Video Article

Chronic Transcranial Electrical Stimulation and Intracortical Recording in Rats

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Abstract

Transcranial electrical stimulation (TES) is a powerful and relatively simple approach to diffusely influence brain activity either randomly or in a closed-loop event-triggered manner. Although many studies are focusing on the possible benefits and side-effects of TES in healthy and pathologic brains, there are still many fundamental open questions regarding the mechanism of action of the stimulation. Therefore, there is a clear need for a robust and reproducible method to test the acute and the chronic effects of TES in rodents. TES can be combined with regular behavioral, electrophysiological, and imaging techniques to investigate neuronal networks *in vivo*. The implantation of transcranial stimulation electrodes does not impose extra constraints on the experimental design while it offers a versatile, flexible tool to manipulate brain activity. Here we provide a detailed, step-by-step protocol to fabricate and implant transcranial stimulation electrodes to influence brain activity in a temporally constrained manner for months.

Video Link

The video component of this article can be found at https://www.jove.com/video/56669/

Introduction

Transcranial electrical stimulation (TES) is a valuable methodological approach to influence brain activity in a temporally constrained manner. Depending on the size and placement of the stimulation electrodes, TES can affect large brain volumes and entrain neuronal populations diffusely[1,2,3]. Transcranial direct current stimulation is already medically approved for the treatment of major depressive disorder[4,5], and many studies focus on showing the cognitive effects of transcranial stimulation in humans[6,7]. Furthermore, promising results were reported regarding the potential of TES in controlling epileptic seizures[8,9].

Despite the intensive research, there are still many open questions regarding the detailed mechanism of action, potential side-effects, and the long-term outcome of applying this method[10,11,12]. Therefore, it is critically important to have a robust, reproducible protocol to investigate the effects of TES in animal models. Given that many disorders (*e.g.*, depression, epilepsy, and schizophrenia) can only be extensively investigated in awake animals, and the nature of these medical conditions usually necessitate long-term treatment, we provide a protocol for chronic implantation of transcranial electrodes in rats. The method presented here can be used for behavioral studies or can be combined with implantation of recording electrodes (*i.e.*, wires, silicone probes, juxtacellular electrodes) or with chronic cranial windows for electrophysiological experiments and imaging studies, respectively. Depending on the experimental design, the timing of the stimuli can be either random or event-triggered to specific behavioral cues, or to the electrophysiological hallmarks of the particular brain states (seizures, theta oscillations)[8,11,13].

It is important to mention that in contrast to the currently used human approach, which uses an embodiment of electrodes placed on the skin, here we show a method that employs subcutaneous implantation right over the surface of the temporal bone, since rats barely tolerate anything placed on their skin which is easily accessible using their paws.

In line with the principles of Replacement, Reduction, and Refinement, due to the chronic nature of implantation, this method helps to reduce the number of animals, since each animal can be recruited in different experimental conditions for months, allowing the use of fewer animals to test various hypotheses.

In the present study, we provide a detailed, step-by-step protocol of transcranial stimulation electrode manufacturing (**Figure 1A-B**) and demonstrate the chronic implantation of these electrodes over the temporal bones of a six-month-old male Long-Evans rat.

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Protocol

All methods described here are in accordance with the directives of the European Communities Council (86/609 ECC) and have been approved by the Ethical Committee for Animal Research at the Albert Szent-Györgyi Medical and Pharmaceutical Center of the University of Szeged (XIV/218/2016).

1. Fabrication of the Stimulation Electrodes

1. To make one stimulation electrode, cut six 10 cm long pieces of miniature hook-up wires, remove 2 cm of the cladding on one end, and 1 cm on the other end.
2. Twist two cables together and solder them at the shorter peeled side.
3. Acquire a strip of three tape packaging of any integrated circuit with SOT-353 case.
4. Stitch the twisted wires through the three holes of the package. The longer peeled segment of the wires should be in the cavities of the package. Position the cables so that the end of the insulation is in line with the edge of the holes.
5. Put the dental cement on the holes to fix the wires.
   Caution: The dental cement must be dense enough to avoid leaking into the cavity of the package.
6. Once the cement is solidified (minimum 10 min), flip the package and put a thin layer of superglue into the hole to form a watertight seal towards the porous cement. Avoid gluing the peeled wires.
7. Twist the cables together on the shorter peeled side and solder them (**Figure 1A**).
8. Grab the longer peeled segment of the wires with the tip of fine tweezers, roll them around, and wrap them into the cavities (**Figure 1B**). Cut excess cable, if necessary.

2. Fabrication of the Recording Electrodes

1. To make one recording triplet electrode (**Figure 1C-D**), cut a one cm long piece of stainless steel tube using high-speed rotary saw. Remove the fin from the ends of the tube with a sharp needle and ensure that the tube is clean of debris and completely permeable.
   NOTE: Do not use pliers, as they will distort the tube.
2. Bend a gold-plated board spacer pin with a minimum length of 3 cm to form a J-shape holder. Cut the longer leg of the 'J' shape, and solder it together to get back the original shape to make a detachable joint between the long linear part, which will be held by the stereotaxic device, and the 'U' shape, which will hold the electrode, and serve as an anchoring point. Glue the tube and the shorter leg of the 'J' holder together. Glue some additional 1 cm long bars, if necessary, for stability.
3. Cut 3 pieces of 2.5 cm long electrode wires with stainless steel, ultrafine scissors. Make sure that it is a clear and sharp cut, and the insulation is intact at the circular surface of the electrode wire. Also make sure that the cut tip is not bent due to the force applied. Bend 1 or 2 mm on one end of each of the wires at different angles (*e.g.*, one left straight, one 45°, and one right angle) to make them distinguishable.
4. Fill the tube with the wires, choose an appropriate spacing of the electrodes for the experiment (*e.g.*, for cortical local field potential (LFP) and current source density (CSD) registration, the recommended protrusion of the tips of the wires from the opening of the tube: 4.5 mm, 4.1 mm, and 3.7 mm, respectively). Note which wire corresponds to which depth for maintaining a proper channel order.
5. Once the wires align right next to each other and are parallel with the tube, to fix the electrode wires, place a single drop of liquid superglue at both ends of the tube with a sharp needle. Make sure that the glue is flowing into the tube due to the capillary effect, but not towards the recording sites at the protruding ends.
6. Prepare an electrode interface board with a proper microconnector compatible with the recording system to be used.
7. Introduce the non-recording, bent ends of the wires into the holes of the electrode interface board corresponding to the desired recording channel. When all the wires are in position, push the gold pins into the holes with tweezers.
   NOTE: Gold pins may be fixed in place using dental cement, but in most cases, it is stable enough even without this safety step.

3. Anesthesia

1. Place the animal in a sealed anesthesia induction chamber and fill it with 4 - 5% of isoflurane in 2 L/min medical air.
2. When the rat is recumbent, remove the animal from the chamber and place it in a stereotaxic frame with an appropriate ventilation nose piece.
3. Set the isoflurane level to 2%, though the rate of airflow should remain the same. Check the level of anesthesia. If there is an absence of the paw withdrawal reflex in response to pinching, continue, otherwise increase the depth of anesthesia.
4. Monitor and maintain the rat's body temperature at 37 ± 0.5 °C with a homeothermic monitoring system. Apply a droplet of paraffin ointment on the eyes to prevent desiccation of the cornea. Repeat this procedure during the surgery several times, if necessary.
5. Subcutaneously inject 0.3 mg/kg atropine to avoid mucus formation in the airways. Repeat this every four hours in case of longer surgeries. Monitor breathing and adjust vaporizer, if needed.

4. Implantation of the Stimulation and Recording Electrodes

NOTE: Autoclave all the necessary surgical instruments and carefully follow the general rules of asepsis and antisepsis during the whole procedure. Avoid touching nonsterile areas outside of the surgical area. Immerse the electrodes in ethyl alcohol (70%) for 30 min before implantation.

1. Remove most of the hair from the scalp with a hair clipper. Apply depilatory cream on the scalp, spread it evenly on the surface, and wait a few minutes. Use a spatula to gently remove the cream and the remaining hair. Rinse the skin with water, then with disinfectant.

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2. Inject 1 - 2% lidocaine (do not exceed a total dose of 7 mg/kg) subcutaneously to numb the skin. Apply a single drop of vet ointment for the eyes (*e.g.*, paraffin).
3. Make a thorough and a long (~2 cm) sagittal incision in the midline with a scalpel, from the forehead to the neck. Dissect the tissues including the periosteum from the skull, then using a chisel or tooth tweezers clean the area between the cristae of the two temporal bones. Keep the skull exposed by retracting the dissected skin using four bulldogs.
4. Gently place fine tweezers between the steep edge of the temporal bone and the muscles and separate them. Make jigging movements to expose as much of the large surface of the temporal bone as possible, preferably from the edge of the occipital bone to the plane of the coronal sutures, without damaging the muscles.
5. Place retractors bitemporally to keep the temporal bones exposed.
6. Rinse the surface of the skull with 1 - 2 mL 3% H_2O_2, then wash it with 1 - 2 mL water.
7. Air-dry the surface of the temporal bones very carefully, mop up the moisture with ocular sticks. Test if the stimulation electrodes fit onto the cleaned vertical skull surface (the upper edge of the stimulation electrode should be in line with the edge of the crista of the temporal bone). Re-adjust the bulldogs and the retractors or shape the stimulation electrodes with scissors, if necessary.
8. Fill the cavities of the stimulation electrodes with electroconductive gel and put a thin layer of glue onto the rim of the electrodes.
9. Place the stimulation electrode on the dry surface of the temporal bone with one accurate movement, and hold it firmly in place for one minute with fine tweezers. Make sure that no moisture is in contact with the glue. Mop up with ocular sticks, if necessary.
   Caution: In case any instability of the electrode is experienced, remove it. After cleaning the bone, repeat this step with a new stimulation electrode.
10. Put dental cement over the edges of the stimulation electrode, while the leaking moisture of the tissues is continuously dried up with ocular sticks. Cover the whole stimulation electrode with cement.
11. After the cement is completely hardened, repeat these steps on the contralateral side.
12. Drill some holes all over the skull for anchoring screws. Drive miniature screws into the holes and put dental cement over them. Use a ~10% smaller diameter drill head compared to the screw diameter.
13. Depending on the purpose of the experiments, either solder a connector to the ends of the cables, place it on the surface, and cover with cement, or continue implanting the recording electrodes and optical fibers, or prepare a cranial window over the skull. In this latter case, solder the connectors and anchor them to the construct only at the end. In case of the need of long-term simultaneous TES and LFP recordings, implant the aforementioned electrode triplets instead of single wires.
    NOTE: This setup allows for the removal of common mode stimulation artefacts[8]. For the details of recording electrode implantation, see previous protocols[14,15].
14. Wash the exposed tissues abundantly with disinfectant. Inject 1 - 2% lidocaine (do not exceed a total dose of 7 mg/kg) subcutaneously.
15. Debride wound edges and close them with simple interrupted sutures around the connector/implant. Disinfect the wound with povidone-iodine.
16. Inject 5 mg/kg carprofene subcutaneously. Repeat this if necessary.
    NOTE: Any other analgesics may be used matching the requirements set by the local ethical permission board.
17. Suspend anesthesia, release the earbars and nosepiece, and put the animal in a postoperative recovery cage to regain consciousness.
18. Ensure that the animal is under close supervision during the first postoperative hours. Continue to monitor the implant area periodically.
    NOTE: The animal should not be left unattended until it has regained sufficient consciousness to maintain sternal recumbency. House the animal individually until full recovery from the procedure.

Representative Results

The implantation of chronic stimulation electrodes (**Figure 1B**) can be combined with additional implantation of recording electrodes (**Figure 1C-D**). Such settings are suitable to form on-demand recording and stimulation systems to interfere with specific brain activities. Here we present representative results of a closed-loop epilepsy detection and intervention system, applied on Long-Evans rats expressing spontaneous seizures (**Figure 2A**)[9]. This strain is known to show the electrographic and behavioral symptoms of absence (petit mal) epilepsy (**Figure 2B**). In case of an epileptic seizure, as the recorded intracortical signals are analyzed in real-time, a trigger is sent to an isolated stimulus generator at the appropriate moment to interfere with the spike-and-wave activity of the brain. In turn, the stimulus generator delivers a charge-balanced, triphasic stimulus through the bitemporal stimulation electrodes in order to interrupt the seizure activity.

Figure 2C-D shows the capacity of the temporally targeted stimuli to interrupt ongoing seizures from week 1 to week 16, demonstrating the robustness and reliability of the implanted stimulation electrodes. To put these results in context, **Figure 2E** displays the recordings of an aborted experiment, where secondary tissue penetrated between the temporal bone and electrode surface due to the improper sealing and cementing of the electrodes (autopsy of the animal confirmed the tissue invasion). Besides increasing the impedance of the stimulation electrodes, the growing tissue is likely to provide an electric shunt. This experiment highlights the utter importance of careful isolation to achieve reliable and reproducible results during stimulation experiments.

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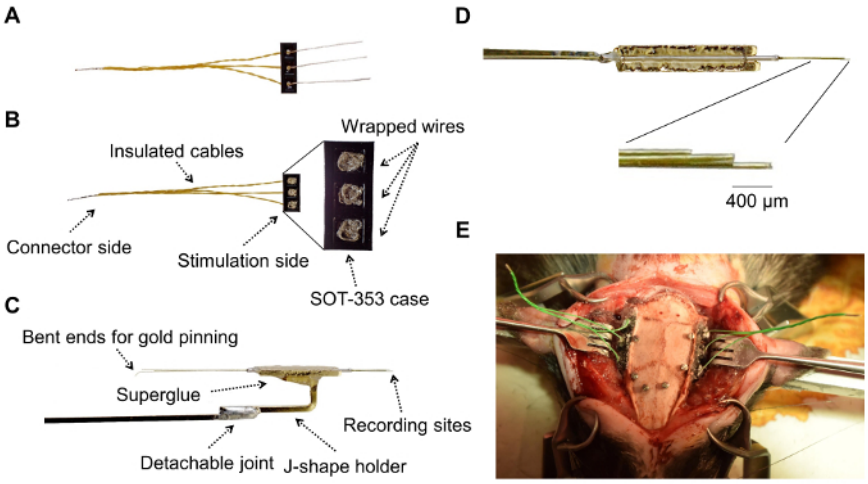

Figure 1: Steps of stimulation and recording electrode fabrication. (**A**) Twisted wires stitched through the holes and fixed to the packaging before wrapping the peeled wires into the cavities. (**B**) Final form of the stimulation electrodes. Inset: wrapped wires inside the packaging; (**C**) Side view of the recording electrodes; (**D**) Top view of the recording electrodes. Inset: Tip of the recording sites, 400 µm spacing. (**E**) Intraoperative picture of the transcranial stimulation electrode placement. The stimulation electrodes are already implanted, together with some of the anchoring screws. Please click here to view a larger version of this figure.

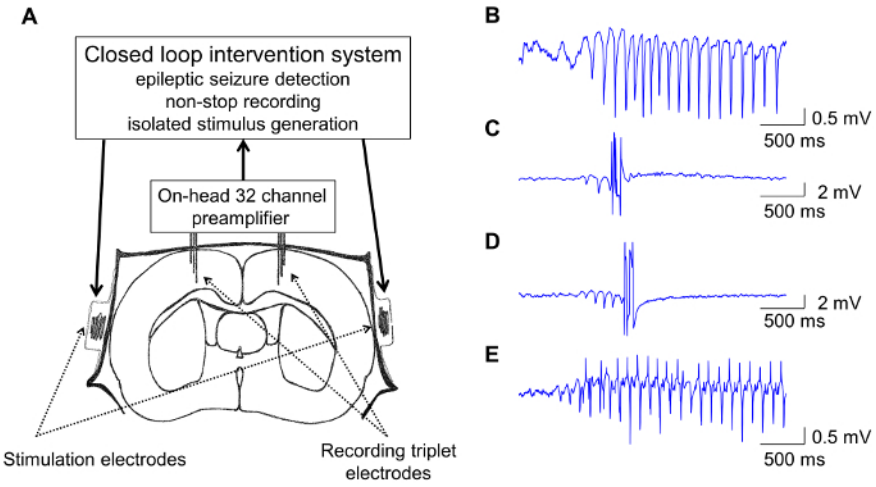

Figure 2: Representative results of closed-loop seizure intervention using the stimulation electrodes of this protocol. (**A**) Closed-loop system overview. Triplet recording electrodes are implanted in the parietal cortex and stimulation electrodes are placed bitemporally on the skull. The rat is equipped with an on-head amplifier and connected to a real-time seizure detection system. (**B**) LFP trace of an uninterrupted spike-and-wave seizure (**C** and **D**) Example LFP traces of seizure intervention on the 1st and 16th week of stimulation. (**E**) Example of failure of seizure interruption in case of tissue growing between the stimulation electrodes and the temporal bone (confirmed by autopsy) Please click here to view a larger version of this figure.

Discussion

The most critical step of this protocol is the gluing of the electrode package on the bone surface. In case of improper sealing, a gap is formed between the electrodes and the bone, and secondary scar tissue can grow into this gap, which lessens the quality of stimulation. The bone surface must be completely dry during the steps of sticking on the package, and in the case of experiencing instability of the electrodes, it must be removed and replaced with a new package to gain the best results.

A limitation of this method is that as the skin is not completely closed after the surgery, there is a relatively higher risk of infection. Post-operative care in the first 4 - 5 days during the recovery with disinfectant solution, and later with powder, helps to prevent infection. In our experience, this treatment facilitates the formation of scar-tissue, which can completely close the wound towards the external world.

Here we presented one of the cheapest, most accessible methods of electrode fabrication, but depending on the special needs of the particular experiments, modification of the conductive material may be necessary, *e.g.*, coating the surface of the cables with non-polarizing electrode interface materials, *e.g.*, PEDOT:PSS. The electrode package can be custom-made, 3D-printed, and modified by the experimenters, in case our recommendations do not match the requirements of a particular study. In our experience, the size of the transcranial electrodes fabricated in this study allow implantations in both male and female rats above 300 g of bodyweight, but the size of the stimulation electrodes can easily be

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reduced by cutting smaller strips in protocol step 1.3. Furthermore, all the glues and dental cements given in the protocol can be replaced with substitutes, considering that the outer layer is in direct contact with the tissues, therefore they should be bio-compatible.

In the present study, we provided a protocol for bitemporal stimulation electrode fabrication and implantation, which is technically simple to perform, cost effective, and reliable over the long-term, allowing electrical stimulation experiments on freely moving rats[9]. As the stimulation electrodes are placed on the temporal bone, the whole horizontal skull surface is preserved for other implantations. This method can be combined with regular electrophysiological[15,16], optogenetic[17], and imaging[18] techniques, providing the possibility of a versatile combination of experimental protocols.

Disclosures

Antal Berényi is the owner and founder of Amplipex Ltd, a company manufacturing large-scale multiplexing biosignal amplifiers. Gábor Kozák and Tamás Földi have nothing to disclose.

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