Recording EEG in immature rats with a novel miniature telemetry system

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Zayachkivsky A, Lehmkuhle MJ, Fisher JH, Ekstrand JJ, Dudek FE. Recording EEG in immature rats with a novel miniature telemetry system. J Neurophysiol 109: 900–911, 2013. First published October 31, 2012; doi:10.1152/jn.00593.2012.—Serial EEG recordings from immature rat pups are extremely difficult to obtain but important for analyzing animal models of neonatal seizures and other pediatric neurological 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. Second, we describe a viable approach for long-term continuous EEG monitoring of naturally reared rat pups implanted with EEG at P6. Third, we have used 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 yr without surgical reimplantation. 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.

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EEG in the Clinic and Animal Models

EEG recordings are an essential test for clinical diagnosis and outcome prediction of various neurological conditions and injuries. EEG is required to detect nonconvulsive seizures, helping to change the clinical treatment of neonatal and pediatric patients (Abend et al. 2011; Connell et al. 1989; Glass et al. 2009; Nash et al. 2011). EEG recordings have been highly effective for prediction of the outcomes of neurological insults such as perinatal hypoxia-ischemia and asphyxia in retrospective human studies (Aso et al. 1990; Garthinkle and Shevell 2011; Hellström-Westas and Rosén I 2005; Holmes and Lombroso 1993; Korotchkova et al. 2011; Murray et al. 2009; Walsh et al. 2011). Although EEG has been used with a high degree of success for neonatal and pediatric seizures and brain injuries in humans, it has been underused in animal models. Rodent animal models are an important tool for preclinical testing of anticonvulsant compounds that are used for treatment of epilepsy. 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 wire-free 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 postnatal day 6 (P6) is technically difficult and would benefit from new approaches. Most of the previous studies examining in vivo EEG or local field potential recordings in immature rats have been conducted using wired recording solutions in animals that were P12 or older (Cuaycong 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; Romijn 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 neurological 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 microtransmitter composed of a physiological amplifier controlling a pulse-width (i.e., frequency) modulation oscillator; and 2) a capacitively-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 EEG 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 4,000/Hz, 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, and a schematic 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 (Figs. 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 in diameter and 10 mm high with a dome-shaped top (Fig. 1). The weight of the transmitter unit was ~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 that 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 ~1 wk 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 stereotoxic frame was sprayed with 70% ethanol. Sterility of surgical tools was maintained with 70% ethanol. Rat pups were stabilized in the stereotoxic 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 (Fig. 2A). The peritoneum was removed using sterile cotton swabs (Puritan Medical Products, 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 diameter (A-M Systems, 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 compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452). Additional cyanoacrylate was applied around the unit and the exposed areas of the compound (Loctite 454) with accelerator (Loctite 7452).

Hynoxia Induction Protocol

Hynoxia 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 solution subcutaneously. Animals were then returned to the dam and allowed to recover.

Kainate Induction Protocol

For the kainate-induced seizure model, P7–P8 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 intraperitoneally 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 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/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 (The 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 2,048 Hanning-window segments based on the Welch method and normalized by $10\times\log_{10}(\text{PSD})$. Power levels at all frequencies in 0.1–60 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 $\geq 10$ s 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 $1.96 \times \text{std}(\text{dB})/\sqrt{n}$ 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 compare
age-dependent EEG changes statistically, 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 chemoconvulsant compound, kainate ($n = 5$). These models presented with different types of seizures (Fig. 3).

Table 1. Statistical analysis of the age-dependent changes in the integrated power of background EEG

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<th>P7_2</th>
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Data are mean integrated power values in microvolts squared across multiple animals ± SD. EEG power was quantified separately in each band by integrating the power under the power spectral density (PSD) curve. 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 postnatal day 7 (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.

Hypoxia-induced seizures had characteristic EEG patterns that began immediately after introduction of the hypoxia gas mixture into the treatment chamber (Fig. 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 by a “shiverlike” behavior of the animal with no classic convulsive features. Intercital, 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 on 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 (Fig. 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 (Fig. 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 spikelike 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 (Fig. 5A). To quantify these apparent changes as a function of age, we calculated the 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 patterns 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 (Fig. 5). At lower-frequency bands (i.e., $\delta$, $\theta$, and $\alpha$), power increased from P7 to P8 and stabilized from P8 to P11 (Fig. 5). In the $\beta$- and $\gamma$-bands, power showed a gradual increase from P7 to P10, with stabilization occurring between P10 and P11 (Fig. 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 (Fig. 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 (Fig. 5).
Long-Term Monitoring

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 (Fig. 7). Several EEG signal dropouts due to the pup orientation were present, but overall signal quality was excellent, and enough data were collected to be able to detect and ultimately to 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 (Fig. 8A), artifacts recorded with the telemetry system were much shorter duration and were less frequent (Fig. 8B). Instances where the receiver antenna did not properly couple with the transmitter on the animal’s skull apparently caused each dropout artifact. The EEG maintained a zero potential until the signal was detected again (Fig. 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 setup 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 (Fig. 8B).

The miniature telemetry system is susceptible to signal dropout 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. 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 dropout causes the signal to shift to zero potential over \(\frac{10}{100}\) ms depending on the EEG potential at the time of dropout. Once the signal is detected again by the receiving antenna, the signal goes from zero potential to the instantaneous EEG potential over \(\frac{50}{100}\) 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 multifrequency high-amplitude bursts that can saturate the signal and make quantitative frequency analysis difficult, the dropout artifacts have minimal impact on the frequencies in the EEG signal. However, during the dropout 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
A mature, with a plateau between P10 and P11. As the animals mature, the power profile of the background EEG 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 with 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. To implant the EEG electrodes, a skull burr hole is normally used. However, classic EEG in humans is a noninvasive 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 results 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 systems that are commonly used 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–P7 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 used the DSI F50-EEE telemetry system for seizure monitoring in the adult animals (Kadam et al. 2010; Williams et al. 2006). 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

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**Fig. 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 β- and γ-bands increases as the animals mature, with a plateau between P10 and P11.

**Table 1.** 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.
intraperitoneal implantation of the transmitter or the use of a “backpack” system is only recommended for animals that weigh \( >175 \) 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; Sherman et al. 1999; Williams et al. 1992). 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 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. Whereas 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 mo 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 mo from a single no. 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 \( >14 \) g; 2) low power requirements that lead to increased battery life; and 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 interhemispheric asynchrony cannot be conducted with the current configuration. Another version of the telemetry system that will allow recording of up to six 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 0.1–120 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, 0.1–120 Hz. No such bandwidth limitations occur in the wired...
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 neurological 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; Folbergrova 1994, 1997; Koh et al. 2004; Koh and Jensen 2001; Lai et al. 2009; Mikati et al. 2007). However, antiseizure 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 electroclinical decoupling that often occurs in this age group (Boylan et al. 2002; Castro Conde et al. 2005; Dzhala et al. 2005; Glass and Wirrell 2009; Glykys et al. 2009; Painter et al. 1999). Rodent models of hypoxia- and kainate-induced neonatal seizures that are monitored with EEG can be used for preclinical testing of antiseizure 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 (Fig. 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/day, 7 days/wk. Intermittent recordings require less time to analyze but 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 (Fitzgerald et al. 2007; Murray et al. 2009; Patel and Edwards 1997; Selton and Andre 1997). Additionally, it can be used to predict the presence of other neurological 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 (Figs. 5 and 6) as a function of age and frequencies in the signal. The age-dependent shift toward 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 neurological conditions such as asphyxia, hypoxia-ischemia, traumatic brain injury, and stroke.

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