TITLE:

The tetanus toxin model of chronic focal neocortical epilepsy and long term continuous EEG/ECoG recording in rodents

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KEYWORDS:

Epilepsy, seizure, focal, electroencephalography (EEG), electrocorticography (ECoG), wireless, telemetry, tetanus toxin, rat.

SHORT ABSTRACT:

Focal neocortical epilepsy (FNE) is a devastating disease with few effective treatments or satisfactory models. We present the most established rodent model of FNE, the tetanus toxin model, and describe how epileptiform activity and seizures can be studied with long-term, wireless electroencephalographic (EEG) or electrocorticographic (ECoG) recordings.

LONG ABSTRACT:

A major challenge in the study of epilepsy is the development of suitable pre-clinical models. While most models mimic temporal lobe eilepsy (TLE), one of the most devastating and treatment-resistant forms of epilepsy is not temporal but neocortical. Neocortical seizures in humans can vary enormously, from frequent, brief bursts of focal, high-frequency activity (*i.e.* epilepsia partialis continua), to long-lasting seizures of focal onset that propagate through the entire brain. Focal neocortical epilepsy (FNE) has classically been studied by applying acute chemoconvulsants directly to the cortex. However, these approaches fail to produce hallmark features of epilepsy: spontaneous seizures and chronic epileptiform activity. Very few FNE models develop chronic seizures, and this has severely hindered research investigating how treatment of the disorder might be improved. Here we present the most established model of FNE, the tetanus toxin (TeNT) model, where seizure foci are introduced by stereotaxic injection of minute quantities of TeNT into the rat neocortex. This treatment produces a gradual (1–2 week) increase in epileptiform or seizure activity that stabilizes and persists for at least 4 weeks. Similar doses of TeNT generate profoundly different patterns of electrographic activity when injected into different cortical regions. This activity can be monitored in freely moving animals using continuous, long-term, wireless EEG or ECoG recordings. The implantation of a transmitter system for EEG/ECoG acquisition is described. Finally, representative results from animals injected with TeNT into the motor or visual cortex are presented, and important methodological considerations for improving reproducibility and animal welfare are discussed. Because the TeNT model has a spatially-restricted seizure focus of known location, when combined with long-term, wireless EEG/ECoG it represents a powerful tool in the study of novel treatments for FNE.

INTRODUCTION:

The overall goal of this approach is to produce a model of FNE with long-lasting, spontaneous epileptiform activity and seizures that can be monitored using continuous EEG/ECoG. The chronic nature of the model makes it ideally suited for investigating novel therapies that might eventually be given to patients suffering from chronic, spontaneous seizures. The use of chronic models is increasingly recognized as essential in translational studies of anti-epileptic or anti-seizure treatments[1]. Pairing the model with long-term, continuous EEG/ECoG provides a quantitative and direct means with which to assess the efficacy of such treatments.

Direct injection of TeNT into the brain was first used to induce chronic experimental epilepsy by Roux and Borrel in 1898[2], and the technique has been widely employed since. In the central nervous system TeNT acts at presynaptic

terminals, cleaving the SNARE protein synaptobrevin to prevent neurotransmitter release[3]. GABAergic synapses are particularly susceptible, and the resulting attenuation of inhibitory neurotransmission is thought to be primarily responsible for the emergence of epileptiform activity[4,5]. This activity, which typically appears 5–10 days after TeNT administration and long-outlasts clearance of the toxin from the brain[6], persists for different lengths of time depending on the brain region injected[7]. Rats receiving TeNT into the visual cortex or hippocampus typically go into remission 6–8 weeks post-injection[8], while injection of TeNT into the motor cortex has produced spontaneous seizures lasting more than 6 months[9]. In this report we restrict our methodology and discussion to injection of TeNT into either the motor or visual cortex of rats.

The TeNT model of FNE is an important tool in a field where the vast majority of pre-clinical research is performed on a small number of models focused almost exclusively on TLE[10]. Most other models of neocortical epilepsy are complicated by epileptogenic zones that change location or seizure activity that does not last long enough to test therapeutic interventions. The advantage of the TeNT model is not only that the location of the seizure focus is known and stable, but within limits, it may be chosen by the researcher. Activity within the focus can be monitored in freely moving animals using long-term, continuous EEG/ECoG recordings. The implantation of transmitter system capable of acquiring such recordings is described.

PROTOCOL:

All procedures outlined below were conducted in accordance with the Animals (Scientific Procedures) Act, 1986. Those wishing to reproduce this protocol should follow their institutional guidelines regarding animal care and the performance of aseptic surgery.

1. Equipment Preparation

1.1) Clean all surgical tools (see Table of Materials) and sterilize using a steam autoclave or equivalent.

1.2) Prepare the EEG/ECoG transmitter (A3028E; Open Source Instruments, Inc. (MA, USA)). Uncoil the exposed tips of the recording and reference electrodes by gently stretching the steel helices using standard pattern forceps. Bend the uncoiled tips to an angle of 90° and cut the bent tips to a length of approximately 2 mm. Soak the entire transmitter with 70% ethanol followed by sterile water and leave to dry.

1.3) Fill 1 ml or 2ml syringes (26 gauge, 3/8 inch needles) with:
- 0.15 ml of 0.3 mg/ml buprenorphine
- 0.2 ml of 5 mg/ml meloxicam
- 2.0 ml of 0.9% w/v sodium chloride saline solution
- *(Optional)* 0.2 ml of 150 mg/ml amoxicillin suspension

These quantities are sufficient for a single 300–400 g rat.

1.4) Place an aliquot of TeNT on ice and transfer to the surgery theatre. **CAUTION:** TeNT is a powerful neurotoxin and should be handled with extreme care. Necessary approvals should be obtained prior to use, and aliquots disposed of in accordance with local government requirements. Those working with TeNT are strongly encouraged to ensure their tetanus immunizations are up to date.

1.5) *Optional (for surgeries involving cannula placement above the site of TeNT injection).* Under the surgical microscope and using a 26 gauge needle as

applicator, coat the outer surface of the cannula's protruding metal shaft with a thin layer of cyanoacrylate tissue adhesive and leave to dry. The cyanoacrylate will insulate the cannula shaft from electrical contact with the recording electrode, avoiding a potential source of noise that might disrupt the EEG/ECoG signal.

2. Animal Preparation

2.1) Retrieve the rat from its housing unit and transfer to the surgery theatre. Weigh the animal, and anesthetize in an induction chamber using 3.0% isofluorane in oxygen.

2.2) When the rat becomes unresponsive, remove it from the induction chamber and use fur trimmers to shave the head and back/flank as depicted in Figure 1A. Remove any loose hairs using small strips of adhesive tape.

2.3) Transfer the rat to a heating pad set to 30–35 °C. Head-fix the animal inside the stereotaxic frame by inserting its snout into the anesthesia nose cone and placing ear bars into each ear canal. Be careful not to insert the ear bars too far as this may damage the tympanic membranes.

2.4) Using a cotton-tipped applicator, apply liquid eye gel to the rat's eyes to prevent them from drying out during surgery.

2.5) Subcutaneously inject the buprenorphine, meloxicam and *(optional)* amoxicillin prepared in step 1.3.

2.6) Lower the isofluorane concentration to 2.5%. The concentration can be gradually lowered to 2.0% over the course of the surgery.

2.7) Cut a hole approximately 3 cm in diameter into a sterile drape and cover the rat so only the top of its scalp is exposed. Using a cotton-tipped applicator, apply diluted povidone-iodine antiseptic solution to the shaved area, wiping away any excess with a fresh applicator to prevent unnecessary drying of the skin.

3. TeNT Injection

3.1) Confirm that the animal is fully anesthetized using a toe-pinch reflex test.

3.2) Using standard pattern forceps and curved-tip iris scissors, cut away an oval-shaped area of scalp to expose the craniometric landmarks bregma and lambda (depicted in Figure 1B). Use the same scissors and a delicate bone scraper to remove any connective tissue covering the surface of the skull. Stem any bleeding using cotton-tipped applicators.

3.3) Place the high-speed stereotaxic drill (with #3, 1.2 mm diameter ball mill carbide drill bit) inside its holder and attach to the stereotaxic frame. Position the tip of the drill bit above bregma and zero the X- and Y- coordinates on the digital display console.

3.4) Move the tip of the drill bit to the following coordinates (relative to bregma) and mark each by drilling 0.1–0.2 mm into the skull.

- For surgeries involving injection of TeNT into the visual cortex:
  - Recording electrode coordinates: +3.0 mm mediolateral (X), −7.0 mm rostrocaudal (Y)
  - Reference electrode coordinates: −1.4 mm X, −4.4 mm Y
  - Fixing screw coordinates: +0.5 mm X, +1.0 mm Y
- For surgeries involving injection of TeNT into the motor cortex:
  - Recording electrode coordinates: +2.4 mm X, +1.0 mm Y
  - Reference electrode coordinates: −2.0 mm X, −1.0 mm Y

Fixing screw coordinates: +1.0 mm X, –4.0 mm Y

3.5) Remove the drill from its holder and finish the burr holes by hand, taking care not to drill into the cortex.

3.6) Using a 26 gauge needle, **very carefully** make a small incision in the *dura mater* at the recording electrode burr hole. This will facilitate insertion of the microsyringe needle into the cortex, an otherwise challenging procedure in adult brains with tough, thick dural membranes.

3.6.1) Successful penetration of the dura may be followed by leakage of small amounts of cerebrospinal fluid and/or blood into the burr hole. Said fluid can be absorbed using a cotton-tipped applicator.

3.7) Secure the microsyringe (with 33 gauge removable needle) inside the microsyringe pump injector and attach to the stereotaxic frame. Dilute the TeNT as required in PBS or saline and withdraw 0.5–2.0 µl of the resulting solution into the microsyringe. The TeNT should be diluted **immediately prior to the injection** of each animal to avoid any unwanted reduction in potency.

3.7.1) We typically inject 12–15 ng of TeNT in 1.0 µl of PBS/saline. However, TeNT potency can vary considerably across suppliers. As such, those wishing to reproduce this model should determine empirically the quantity of TeNT that generates adequate epileptiform/seizure activity without significant mortality.

3.8) Position the tip of the microsyringe needle above the recording electrode burr hole. Lower the needle until the tip touches the surface of the cortex, and zero the Z- coordinates on the stereotaxic frame digital display console.

3.9) Slowly lower the tip of the microsyringe needle to an injection depth of 1.0 mm, corresponding to cortical layer 5.

3.9.1) In our hands, injection of TeNT into more superficial cortical layers has produced severe epileptiform/seizure activity associated with increased mortality. However, this effect has not yet been systematically characterized.

3.10) Inject the TeNT at a rate of 100 nL/min. Avoid disturbing the surgery trolley/table during the injection; movement or vibration of the microsyringe needle while inserted into the cortex can cause significant structural damage.

3.11) After the injection is complete, leave the needle in place for 5–10 minutes before slowly withdrawing. This delay minimizes backflow of TeNT along the injection tract.

4. Transmitter Implantation

4.1) Reposition the sterile drape so the shaved area of the rat's back/flank is exposed. Apply diluted povidone-iodine antiseptic solution to this area with a cotton-tipped applicator, wiping away any excess with a fresh applicator to prevent unnecessary drying of the skin.

4.2) Using blunt-tip mayo scissors, make a 3-4 cm horizontal incision in the middle of the shaved area. If the shaving was performed correctly, the incision should be located about half-way up the rat's back, extending from the midline down the right flank (Figure 1A).

4.3) Using the same scissors, create a subcutaneous cavity approximately 5 cm in diameter to the left of the incision. The cavity is created by separating the skin from the underlying

connective tissue. This can be achieved by repeatedly inserting **closed** scissors into the incision and opening them as gentle forward pressure is applied. The scissors should always be removed from the cavity **before** they are closed to avoid unwanted cutting of connective tissue.

4.3.1) The subcutaneous cavity provides a pocket in which the body of the transmitter will sit. It should be large enough to allow the transmitter to settle in a position that is comfortable for the animal, not causing pain or restricting movement. Care should be taken to ensure the body of the transmitter will not settle directly below the back/flank incision.

4.4) Using the same technique as above, create a subcutaneous tunnel that runs from the cavity to the exposed skull. The tunnel should be as narrow as possible and exit behind the right ear.

4.4.1) The subcutaneous tunnel provides a tract in which the recording and reference electrode wires will sit. In our experience, running the tunnel over the neck at the midline (as opposed to behind the right ear) leads to increased strain on the electrode wires as the animal moves its head. This can lead to signal loss and be a source of discomfort to the rat.

4.5) Gently grip the tips of the recording and reference electrode wires with a Providence Hospital hemostat. Insert the body of the transmitter into the subcutaneous cavity and use the hemostat to gently push the tips of the electrodes wires through the subcutaneous tunnel to the exposed skull. Release the tips and withdraw the hemostat from the tunnel.

4.6) Use Dumont #5/45 forceps to pick up a 0-80 (1.6 mm long) stainless steel screw by its thread. Place the tip of the thread into the fixing screw burr hole and screw into the skull using a slotted screwdriver, slowly releasing the forceps as the thread takes.

4.7) Pick up another screw using the Dumont #5/45 forceps. With the other hand, use the curved-tip Dumont #7 forceps to insert the bent tip of the reference electrode wire into the cortex at the reference electrode burr hole. Place the tip of the screw thread into the burr hole to temporarily secure the electrode tip in position. While holding the screw in place, put down the forceps holding the reference electrode wire and pick up a slotted screwdriver. Screw the screw into the burr hole as described above.

4.8) For surgeries **not** involving cannula placement above the site of TeNT injection, repeat step 4.7 for implantation of the recording electrode, and move directly to step 4.11.

4.9) *Optional (for surgeries involving cannula placement above the site of TeNT injection)*. Secure a cannula in the cannula holder and attach the holder to the stereotaxic frame. Position the tip of the cannula above the recording electrode burr hole.

4.10) *Optional (for surgeries involving cannula placement above the site of TeNT injection)*. Use the curved-tip Dumont #7 forceps to insert the bent tip of the recording electrode wire into the cortex at the recording electrode burr hole. Secure the electrode tip in position by lowering the metal shaft of the cannula into the burr hole until the plastic pedestal lies flat on the surface of the skull.

4.11) If necessary, use standard pattern forceps to grip the electrode wires near the body of the transmitter and gently pull any loose wires back into the subcutaneous tunnel. As little wire as possible should be showing at the exposed skull.

4.12) Thoroughly dry the exposed skull with a cotton-tipped applicator, and secure all screws (and cannula if applicable) in place by applying several drops of cyanoacrylate tissue adhesive to the head wound. A thin layer of adhesive should cover any area exposed by surgery, including skull and soft tissue. Avoid accidental application of adhesive to the skin or fur surrounding the head wound.

4.13) In a 12-well cell culture plate or equivalent container, mix the liquid and powder components of the rapid-set dental cement using the wooden end of a cotton-tipped applicator. Apply dental cement to all areas covered by the tissue adhesive. If applicable, avoid dripping cement on the opening or plastic pedestal of the cannula. Cement on the pedestal can prevent the cannula stylet (dummy cap) from being properly inserted.

4.14) Wait a few minutes for the dental cement to dry, and apply additional layers as necessary. Several layers may be needed, and the cement should be freshly mixed each time. The aim is to create a flat, smooth headpiece with as few sharp edges as possible. Again, care should be taken to avoid accidental application of cement to the skin or fur surrounding the head wound.

4.15) While waiting for the dental cement to dry, use a hemostat, standard pattern forceps and an absorbable suture to close the wound on the rat's back/flank. Apply lidocaine ointment to the closed wound using a cotton-tipped applicator.

4.16) Subcutaneously inject the saline prepared in step 1.3.

4.17) *Optional (for surgeries involving cannula placement above the site of TeNT injection).* Gently screw the cannula stylet onto the plastic pedestal of the cannula. The stylet fills the cannula shaft, preventing it from clogging before a cannula-guided injection can be performed.

4.18) Turn off the anesthesia equipment, remove the rat from the stereotaxic frame and leave on the heating pad to recover. At the first signs of stirring, return the animal to its cage and observe carefully as the anesthesia wears off.

5. Post-operative Care and Housing

5.1) Following surgery, rats should be housed in standard conditions with unrestricted access to food and water. Pica may be observed in animals treated with buprenorphine, and caution should be taken when providing bedding that may be hazardous if eaten (e.g. paper towels). Co-housing may be considered to improve welfare during recovery, but animals should be singly housed after 3-4 days to prevent fighting upon the emergence of epileptiform/seizure activity.

5.2) Several hours after surgery, and at least once a day for 10 days thereafter, closely observe the rat for any signs of pain, movement difficulties, weight loss, or reduction in food or water consumption. The dental cement headpiece should also be carefully checked, and the animal culled immediately if it is removed or either of the electrode wires is exposed. The transmitter can be recycled provided the silicone/epoxy housing of its body, antenna and electrode wires is not compromised.

5.2.1) Movement difficulties and rapid weight loss occur in a small proportion of rats from both models, although they are more common in the motor cortex model (approximately 20% compared to 5% for the visual cortex model). Weight loss can occasionally be mitigated by subcutaneous administration of meloxicam and/or saline. Supplementing the diet with soft (gel) food is also advisable. In our experience, rats that lose more than 12% of their pre-surgery body weight are unlikely to recover and should be culled.

[Place Figure 1 here]

6. EEG Recording

The protocol for acquiring EEG/ECoG recordings will vary depending on the transmitter and detection system used. The setup below is compatible with the A3028E transmitter described above, utilizing hardware and software purchased from Open Source Instruments, Inc. (MA, USA) (Figure 2). Detailed specifications can be found at http://opensourceinstruments.com/.

6.1) Switch the transmitter on by passing a magnet over the implanted transmitter body.

6.2) Place the rat's cage in a suitable Faraday enclosure containing one or several loop antennae. The Faraday enclosure limits local radio-frequency interference that might disrupt transmission of the EEG/ECoG signal.

6.3) Connect each loop antenna to the (octal) data receiver (situated outside the Faraday enclosure) using a Bayonet Neill-Concelman (BNC) cable. If required, a BNC adapter with insulting flange can be fixed permanently into the Faraday enclosure wall, and two BNC cables used to connect the antenna to the adapter and the adapter to the receiver.

6.4) Connect the data receiver to the digitizer (long-wire data acquisition (LWDAQ) driver) using another BNC cable, and the digitizer to a computer using a category 6 (Ethernet) cable.

6.5) EEG/ECoG is acquired using LWDAQ software (download: http://alignment.hep.brandeis.edu/Software). Full documentation, including instructions for data acquisition and the automated detection of epileptiform/seizure events, is provided at: http://opensourceinstruments.com/Electronics/A3018/Neuroarchiver.html.

6.6) *Optional.* For simultaneous video-EEG/ECoG recordings, the rat's cage can be rigged with a suitably sized closed-circuit television (CCTV) camera. A camera equipped with infrared cut-off filter removal for recording at night or in low-light conditions is recommended. Video and EEG/ECoG should be acquired using the same computer to facilitate accurate time-stamping between the two sets of recordings.

[Place Figure 2 here]

REPRESENTATIVE RESULTS:

In the motor cortex, injection of 12.5 ng of TeNT evokes high-frequency (70–160 Hz) epileptiform events typically lasting less than 1 s (Figure 3A). These events begin a few days after toxin injection and persist for at least 6 weeks. The frequency of events varies, but on average there are around 500 per day[11].

Secondary generalised seizures can be observed with higher doses of tetanus toxin (> 15 ng)[11], although these doses may result in dystonia of the contralateral front or hind paws and an increased mortality rate.

In contrast to the motor cortex where secondary generalized seizures are rare, injection of 15 ng of TeNT into the visual cortex produces frequent, focal-onset, generalized seizures (Figure 3B). These commence a few days after toxin injection and are much better tolerated than motor cortex seizures. Seizure duration is variable, ranging from 10–300 s in length with the majority lasting 50–100 s. Seizure frequency is also highly variable, ranging from less than 1 to more than 100 per day. A high seizure frequency persists for 5–6 weeks, after which the frequency begins to decline and many animals go into remission.

Detecting genuine behavioral correlates of electrographic seizures is a major challenge when modelling FNE. For the motor cortex model, bursts of high frequency epileptiform activity have been correlated with electromyographic activity in the contralateral paw[9]. For the visual cortex model, simultaneous video-EEG recordings reveal that long-lasting electrographic seizures (> 30 s) are occasionally associated with behavioral manifestations including rearing and "wet dog shakes", which appear as the seizure generalizes (Figure 4 or Animated/Video Figure 1). However, a significant proportion of visual cortex electrographic seizures are not accompanied by overt behaviors.

[Place Figure 3 here]
[Place Figure 4 (Animated/Video Figure 1) here]

Table and figure legends:

Figure 1: Surgical implantation of the wireless EEG/ECoG transmitter. A) Photograph depicting the areas of the head and back/flank that should be shaved prior to surgery. The black dotted lines denote the approximate eventual locations of the scalp and back/flank incisions. **B**) Photograph depicting the size and position of the area of scalp that should be cut away to reveal the craniometric landmarks bregma and lambda (labelled). **C**) Diagram highlighting the approximate implantation locations of the transmitter body, recording electrode, reference electrode and fixing screw in an animal injected with TeNT into the visual cortex. Dashed lines denote the subcutaneous placement of the transmitter body and electrode wires. **D**) Cross-sectional diagram of the recording electrode implantation site for surgeries including (right) and not including (left) placement of a cannula above the site of TeNT injection.

Figure 2: EEG/ECoG acquisition. A) Photograph of an implanted rat one day after surgery. The dental cement headpiece and sutures on the animal's back/flank are clearly visible. **B**) CCTV camera used for simultaneous video recording. **C**) The EEG/ECoG recording system, including Faraday enclosure, loop antenna, (octal) data receiver, LWDAQ driver (digitizer) and computer. The loop antenna is turned sideways for clarity but should be positioned between the cages for optimal reception in the set-up shown. Although not clearly visible, each animal's cage lid is fitted with the camera displayed in panel B.

Figure 3: Sample ECoG recordings. A) Typical bursts of high frequency epileptiform activity following injection of 12.5 ng of TeNT into the motor cortex. **B**) A representative focal-onset, generalized seizure following injection of 15 ng of TeNT into the visual cortex.

Figure 4 (Animated/Video Figure 1): Simultaneous video-ECoG recordings. Simultaneous video (upper) and ECoG (lower) recordings of a focal-onset, generalized seizure from a rat injected with 15 ng of TeNT into the visual cortex.

DISCUSSION:

In our hands the TeNT model of FNE is highly reproducible. The model is, however, extremely sensitive to variation in technique, and even small changes in the protocol can adversely influence experimental outcomes. Here we summarize the main issues we have encountered and provide recommendations for how they might best be avoided. Preliminary observations regarding different sites of TeNT injection and rodent strains are also discussed.

In cases where focal treatments are to follow the establishment of chronic seizures, it may be tempting to administer TeNT via a pre-implanted cannula in order to fully align the treatment with the focus. Unfortunately we have found that injection of TeNT via a cannula often allows small amounts of toxin to enter the bloodstream or cerebrospinal fluid (CSF). This can lead to symptoms consistent with conventional tetanus exposure. When this occurs, the experiment must be ended for humane reasons. Consequently, we strongly discourage delivery of TeNT via cannula.

Our data suggest that similar doses of TeNT can generate profoundly different patterns of electrographic activity when injected into different brain regions. TeNT injection may also be better tolerated in some regions than others. For example, while in visual cortex injection of 15 ng TeNT produces long-lasting, well-tolerated seizures, delivery of 12.5 ng to the motor cortex generates full seizures only very rarely, on a background of more or less constant high frequency activity. When the frequency of seizures arising from the motor cortex is increased by raising the amount of TeNT to 15 ng, marked dystonia of the contralateral front or hind paws is often observed, and there are significant increases in post-seizure mortality. While we have not systematically investigated seizure severity after TeNT injection into other brain regions, we suggest such exploration is approached with caution. In a small pilot study we found seizures originating in the parietal cortex to be poorly tolerated. The depth of TeNT injection may also be important. The current protocol focuses on injection into cortical layer 5; preliminary experiments suggest layer 2/3 may be more sensitive, perhaps due to increased leakage of TeNT into the CSF.

TeNT potency varies considerably between different suppliers. Due to the relatively narrow range of doses that will generate adequate epileptiform/seizure activity without significant mortality, the challenge of obtaining a consistent supply of TeNT is potentially concerning from an animal welfare standpoint. These welfare concerns have understandably delayed adoption of the model by the wider epilepsy field. We recommend that groups seeking to implement the model purchase TeNT in bulk, preferably from a single batch, and carry out small pilot studies to determine a well-tolerated dosage range for each batch. More generally, we suggest that researchers utilizing rodent models of epilepsy adopt recently-outlined strategies to maximize animal welfare during their experiments[12].

The TeNT model does not appear to be strongly modified by background strain. We have successfully generated chronic focal neocortical epilepsy in Sprague Dawley, Long-Evans and Lister hooded rats, as well as in C57BL/6J mice. Our numbers of mice are low and these experiments must be considered preliminary, but other groups have reported similar success[13,14]. Thus far we have only carried out studies in male animals. We have found that younger males appear more sensitive to TeNT, and have weighted our work to larger (>275 g)

adults. The estrus cycles of female animals can alter seizure frequency and this should be carefully considered before undertaking any study that involves them.

We have used the terms “EEG” and “ECoG” interchangeably throughout this report. This is because the position of the recording electrode has varied during our studies. Much of our initial work involved recording EEG with the electrode wire physically attached to a screw that did not penetrate the cortex. However, upon introduction of the cannula we began inserting the bent tip of the recording electrode directly into the cortex to increase the power/amplitude of our recordings. This configuration should technically be referred to as “ECoG”, not “EEG”.

Future work with the TeNT model may identify novel therapies for FNE, a disease that is frequently treatment-resistant. In addition, targeting different regions or layers of the cortex with TeNT may provide new insights into the propagation and network basis of seizures, uncovering the mechanisms by which cortical architecture impacts the spread of epileptiform activity.

ACKNOWLEDGEMENTS:

We thank G. Schiavo (UCL Institute of Neurology) for the gift of TeNT, and C. Hills (UCL Institute of Neurology) for her comments on the manuscript. This work was supported by the Medical Research Council, the Wellcome Trust, the European Research Council, the Royal Society, the Guarantors of *Brain*, the Brain Research Trust, and Epilepsy Research UK.

DISCLOSURES:

The authors declare no competing interests, financial or otherwise.

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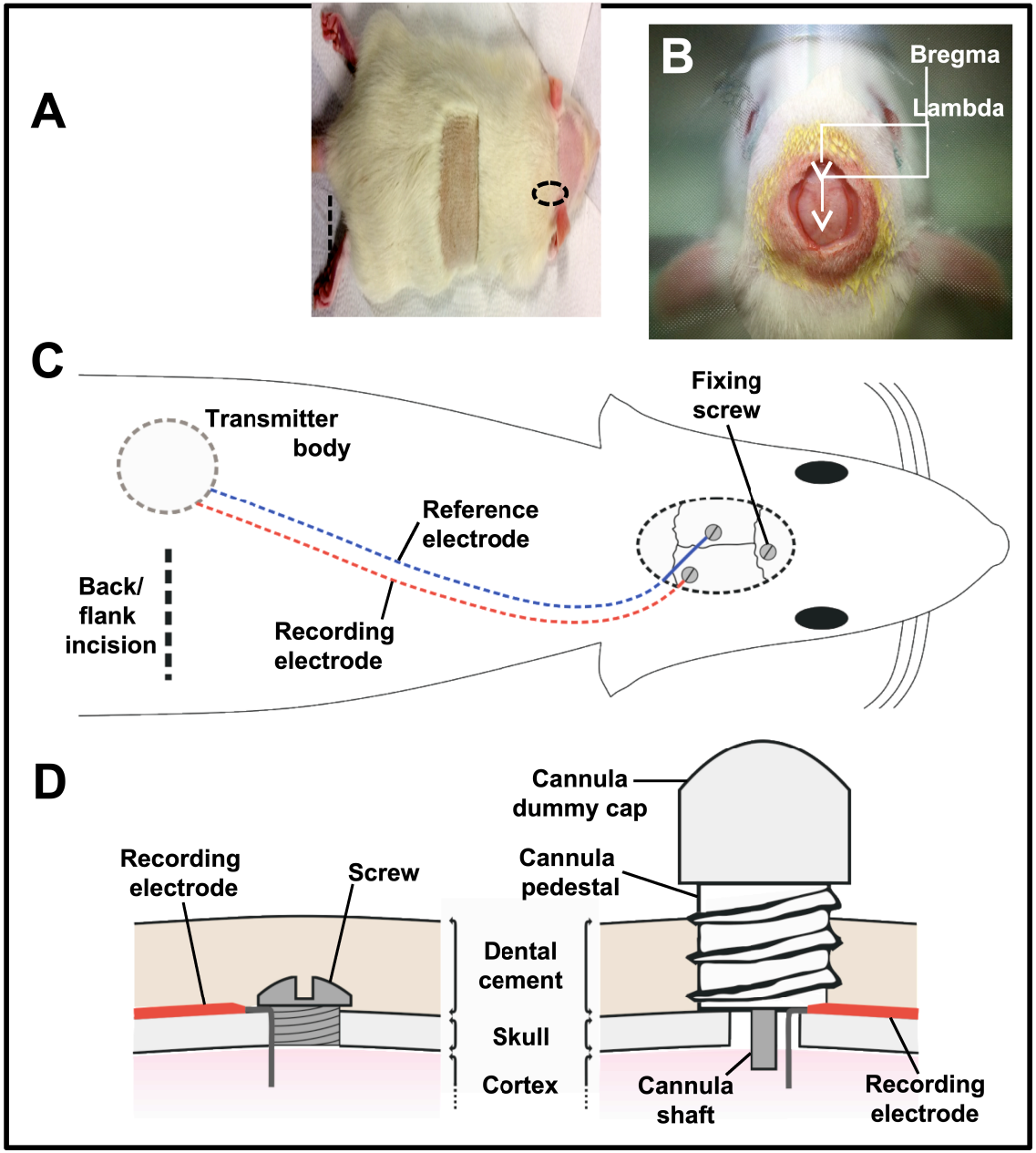
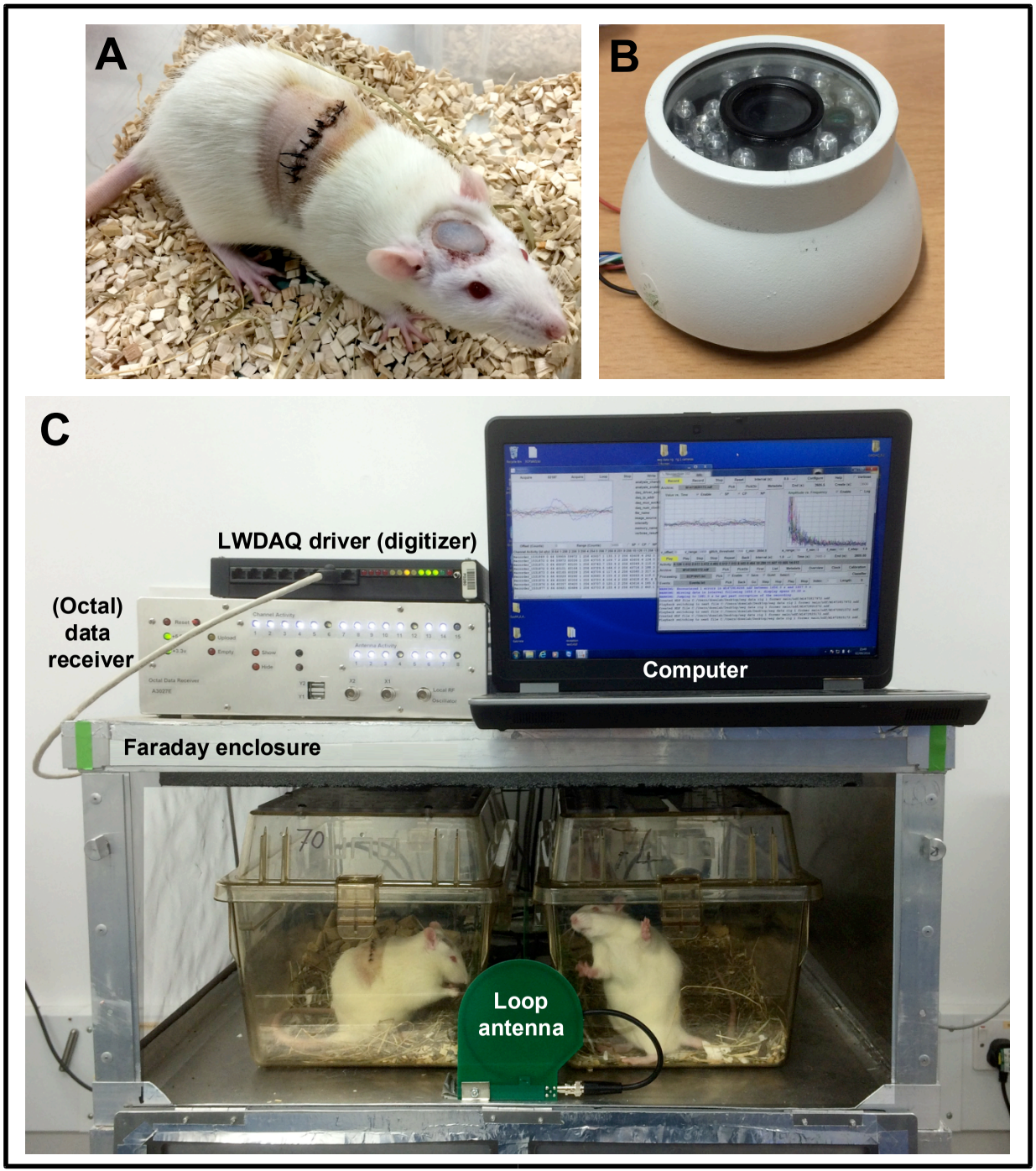
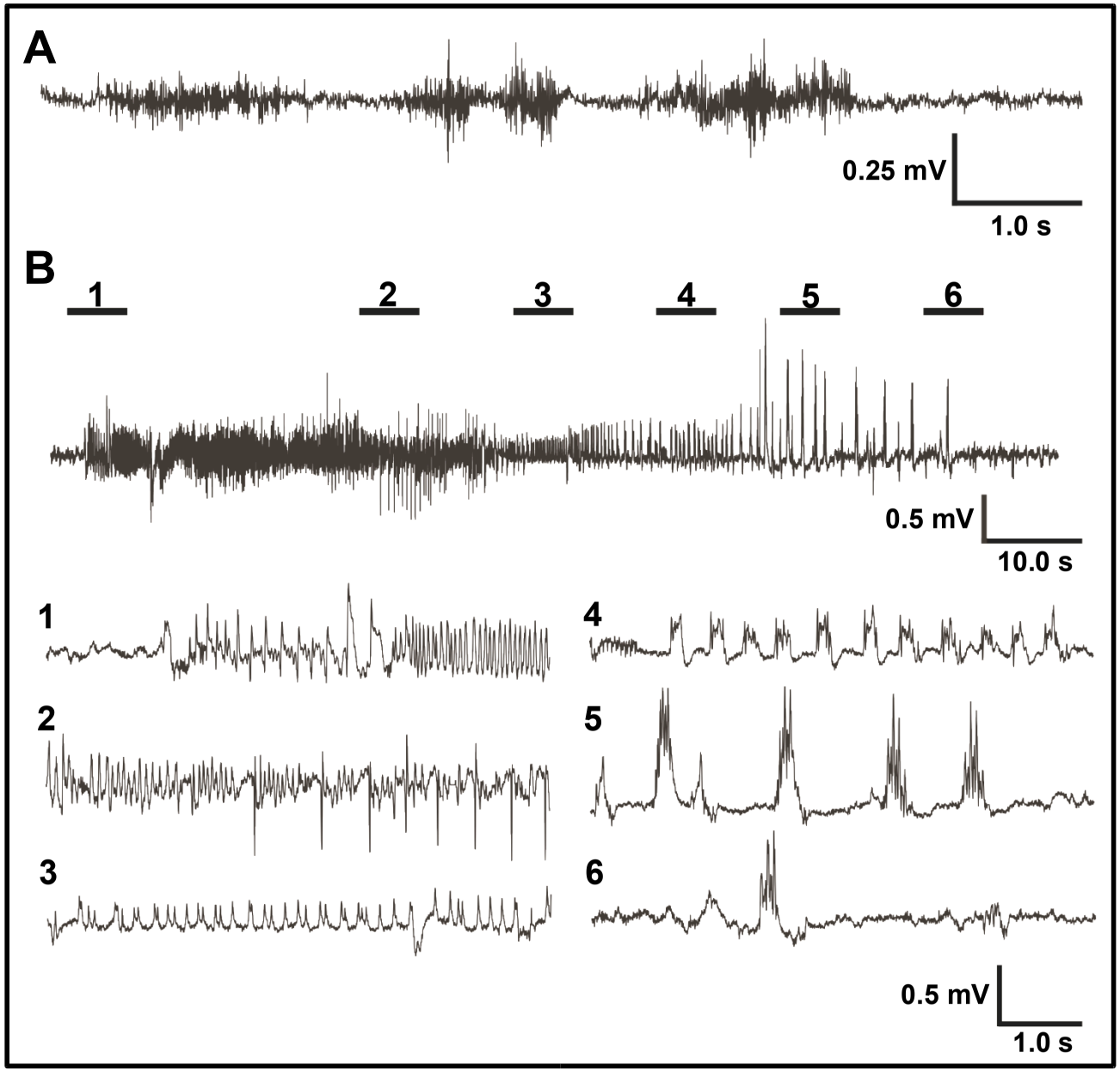
TABLE OF SPECIFIC MATERIALS/EQUIPMENT:

See separate excel file.


A
B
Bregma
Lambda
C
Transmitter body
Fixing screw
Reference electrode
Back/ flank incision
Recording electrode
D
Cannula dummy cap
Recording electrode
Screw
Cannula pedestal
Dental cement
Skull
Cortex
Cannula shaft
Recording electrode


A
B
C
LWDAQ driver (digitizer)
(Octal)
data
receiver
Computer
Faraday enclosure
Loop
antenna


A
0.25 mV
1.0 s
B
1
2
3
4
5
6
0.5 mV
10.0 s
1
2
3
4
5
6
0.5 mV
1.0 s