Recording EEG in immature rats with a novel miniature telemetry system.

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Abstract

Serial electroencephalographic (EEG) recordings from immature rat pups are extremely difficult to obtain, but important for analyzing animal models of neonatal seizures and other pediatric neurologic conditions as well as normal physiology. In this report, we describe the features and applications of a novel miniature telemetry system designed to record EEG in rat pups as young as postnatal day 6 (P6). First, we have recorded electrographic seizure activity in two animal models of neonatal seizures, hypoxia- and kainate-induced seizures at P7. Third, we describe a viable approach for long-term continuous EEG monitoring of naturally-reared rat pups implanted with EEG at P6. Second, we have utilized serial EEG recordings to record age-dependent changes in the background EEG signal as the animals matured from P7 to P11. The important advantages of using miniature wireless EEG technology are (1) minimally invasive surgical implantation; (2) a device form-factor that is compatible with housing of rat pups with the dam and littermates; (3) serial recordings of EEG activity, and (4) low power consumption of the unit, theoretically allowing continuous monitoring for up to 2 years without surgical re-implantation. The miniature EEG telemetry system provides a technical advance that allows researchers to record continuous and serial EEG recordings in neonatal rodent models of human neurological disorders, study the progression of the disease, and then assess possible therapies using quantitative EEG as an outcome measure. This new technical approach should improve animal models of human conditions that rely on EEG monitoring for diagnosis and therapy.
Introduction

**EEG in the clinic and animal models**

Electroencephalographic (EEG) recordings are an essential test for clinical diagnosis and outcome prediction of various neurologic conditions and injuries. EEG is required to detect non-convulsive seizures, helping to change the clinical treatment of neonatal and pediatric patients (Abend et al., 2011; Nash et al., 2011; Connell et al., 1989; Glass et al., 2009;). EEG recordings have been highly effective for prediction of the outcomes of neurologic insults such as perinatal hypoxia-ischemia (HI) and asphyxia in retrospective human studies (Korotchikova et al., 2011; Holmes and Lombroso, 1993; Aso et al., 1990; Murray et al., 2009; Hellstrom-Westras and Rosen, 2005; Garfinkle and Shevell, 2010; Walsh et al., 2011). While EEG has been used with a high degree of success for neonatal and pediatric seizures and brain injuries in humans, it has been under-utilized in animal models. Rodent animal models are an important tool for pre-clinical testing of anti-convulsant compounds that are used for treatment of epilepsy. In order to improve overall validity of neonatal animal models of human disorders, it is critical to use methods and techniques similar to those that are used to diagnose and manage human patients in the clinic. To address these issues, we describe here a wireless EEG telemetry system that is highly effective in immature rodents.
EEG in immature animals

Obtaining high-quality, serial EEG recordings from immature rodents as young as P6 is technically difficult and would benefit from new approaches. Most of the previous studies examining in vivo EEG or local field potential (LFP) recordings in immature rats have been conducted using wired recording solutions in animals that were postnatal day 12 (P12) or older (Cyacong et al., 2011). Several published studies suggest that the P7-P12 rat pup is the developmental age that corresponds to a full-term human neonate (Quinn, 2005; Romjin et al., 1991). However, the largest burden from neonatal seizures and other neurological abnormalities exists in the premature infant population (Cummins et al., 1993). This raises the importance of modeling the disorders in rat pups at a younger age (i.e., P6-P9). Working with rat pups in this age group is difficult, and requires specialized surgical, recording and rearing strategies. To evaluate an animal model, the entire disease process including the acute period, progression and outcome, should be analyzed quantitatively; thus, serial recordings from the same animals are critical for translational analyses. Making serial EEG recordings would not only enable the ability to examine the acute period, but also would allow quantitative evaluation of progression of the disease after an injury or following an intervention. Accomplishing the goal of making serial recordings starting at P6 required several unique technical developments and solutions.

Significance of wireless EEG in immature rodents
Here we describe design features of a miniature EEG telemetry system and surgical techniques that make it compatible with use in immature rat pups as young as P6. We use two models of neonatal seizures in P7 rat pups to record electrographic seizures, and describe age-dependent features of the normal EEG. Additionally, we show long-term monitoring approaches that enable continuous serial monitoring of animals from pup to adult ages. We show that animal models of neurologic conditions do not have to be limited to behavioral outcome measures; instead, EEG can be used for longitudinal, quantitative electrophysiological analysis.

Materials and Methods

Wireless Transmitter and Receiver

The requirements for making EEG recordings in rat pups dictate that the device be small, have a low profile, and have minimal power requirements. To accomplish the low-power and small form-factor demands, we used the following design. The device, as presently designed and used, consists of two fundamental components: (1) a micro-transmitter comprised of a physiological amplifier controlling a pulse-width (i.e. frequency) modulation oscillator, and (2) a capacitive-coupled receiver, which includes a frequency-to-voltage converter that recovers the original EEG signal. The recording input is two leads connected to an amplifier. The amplitude of the EEG signal modulates the pulse width of a
square-wave oscillator, which is transmitted via capacitive coupling to the antenna. A high-impedance receiver then detects, amplifies, and filters the EEG signal. The receiver consists of an integrator (or a frequency-to-voltage converter) and a band-pass filter, which recovers the original AC signal from the transmitter. The bandwidth of the device is 0.1 – 120 Hz, which is suitable for many experiments recording EEG signals for long-term monitoring. The gain of the amplifier is 4000x, so a 1 mV EEG signal is 4 V on the analog output of the receiver base. The amplifier design is patent-pending by the University of Utah Research Foundation, a schematic of which can be found in the online application, US 20100222686A1.

Device form factor

Form factor is an important consideration in a device designed for use in immature rats (Figures 1 and 2). The implanted device needs to be stabilized without the use of skull screws. Stability and durability are critical because rat pups younger than P21 are normally housed and reared with their littermates and the dam, who will remove extraneous objects from pups. To achieve the required durability, the transmitter was encased in optically clear epoxy (Epo-tek 301; Billerica, MA). The mold was designed in the shape of a cylinder that is 10 mm diameter and 10 mm high, with a dome-shaped top (Figure 1). The weight of the transmitter unit was approximately 1 g. This shape was the most dam-resistant and was easy to affix to the top of the rat-pup skull. The bottom of the transmitter which contacts the skull is slightly concave; this shape increased the contact
area with the slightly convex rat skull. The increased contact area improved the effectiveness of fixation by cyanoacrylate glue, eliminating the need for skull screws.

Animals

All surgical procedures were performed under protocols approved by the University of Utah Animal Care and Use Committee. Pregnant Sprague-Dawley adult female rats (14 days gestation) were received from Charles-River (Wilmington, MA). Pups were delivered in the animal facility approximately 1 week after the arrival of the pregnant female (University of Utah, Salt Lake City, UT). The litter size varied from 8 to 10 pups. Animals were housed with the dam and littermates, and at P6 they were implanted with the transmitter of the miniature telemetry system. The weight of the pups at the time of implantation was 14-16 g.

Surgical implantation of the transmitter unit

During the surgical procedure, animals were initially anesthetized with 4% isoflurane (MWI Veterinary Supply, Meridian, ID) and maintained at 2% during the procedure. Surgical equipment and ear bars were autoclaved and the stereotaxic frame was sprayed with 70% ethanol. Sterility of surgical tools was maintained with 70% ethanol. Rat pups were stabilized in the stereotaxic frame with ear bars designed for small animals (David Kopf Instruments). An incision
was made across the midline of the scalp using a scalpel (no. 15, Bard-Parker Safety Lock, Becton Dickinson and Company, Franklin Lakes, NJ). After the incision was made, the skin was pulled aside and clamped with hemostats to ensure access to the surgical field on the top of the skull (Figure 2A). The periosteum was removed using sterile cotton swabs (Puritan Medical Products Company, Guilford, ME) and areas of surface bleeding were cauterized with a fine-tip, low-temperature cautery pen (Bovie Medical, Clearwater, FL). Two electrode holes were made using a dental drill with a 0.7 mm burr (Fine Science Tools), -2 mm from bregma, 2 mm lateral from midline of the skull, and separated by 2 mm in the anterior-posterior direction. The electrodes in the transmitter were uncoated stainless steel of 127 µm dia. (A-M Systems, Inc., Carlborg, WA). Wires were cut-to-length during surgery such that the tip of the wire extended through the burr holes in the skull, while minimizing pressure on the dura (0.5 – 1 mm; depending on the curvature of the bottom part of the transmitter). The unit was then attached to the surface of the skull using cyanoacrylate gel compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the skull to stabilize the implant. The area was rinsed with warm sterile saline and the skin was sutured with Vicryl 4-0 coated polyglactin 910 suture (Ethicon) (Figure 3C). Anesthesia was terminated and the animals were injected with 0.5 mL of lactated Ringers (sub-cutaneous), and superficially treated with 0.5 mL of 0.5% local anesthetic (Marcaine). The entire surgical procedure was kept to <10 min. Pups were then allowed to recover with the dam and littermates for 24 h prior to treatment.
For wired recordings, rat pups were implanted at P7. The animals were anesthetized with 2% isoflurane, and an incision was made on the top of the scalp. Three burr holes were drilled for electrodes (MS333-3-B-SPC, Plastics One, Roanoke, VA), and an additional three were drilled for stabilizing skull screws (F303SS STAP; Morris, Southbridge, MA). The implant was secured on the skull using dental acrylic cement. The EEG signal was recorded using an electrical tether into a commutator, and amplified with a Biopac amplifier (EEG100C; 5000x) before being digitized (Biopac, MP150).

**Hypoxia recording chamber**

Hypoxia in P7 rat pups was induced in a custom in-house designed hypoxia chamber with integrated telemetry receiver bases. The chamber was designed with a clear acrylic housing (65 x 33 x 41 cm), a hinged lid and a stable aluminum platform where the capacitive-coupled receiver “bases” were placed. Each chamber could house up to three receiver bases. A feedback-controlled proportion-integrate-derivative (PID) temperature controller unit with thermocouple regulated the temperature inside of the chamber. Temperature was held at 37°C for the duration of recordings. The PID controller modulated a fan with heating elements located below the platform. The fan circulated warm air in the chamber by unidirectional flow. Chamber temperature was additionally verified by an independent thermocouple and logged to the recording computer (Vernier Instruments, Beaverton, OR). Each animal was placed in a sealed acrylic treatment chamber that rests on a receiver base, and the gas mixture was
administered at a positive pressure rate of 1 L/min through a manifold. The
design of the recording chamber allowed for parallel recordings of multiple
animals. Because each chamber had separate gas inputs under positive
pressure, multiple gas mixtures could be used within each of the housings. EEG
signals from multiple receivers were digitized by an analog-to-digital converter
(Biopac MP150, Biopac Systems, Goleta, CA), sampled at 500 Hz and stored on
a computer using Acknowledge 4.1.1 software (Biopac, Goleta, CA). The
recordings were conducted during the light phase of the light-dark cycle. Animals
were placed into the chamber and were allowed to settle for 5 min, after which
the EEG recording was enabled. Rat pups were behaving normally and were
awake and moving during the 30 min of the analyzed EEG epochs. No EEG
changes were obviously linked to the sleep-wake cycle were detected during the
30 min of the recording.

Hypoxia induction protocol

Hypoxia was induced in rat pups at P7 after recovery from implantation of
the device at P6. Animals were placed in the recording chamber with normoxic
air, and baseline activity was recorded for 30 min. After the baseline recording, a
hypoxia mixture of 8% oxygen, 92% nitrogen was introduced into the chamber.
The duration of hypoxia administration was 2 h. EEG was recorded continuously
during the time when animals were hypoxic. After treatment, the pups were given
0.5 mL of lactated Ringer’s solution subcutaneously. Animals were then returned
to the dam and allowed to recover.
Kainate induction protocol

For the kainate-induced seizure model, P7-8 rat pups were used. The treatment and recordings were conducted in the previously described chamber with normoxic air and chamber temperature held at 37 °C. Baseline EEG was recorded for 30 min. Kainate was dissolved in sterile saline and injected intraperitoneal (IP) at 2 mg/kg. Another dose of 1 mg/kg was administered after 40 min. EEG was recorded for 3 h after the first administration of kainate. Pups were then given 0.5 mL of lactated Ringer's solution subcutaneously and were allowed to recover with the dam and littermates.

Long-term recording protocol

Two methods were used for long-term recordings. For the first method, animals were monitored in 2 h epochs every day from P7 until P11 in the previously described recording chamber. For the second method, one rat pup implanted with the wireless transmitter was placed in a cage with its dam and littermates. The cage was then placed on an adult-sized EEG receiver that allowed for 24-h per day monitoring in the care of the dam.

Quantitative EEG Analysis

For each animal, 30 min of EEG data were selected from each day of the recording, 5 min after animals were removed from the dam and placed in the chamber. Signal dropout artifacts were manually removed from the EEG. The
Data files were then converted into MATLAB format (Mathworks, Natick, MA). Discrete Fourier Transforms (DFTs) were performed to analyze EEG data in the frequency domain from 0 to 60 Hz. Power spectral densities (PSDs) were estimated from the DFT using 2048 Hanning-window segments based on the Welch method and normalized by $10 \times \log_{10}(PSD)$. Power levels at all frequencies in 0.1 to 60 Hz were plotted with 95% confidence intervals. To compute integrated EEG power, the area under the PSD curve was integrated in the frequency ranges defined by EEG bands: delta 0.1-4 Hz, theta 4-8 Hz, alpha 8-13 Hz, beta 13-30 Hz, gamma 30-60 Hz (Krauss and Fisher, 2006; Stockard-Pope et al., 1992). The characteristic to denote seizures was a discharge length of at least 10 sec that was never present in the controls, accompanied by an abnormal convulsive behavior. Video recordings were performed in some, but not all of the animals to determine behaviors consistent with exact pattern of EEG discharges. When video was not recorded, an observer was present; EEG was recorded in all of the studied animals.

**Statistical Analysis**

The 95% confidence was calculated as $1.96 \times \text{stdev}(dB)/\sqrt{n}$ where 1.96 was the “critical value” for 95% confidence, dB was the distribution of power at a particular frequency from the animal set, and n was the number of animals in the data set. To statistically compare age-dependent EEG changes, one-way ANOVA was performed on the integrated EEG power data (Table 1).
Results

Neonatal seizures in hypoxia- and kainate-induced models

The telemetry device allowed us to record EEG activity from two different models of neonatal seizures. Seizures were induced by lowering the concentration of oxygen to 8% (i.e., hypoxia, n = 12) or by administering a chemo-convulsant compound, kainate (n = 5). These models presented with different types of seizures (Figure 3).

Hypoxia-induced seizures had characteristic EEG patterns that began immediately after introduction of the hypoxia gas mixture into the treatment chamber (Figure 3A). The EEG discharges during 2 h of hypoxic treatment included high-amplitude, low-frequency bursts, which were accompanied by classic tonic-clonic convulsive behaviors. Additionally, lower-amplitude, higher-frequency discharges were present, accompanied with a “shiver-like” behavior of the animal with no classic convulsive features. Inter-ictal, short, high-amplitude discharges were present between convulsions, but did not show a specific behavior at the time of the discharge. The behaviors and abnormal EEG patterns ceased upon introduction of normal air environment into the treatment chamber. The behavioral and EEG findings were similar in all of the treated animals (12/12).

Kainate elicited a different EEG and behavioral pattern in rat pups than in adults. Seizures began 15-30 min after an injection of a 2-mg/kg dose of kainate. The EEG began with high-frequency discharges that continued to be abnormal for up to 4 h after the first seizure (Figure 3B). All animals injected with kainate
showed similar discharge patterns. Clinical correlates of the seizure activity detected on EEG included myoclonic jerks, tonic stiffening, limb clonus and behavioral arrest. Behavioral and electrographic seizures were present in 5/5 animals that were injected with kainate. Additionally, the kainate-induced seizure activity was detectable when injected at P15 (Figure 4) when electrodes were implanted with no burr holes at P7.

**Age-dependent features of the background EEG**

The recording-electrode configuration enabled us to record not only seizures, but also normal EEG patterns. The spontaneous electrical activity in the normal EEG showed waves of spike-like activity. This pattern of activity was present in all of the control animals. As the animals matured, the EEG became more continuous, with pattern of electrical activity at a higher frequency (Figure 5A). To quantify these apparent changes as a function of age, we calculated the power spectral density (PSD) to estimate the power of classically defined EEG bands (delta: 0.1-4 Hz, theta: 4-8 Hz, alpha: 8-13 Hz, beta: 13-30 Hz, gamma: 30-60 Hz) as the animals matured from P7 to P11 (n=10, serial recordings). The characteristic pattern of activity in these EEG bands included diffuse patterns in the signal without discrete oscillations. When examined visually, the EEG signal had qualitative changes as the animals matured. These changes could be quantified using PSD (Figure 5). At lower frequency bands (i.e., delta, theta, alpha), power increased from P7 to P8 and stabilized from P8 to P11 (Figure 5). In the beta and gamma bands, power showed a gradual increase from P7 to
P10, with stabilization occurring between P10 to P11 (Figure 5). Age-dependent changes in the signal were detected in all of the EEG bands by integrating the power under the PSD curve in each of the bands and finding the mean of integrated power between animals of each age group. The profile of integrated power follows the above-described PSD profile (Figure 6; Table 1). This pattern of activity is present in all of the control animals. As the animal matures, the EEG becomes more continuous, and higher frequency patterns become more apparent (Figure 5).

**Long-term monitoring**

In order to study seizures and other spontaneous EEG events in animal models of epilepsy, 24-h continuous monitoring is necessary. However, these experiments are difficult because the rat pup is absolutely dependent on the dam for survival and normal development. For a proof-of-concept experiment for 24 h monitoring, we implanted two rat pups with the EEG telemetry at P6 and housed them separately with their respective dams and littermates. The animals were then placed on a receiver base designed for an adult animal under standard animal housing conditions. We then recorded EEG for a period of 48 h continuously (Figure 7). Several EEG signal “dropouts” due to the pup orientation were present, but overall signal quality was excellent and enough data was collected to be able to detect and ultimately quantitate EEG abnormalities. None of the animals had spontaneous seizures. Over the course of the experiment, the other animals that were housed in the same cage did not damage the implant.
Overall, electrical and movement artifacts recorded with the telemetry system were minimal. Compared with wired recordings (Figure 8A), artifacts recorded with the telemetry system were much shorter duration and were less frequent (Figure 8B). Instances where the receiver antenna did not properly couple with the transmitter on the animal’s skull apparently caused each “drop out” artifact. The EEG maintained a zero potential until the signal was detected again (Figure 8C).

Discussion

Telemetry vs. Wired Recording Techniques

Artifacts

The connector, commutator and wire are important noise sources in tethered systems, requiring grounding, shielding and performing recording in a faraday cage to reduce the contribution of the noise. This greatly increases the complexity of the recording set-up, while increasing the number of possible points where noise can be introduced into the system. An additional important consideration unique to P6-P7 rat pups is the soft and flexible skull bones. The tether forces probably act like a lever, amplifying the bending of the electrodes relative to the skull as the animal moves around the cage. These forces likely
cause flexing of the bones in the skull, which may result in major movement-
related electrical artifact (Figure 8B).

The miniature telemetry system is susceptible to signal drop-out artifacts,
where the electric field generated by the transmitter is smaller than the receiver
antenna is able to detect. These occur when the animal is positioned sideways
with the transmitter antenna parallel, or 90° out of phase, to the receiver antenna.
Other artifacts can occur when the animal contacts the metal water spout, or
when the animal comes in contact with the metal wire-top of the cage. In order to
have minimal impact on the signal, we designed the receiver base to cancel
these artifacts by clamping the signal to 0 V when the contact between the
antennae is lost. A drop-out causes the signal to shift to zero potential over ~10
ms depending on the EEG potential at the time of drop-out. Once the signal is
detected again by the receiving antenna, the signal goes from zero potential to
the instantaneous EEG potential over ~50 ms, depending on the EEG potential.
Because of the time constant, the artifact created by clamping the potential to
zero is unlikely to alias across frequencies due to the absence of sharp
transients. Unlike movement artifacts in the wired system, which appear as multi-
frequency high-amplitude bursts that can saturate the signal and make
quantitative frequency analysis difficult, the drop-out artifacts have minimal
impact on the frequencies in the EEG signal. However, during the drop out
artifacts, the EEG signal is not detectable, making a false-negative result
possible if an animal had a seizure or abnormal EEG discharge during the
artifact. This is unlikely because convulsions are often associated with robust
movement, making the probability low that the animal will remain in a position with signal “drop out” over the course of the seizure. Furthermore, these signal “drop outs” tend to be limited to a few seconds. Because this effect is binary (full signal vs. signal clamped to zero), it will not affect the analysis. The EEG amplitude of the wirelessly transmitted signal never fluctuates due to the position of the antenna. Reduction of electrical artifacts and hardware-enabled strategies that would reduce their impact on the quality of the EEG signal is a substantial advantage of the miniature telemetry system.

Surgical procedures

The small, self-contained form factor of the package and the reduced requirements for reinforcing the implant to the skull enabled us to design surgical procedures that are less invasive and require shorter periods of anesthesia. Tethered systems are usually stabilized on the surface of the skull using multiple stainless-steel skull screws and dental cement (Ekstrand et al., 2011; Lehmkuhle et al., 2009). Implantation of the telemetry unit with the intracranial electrodes requires two burr holes for the electrodes and a small amount of cyanoacrylate gel glue that binds the implant to the bones of the skull, in contrast to most wired implants that require three or more burr holes for skull screws, in addition to the burr holes for the electrodes. The wireless telemetry system can be implanted in 10-15 min, compared to 45 min to 1 h required to implant a typical wired unit. Less-invasive, shorter surgeries improve animal survival and recovery time. One of the traumatic procedures with most EEG recording techniques is the surgical
implantation of the electrode wires into the brain. The wires and surgery cause trauma from the procedure itself that could confound the EEG signal. The trauma includes bleeding, disruption of the blood-brain barrier, and increased possibilities of infection by compromising the integrity of the skull. In order to implant the EEG electrodes, a skull burr hole is normally used. However, classical EEG in humans is a non-invasive technique where electrodes are placed on the scalp. In an attempt to reduce the trauma of making burr holes in the skull and placing electrodes through the holes, we performed a proof-of-concept experiment to develop a hole-free implantation method by placing electrodes on the surface of the skull without burr holes. This result provides an important future avenue of development for minimally invasive recording techniques, which should allow better comparisons between clinical data and result from the animal models.

Clinically, EEG electrode placement is regimented and consistent between patients and studies. However, EEG techniques are not well developed in studies that use animal models of neonatal seizures. This has the potential to limit the reproducibility between studies. Here, we have attempted to standardize the electrode placement by positioning the differential pair over one hemisphere with consistent, stereotaxically-defined locations that can be used with or without burr holes. Our results suggest that this proof-of-concept is valid and can record both seizures and background activity. This configuration is an animal model approximation of central-parietal, differential-pair electrode placement used in commercial amplitude-integrated EEG system that are commonly utilized.
clinically on neonates for monitoring of cerebral activity (El-Dib et al., 2009). We propose that our configuration could be a useful baseline standard for EEG recordings in P6-7 rat pups.

Size and power requirements

Although the telemetry device described here is unique, other telemetry systems are currently available. A long-standing challenge is the size of the transmitter and the method of implantation. Prior studies in our laboratory utilized the DSI F50-EEE telemetry system for seizure monitoring in the adult animals (Williams et al., 2006; Kadam et al., 2010). The DSI system is useful for EEG monitoring in adult animals, but no device currently exists that would enable high-quality, serial, minimally invasive recordings in pups as young as P6. The size of the DSI system makes it impossible to be used in immature rat pups. The weight of the DSI transmitter is 11.5 g and the volume is 5.5 cc. The required intraperitoneal implantation of the transmitter or the use of a “backpack” system is only recommended for animals that weigh over 175 g. The weight of the miniature telemetry device described in this manuscript is 1 g and the volume is 1 cc. The device is mounted directly to the skull, and has been routinely used in rat pups that weigh 14 g. Most physiological and/or translational research on neonatal and immature animals has used large animals, such as pigs, dogs or goats (Bjorkman et al., 2010; Williams et al., 1992; Sherman et al., 1999). The system described here enables the use of much smaller animal models such as the rat pup. The use of such a device would enable studies beyond monitoring of
spontaneous seizures in the adult period, and new focus on such topics as developing treatments for neonatal and pediatric status epilepticus, perinatal hypoxic-ischemic encephalopathy, and other devastating pediatric conditions.

Power requirements are another inherent disadvantage of telemetry systems. While wired systems are inherently passive, telemetry requires the use of batteries, a power source, or a transducer to power the transmitter. This limits the useful life of most transmitters to 6-12 months, or even hours/days/weeks in some cases. The telemetry system described here uses capacitive coupling for transmission of the signal, which limits the power draw to 8 $\mu$A at 1.5 V. The low power draw enables the useful life of the transmitter to be up to 24 months from a single #303 silver-oxide battery, theoretically allowing continuous monitoring from an immature age until death in most rodents. Thus, the miniature telemetry device has several important advantages over currently available techniques: (1) ability to use in the immature animals with weight above 14 g; (2) low power requirements that lead to increased battery life; (3) minimally-invasive surgical techniques to implant the device.

*Single channel per animal per cage*

The current iteration of the miniature telemetry system design allows for recording of one channel of EEG from one animal per receiver base. This design limitation requires single housing of the animals or housing one animal implanted with the transmitter per cage. Studies that require chronic 24-h monitoring of EEG in rat pups allow recording from only one pup in the litter. Thus, a large
number of litters are required to conduct a statistically well-powered study. The telemetry system currently offers only one channel of differential EEG recording. This limits the possible electrode configurations to differential recording within one brain hemisphere, between the two hemispheres, or to one hemisphere with reference to a disparate region such as the cerebellum. This in turn restricts the application of the device to detection of seizures or recording background EEG from one hemisphere. Studies that examine seizure propagation or inter-hemispheric asynchrony cannot be conducted with the current configuration. Another version of the telemetry system that will allow recording of up to 6 channels of data from multiple animals in a single cage is currently under development. The new configuration will allow experiments that are not possible with the current iteration of the wireless EEG system.

Bandwidth limitations

Our current miniature telemetry system is bandwidth-limited to 0.1 Hz at the low end and to 120 Hz at the high end of the frequency range. If the signal frequency falls outside the range of 0.1 to 120 Hz, the signal is attenuated at 12 dB per octave at the high end (>120 Hz) and 6 dB per octave at the low end (<0.1 Hz). This feature of the device limits the amount of noise amplified at the receiver base and enables recordings in electrically noisy environments, such as the typical animal facility. However, due to the bandwidth limitations, the telemetry system is not suitable for studying fast, high-frequency events, such as action potentials, high-frequency oscillations and the electrocardiogram. Instead,
the miniature telemetry system is optimized for best performance while recording EEG in the classically defined EEG bands – 0.1 to 120 Hz. No such bandwidth limitations occur in the wired systems. An increase in bandwidth to 4 KHz would address this issue, however it would lead to a decrease in transmitter lifetime.

Applications of the telemetry system

Neonatal seizures

The miniature wireless telemetry unit could be used to address many important research applications. In this study, we used the telemetry system to record neonatal seizures in hypoxia and kainate models. Neonatal seizures are a common and serious neurologic condition with poor response to pharmacotherapy. Studies that test new therapeutic approaches for translational drug discovery typically use behavioral seizure scores as the primary outcome measure, without recording EEG (Aujla et al., 2009; Koh et al., 2004; Lai et al., 2009; Mikati et al., 2007; Koh and Jensen, 2001; Folbergrova, 1997, 1994). However, anti-seizure compounds often have sedative effects, making behavioral analysis difficult if not meaningless. Additionally, behavioral monitoring can only detect clinical seizures, ignoring electrographic seizures that do not present with a behavior. Electrographic seizures are a serious concern in neonates due to the electro-clinical decoupling that often occurs in this age group (Glass and Wirrell, 2009; Dzhala et al., 2005; Glykys et al., 2009; Castro Jr. et al., 2005; Boylan et al., 2002; Painter et al., 2009). Rodent models of hypoxia- and kainate-induced
neonatal seizures that are monitored with EEG can be used for pre-clinical
testing of anti-seizure drugs and other therapies given the ability to analyze the
EEG quantitatively. The EEG telemetry system allows detection of both
convulsive and electrographic seizures, making determination of drug efficacy
more accurate and relevant to the human condition, a key translational
component for drug discovery.

The kainate-induced discharges recorded in P7 using the miniature
telemetry device were substantially different from those seen at P10 and in the
adult animals. Kainate-induced seizure behavior at P7 in our study was similar to
that described at P10 by Dzhala et al., (2005) and Raol et al., (2009), which
included scratching, jerky movement, turning on the side and tail shaking.
However, in our recordings, the EEG signal was not as stereotypical and
organized as previously described. This finding is not completely surprising,
given the age-dependent differences between animals at P7 vs. P10, and the
developmental switch in activity from immature to adult that occurs around P10
(Ben-Ari et al., 2007). Therefore, it is likely that if the baseline background activity
in the brain was different, the kainate-induced seizure patterns would be different
as well.

Continuous uninterrupted recordings

A unique feature of the telemetry system is the ability to monitor EEG
continuously (Figure 7). This feature is particularly important in experimental
designs that involve, for example, epileptogenesis and progression of epilepsy.
Currently, electrographic epilepsy research uses two approaches: *intermittent monitoring*, where recordings are conducted for several hours during the day, and *continuous monitoring* where recordings are conducted uninterrupted 24 h per day, 7 days per week. Intermittent recordings require less time to analyze, but they are susceptible to false-negatives by “missing” seizures during the periods between recording sessions. This is especially critical when considering that seizures often tend to “cluster”. This strategy is suitable for studying the acute period associated with brain injuries, or for experiments that require high numbers of animals with high throughput. Continuously monitored recordings require more analysis yet provide a more comprehensive picture of seizure frequency and duration. Continuously monitoring the EEG is more suitable for tracking disease progression and/or therapy effectiveness, particularly when seizure frequency is low. The miniature telemetry system should enable long-term monitoring of rodents from the neonatal period up to when they mature into adults.

Quantitative approaches and background EEG

The miniature telemetry system provides recordings of background (or normal) EEG with few artifacts. Clinically, background EEG has been used to predict outcome of various neonatal conditions, including *in utero* asphyxia and hypoxia-ischemia (Selton and Andre, 1997; Murray et al., 2009; Patel and Edwards, 1997; Fitzgerald et al., 2007). Additionally, it can be used to predict the presence of other neurologic insults, such as grading of concussions (Mizrahi
and Kellaway, 1984). However, in animal models, the concept of using background EEG as a dependent variable has not been examined. Here we show that by using the quantifiable background EEG patterns, we can track maturation of rat pups from P7 to P11 (Figures 5 and 6) as a function of age and frequencies in the signal. The age-dependent shift towards higher frequencies as a function of the degree of maturation has been previously reported in human preterm infants (Niemarkt et al., 2011). This proof-of-concept research enables studies of clinically described background EEG abnormalities in animal models of neurologic conditions, such as asphyxia, hypoxia-ischemia, traumatic brain injury and stroke.

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Disclosures

Dudek F.E., Lehmkuhle M.J., and Fisher J.H. have equity interest in and receive salary and/or consultant fees from Epitel, Inc.

Author Contributions:

Zayachkivsky, A. designed and performed the experiments, performed data analysis, wrote the paper.

Lehmkuhle, M.J. designed the experiments, performed data analysis, wrote the paper

Fisher, J.H. invented the miniature telemetry system

Ekstrand, J.J. supervised the project and revised the paper

Dudek F.E. supervised the project, designed the experiments and wrote the paper
Reference List


Folbergrova J. NMDA and not non-NMDA receptor antagonists are protective against seizures induced by homocysteine in neonatal rats. Exp Neurol 130: 344-350, 1994.


**Figure 1: Form-factor design of the miniature telemetry transmitter.** The transmitter consists of two boards with electronic components, a battery, and a flat copper antenna located at the top of the unit. The assembly is encased in a cylindrical epoxy housing. Two electrodes protrude from the housing, with a differential recording configuration (A minus B). The transmitter is implanted on the top of the skull with cut-to-size electrodes located over the surface of the dura.

**Figure 2: P6 rat pup implanted with the miniature telemetry device.** The transmitter unit was surgically implanted at P6. The transmitter unit is small in size (<1 cc, <1 g), and is designed to fit the top surface area of the skull in the immature rat pup. The surgery is performed in a sterile field with a stereotaxic frame. An incision is made in the scalp of the animal (A); the burr holes are drilled and the transmitter unit is fixed on the surface of the skull with cyanoacrylate gel and accelerator (B); and then, the skin is sutured around the base of the implant (C). The form factor of the transmitter is compatible with co-housing of the implanted animals with the dam and littermates (D).

**Figure 3: Typical hypoxia- and kainate-induced seizure activity recorded with the telemetry device.** During hypoxia treatment (A), 30 min of baseline was recorded (1) and an 8% oxygen/92% nitrogen gas mixture was administered into the chamber. Hypoxia induced robust electrographic seizure activity throughout the administration of the gas (2, 3, 4). In the kainate model (B), 30 min of baseline was recorded and 2 mg/kg kainic acid was injected IP, with an additional 1 mg/kg after 40 min. Robust seizure activity was recorded after administration of the kainate. Recordings from an untreated control are included for comparison (C).
Figure 4: Kainate induces seizure activity in a P15 rat pup recorded without burr holes. Animal was implanted at P6 without using electrode burr holes. The electrode wires were placed on the surface of the skull. The animal was treated with 2 mg/kg kainate at P15. Clearly identifiable seizure activity was recorded successfully with this electrode configuration.

Figure 5: Age-dependent changes of the background EEG frequencies. Serial background EEG was recorded from rat pups every day starting at P7 and ending at P11 (A). Power spectral density (PSD) was estimated in the EEG bands. Mean values were plotted with 95% confidence intervals across n=10 animals (B). As the animals mature, the power profile of the background EEG changes. A marked increase in power is present from P7 to P8 in all of the frequency bands. The power in the beta and gamma bands increases as the animals mature, with a plateau between P10 and P11.

Figure 6: Age-dependent changes in the integrated power of background EEG. EEG power was quantified separately in each band by integrating the power under the PSD curve and plotted as mean and standard deviation in µV² (n=10). The age-dependent changes are apparent during animal maturation. Two recordings were conducted at P7 to verify stability and evaluate the same-day variability of the signal. Integrated power was compared with ANOVA (see Table 1).

Figure 7: Continuous 48-h monitoring in a P7-P8 rat pup. A rat pup was implanted with the telemetry unit at P6 and housed with the dam and littermates in a receiver base designed for an adult animal. A continuous recording of 48 h was made from the animal. Arrows indicate telemetry signal “drop-outs.” This proof-of-concept experiment shows the possibility of conducting continuous uninterrupted recordings in the immature rats.

Figure 8: Typical artifacts in a wired and telemetry EEG recording. The wired recording is extremely susceptible to movement artifacts that cause the
tether to shift, applying torque forces to the skull of the animal. Because the skull is extremely thin and flexible, these movements probably cause large-amplitude artifacts in the signal (A). Telemetric recording result in a much cleaner signal (B). “Dropout” artifacts (C) occasionally occur in the telemetry recording when the transmitter does not properly couple with the receiver.

Table 1: Statistical analysis of the age-dependent changes in the integrated power of background EEG. EEG power was quantified separately in each band by integrating the power under the PSD curve. Data are mean integrated power values in µV^2 across multiple animals with standard deviations. The values were compared using ANOVA between animals (n=10). Integrated power of background EEG was averaged in each age group and was compared between age groups beginning with P7 until P11. Significant p-values are indicated in bold (p>0.05 to reject). Two recordings were conducted 6 h apart on P7 (indicated by P7_1 and P7_2). The largest age-dependent changes occurred in the beta and gamma EEG bands.
A

Start

1 2 3 4

1 2 3 4

Kainate 2 mg/kg 1 mg/kg

B

Untreated control
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