Notice that the center part of the male pin has an element with slightly larger OD relative to the rest of the pin.
5. Cut out a first piece of 0.050" heat shrink tubing, long enough to cover the asterisked end but not extend beyond the edge of the pin. Use a heat shrink gun to fit the heat shrink on the asterisked end snugly, applying uniform heat to ensure good fit (at this point, the central portion of the male pin should be elevated only slightly relative to the OD of the heat shrink on the asterisked end) (Figure 4C).
6. Cut out a second piece of 0.050" heat shrink tubing, long enough to cover to the asterisked end edge and the elevated central portion of the male pin, but again not extending beyond it. Fit uniformly using the heat gun.
7. Create the array electrodes by unspooling some Pt-Ir wire and cutting four 3 cm pieces.
8. Remove about 2 mm of insulation from one end on each piece of array wire.
9. Cut two pieces of larger diameter 0.066" heat shrink tubing of approximately about 2 mm in length.
10. Slide the two unheated 0.066" heat shrink pieces over the asterisked end. This should leave a small space to insert the array wires (Figure 4D). Using a helping hand, position each array wire in a square formation (viewing from the top down with the pin in the middle) (Figure 4E and 4F). Insert the four array wires so that they extend more than 3 mm beyond the tip of the asterisked

end.

11. Apply slight heat to the third piece of heat shrink tubing to reduce the space available, reposition the array wires into their final positions, and finish the shrink by applying full and uniform heat (ensure the relative length of each array wire is the same).
12. Mix a small portion (1/4 of a pea sized portion is sufficient) of Parts A and B of the epoxy adhesive together on a disposable surface, and apply generously to cover all three portions of heat shrink and permanently fix the electrodes in their final position [(take care not to cover the actual tips of the electrodes where they will be inserted into the brain (Figure 4G); the other end of the epoxy should not go beyond the edge of the central portion of the male pin]. Wait 24 h for the epoxy to settle, then make final adjustments to the array electrodes so that the leads are parallel to one another.
13. Cut the asterisked end of the array's electrodes to the desired final length (Figure 4H) (this depends on the expected depth of the electrodes in the brain and targeted structures; ensure that all four electrodes in the array are uniform). Ensure that a bit of each tip has insulation removed to facilitate neurostimulation once the array is implanted in the brain.
14. Coat the *other end* (opposite to the side going into the brain) of the array electrodes with a small bit of solder to facilitate attachment to the head connector during the surgery.
15. Insert the male end of the array pin into the female socket holder, and store electrode side up, by sticking the mount in a small piece of sturdy foam. Use caution when manipulating the array, and store in a protected location where foot/hand traffic is minimal, to prevent accidental trauma with the sharp electrodes. A finished array is depicted in Figure 4I.

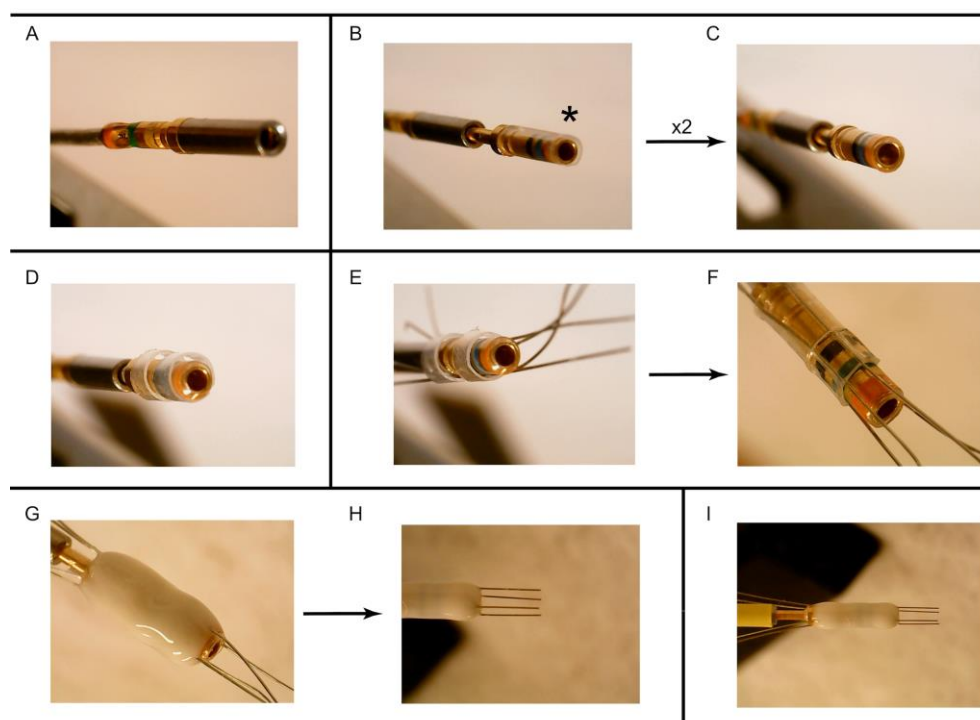


Figure 4. Cortical Stimulation Array Assembly.

Refer to protocol steps for figure panel annotations.

B. Making Part B: Head Connector and EMG Electrodes (Considerations in Brackets)

1. Start with obtaining the necessary components (see list of equipment) and clearing the workbench space if necessary.
2. Select the spool of insulated stainless steel wire (Figure 5A), and use scissors to cut one short length of wire (approximately 5 cm) for the EMG reference electrode.
3. Continue cutting the wire in pairs of the same length, corresponding to the desired muscles [more distal muscles will require longer wires to ensure comfort and prevent movement tension after implant, but generally, no longer than 10 cm for the forelimb extensor muscles. The size of the rat will also need to be taken into consideration, with larger rats requiring longer wires to accommodate their larger frame. If in doubt, err on the side of longer than necessary at this stage – the wires can be trimmed at their free (unsoldered) end later on].
4. Using a scalpel blade on a flat block of Teflon®, and under a microscope at low magnification and sufficient lighting, *gently* remove about 2 mm of insulation (dashed arrow in Figure 5B) from each end of each stainless steel wire, to permit soldering (Figure 5B) (with practice, this will be possible without fraying of the threaded wire). The EMG reference wire should have about 2 cm of insulation removed from one end (Figure 5C). When inserted in the rat's back, this will help ensure that a cleaner differential can be calculated.
5. Using serrated scissors, cut four short (approximately 2 cm) lengths of thin gauge electrical wire, and remove approximately 2 mm of the insulation from both ends of each piece using a wire stripper. The components should be ready for assembly now (Figure 5D).
6. Verify the plan for the implant, which will dictate which location to solder each EMG and cortical wire. A sample arrangement is provided in Figure 2, but feel free to adapt the pattern to your specific project.
7. Place the Samtec® connector in a helping hand, male side down (with the exposed underbelly of the connector up) in preparation for soldering. Use the notch (dashed arrow in Figure 5D) to orient yourself to the respective pins on the Connector.
8. Solder each wire to the appropriate pins on the underside of the Samtec® connector, ensuring that no shorts are introduced (Figure 5E, 5F). If necessary, test each connection and Samtec® pin using a multimeter. EMG wire pairs should be marked with sets of one, two or three pairs of suture knots tied along their lengths, each pair corresponding to two pins on the Samtec® connector, to ensure they can be routed to the correct muscles during the surgery.
9. Use a fine thread or a spare piece of suture to tie the wires together near their base, and redirect them in one common exit direction prior to applying epoxy. The wires should be bent in a common direction and not exit the Samtec® connector perpendicularly, in order to minimize the height of the eventual implant (Figure 5G).
10. Mix equal parts epoxy precursor in a weigh boat or other disposable surface (half a pea-sized amount of each component) and apply using a small spatula to the soldered underside of the

connector to fix the connections permanently (Figure 5H) [NB: Take special care not to apply too much, such that the epoxy drips into the other (connecting) side of the implant. This will prevent a reliable and easy connection with the Samtec® female connector in the cable. However, ensure that the exposed pins on the soldering side of Samtec® connector are fully covered].

11. Leave the epoxy to set for 24 h and reapply a second more generous coating of epoxy to finish the implant, at least 24 h (preferably 48 h) prior to the actual surgery (Figure 5I).
12. Gently twist each pair of EMG wires together to facilitate implantation and reduce noise.
13. The morning of the surgery, place the implants in a shallow bowl of 70% ethanol to disinfect them, and remove the thread prior to implantation. We recommend making fresh versions of both implants for each surgery.

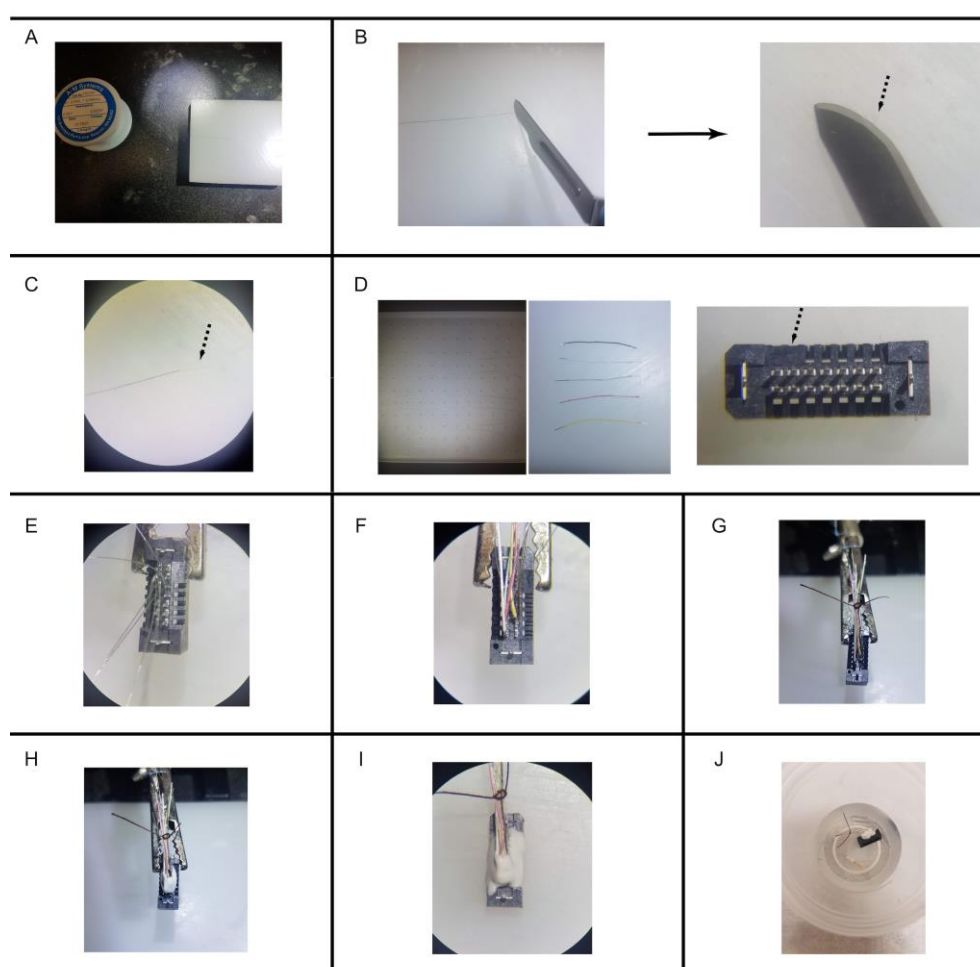


Figure 5. Head Connector and EMG Electrode Assembly.

Refer to protocol steps for figure panel annotations.

C. Implant Surgery (Considerations in Brackets)

1. Pre-operative Care and Induction of Anesthesia

- a. Weigh the rat and open a new log file or notebook page for recording peri-operational details.
- b. Prepare the surgical workspace. We use a water bed heater set at 38°C with a sterile disposable liner on top. We also have a Zeiss microscope on the same table, and a stereotaxic frame for rats within close reach.
- c. Deeply anaesthetize the rat with the agent of choice [Surgery can be completed under isoflurane or the appropriate dosage per kilogram of body weight of ketamine-xylazine (KX) anesthesia, but isoflurane inhalation is recommended because it provides a more reliable anesthesia and analgesia, and is easily adjustable during the surgery)]. Use the paw pinch reflex to assess the depth of anesthesia.
- d. After sedation, position the rat on its belly, with the forelimb selected for EMG implantation directly facing the experimenter [for illustration purposes we will select the right paw. If the right forelimb muscles are the target for excitability measurement, then perform a craniectomy and insert the array in the left caudal forelimb area (CFA); reverse the laterality if the left forelimb is the target].
- e. Apply eye gel to ensure lubrication, inject lidocaine/bupivacaine solution at the incision sites for local analgesia, and carprofen as a systemic analgesic. Hydrate the rat with appropriate amounts of Lactated Ringer solution (approx mmol/L Na – 130, K – 4, Ca – 1.4, Cl – 109, Lactate – 28, mOsmol/L – 272, pH 6.5) peri-operatively.

2. Insertion of EMG Electrodes

- a. Shave the rat's head and forelimb areas.
- b. Prepare a sterile field.
- c. Sanitize the forelimb area using chlorhexidine.
- d. Don a set of sterile gloves.
- e. Make a short (5 mm) incision using a new scalpel blade on the proximal dorsal forelimb surface.
- f. Undermine the skin in a small radius around the incision site using blunt scissors, to expose the extensor carpi radialis (ECR) muscle.
- g. Make a second incision to access the biceps (Bi) muscles.
- h. Make a third 0.5 cm incision on the shoulder ipsilateral to the target forelimb, over the trapezius (Trap) muscle. Make a final 0.5 cm incision on the scalp, just rostral to the ears.
- i. Next, use fine hemostatic forceps to carve a subcutaneous tunnel from the forelimb to the scalp incisions; retrieve and gently pull back the EMG wires, from the scalp incision to the forelimb exit site. Leaving the pair of EMG wires in place, repeat the process to pull the two remaining pairs to their respective destinations.
- j. To implant the EMG wires in the targeted muscles, use the tail end of a 6-0 polypropylene suture to tie a knot near the tip of the exposed EMG wire (Figure 6A).
- k. Then, using a needle holder, insert the suture needle deep into the targeted muscle belly,

and pull it back out of the muscle about 2-3 mm from the entry point, in a direction perpendicular to the muscle fibers (Figure 6A, suture movement as in green arrow). Gently thread the suture until the tip of the EMG electrode penetrates into the muscle belly, while holding the tail end of the suture to prevent it from entering the muscle.

- l. While ensuring the electrode tip remains buried inside the muscle, tie back the tail of the suture with the main thread across the muscle (Figure 6B; green arrow indicates two areas to tie together), not too tight so as to avoid unnecessary pressure on the muscle and hinder blood flow. The end result is depicted in Figure 6C.
- m. Steps C2j-C2l are repeated for a second EMG wire, approximately 2 mm proximal or distal to the first, in order to perform differential recording.
- n. After both wires are securely implanted, anchor the pair of wires in a bundle to the muscle fascia a few mm away from their insertion point, through a separate polypropylene suture.
- o. Finally, flush the surgical area with saline and close the wound with a 5-0 nylon suture.
- p. Repeat the procedure as needed for other target muscles. Take note that polypropylene, or other braided, flexible, and non-absorbent suture materials, are best for internally securing the EMG wire, and a nylon monofilament suture is best for closing the skin on the surface.
- q. Afterwards, place the rat in a stereotaxic frame equipped with a mask for gas anesthesia and a micromanipulator for the stereotaxic insertion of electrodes into the cortex, once it is exposed.

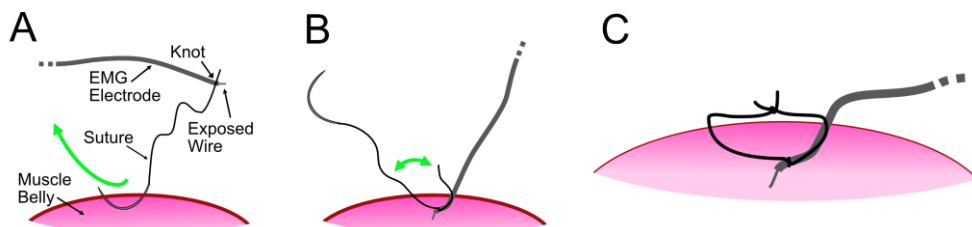


Figure 6. Insertion and Fixation of EMG Electrodes.

Refer to protocol steps for figure panel annotations.

3. Insertion of Cortical Array

- a. At this point, the isoflurane can be routed through the mask secured in the stereotactic frame (carefully monitor for any whisker movements or paw withdrawal reflex and adjust anesthesia delivery as required).
- b. Start the craniectomy by extending the scalp incision made earlier for the EMG wire bundle entry point [the incision should only be long enough to comfortably insert a Barraquer wire speculum (retractor) with slight tension].
- c. Clear the surface of the skull by removing the periosteum covering the surface (it can also be dried using autoclaved cotton swabs).
- d. Once the skull surface is sufficiently clean and exposed, mark bregma and delineate the area for drilling [this craniectomy just needs to be large enough (approximately 2 × 2 mm)

to comfortably insert the cortical array in the desired location (posterior and medial electrode placed approximately 0.2 mm anterior and 3.0 mm lateral to bregma to target CFA)] – see Ting *et al.* (2020) for further details, and Figure 7A for a schematic summarizing this, as well as the following four steps.

- e. Insert three self-tapping skull screws on the contralateral side to the planned craniectomy near the lateral ridges of the skull surface, and two on the ipsilateral side (leave the closest burr hole to the craniectomy open to permit manoeuvrability of the dremel®), to provide mechanical anchors for the dental cement to support the cortical implant and connectors. The size of the skull screws is such that they can be secured within the bone and leave the top of the screw approximately 1 mm above the surface of the skull, acting as an anchor for the acrylic. Be careful not to screw in too deep however.
 - f. Insert the skull screws before starting the craniectomy, to prevent any debris from falling onto the dura. Using a 0.7 mm burr on a rotary tool (Dremel®), perform a craniectomy over the CFA.
 - g. After removing the skull flap, attach the cortical array to the holder (ensure the electrodes are parallel to each other and to the holder, to ensure maximum accuracy).
 - h. Insert the final skull screw in the burr hole closest to the craniectomy site.
 - i. Using the stereotaxic apparatus, carefully descend the electrodes into the cortex (ensure that the tips of the electrodes insert cleanly through the dura – expect that there will be some resistance and dimpling before all the electrodes pass through) (see Figure 7B summarizing this step and the five following ones).
 - j. Once all the electrode tips have pierced the dura, wait one or two minutes while the cortical surface rises back to its original position. Gently bring the electrode tips to their final position at 1.5 mm below the cortical surface.
 - k. Mix and use a pea-sized portion of quick-setting silicone array (Kwik-Cast®) to cover the dural surface around the implant, using a mini-spatula to fill the gap in the craniectomy.
 - l. Within five minutes, the silicone will solidify, covering any exposed dura.
 - m. In order to secure the cortical array permanently, use dental cement. Stir the mixture to a slightly runny consistency, and use a spatula to connect the epoxy body of the cortical array to the closest anchoring skull screws (take care for drops of dental cement not to fall on the surrounding surfaces, and especially on the rat's eyes, fur, *etc.*).
 - n. After a few minutes, the dental cement will harden, and the electrode holder can be safely removed, leaving the cortical array in place. There will still be four wires extending upwards, however, which will be connected shortly.
4. Soldering to Connector
- a. Next, use a stereotaxic holder to hold the Samtec® connector (we used a custom-made clamp mounted on a micromanipulator).
 - b. Position the Samtec® connector about 1 cm above the surface of the skull, oriented rostro-caudally lengthwise.

- c. Then, prepare a soldering setup.
 - d. Ensure the soldering connections match the pinout specified *a priori*, and that you record the position of each electrode in the cortical array relative to the pinout (for example, Samtec® pin 3 is connected to the rostral medial electrode, *etc.*).
 - e. Using fine solder, solder each wire extending upwards from the cortical array to the designated wires on the Samtec® connector, first placing a piece of fine heat shrink on one wire, so the new connection can be promptly insulated with a heat gun after the solder has set (see Figure 7C for an illustration of these connections). Take care not to burn the fine wires by applying the iron for longer than is necessary. A very fine coating of flux may facilitate this connection; use a small gauge solder wire. Only a small drop is necessary per connection.
 - f. Use a heat gun with a narrow air stream to insulate the electrical connection. Do not aim the stream of hot air toward the animal, or near its eyes, nose, mouth or whiskers in particular. Repeat for each cortical wire; ground wire should be soldered to a posterior skull screw.
 - g. Once all the correct electrical connections have been made, lower the Samtec® connector closer to the skull surface (in practice, it fits well immediately adjacent to the A-M Systems® male pin that extends upwards from the cortical array; see Figure 7C for relative positioning of the connector and electrodes).
 - h. Then, use fresh dental cement to fix the Samtec® connector permanently to the skull, using the skull screws as anchor points, while embedding all the wires so none are left exposed (take special care *not* to drip wet dental cement into the Samtec® connector, which can be very difficult to remove and renders a good electrical connection almost impossible after the surgery. A small piece of protective tape is useful here). See pink area in Figure 7C.
 - i. Close the open wound by carefully applying dental cement into the surrounding area and sculpting the cement surface so that there are no sharp edges.
 - j. Trim the skin on the perimeter of the implant to remove excess and aid recovery.
 - k. Implant placement is completed!
5. Post-Operative Care
- a. Clean any residual blood from the surface of closed incisions on the limbs using saline and some gauze; also clean the perimeter of the implant on the head and ensure there is no residual blood or scab formation.
 - b. Trim the nails on the rat to prevent self-injury from scratching. House the rat singly in a clean cage.
 - c. Allow the rat to recover with institutional animal care best practices (give Lactated Ringer's solution for hydration and atipamezole to counteract the effects of the ketamine-xylazine and speed up recovery). Remaining skin sutures can generally be removed seven to ten days after surgery.
 - d. Carprofen, or another non-steroidal anti-inflammatory, should also be administered up to 48

hours after the surgery to relieve pain and inflammation.

- e. Allow at least three days (generally up to one week) of undisturbed recovery prior to testing the chronic implant. Monitor daily for signs of infection and treat accordingly. In case of infection, consult the veterinary staff at your institution, who can advise and carry out the appropriate treatment plan for recovery. A high degree of care to maintain aseptic conditions during the implant surgery will minimize the risk and occurrence of infection.

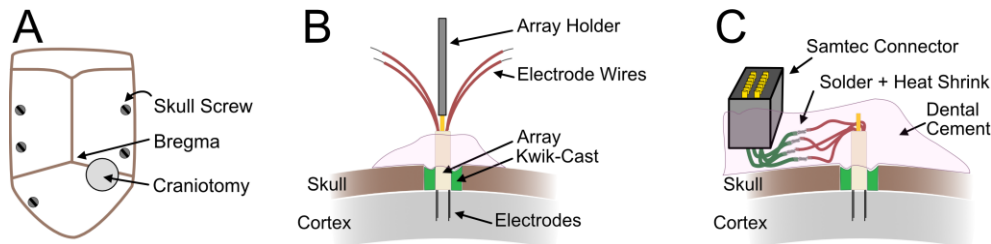


Figure 7. Insertion of Cortical Array.

Refer to protocol steps for figure panel annotations.

D. Making the Connector Cable between Rat and Commutator (Considerations in Brackets)

1. Start with obtaining the necessary components (see list of equipment) and clearing the workbench space if necessary (Figure 8A).
2. Cut the Invivo1® spring shielding to a desired length, we have found that approximately 40 cm works well.
3. Cut appropriate lengths of the Samtec® female connector wires and InVivo1 exposed wires, such that soldered together, their combined length is slightly longer than the length of the InVivo1® spring shielding.
4. Create or revisit the desired mapping between Samtec® male pinout and InVivo1® pins (refer to their respective datasheets). A sample map is provided in Figure 2.
5. Carefully feed all the wires from the Samtec® female connector into the metal spring shielding (Figure 8B) (be careful not to lose any wire in the spring structure).
6. Expose approximately 3 mm from each wire tip to facilitate soldering.
7. For each wire, slide a large enough piece of heat shrink down the wire before soldering.
8. Using a helping hand, solder each wire to the correct matching one in the plan (as there are 14 pins in the Samtec® connectors and 12 pins in the Invivo1®, two pins will be left unsoldered in the Samtec® connector) (Figure 8C).
9. Slide the heat shrink over the new electrical connection and secure using a heat gun.
10. Repeat Steps D6 to D9 for each available wire.
11. Verify each electrical connection between the two connecting ends of the cable using a voltmeter.
12. Mix some epoxy parts A and B, and apply liberally to the loose wire assembly on approximately the final centimeter of the InVivo1® connector (Figure 8D) (this helps with wire management). Wait 24 h for the epoxy to set.
13. Apply some silicone adhesive liberally over this epoxy, and as well as the loose wire assembly

leading into the Samtec® end of the cable (Figure 8E) (a general guide is to apply until the edge of the wire shielding). Wait 24 h for this adhesive to set – the surface should not be sticky once that happens.

14. Make a duplicate second cable; aside from the implant in the rat itself, this is the element of the system most prone to malfunction, and so it is prudent to have a backup to ensure scientific progress is not unduly impeded. A finished cable is depicted in Figure 8F.

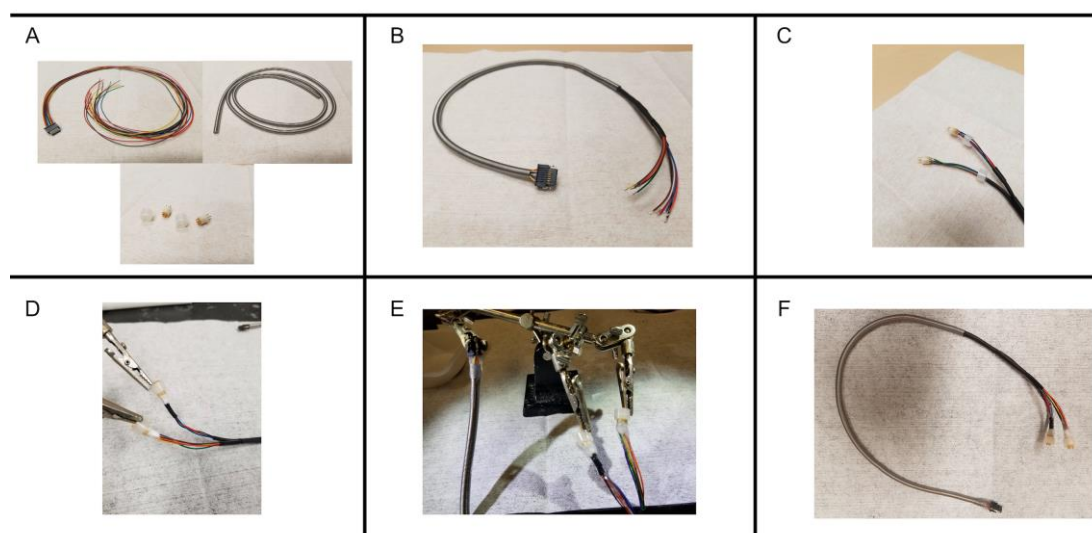


Figure 8. SAMTEC to InVivo1 Connector Cable Assembly.

Refer to protocol steps for figure panel annotations.

Data analysis

A. Hardware and Software Interface for Closed-Loop Assessment of CSE

Software was written for closed-loop assessment of corticomotor excitability and neurostimulation (https://github.com/ethierlab/PAS_interface_stim). The interface captures the EMG activity in the target muscle, extracts the envelope of the signal, classifies whether the amplitude of EMG activity is within a pre-determined window, and if so, whether it entered that window from a lower (increasing EMG) or higher (decreasing) activity level. This facilitates the decision to dynamically trigger cortical stimulation only when EMG levels are within a desired range and with a certain activity pattern, respectively, both of which can be defined by the user (Figure S1 in [Appendix](#)). In our setup, the EMG signals are amplified and filtered using Brownlee Precision Model 400 differential amplifiers (gain of 100, bandpass filtered between 10 Hz and 1.5 kHz with a 60 Hz notch filter). The processed EMG signal from the target muscle is routed to an analog port of a NI-USB 6212 digital acquisition (DAQ) card, connected to a workstation computer. The MATLAB interface, which can be found in our GitHub repository, plots and updates the EMG signal in pseudo-real time. The interface allows the user to specify the EMG-based criteria which should trigger stimulation. The superior envelope of the EMG signal is calculated using the MATLAB *envelope()* function. Cortical stimulation is

invoked when the envelope rises within a user-specified range relative to a defined noise baseline, and is maintained within that range for a specified duration. When the specified conditions are met, a transistor-transistor logic (TTL) signal is sent by the output ports of the DAQ system, in order to activate an AM Systems® 2100 stimulator connected to the cortical electrodes. The stimulator therefore generates trains or single current pulses according to user-defined EMG parameters.

B. User Interface and Codebase

The graphical user interface is versatile and facilitates easy customization of the stimulation protocols and parameters. A reference guide to the experimental interface is provided in the [Appendix](#). Unique electrical and optical stimulation protocols can be programmed directly into this system, and stimulation can be conditioned based on EMG activity (*i.e.*, EMG-controlled closed-loop neurostimulation). More scientific details about why this is desirable are in the companion paper.

C. Data Analysis Workflow

We have also developed a standalone program for analyzing and visualizing evoked potentials during CSE assessment (<https://github.com/ethierlab>, PAS, Plotting, and Data_Processing Repositories). Data analysis for experiments may follow a stereotyped workflow, as below, but is highly modular and elements of the analysis program can be used independently if needed. Develop a folder structure which is effective for you, to keep everything organized. The following instructions are current as of writing, but the software is being continually improved and developed, so if there are additional features which are not described below or if there are any bugs, please contact us and we would be happy to assist.

1. Download the latest PAS Analyzer Code.
2. Save the raw data in individual LabChart files (.adicht, ADInstruments, Inc.) after each probe. In order to be effective at isolating snippets of EMG signal relative to stimulation, PAS Analyzer requires that there be at least one data channel (typically with EMG), and at least one stimulation channel dictating when electrical current or optical light was delivered. This can be easily done by connecting a BNC cable from the stimulator to the LabChart Hardware Unit, and selecting a high sampling frequency (10 kHz works well for sub-millisecond single electrical pulses). During experimental setup within LabChart, remember to label the channels in a clear and concise manner so that the respective data can be easily identified during analysis.
3. Export these LabChart (LC) files into MATLAB format, with the desired channels and data settings. Save in a different folder for analysis.
4. Open and run the PAS Analyzer.m file in MATLAB. This will open a graphical user interface (GUI) where the data can be processed without use of the command line or coding.
5. The PAS Analyzer re-organizes each dataset into human-readable simplified format (Ethier Lab Format, ELF) and imports them into the GUI for processing. Start this process by clicking "Import LC" and selecting the folder of the exported MATLAB files from step 3.
6. Another window will pop up. Create and select a new folder to store the converted MAT files in

ELF format.

7. Look in the command line. For each probe file which was exported, all the channels within that file will be listed and number coded. Enter, in vector format, the desired channels to analyze as dependent variables, and subsequently the corresponding stimulation channels to extract the EMG snippets (in the current iteration of the software, only one stimulation channel can be selected at a time, but the process can be repeated for multiple channels if required).
8. Once this process is completed, each probe file will be listed as a row in the table to the left. Most of the other buttons in the GUI will use the selected dataset or require selecting a series of datasets (for plotting bar graphs across probes, for instance).
9. Specify the initial parameters for stimulation-by-stimulation analysis of the data in the selected probe, using the text boxes to the right. Options available include selecting one or more data channels, the time before stimulation to include, the time after stimulation, and the hardware gain setting applied in the amplifier, which will then calibrate the dependent data to the correct units in microvolts.
10. The generic processing pipeline first involves plotting each MEP individually and manually verifying the quality of the response. MEPs contaminated with stimulation or other artifacts can be marked as such (artifact button) and can be excluded from further analyses. Flagged MEPs are preserved in the data files and can be restored for future auditing or re-analysis purposes. For analyses requiring the exclusion of non-MEP data segments, an option (MEP -) is available as well.
11. Once all the stimulations in the particular probe file have been screened, a pop-up window will ask the user if they would like to update the data structure with the screening results. If the user selects "update", the excluded MEPs will be removed from further calculations. If the user selects "cancel", no changes to the data structure will be applied. Prematurely selecting "close" during the MEP validation will bring up the same dialog, presenting the user the opportunity to quit the subprogram without saving any changes.
12. After each probe is validated, the average EMG trace can be plotted for each probe block by selecting "plot trace". This plots the mean response as well as shades in ± 1 standard deviation (SD) from the mean, for all MEPs in the probe block after validation. This will give the user a sense of the average excitability during the assessment, and helps to determine the relevant time range encompassing the average MEP response, which will be required next as a parameter to quantify MEP responses for the entire experiment.
13. Follow with validation of all the probes in the experiment if desired, by selecting the appropriate data block and repeating the process. Validation is not a prerequisite to do further computations on the data from a software perspective, and in some cases (when the signal-to-noise ratio is high, such as experiments done under anesthesia) it is not required. However, in awake experiments, when stimulation may coincide with ongoing muscular activity from free behavior, it may be desirable based on the scientific questions to do a more careful screening, to enhance the strength of the study conclusions.

14. Specify a particular analysis timeframe by entering two positive numbers corresponding to the lower and upper millisecond bound, in vector format under “measurement time window”. Specifying a nonzero lower bound is helpful, for instance, when there is a pronounced stimulation artifact, to exclude that part of the waveform from analysis of response size.
15. The average MEP response for each data block can now be calculated by selecting all the rows in the table and clicking “measure”. The calculated values will populate the table columns to the right of each datablock name.
16. Three calculations are made on MEPs of each block of the experiment, using the predetermined analysis time window: 1) the mean peak-to-peak MEP amplitude, 2) the mean integral of single MEPs, and 3) the integral of the averaged MEP waveform. Qualitatively, these three approaches usually provide relatively similar information, but each have their limitations. We have found peak-to-peak values to provide fewer representative measures when MEPs are multi-modal, presenting multiple positive and negative peaks. Integrals of average MEPs are suboptimal when MEPs tend to vary in latencies. Both the latter and the mean of MEP integrals can provide erroneous values if the mean of the EMG signal shifts away from zero for any reasons (DC offset in EMG signal). Nevertheless, one should start with the mean peak-to-peak value as this measure is simple to interpret, is generally representative of the MEP size, and is the literature standard.
17. Once the MEPs are quantified, click on the “bar plot” button to summarize the results using bar graphs representing any of the above three calculation approaches (see Ting *et al.*, 2020, Figure 3). The bar labels can be edited directly within the GUI table, and the order of bars in the plot can be specified using the subsequent dialog box. The standard error of the mean (SEM) can be plotted as well. These plots can be further processed, refined, and saved using built-in MATLAB functions.
18. Enter basic metadata about the experiment, including “rat name”, “date (of experiment)”, and “Exp(erimental) condition”. This information will be saved alongside the other data.
19. The validated and processed data can then be saved in MATLAB format using the “save as” button, and easily retrievable via the “load mat” button should the experimenter wish to reload the data to regenerate one or more plots. All data within the PAS analyzer can be cleared at any time using the “clear” button below the table.
20. From there, data can easily be exported to statistical analysis and visualization tools such as R, SPSS, or Minitab for publication purposes.

D. Conclusion

We demonstrate here a method of assaying corticospinal excitability using electrical stimulation to the motor cortex of awake rats. In documenting this development, we hope to release a platform upon which to improve. Future iterations of this technique may employ optogenetic techniques to stimulate the corticospinal tract neurons specifically, and increase the specificity of the intervention and results.

Notes

A. Troubleshooting Common Issues

1. Poor EMG data recording quality

With this setup, the most likely scenario is a faulty tethering cable from mechanical stress. Double check that each individual pin in the cable maintains sufficient electrical contact between the Samtec® connector, which attaches on the rat head, and the InVivo1® connector, which is connected directly to the commutator. With a well-constructed cable this is less likely to be a recurrent problem.

2. Connected rat escaping from the cage

Drill a hole ~1 cm² in a cage filter cover and feed the connector cable through the hole.

3. Implant deterioration

Initially, we observed that the two main reasons for implant failure involved oedema and infections at EMG implant sites and the loss of EMG signals, and the ability to activate muscles at low stimulation intensity. Careful disinfection of EMG wires and connectors in 70% alcohol, combined with thorough aseptic surgical methods, and meticulous wound closure greatly reduced the occurrence of infections. Minimizing skin undermining to avoid creating pockets of fluids may also help mitigating oedema and infections. The addition of an anchoring suture near the EMG wire insertion point, to help prevent wires from pulling out of the muscle, improved the stability of muscle recording and stimulation. Although occasional electrode failure or signal deterioration is expected, following these procedures allowed us to create stable implants in rats which could last up to several months.

B. Next Steps

Future iterations of a CSE assessment system should focus on specificity of stimulation and adding constraints to stimulation conditions, while preserving a high degree of physical freedom when no stimulation is required. Electrical stimulation recruits muscle fibers in the reverse order compared to normal muscle activation, as would be predicted by the Hennemann Size Principle (Dehail *et al.*, 2008), and electrical stimulation in the brain is non-specific to corticospinal neurons and will activate all excitable cells within a given radius touched by current spread. The first improvement can be addressed using optogenetics, to target specific neuronal populations in the corticospinal system. We have successfully adapted this system for closed-loop optogenetic assessment of corticospinal excitability. The second issue may be solved with behavioral training on a stereotyped task. We have had initial success training rats on an isometric pull task on the Mototrak apparatus (Hays *et al.*, 2013), which despite being time intensive (roughly a month of twice daily 30 min training sessions to obtain baseline performance), can easily be merged with our platform such that CSE assessment takes place during specific corticomotor states. Operationally, one can envision a multifactorial system where probe stimulation is only delivered when the EMG is within a certain range, as already realized, but with the additional requirement that the force profile be within a

predefined range as well, corresponding to a certain phase of the pull movement. Our platform is readily extensible for new stimulation paradigms, or conditional stimulation of multiple areas of the brain.

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Competing interests

There are no conflicts of interest or competing interests.

Ethics

All animal procedures were performed in accordance with the regulations of the Université Laval animal care committee (CPAUL# 2015-131 and 2019-007, valid from 12/2015 to 01-2022).

References

1. Alstermark, B., Ogawa, J. and Isa, T. (2004). [Lack of monosynaptic corticomotoneuronal EPSPs in rats: disynaptic EPSPs mediated via reticulospinal neurons and polysynaptic EPSPs via segmental interneurons.](#) *J Neurophysiol* 91(4): 1832-1839.
2. Brown, A. R. and Martinez, M. (2019). [From cortex to cord: motor circuit plasticity after spinal](#)

- [cord injury](#). *Neural Regen Res* 14(12): 2054-2062.
3. Christiansen, L. and Perez, M. A. (2018). [Targeted-Plasticity in the Corticospinal Tract After Human Spinal Cord Injury](#). *Neurotherapeutics* 15(3): 618-627.
4. Dan, Y. and Poo, M. M. (2004). [Spike timing-dependent plasticity of neural circuits](#). *Neuron* 44(1): 23-30.
5. Dancause, N. and Nudo, R. J. (2011). [Shaping plasticity to enhance recovery after injury](#). *Prog Brain Res* 192: 273-295.
6. Dehail, P., Duclos, C. and Barat, M. (2008). [Electrical stimulation and muscle strengthening](#). *Ann Readapt Med Phys* 51(6): 441-451.
7. Feldman, D. E. (2012). [The spike-timing dependence of plasticity](#). *Neuron* 75(4): 556-571.
8. Hays, S. A., Khodaparast, N., Sloan, A. M., Hulse, D. R., Pantoja, M., Ruiz, A. D., Kilgard, M. P. and Rennaker, R. L., 2nd (2013). [The isometric pull task: a novel automated method for quantifying forelimb force generation in rats](#). *J Neurosci Methods* 212(2): 329-337.
9. Kleim, J. A., Boychuk, J. A. and Adkins, D. L. (2007). [Rat models of upper extremity impairment in stroke](#). *ILAR J* 48(4): 374-384.
10. Lemon, R. N. (2008). [Descending pathways in motor control](#). *Annu Rev Neurosci* 31: 195-218.
11. McGie, S. C., Masani, K. and Popovic, M. R. (2014). [Failure of spinal paired associative stimulation to induce neuroplasticity in the human corticospinal tract](#). *J Spinal Cord Med* 37(5): 565-574.
12. Mishra, A. M., Pal, A., Gupta, D. and Carmel, J. B. (2017). [Paired motor cortex and cervical epidural electrical stimulation timed to converge in the spinal cord promotes lasting increases in motor responses](#). *J Physiol* 595(22): 6953-6968.
13. Rothwell, J. C. (2016). [Can Motor Recovery in Stroke Be Improved by Non-invasive Brain Stimulation?](#) *Adv Exp Med Biol* 957: 313-323.
14. Sale, M. V., Ridding, M. C. and Nordstrom, M. A. (2007). [Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation](#). *Exp Brain Res* 181(4): 615-626.
15. Serradj, N., Agger, S. F. and Hollis, E. R., 2nd (2017). [Corticospinal circuit plasticity in motor rehabilitation from spinal cord injury](#). *Neurosci Lett* 652: 94-104.
16. Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R. and Classen, J. (2000). [Induction of plasticity in the human motor cortex by paired associative stimulation](#). *Brain* 123 Pt 3: 572-584.
17. Suppa, A., Quartarone, A., Siebner, H., Chen, R., Di Lazzaro, V., Del Giudice, P., Paulus, W., Rothwell, J. C., Ziemann, U. and Classen, J. (2017). [The associative brain at work: Evidence from paired associative stimulation studies in humans](#). *Clin Neurophysiol* 128(11): 2140-2164.
18. Tarri, M., Brihat, N., Gasq, D., Lepage, B., Loubinoux, I., De Boissezon, X., Marque, P. and Castel-Lacanal, E. (2018). [Five-day course of paired associative stimulation fails to improve motor function in stroke patients](#). *Ann Phys Rehabil Med* 61(2): 78-84.
19. Taylor, J. L. and Martin, P. G. (2009). [Voluntary motor output is altered by spike-timing-dependent changes in the human corticospinal pathway](#). *J Neurosci* 29(37): 11708-11716.

20. Ting, W. K., Huot-Lavoie, M. and Ethier, C. (2020). [Paired Associative Stimulation Fails to Induce Plasticity in Freely Behaving Intact Rats](#). *eNeuro* 7(2): ENEURO.0396-19.2020.
21. Urbin, M. A., Ozdemir, R. A., Tazoe, T. and Perez, M. A. (2017). [Spike-timing-dependent plasticity in lower-limb motoneurons after human spinal cord injury](#). *J Neurophysiol* 118(4): 2171-2180.
22. Zanos, P. and Gould, T. D. (2018). [Mechanisms of ketamine action as an antidepressant](#). *Mol Psychiatry* 23(4): 801-811.
23. Zeiler, S. R. and Krakauer, J. W. (2013). [The interaction between training and plasticity in the poststroke brain](#). *Curr Opin Neurol* 26(6): 609-616.
24. Zhang, X. Y., Sui, Y. F., Guo, T. C., Wang, S. H., Hu, Y. and Lu, Y. S. (2018). [Effect of Paired Associative Stimulation on Motor Cortex Excitability in Rats](#). *Curr Med Sci* 38(5): 903-909.