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Optimized methods for epilepsy therapy development using an etiologically realistic model of focal epilepsy in the rat

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ABSTRACT

Conventionally developed antiseizure drugs fail to control epileptic seizures in about 30% of patients, and no treatment prevents epilepsy. New etiologically realistic, syndrome-specific epilepsy models are expected to identify better treatments by capturing currently unknown ictogenic and epileptogenic mechanisms that operate in the corresponding patient populations. Additionally, the use of electrocorticography permits better monitoring of epileptogenesis and the full spectrum of acquired seizures, including focal nonconvulsive seizures that are typically difficult to treat in humans. Thus, the combined use of etiologically realistic models and electrocorticography may improve our understanding of the genesis and progression of epilepsy, and facilitate discovery and translation of novel treatments. However, this approach is labor intensive and must be optimized. To this end, we used an etiologically realistic rat model of posttraumatic epilepsy, in which the initiating fluid percussion injury closely replicates contusive closed-head injury in humans, and has been adapted to maximize epileptogenesis and focal non-convulsive seizures. We obtained week-long 5-electrode electrocorticography 1 month post-injury, and used a Monte-Carlo-based non-parametric bootstrap strategy to test the impact of electrode montage design, duration-based seizure definitions, group size and duration of recordings on the assessment of posttraumatic epilepsy, and on statistical power to detect antiseizure and antiepileptogenic treatment effects. We found that use of seizure definition based on clinical criteria rather than event duration, and of recording montages closely sampling the activity of epileptic foci, maximize the power to detect treatment effects. Detection of treatment effects was marginally improved by prolonged recording, and 24 h recording epochs were sufficient to provide 80% power to detect clinically interesting seizure control or prevention of seizures with small groups of animals. We conclude that appropriate electrode montage and clinically relevant seizure definition permit convenient deployment of fluid percussion injury and electrocorticography for epilepsy therapy development.

Introduction

Epileptic seizures can be controlled in a majority of patients by chronic administration of one or more of over two dozen anti-seizure drugs (ASD) now on the market (Löscher et al., 2013). However, no non-surgical treatment has ever been found to cure epilepsy, and no treatment has been found to prevent it, or modify its course in those at risk. Over one third of epilepsy patients suffer inadequate seizure control — a proportion that has not changed appreciably despite the introduction of numerous ASDs over the past three decades (Löscher and Schmidt, 2011; Temkin, 2009).

Most of the treatments available today were identified on the basis of their ability to acutely suppress behavioral endpoints of seizure activity evoked by various forms of electrical or chemical stimulation (Löscher et al., 2013; Löscher and Schmidt, 2011, 2012; Smith et al., 2007; White, 1998) and are all thought to act on various neuronal and synaptic mechanisms to nudge the balance between neural inhibition and excitation, thereby preventing the spontaneous abnormal hypersynchronous neuronal activity with associated behavioral changes that define epileptic seizures (Fisher et al., 2005). However, current ASDs share critical shortcomings. First, they do not prevent epilepsy, but control seizures to provide symptomatic relief. While several clinical trials have been conducted to test the antiepileptogenic effect of ASDs based on the hypothesis that "seizures beget seizures" and, thus, that ASDs should modify epileptogenesis, these trials have all failed to identify an effective treatment (Temkin, 2009). Second, these drugs do not cure epilepsy, and must be taken chronically to control sporadic seizures. The resulting global damping of neuronal excitability often produces chronic aversive or debilitating side effects (Gilliam et al., 2004). Third, the expanding ASD pharmacopeia remains incapable of controlling seizures in over a

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third of epilepsy patients, the vast majority of whom predominantly suffers from non-convulsive focal seizures (Juul-Jensen, 1986; Mattson et al., 1996; Semah et al., 1998). This situation is arguably attributable to drug development strategies that fail to incorporate either the epileptogenic mechanisms that mediate the development of acquired human epilepsies, or the ictogenic mechanisms that cause the epileptic focus to suddenly precipitate spontaneous seizures in patients. Since, with few specific exceptions, the mechanisms of human ictogenesis and epileptogenesis are not yet known, the problem of identifying better epilepsy treatments can be distilled to one of identifying and deploying animal models that prominently incorporate these yet-to-be-identified mechanisms.

To this end, new syndrome-specific models have been developed based on etiologically realistic epileptogenic insults such as viral encephalitis, stroke, febrile seizures, perinatal hypoxia and head trauma (D'Ambrosio et al., 2004; Dubé et al., 2010; Kelly et al., 2001; Rakhade et al., 2011; Williams and Dudek, 2007). These models have the advantages that: 1) they feature chronic spontaneous recurrent seizures (CSRSs), which are the hallmark of epilepsy, 2) they include focal non-convulsive CSRSs, which are known to be difficult to treat with current ASDs, 3) the realism of the initiating injury ensures that mechanisms that operate in the corresponding patient populations are recruited, and 4) the patient populations best suited for clinical tests of treatments discovered using these models are readily identifiable. However, in contrast to evoked motor seizures, which appear predictably and can be reliably and conveniently assessed in a short period of time by simple behavioral observation, spontaneous nonconvulsive seizures generally require prolonged monitoring because they are unpredictable, and also require expert ECoG analysis for accurate detection and evaluation. To further complicate matters, high inter-subject variability in seizure frequency is a consistent feature of all acquired CSRSs models developed to date, which tends to result in larger group sizes and increased labor and cost.

Because the combination of sensitive ECoG monitoring and of etiologically realistic models represents a promising strategy for discovery of novel epilepsy treatments that could translate to readily identifiable patient populations, there is an urgent need to determine the most effective ways to deploy them. Investigators have taken a variety of approaches to the ECoG monitoring and reporting of seizure data in CSRS models. Electrical activity may be recorded with two or more electrodes on the surface of the brain and/or from deep brain structures. Recording montages are most often designed without knowledge of the location(s) of epileptic foci, which are poorly documented in most CSRS models, and investigators often report only seizures that exceed various duration based criteria or that result in Racine scale 4–5 behavioral seizures. These practices are relatively convenient and economical, but bias observation in favor of tonic–clonic and other generalized seizures at the expense of focal non-convulsive seizures (D'Ambrosio and Miller, 2010). In addition, animals may be recorded either continuously or periodically for durations ranging from hours to months, based on each lab's experience with what might be adequate for the task at hand. Such differences in experimental approach seriously complicate comparison of studies from different laboratories and, critically for therapy development, affect the quality of the assessment of both epileptic syndromes and their treatments. Yet, the impact of these procedural variations on therapy development has never before been systematically investigated.

Here we examine these different experimental approaches in a rostral parasagittal fluid percussion injury (rpFPI) model of posttraumatic epilepsy (PTE). This is an etiologically realistic syndrome specific PTE model based on an experimental brain insult, FPI, that has long been regarded as a realistic model of contusive closed head injury in man (Thompson et al., 2005). We have previously optimized this model with respect to the location and severity of injury to produce rapid and reliable epileptogenesis with low acute mortality (Curia et al., 2011, in press; D'Ambrosio et al., 2009), and have identified the location of the early epileptic focus that develops after injury. This has been mapped with grid and depth electrode recordings (D'Ambrosio et al., 2005, 2009), as well as by focal cooling of the perilesional neocortex (D'Ambrosio et al., 2013). The localization of the neocortical epileptic focus permits use of a recording montage that closely monitors its activity. Detailed characterization of the electrobehavioral seizures generated by this neocortical epileptic focus has permitted the use of realistic seizure detection criteria that are based on clinical practice and are consistent with the ILAE definition of epileptic seizure (D'Ambrosio and Miller, 2010). We also have established statistical methods to efficiently detect effects of investigational ASDs in the presence of non-responders (Eastman et al., 2010, 2011). These studies were based on periodic recordings (24–72 h/week) as a compromise to keep costs down while still sampling much of the variability in seizure frequency. We now use seizure data from rpFPI rats recorded continuously for a full week 1 month after injury, in bootstrapped nonparametric statistical power analyses to estimate a cost-effective duration of recording, as well as the effects of different recording montages and seizure definitions on statistical power to detect antiepileptogenic or disease modifying (AEG/DM) or anti-seizure (AS) effects. The results show how to optimize the deployment of rpFPI for epilepsy therapy development.

Methods

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experiments were approved by the University of Washington Institutional Animal Care and Use Committee (Animal Welfare Assurance #A3464-0 l). All surgery was performed under halothane anesthesia, and all efforts were made to minimize suffering.

Animals

Outbred male Sprague-Dawley rats (Charles River, Hollister, CA) were administered head injury at 32–36 days of age. Rats were housed 2 to 3 per cage prior to epidural electrode implantation, and individually afterward. Animals were kept in a specific-pathogen-free facility with controlled light (12 h light-dark cycle), temperature and humidity, and ad libitum access to food and water.

Surgical procedures

rpFPI and epidural electrode implantation were performed as detailed previously (D'Ambrosio et al., 2013; Eastman et al., 2010). For rpFPI, 17 animals were anesthetized (4% halothane), intubated and mechanically ventilated (1–1.5% halothane, 30% O_2 and air). Rectal temperature was monitored and maintained at 37 °C with a heat pad. A 3 mm burr hole was drilled centered at 2 mm posterior to bregma and 3 mm from the midline over the right convexity. Animals were disconnected from the ventilator and a pressure pulse (8 ms, 3.5 atm) was delivered with the FPI device (Scientific Instruments, University of Washington) and measured by a transducer (Entran EPN-D33-100P-IX, Measurement Specialties, Hampton, VA). Mechanical ventilation was resumed 10 s after injury to standardize posttraumatic apnea and hypoxia, and terminated when spontaneous breathing resumed. All animals survived the injury.

Epidural electrodes were implanted 14–15 days after injury. Briefly, 1 mm diameter stainless-steel screw electrodes were implanted through 0.75 mm diameter guiding craniotomies. The full ECoG montage consisted of five epidural electrodes (Fig. 1A): a reference electrode placed midline in the frontal bone and two electrodes per parietal bone, placed at coordinates bregma 0 mm and −6.5 mm, 4.5–5 mm from the midline. Three anchoring screws (one frontal and two occipital) were implanted to help secure the headset. All electrodes were connected through insulated wire to gold-plated pins in a plastic pedestal