Video Article

Non-restraining EEG Radiotelemetry: Epidural and Deep Intracerebral Stereotaxic EEG Electrode Placement

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Abstract

Implantable EEG radiotelemetry is of central relevance in the neurological characterization of transgenic mouse models of neuropsychiatric and neurodegenerative diseases as well as epilepsies. This powerful technique does not only provide valuable insights into the underlying pathophysiological mechanisms, *i.e.*, the etiopathogenesis of CNS related diseases, it also facilitates the development of new translational, *i.e.*, therapeutic approaches. Whereas competing techniques that make use of recorder systems used in jackets or tethered systems suffer from their unphysiological restraining to semi-restraining character, radiotelemetric EEG recordings overcome these disadvantages. Technically, implantable EEG radiotelemetry allows for precise and highly sensitive measurement of epidural and deep, intracerebral EEGs under various physiological and pathophysiological conditions. First, we present a detailed protocol of a straight forward, successful, quick and efficient technique for epidural (surface) EEG recordings resulting in high-quality electrocorticograms. Second, we demonstrate how to implant deep, intracerebral EEG electrodes, *e.g.*, in the hippocampus (electrohippocampogram). For both approaches, a computerized 3D stereotaxic electrode implantation system is used. The radiofrequency transmitter itself is implanted into a subcutaneous pouch in both mice and rats. Special attention also has to be paid to pre-, peri- and postoperative treatment of the experimental animals. Preoperative preparation of mice and rats, suitable anesthesia as well as postoperative treatment and pain management are described in detail.

Video Link

The video component of this article can be found at http://www.jove.com/video/54216/

Introduction

Radiotelemetry is a most valuable methodological approach for measuring a variety of behavioral and physiological parameters in conscious, unrestrained animals of various sizes, particularly in the context of EEG, ECG, EMG, blood pressure, body core temperature or activity measurements [1-7]. Theoretically, any species can be analyzed using implantable EEG radiotelemetry from laboratory rodents such as mice and rats to cats, dogs, pigs and primates [3,8]. Even fish, reptiles and amphibians are subject to radiotelemetric investigation [9]. Over the last two decades, implantable EEG radiotelemetry has proven to be valuable in the characterization of various transgenic animal models of human diseases, such as epilepsies, sleep disorders, neurodegenerative and neuropsychiatric disorders [7,10-12]. In the past, numerous methodological approaches collecting physiological data including biopotentials from mice and rats have been described. Worn in jacket recorder systems, physical restraint methods, non-implanted radiotransmitters and tethered systems have received the main attention in the past [13,14]. Nowadays, various systems for radiotelemetric implantation are commercially available. However, a literature screen also revealed 29 publications that describe the development of self-made radiotelemetric systems [15-40]. Whereas home-made systems are likely to be less expensive and more user adapted, commercially available systems are straight forward, relatively easy to install and can be setup quickly.

Implantable EEG radiotelemetry has a number of advantages compared to competing techniques such as physical restraint methods, worn in jacket systems or tethered approaches. The latter are restraining by definition, *i.e.*, the animal is unable to move or its normal behavior is impaired. It might even be necessary to anesthetize the animal for acquisition of reliable data. Modern tethered systems however are likely to be less restraining, but this needs to be scientifically validated. Radiotelemetry on the other hand allows animals to exhibit their full repertoire of behavior without spatiotemporal restrictions and thus, is thought to be superior to restraining approaches and be more predictive of the results that could be acquired in humans [1,3]. It is known for quite a while that restraining approaches can dramatically alter fundamental physiological parameters, *e.g.*, food intake, body core temperature, blood pressure and heart rate and physical activity for example [3]. Tethered systems represent one still widely used classical restraining approach [13,14]. The electrodes which are either epidural or deep electrodes are generally connected to a miniature socket which is anchored to the skull. The socket itself is exposed for attachment of a cable that allows relatively free movement of the animal. Although nowadays tethered systems have become extremely filigree and highly flexible, one of its major disadvantages is, that it is still semi-restraining. Besides, there might be a risk of infection at the electrode implantation site as the animals tend to manipulate any external devices originating from their body (head). Although wireless radiotelemetry technology in various species has already been described in the late 60s and has thus existed for decades, it has only recently become affordable, reliable, and relatively easy-to-

use [10,41,42], particularly in small laboratory rodents such as mice and rats. Small, miniature implantable EEG transmitters are now commercially available and can be implanted in mice greater than 20 g (~10 weeks). Thus, the electrophysiological characterization of transgenic mouse models in particular has become a predominant field of application of implantable EEG radiotelemetry these days. Animal size is no longer an absolute experimental restriction whereas the life-span of the transmitters' battery indeed is. Despite its limited life-time, implantable transmitter systems are capable of minimizing most disadvantages related to potential recording-associated stress by restraining systems. Rodents can present their complete armamentarium of physiological behavior including resting, locomotor activity (exploration) and sleep (REM, slow-wave sleep) [43,44]. Importantly, implantable radiotelemetry can strongly reduce animal use [3]. Currently, there is an intense discussion on how to limit the number of experimental animals in science and reduce their suffering. Clearly, animal experimentation and animal models of human and animal diseases are essential for our understanding of the bottom-line pathophysiology and subsequent progress in therapy. Furthermore, animal experiments are critical in drug research and development. They do substantially contribute to preclinical/toxicological studies in drug licensing thus committing to both human and animal care. It's noteworthy, that currently no alternatives are yet available to animal research to understand the complex pathophysiological mechanisms which would be otherwise impossible to be elicited. At the same time, the 3R, *i.e.*, replacement, reduction and refinement strategy in the EU and the USA strongly encourages research into complementary and alternative methods. Radiotelemetry is an important example of a successful 3R strategy as it can reduce the number of experimental animals and their suffering compared to other techniques.

Here we provide a detailed and contiguous step-by-step approach to perform a subcutaneous pouch implantation of a radiofrequency transmitter in both mice and rats. This first sequence is followed by a description of stereotaxic epidural and deep intracerebral EEG electrode positioning. Special attention is paid to housing conditions, anesthesia, peri- and postoperative pain management and possible anti-infective treatment. The focus is on the computerized 3D stereotaxic approach to reliably target epidural and deep intracerebral structures. We also comment on frequent experimental pitfalls in EEG electrode implantation and strategies for reduction of trauma and optimization of pain management during postoperative recovery. Finally, examples of surface and deep EEG recordings are presented.

Protocol

Ethics Statement: All animal experimentation was performed according to the guidelines of the local and institutional Council on Animal Care (University of Bonn, BfArM, LANUV, Germany). In addition, all animal experimentation was carried out in accordance with superior legislation, *e.g.*, the European Communities Council Directive of 24 November 1986 (86/609/EEC) or individual regional or national legislation. Specific effort is made to minimize the number of animals used and their suffering.

1. Experimental Animals

1. Selection of experimental animals and species
    1. Perform radiotelemetric studies in rodents, *i.e.*, mice and rats that fulfil the requirements of homology, isomorphism and predictability related to a specific human disease [7,9,45,46].
    Note: Miscellaneous mouse and rat strains available can severely differ in basic physiological and pathophysiological characteristics [47-49].
    2. Consider or evaluate physiological and pathophysiological characteristics of mouse/rat strains prior to performing subsequent electrophysiological experiments, *e.g.*, response to applicable dosages of anesthetics, sleep architecture and seizure susceptibility [50,51].
    3. Note gender specific characteristics in study design. The estrous cycle can strongly affect central rhythmicity, its circadian dependency, sleep and seizure activity [52-54]. Thus, perform gender specific analysis.
    Note: If financial and experimental capacity is limited, restriction to male mice is advised.

2. Animal housing and handling
    1. House mice and rats in filter-top cages or even better in individually ventilated cages.
    2. Transfer mice from the animal facility to ventilated cabinets placed in special lab rooms exclusively dedicated to implanted animals and their subsequent recording (**Figure 1**).
    3. For acclimatization after ground transportation, place animals for one week in a ventilated cabinet under standard conditions, *i.e.*, 21 ± 2 °C ambient temperature, 50 - 60% relative humidity, and a conventional 12 hr light/dark cycle.
    4. Prior to surgical implantation, house mice in groups of 3 - 4 in clear polycarbonate cages type II (26.7 cm x 20.7 cm x 14.0 cm, area 410 cm^2) with *ad libitum* access to drinking water and standard food pellets. Use clear polycarbonate cages type III (42.5 cm x 26.6 cm x 18.5 cm, area 800 cm^2) for rats.
    5. Do not separate/isolate animals at this stage as isolation can cause stress influencing experimental results later on. However, following surgical instrumentation, house animals separately as the animals tend to manipulate wound stiches/sutures or metal clips (see below).
    6. Avoid open housing conditions as they are judged inappropriate for a variety of scientific questions, *e.g.*, sleep studies.
    7. Use mouse and rat specific equipment so that neither mice nor rats can sense the presence of each other as this poses additional stress to the animals.

2. EEG Radiotelemetry System

Note: The protocol described is based on a commercially available telemetry systems used for surface and deep intracerebral EEG recordings (**Figure 2**).

1. Use a radiofrequency telemetry implant suitable for implantation in mice or rats, *e.g.*, a one-channel transmitter or a two-channel transmitter.

Note: Both transmitters are capable of measuring various biopotentials, *i.e.*, electroencephalogram (EEG), electrocardiogram (ECG), electromyogram (EMG), but also physical activity and temperature. They have a magnetically actuated on-off mechanism. The transmitter and sensing leads are provided sterile. If the transmitter is to be re-used follow the manufacturers' instructions for resterilization.

2. For high-frequency gamma analysis (up to 500 Hz) for example, choose transmitters with higher nominal sampling rate (f, up to 5,000 Hz) and transmitter bandwidth (B, up to 500 Hz). In particular, consider the Nyquist-Shannon sampling limit, *i.e.*, EEG data can be analyzed up to an absolute maximum of f/2, but not beyond. For reliable frequency analysis, a frequency bandwidth (B) of f/10 - f/5 is recommended.
Note: The scientific question to be addressed must meet the technical specifications of the transmitter.

3. Anesthesia and Pain Management

1. Use isoflurane inhalation narcosis.
    1. Place the animal in an "induction chamber" filled with 4 - 5% isoflurane and 0.8 - 1% oxygen or carbogen (5% CO_2 and 95% O_2) L/min. Maintain the desired depth of anesthesia with a silicon facemask providing a flow 1.5 - 3.0% isoflurane and 0.8 - 1% oxygen or carbogen L/min (**Figure 3A**).
    Note: The appropriate isoflurane concentration varies according to body weight (distribution volume), age, sex and genetic background of the animal. If gas anesthesia equipment is not available, *i.e.*, "induction chamber", carbogen or oxygen supply, flowmeter, isoflurane vaporizer, scavenging system, see section 3.2. A withdrawn by suction system (scavenging system, **Figure 3A**) is to be installed to avoid isoflurane exposure of the experimenter (the tubing is not shown in the video document for demonstration).

2. When inhalation aneesthetics are not an option, perform anesthesia by injectable anesthetics. Prepare a combination of esketamine hydrochloride (rodent dosage 100 mg/kg) and xylazine hydrochloride (rodent dosage 10 mg/kg) in 0.9% NaCl and inject the animal intraperitoneally based on its body weight.
3. Observe the animals carefully for depth of anesthesia using tail pinch, foot pinch and by monitoring respiration rate (mice 150 - 220 breaths/min; rats 70 - 115 breaths/min). Check for possible gasping.
Note: Different mouse and rat lines can exhibit different sensitivities to anesthesia. The same holds true for transgenic mouse models.
Note: Endotracheal intubation is not a must in rodents. In fact, intubation increases the risk of damage to the trachea.

4. Surgical Instrumentation — General Aspects

1. Apply supplemental warmth during and post-surgery using recirculating warm water blankets, electrical warming plates, heat lamps, forced warm air units or pocket warmers to maintain body core temperature. Maintain the latter at 36.5 - 38.0 °C (98.6 - 100.4 °F).
Note: Small rodents are predisposed to hypothermia due to their high ratio of body surface (mouse, 10.5 x (weight in g)$^{2/3}$; rats, 10.5 x (weight in g)$^{2/3}$) to body volume.
2. Avoid corneal desiccation and cover eyes with petroleum-based artificial tear ointment or dexpanthenol (see video document) during the whole implantation process and early recovery until the blinking reflex is totally restored.
3. Autoclave surgical instruments (see **Table of Materials**) for sterilization or place them in disinfectants.
Note: An elegant and fast way is the usage of a heat-based surgical instrument sterilizer with glass beads.
4. Have a binocular surgical magnification microscope and a cold light source available for intense illumination via flexible or self-supporting, movable light guides.
5. Wear a clean laboratory coat, a facemask, a head cover and sterile gloves.
Note: Optimal supplies and instruments may vary from lab to lab and must meet lab-specific and institutional requirements.

5. Surgery — Transmitter Placement

1. Remove the body hair from the scalp from fully anesthetized mice/rats using a shaver. Clean the shaved area using a disinfectant, *e.g.,* 70% ethanol and an iodine based scrub. Avoid skin irritation or inflammation due to excessive exposure. Place the animal in prone position on a heating blanket to maintain body temperature during anesthesia.
2. Using a scalpel, make a midline incision on the scalp from the forehead (so that the bregma craniometric landmark becomes visible) to the neck (so that the trapezoid muscle becomes visible). Starting from the nuchal incision site and using a surgical scissor, open a subcutaneous pouch along the lateral flank of the animal by blunt dissection.
3. Inject 1 ml 0.9% NaCl in the subcutaneous pouch. Place the transmitter with the sensing leads oriented cranially inside the subcutaneous pocket at the flank close to the ventral abdominal region. If the transmitter has a suture tab, fix the transmitter at the dorsal/lateral skin using one or more stiches (over-and-over sutures).
Note that fixation of the transmitter is not a must. Pay special attention to preventing contamination of the surgical site and transmitter implant. Drapes should be used to properly isolate sterile from non-sterile areas.
4. For post-operative care and pain management, see section 8.

6. Stereotaxic Surface Electrode Implantation

1. Place the animal on the stereotaxic frame under anesthesia and carefully position the head with the help of the bars and the nose clamp so that the bregma and lambda craniometrics landmarks of the skull are at the same level (**Figure 3B**). Do not damage the inner ear using ear bars. Cover ear bars with cotton balls if necessary. This precautions allows for tight fixation of the head within the stereotaxic frame.
2. Clean the periosteum with cotton tips without damaging the temporal and occipital muscles. Pre-treat the superficial thin layer of the skull with 0.3% H_2O_2 for the mouse skull and 3% H_2O_2 for the rat skull. This procedure clearly exposes cranial suture and craniometrics landmarks such as bregma and lambda (**Figure 4B, C**).

3. Use a special, fully equipped stereotaxic setup for mice and rats including stereotaxic frame with ear bars and nose clamp size-adapted for mice and rats, respectively. Ensure that the stereotaxic frame includes a gas anesthetic mask with connections to the isoflurane evaporator and the isoflurane scavenger module.
   Note: A computerized 3D stereotaxic setup with a specific mouse and rat brain coordinate software including a user interface for navigation and 3D atlas, allowing axial, coronal and sagittal views is recommended.
4. Mount a precision drill on the vertical arm of the stereotaxic frame. Use a mounted pencil or pen on the vertical arm leaving a tiny mark at the coordinates of choice on top of the skull if no computerized stereotaxic system is available.
5. Drill holes carefully taking into consideration that mice and rats severely differ in the neurocranial bone thickness. In addition, note that the thickness of the murine cranial bones strongly depends on the localization, *e.g.,* in mice, *os frontale*: midline section: 320-390 µm, lateral section: 300 - 430 µm; *os parietale*: midline section: 210 - 250 µm, lateral section: 200 - 210 µm; *os occipitale*: midline section: 600 - 730 µm, lateral section: 380 - 420 µm).
6. Drill holes pressure-free at maximum velocity.
   Note: This avoids a tonic applanation of the skull, which may result in a sudden breakthrough of the drill head and potential damage mainly in the cortical field. For craniotomy, a neurosurgical high-speed precision motor drill system is highly recommended.
7. Drill burr holes at the coordinates of choice with typical drill head diameter of 0.3 - 0.5 mm.
   Note: The diameter of the holes might be smaller depending on the electrode diameter. As a general rule, the smaller the diameter, the less damage is produced.
8. Bend the tip of the transmitters' sensing lead which serves as an epidural electrode and place it directly on the dura mater in the hole at the coordinates of choice. Alternatively, use cortical screws and mechanically attach them to the sensing leads of the transmitter (**Figure 4A**).
9. For recordings from the surface, *e.g*., the murine motor cortex M1/M2, position the electrode, *e.g.,* at: cranial 1 mm, lateral 1.5 mm (left hemisphere). Place the epidural reference electrode on the cerebellar cortex: bregma -6 mm, lateral of bregma 1 mm (left hemisphere) or bregma -6 mm, lateral of bregma 1 mm (right hemisphere) (**Figure 4D**).
   Note: The cerebellum serves as a reference as it is an electroencephalographically silent region. Stereotaxic coordinates can be derived from standard stereotaxic atlases for mice and rats.
10. Fix electrodes with glass ionomer dental cement (water-based), which is extremely hard and gives strong adhesion to the underlying neurocranium.
    Note: If glass ionomer dental cement is used, no anchoring screws are necessary to secure the electrodes.
11. Leave the cement to dry for 5 min. Close the scalp using over-and-over sutures with non-absorbable 5-0/6-0 suture material. Alternatively, skin glue can be used. Closely monitor the quality of EEG recordings based on the electrode implantation site. Note: Ossification from the drilled holes can occur that has the capability to lift up the electrodes with time. This can result in reduced EEG quality due to EMG and ECG contamination and can thus limit the optimum recording duration.
12. For post-operative care and pain management, see section 8.
13. Validate EEG electrode position post mortem.
    1. For euthanasia, place the animal(s) in an incubation chamber and introduce 100% carbon dioxide. Use a fill rate of 10% - 30% of the chamber volume per minute with carbon dioxide added to the existing air in the incubation chamber. This is appropriate to achieve rapid unconsciousness with minimal distress to the animals.
       Note: Avoid sudden exposure of conscious animals to carbon dioxide concentrations >70% as this has been shown to be distressful.
    2. Observe each mouse/rat for lack of respiration and faded eye color. Maintain CO_2 flow for a minimum of 1 min following respiratory arrest. Expected time to unconsciousness is usually within 2 to 3 min.
    3. If both signs are observed, then remove the rodents from the cage; otherwise continue exposing them to CO_2. If unconsciousness has not occurred within 2 to 3 min, check the chamber fill rate.
    4. To verify the correct electrode placement, extirpate brains post mortem, *e.g*., following CO_2 euthanasia and fix them in 4% paraformaldehyde in PBS (pH 7.4) overnight. Alternatively, perform cardiac perfusion of the animals using ice-cold phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde (PFA) solution dissolved in PBS.
    5. Postfix brains for 2 - 4 hr in 4% PFA at RT followed by cryoprotection in 30% sucrose in PBS and store brains at 4 °C till further processing.
    6. Using specimen matrix for cryostat sectioning, freeze brains onto a stereotactic block and cut 60 µm coronal slices using a cryostat. Mount slices onto glass slides, air dry, and stain with Nissl blue using standard techniques to visualize the branch canal and former electrode position.
       Note: This approach also reveals whether the surface electrodes have been placed to deep accidentally by leaving a minor impingement on the top of the cortex.

7. Stereotaxic Deep Intracerebral EEG Electrode Implantation

1. Pre-treat the scalp and skull of the animal as described in sections 6.1 - 6.2. Select the type of deep electrodes carefully, taking in consideration its material characteristics, *e.g.*, diameter and impedance and possible connection to the transmitter's sensing leads.
   Note: Parylene coated steel and tungsten electrodes are commonly used. The electrode characteristics have to fit the individual experimental needs. If the electrodes are not provided sterile, they should be incubated in 70% ethanol before use. As the electrodes are coated for this experimental purpose, a heat-based sterilization is not applicable.
2. Drill holes at the coordinates of choice as described in section 6 using the stereotaxic system. To target the murine CA1 region for example, which serves as an intensively investigated brain area, place the differential electrode at the following coordinates referring to bregma: caudal 2 mm, lateral 1.5 mm (right hemisphere) and dorsoventral (depth) 2 mm. Place an epidural reference electrode on the cerebellar cortex, *e.g.*, bregma -6 mm, lateral of bregma 1 mm (left or right hemisphere) (**Figure 4D, E**).
   Note: The cerebellar electrode serves a pseudo-reference electrode on the silent region of the cerebellum. Stereotaxic coordinates can be derived from standard stereotaxic atlases for mice and rats.
3. Shorten the deep electrodes to the required length depending on how deep into the brain they will be inserted. Connect the extracranial part of the electrode to the stainless steel helix of the transmitter lead by bending both sections to a 90° angle in between.

4. Clip the deep electrode to the sensing lead of the transmitter mechanically. Do not solder whenever possible as this can induce significant noise in the EEG recording. Expose the stainless steel helix of the transmitter lead by removing a short section of the outer silicone isolation at the tip of the transmitter lead using a sterile scalpel blade.
5. Rewire the lead of the transmitter to the deep brain electrode. Ensure a suitable and stable connection of both components (**Figure 4F**). Attach the implanted electrode (which is mechanically connected to the transmitter lead) to the vertical arm of the stereotaxic device.
6. Fix the electrode with glass ionomer dental cement (water based), which is extremely hard and gives strong adhesion to the underlying neurocranium. Leave the cement to dry for 5 min. Close the scalp using over-and-over sutures with non-absorbable 5-0/6-0 suture material. Alternatively, skin glue can be used.
7. Closely monitor the quality of EEG recordings based on the electrode implantation side.
   Note: Ossification from the drilled holes can occur that has the capability to lift up the electrodes with time. This can result in reduced EEG quality due to EMG and ECG contamination and can thus limit the optimum recording duration. This is of special relevance for deep electrode placement.
8. For post-operative care and pain management, see section 8.
9. Validate EEG electrode placement post mortem as described in section 6.13.

8. Post-operative Care and Post-operative Pain Management

1. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbency.
2. Do not return an animal that has undergone surgery to the company of other animals until fully recovered.
3. For post-operative pain management, choose a drug of one of the following groups: narcotic opioids, opioid agonists / antagonists, α_2-agonists, local anesthesia and nonsteroidal anti-inflammatory drugs (NSAID). [55-60] Please note that due to the severity of the surgery a 3 day analgesic treatment is recommendable.
   1. If using buprenorphine, administer the following dose: mouse: 0.05 - 0.1 mg/kg, i.p., s.c., every 6 - 12 hr; rat: 0.01 - 0.05 mg/kg, i.p., s.c., every 8 - 12 hr.
   2. If using butorphanol, administer the following dose: mouse: 1.0 - 5.0 mg/kg, s.c., every 4 hr; rat: 2.0 - 2.5 mg/kg, s.c., every 4 hr.
   3. If using tramadol, administer the following dose: mouse, rat: 10 - 30 mg/kg, i.p.
   4. If using flunixin, administer the following dose: mouse: 2.5 mg/kg, s.c., every 12 hr; rat: 1.1 mg/kg, s.c., every 12 hr.
   5. If using ketoprofen, administer the following dose: mouse: 5 mg/kg, s.c., every 12 - 24 hr; rat: 5 mg/kg, s.c., every 12 - 24 hr.
   6. If using metamizole, administer the following dose: mouse, rat: 100 mg/kg, i.p., every 8 hr.
   7. If using meloxicam, administer the following dose: mouse, rat: 1 mg/kg s.c., every 24 hr.
   8. If using carprofen, administer the following dose: mouse: 5-10 mg/kg, s.c., every 12 - 24 hr; rat: 2.5 - 5.0 mg/kg, s.c., every 12 - 24 hr.
   9. If using acetaminophen, administer the following dose: mouse: 300 mg/kg, p.o., every 4 hr; rat: 100 - 300 mg/kg, every 4 hr.
   10. If using lidocaine (as adjunct analgesic), administer the following dose: mouse, rat: 1 - 4 mg/kg s.c.
4. When using carprofen (rodent dosage 5 - 10 mg/kg s.c., diluted in 0.9% NaCl) for long-lasting post-operative pain management, perform the initial injection 10 - 15 min before the end of the surgical instrumentation and repeat for two subsequent days once a day.
5. Postoperatively, feed moistened pellets in order to facilitate food uptake. Carefully observe food (~15 g/100 g/d; ~5 g/24 hr) and water (~15 ml/100 g/d; ~5 ml/24 hr) consumption.
6. Monitor animals closely for the return of their normal postures and behaviors.
   Note: Systemic administration of antibiotics such as enrofloxacin or trimetroprim-sulphonamides is often recommended but not an absolute must unless inflammatory signs of meningitis or encephalitis at the sites of implantations are detected.
7. Give mice at least 10 to 14 additional days to fully recover before starting EEG recordings for further analysis.
   Note: Specific experimental tasks may require longer recovery periods.
8. Follow-up postoperative recovery after implantation by evaluating postsurgical development of body weight. A maximum reduction in body weight is normally observed around day 4 - 5 post surgery followed by a slight, but steady increase of weight during a 10 - 14 day recovery period.

Representative Results

This section illustrates examples obtained from surface and deep, intracerebral EEG recordings. Initially it should be stated that baseline recordings under physiological conditions are mandatory prior to subsequent recordings following *e.g.,* pharmacological treatment. Such baseline recordings may provide valuable information about functional interdependence of brain rhythmicity with different behavioral states or sleep / circadian rhythmicity. Here, we show examples of recorded seizure activity following acute administration of proconvulsive / psychoenergetic drugs. As outlined above, a common field of application in EEG radiotelemetry is epilepsy research. Epilepsy models include acute and chronic pharmacological models as well as genetic (transgenic) models of epilepsy. Here we demonstrate acute models of non-convulsive absence-like seizures induced by i.p. administration of R/S-baclofen at 20 mg/kg and bicucullinemethobromide at 10 mg/kg. Pharmacodynamically, baclofen is a GABA(B) receptor agonist that increases K^+ efflux out of the cell both pre- and postsynaptically whereas bicuculline is a GABA(A) antagonist that inhibits Cl^- influx into the cell. Activation of GABA(A) receptors results in the initiation and maintenance of hyperoscillation and hypersynchronization within the thalamocortical-corticothalamic circuitry. **Figure 5B, C** displays epidural EEG recordings following the i.p. administration of R/S-baclofen (20 mg/kg) and bicucullinemethobromide (10 mg/kg). Systemic administration of 4-aminopyridine (4-AP) at a dosage of 10 mg/kg i.p. or pentylenetetrazole (PTZ) can provoke generalized tonic-clonic seizures in mice and rats. Following 4-AP or PTZ injection, animals show a typical temporal sequence of motoric actions that dose-dependent in severity, *i.e.*, intensity and duration. Seizures normally start from a hypoactive state, followed by a mild, partial myoclonus that mainly affects the face with vibrissal twitching, the head and/or the forelimbs. This partial seizure state can than generalize into a myoclonus characterized by loss of upright posture or a whole body clonus involving all four limbs. The latter is characterized by jumping, wild running and finally, a tonic extension of the hindlimbs. A typical epidural EEG recording following 4-AP administration (10 mg/kg) is depicted in **Figure 5A**. This epidural type of recording is capable of eliciting the early stages of seizure development, *i.e.*, myoclonic head movement, jerks of face and forelimbs) with high precision. Although there is a high degree of motoric seizure activity associated with high EMG, *i.e.*, muscle activity, only minimal EMG contamination of EEG recordings is observed. As becomes obvious in **Figure 5A**, the sporadic spike activity (*) is followed by a generalized clonus with a typical spike/polyspike/spike-wave pattern (1) followed by a subsequent episode of continuous spike activity. Note that EMG contamination is hardly detectable. Although the recording segment is characterized by enhanced muscle activity due to the whole body clonus, the spike activity originating from the brain is prominent and EMG contamination is extremely low. This example proves that the proposed experimental approach is capable of recording EEG signals selectively even under generalized seizure conditions, when EEG signals might expected to be masked by EMG artifacts. Note that drug injection regimes as described here always require recordings prior to injection, under injection and following pharmacological administration. Controls should include sham-injected / vehicle injected animals.

A typical intracerebral brain target is the hippocampus, *e.g.,* the CA1 region. Hippocampal seizure activity can be induced by kainic acid (KA) or N-methyl-D-aspartate (NMDA). The non-NMDA receptor agonist KA is generally administered intraperitoneally at a dose of 10-30 mg/kg. Hippocampal seizures represent an important seizure subgroup that can be acutely induced by various glutamate receptor agonists. Using the deep electrode implantation procedure described above, KA induced hippocampal seizures can be recorded with high precision (**Figure 5D**). Besides KA, hippocampal seizures can also be induced by i.p. administration of NMDA at a dose of 150 mg/kg. As in KA treated animals, NMDA treated mice, develop seizures through a sequence of paroxysmal scratching, hypermotility and circling, tonic-clonic convulsions, and, occasionally, death.

Figure 6 illustrates examples of simultaneous cortical (epidural) and hippocampal (deep) EEGs in a most popular chronic hippocampal seizure model, *i.e.*, the pilocarpine model of mesial temporal lobe epilepsy (mTLE) in rats. It should be noted that EEG artifacts can sometimes mimic ictiform discharges (**Figure 7**). Thus special attention has to be paid to reduce ECG, EMG and externally induced EEG signal disturbance. It should be noted that the implantation procedure described here allows for maximum reduction in EEG signal contamination. Artifacts either result from external electrical devices which can be shielded by, for example, a Faraday cage or by ossification processes around the drilled holes that tend to lift the electrodes out of the brain. The latter is a time-dependent process that marks an experimental limitation of the technique. It should be noted that seizure recording and analysis is not the only field of application of the techniques described here. Surface and deep intracerebral EEG recordings can be used for complex time-frequency analysis, *e.g.,* in animal models of neuropsychiatric diseases and for sleep studies for example.

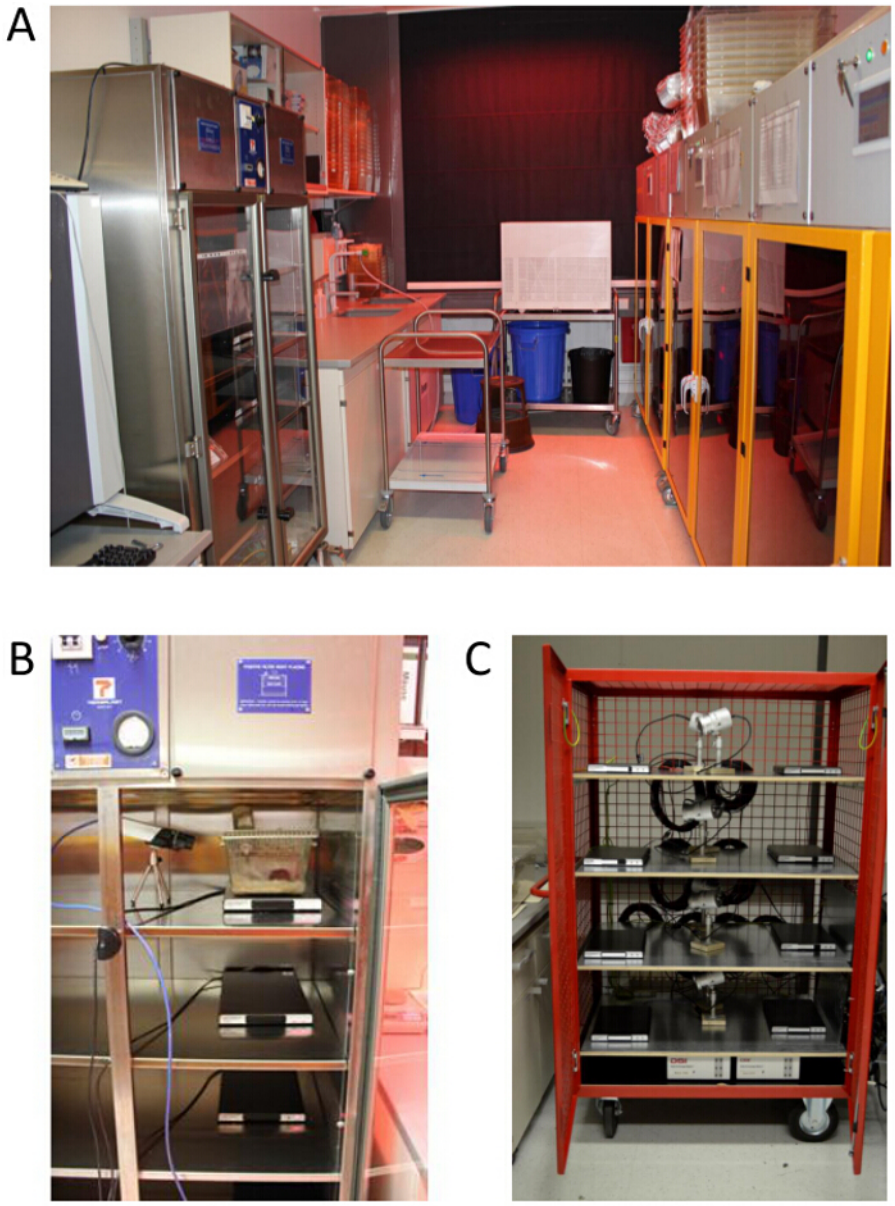

Figure 1: Housing Conditions in Radiotelemetry. *In vivo* studies in different mouse or rat lines or pharmacological or transgenic lines of human diseases require high standardization to minimize intra-individual variability and potential bias arising from confounding factors. Proper housing conditions are a prerequisite for high-quality recordings and valid telemetric results. Open housing conditions on lab shelves are not suitable for recording. Instead recording should be performed inside an animal facility, or in ventilated cabinets (**A**). Ideally, ventilated cabinets are not only used for pre-surgical and postsurgical housing and recovery, but also for EEG recording (**B**) as this guarantees stability of environmental conditions and lack of disturbance. If recording cannot be performed in a ventilated cabinet, they should be done in a Faraday cage inside an environmentally controlled animal room (**C**). Please click here to view a larger version of this figure.

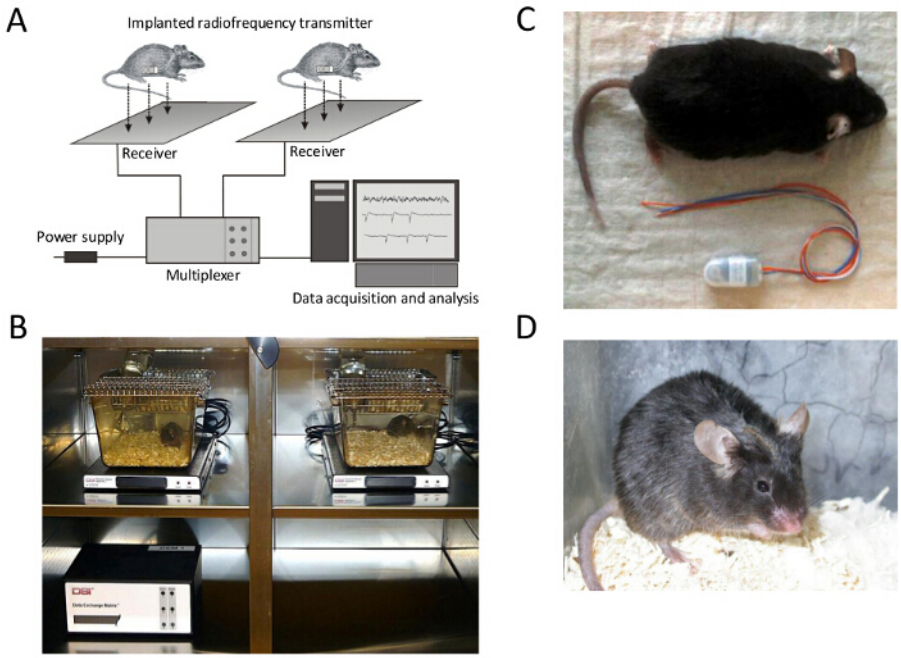

Figure 2: Standard EEG Radiotelemetry System and Radiofrequency Transmitters. Besides self-made systems, a number of commercially available systems are on the market. The basic setup of such system is depicted in (**A**). The system consists of a radiofrequency transmitter, the receiver plate, a data exchange matrix serving as a multiplexer, and the data acquisition, processing and analyzing core unit. For frequency analysis, seizure detection and sleep analysis specific software modules are offered. Multiple types of transmitters are available depending on which species is supposed to be investigated and depended on the scientific question. **B**) Implanted mice, receiver plates and a multiplexer placed inside a ventilated cabinet for standardized recording conditions. **C**) An adult C57Bl/6J mouse and a 2-channel radiofrequency transmitter. **D**) Dorsal view of the skull 4 weeks after electrode implantation and fixation using glass ionomer cement (reprinted from [61] and [62] with permission). Please click here to view a larger version of this figure.

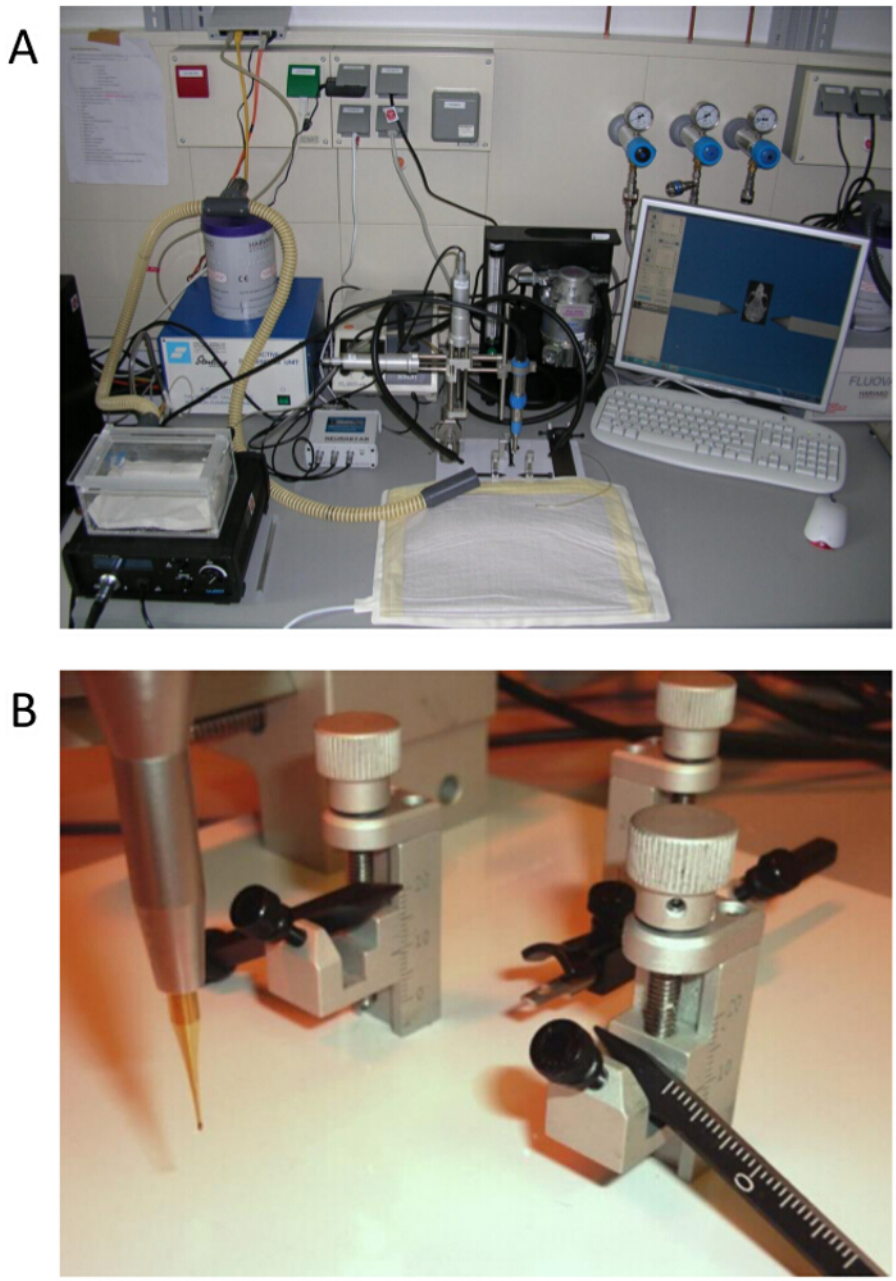

Figure 3: Anesthesia and Stereotaxic Setup for Mice and Rats. A) Gas anesthesia system using isoflurane. A precision high-speed dental drill is mounted on a 3D stereotaxic device for mice and rats respectively. Supplemental warmth is given using a heating pad. **B**) Close-up of drill, stereotaxic ear bars and nose clamp (reprinted from [62] with permission). Please click here to view a larger version of this figure.

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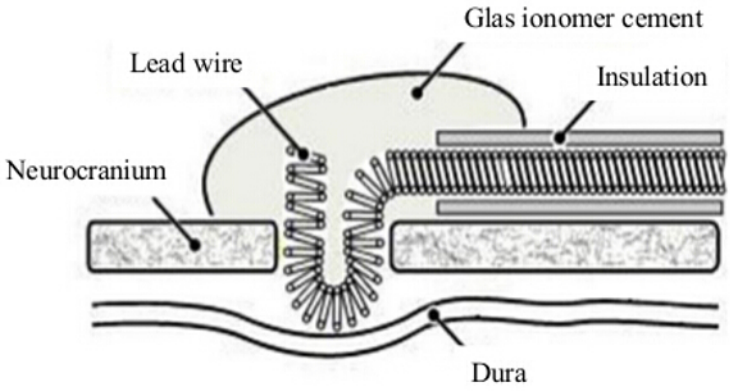
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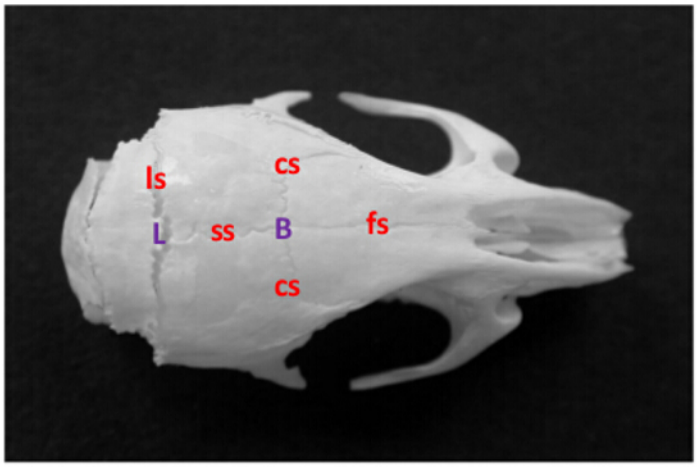
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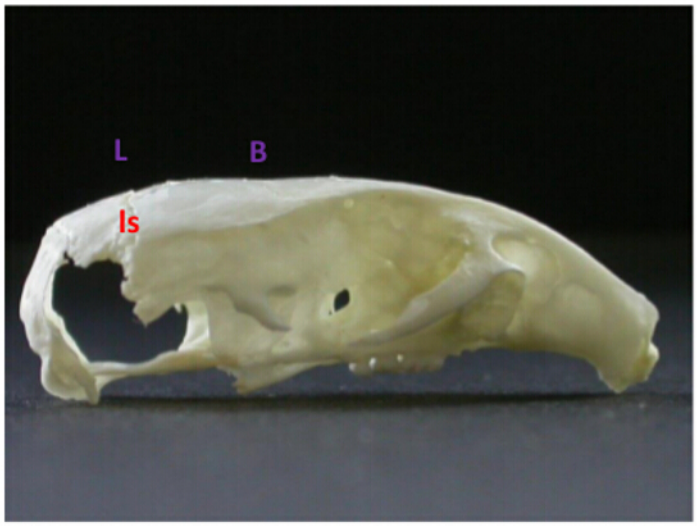
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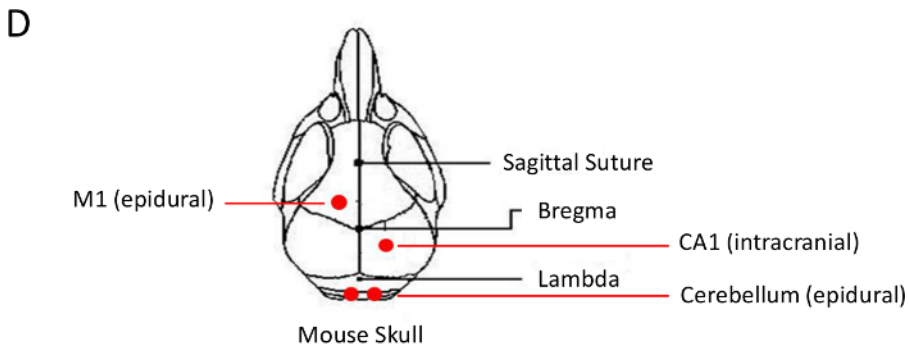

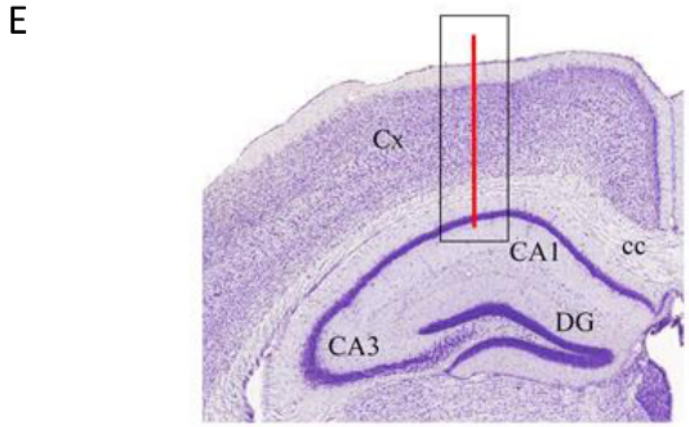

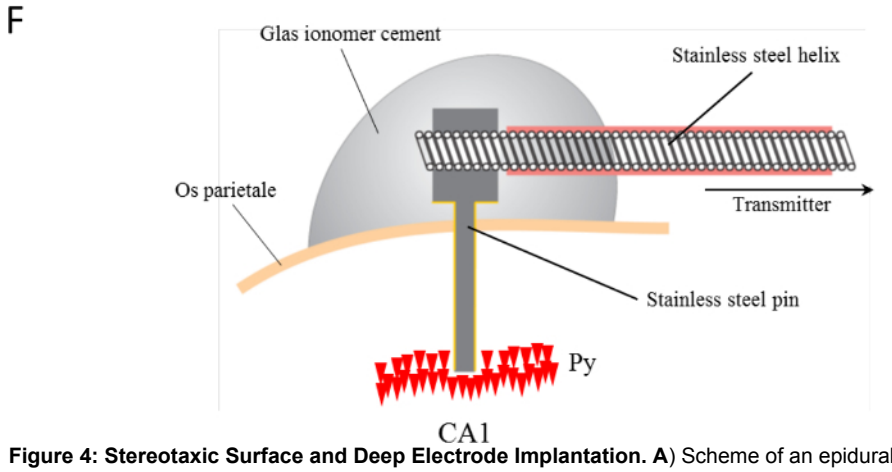

Figure 4: Stereotaxic Surface and Deep Electrode Implantation. A) Scheme of an epidural electrode placement in mice and rats. **B**) Anatomic structures and landmarks of the murine skull. Apical view of a C57Bl/6J mouse skull which has been prepared in 0.3% H_2O_2. Note cranial bones (os frontale (of), os parietale (op), os occipitale (oo)) and sutures (sutura frontalis (sf), sutura sagittalis (ss), sutura coronaria (sc), and sutura lambdoidea (sl)) which determine the major anatomic landmarks bregma (B) and lambda (L). **C**) Lateral view of a C57Bl/6J mouse skull. **D**) One epidural, differential electrode is placed on the motor cortex (M1), an additional intrahippocampal differential electrode is placed in the CA1 region of the hippocampus. Both pseudo-reference electrodes are localized on the cerebellum. **E**) Coronal section (scheme) illustrating the localization of the deep, intracranial electrode for recording the electrohippocampogram. **F**) Close-up of the deep EEG electrode, the sensing lead of the radiofrequency transmitter and their arrangement on top of the murine skull (reprinted from [61] and [62] with permission). Please click here to view a larger version of this figure.

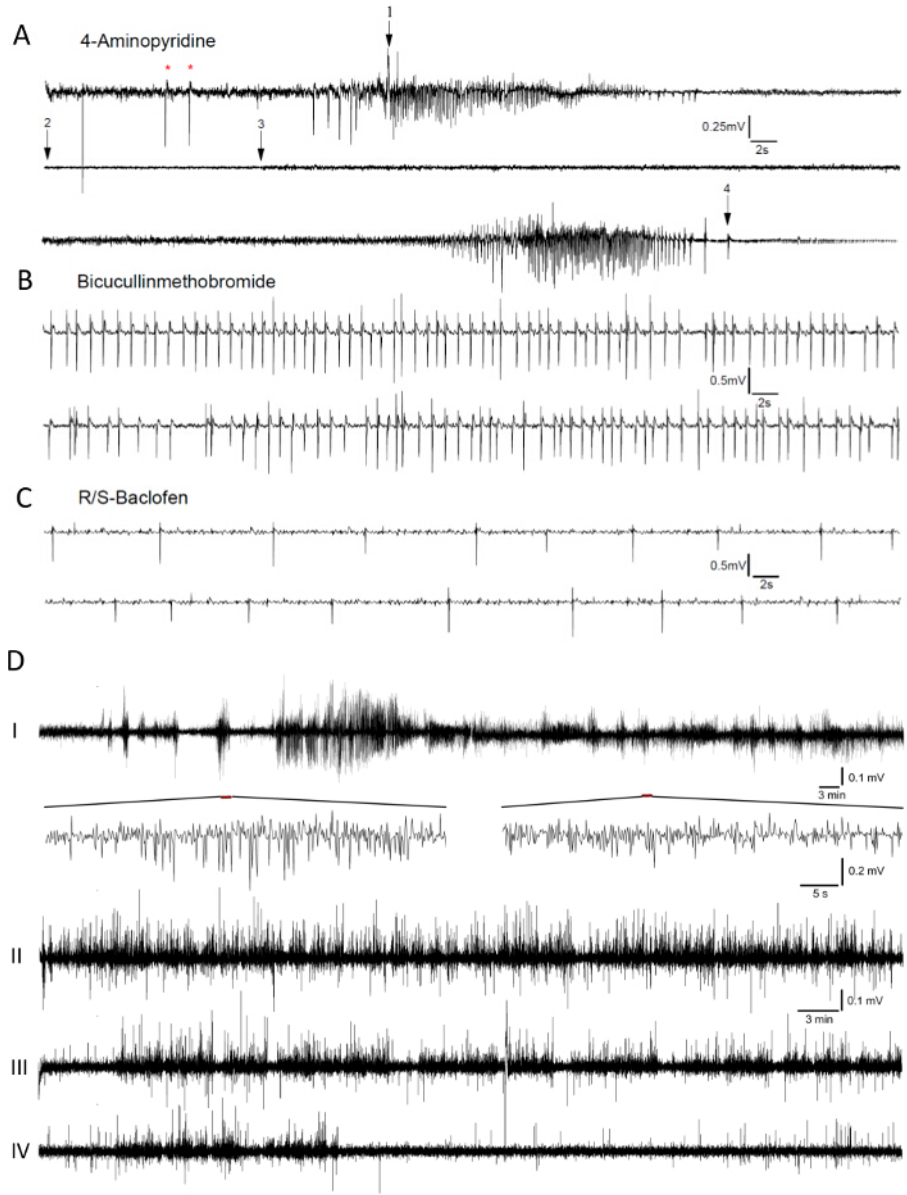

Figure 5: Pharmacological Induction of Epileptic Discharges. A) Surface EEG recording displaying ictal discharges after i.p. administration of 4-aminopyridine (4 AP, 10 mg/kg). Sporadic spikes (*) evolve into a transitory episode of continuous spiking (1), resulting in an EEG depression (decreased amplitude, 2-3). Shortly after this period a second spike-train concomitant to the development of a generalized tonic-clonic seizure with wild running and jumping becomes apparent which finally results in a tonic extension of the hindlimbs (4) and death. The remaining tiny signal following brain death represents an ECG (R-spike) contamination. **B**) After i.p. administration of bicucullinemethobromide (BMB, 10 mg/kg) mice show trains of characteristic spikes and spike waves. **C**) Administration of baclofen (20 mg/kg) resulting in sporadic occurrence of spiking activity. **D**) Intrahippocampal electroencephalographic (EEG) recordings following i.p. administration of KA (30 mg/kg). I: deep CA1 recording from a C57Bl/6J mouse for 2 hr immediately after KA administration. At 30 mg/kg KA contiguous hippocampal seizure activity is observed occasionally interrupted by postictal depression (arrows). Ictal discharges are characterized by spike and/or spike-wave activity (see insets) in the delta- and theta-wave range (4-8 Hz). II-IV: At days 1, 3, and 5 post-injection 1h CA1 EEG recordings illustrate declining but still continuous ictal discharges related to neuronal excitotoxic degeneration (reprinted from [61] and [62] with permission). Please click here to view a larger version of this figure.

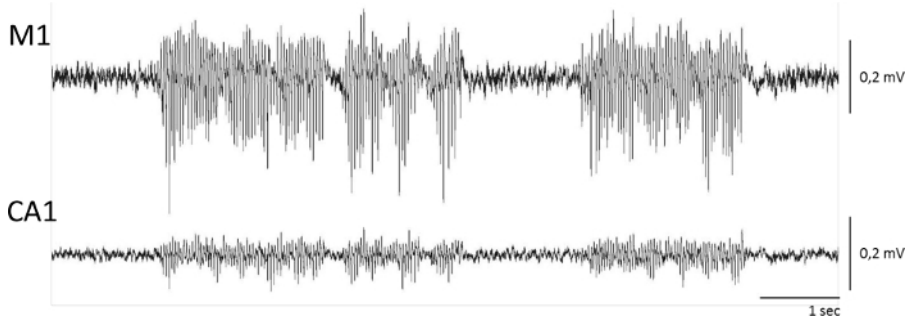

Figure 6: Radiotelemetric EEG Recording in a Rat Model of Mesial Temporal Lobe Epilepsy. Limbic seizures are pharmacologically induced via a pilocarpine injection regime. This figure illustrates synchronous recording from the primary motor cortex (M1) as well as the hippocampal CA1 region from a rat at the age of 3 months. Ascending and descending spike / poly-spike trains are present in both deflections (reprinted from[62] with permission). Please click here to view a larger version of this figure.

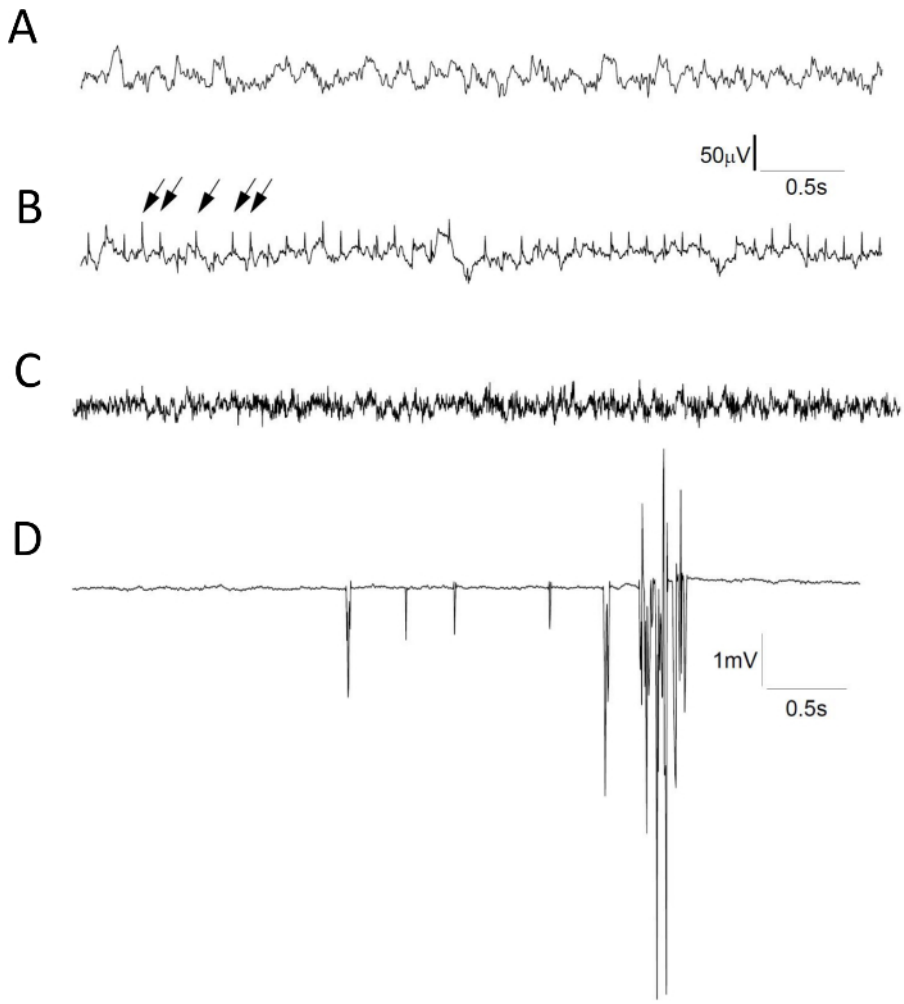

Figure 7: Electrocardiographic/Electromyographic and System Artifacts Contaminating the EEG (deep electrodes (A-C), surface electrodes (D), vertical bar: 50 µV in A-C, 1 mV in D). **A**) Intrahippocampal EEG recording from a control mouse. **B**) Damaged silicone insulation of the sensing leads as well as ossification processes originating from the edge of drilled holes can result in dramatic contamination of electroencephalographic recordings. Note the regular pattern of interfering ECG signal, *i.e.*, R-spikes (arrows). Importantly, ECG contamination cannot be completely avoided but the implantation procedure presented here will reduce it to a minimum. **C**) Electromyographic contamination of the EEG characterized by high frequency activity. **D**) Artifacts can also originate from cross-talk between receiver plates or from electrical noise evolving from room lights or various other electrical devices that are close to the receiver plates. An effective way of preventing the system picking up noise is to shield receiver plate and home cage using a ventilated cabinet or a Faraday cage (reprinted from [61]and [62] with permission). Please click here to view a larger version of this figure.

Discussion

Implantable EEG radiotelemetry is of central relevance as it is a non-restraining technique allowing experimental animals to perform their full repertoire of behavior[1,3]. This is of major interest as the telemetric approach enables not only spontaneous EEG recordings but also recordings under cognitive tasks and circadian analytical setups, such as T-maze, radial maze, water maze, sleep deprivation tasks or whenever an EEG recording is necessary or helpful during complex cognitive or motor activity.

This protocol describes epidural surface and deep intracerebral EEG electrode implantation in mice and rats and connection to an implantable EEG radiofrequency transmitter. Critical steps within the procedure comprise pre-surgical issues, *i.e.,* selection of species and strain, housing conditions, anesthesia and pain management. A critical literature screen reveals that the latter can serve as confounding factors that contribute to divergent results in various research approaches. For example, the choice of experimental species, *e.g.,* mice versus rats and even strains can totally alter experimental results. The same holds true for gender. In general, a gender specific grouping and analysis is highly recommended. If this is not possible, genders should be balanced at least. If experimental conditions are not strictly harmonized or controlled, acquired data are either not comparable or simply invalid.

The stereotaxic implantation procedure described here provides a reliable tool to record high-quality EEGs from both the surface and deep intracerebral structures. Critical steps of the implantation procedure include the drilling process. It should be performed at maximum speed (RPM) with minimum pressure. Though high drilling speed generates heat, minimum pressure guarantees that subcortical structures are not thermally damaged. Minimum pressure is essential to avoid a sudden breakthrough of the skull and subsequent damage of the underlying cortex. In addition, special care has to be taken not to damage a meningeal artery or a dural sinus. In mice, the skull is rather transparent due to its small thickness. Therefore, meningeal arteries and sinuses can be identified to avoid damage. In case of bleeding the early and late prognosis is bad in general and it is questionable whether such an animal meets the inclusion criteria for a reliable study. We recommend sacrificing such animals.

In our experience, high-quality EEG recordings using the described approach can be performed up to 4 weeks. Due to ossification processes originating from the drilled holes within the calvaria, electrodes tend to be lifted up resulting in ECG and EMG contamination. It should further be considered that targeting a specific surface or deep, intracerebral structure relies on stereotaxic coordinates from brain atlases. These stereotaxic brain maps are normally related to a specific mouse or rat strain of a specific age. It has to be noted critically that different mouse and rat strains can exhibit differences in age specific size of the body and the skull. Thus, there are inter-strain and intra-strain differences as regards the basic craniometrics landmarks bregma and lambda. This issue poses a specific challenge if one wants to perform surface and deep electrode recordings from young mice and rats that are still developing, *i.e.*, display skull and brain growth. In this case, a reliable long-term recording from the position of choice is hardly possible.

In order to make the craniometric landmarks visible a bleaching procedure is recommended. Care must be taken to limit the incubation time of H_2O_2 as it can otherwise penetrate the skull and do oxidative damage to the cortex.

Finally, it's important to note that commercial EEG radiotelemetry systems can be combined with other electrophysiological setups as well. We recently established the combination of radiotelemetric EEG recording with an auditory evoked potential setup in mice. This sophisticated approach allows, for example, to perform endophenotyping and to identify and characterize transgenic mouse models of schizophrenia, *e.g.,* by application of the double-click paradigm and analysis of P50/N100 potentials. In general, the technical link between EEG radiotelemetry and evoked-potentials is likely to be a promising approach in the future.

Disclosures

The authors have nothing to disclose.

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