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Appraisal of state-of-the-art

Assessment of seizure risk in pre-clinical studies: Strengths and limitations of the electroencephalogram (EEG)

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1. Introduction

Many compounds under development or with established efficacy are linked to lower thresholds and increased seizure risk. Two recent surveys have examined possible drug-induced seizures in clinical populations using the WHO adverse drug reaction database (Kumlien & Lundberg, 2010) and the psychopharmacologic clinical trials in the FDA approval reports database (Alper et al., 2007). These studies documented increased seizure risk associated with neuroleptics, anti-depressants, anti-migraine, anti-Parkinson, and anxiolytic drugs, as well as with more than a dozen neurotropic compounds that do not fit into these categories (i.e., acetylcholinesterase inhibitors). Although seizures are often associated with compounds targeting the central nervous system (CNS), they can also be evoked by non-neural therapeutic targets (e.g., respiratory, cardiovascular) or by metabolic changes such as lowered glucose levels (Easter et al., 2009; Ruffmann et al., 2006).

The seizure potential of a compound depends on a combination of its ability to cross the blood brain barrier, the potential action of metabolites, the exposure levels and speed at which the compound reaches the brain, and the species under investigation (Easter et al., 2009). The link between investigational drugs and increased seizure risk supports a thorough examination of known biomarkers for both frank seizures and lowered seizure threshold in pre-clinical studies. These studies should note behaviors that may antecede seizures (e.g., myoclonic movements, rigidity), characterize the type of seizure observed (e.g., generalized, partial), note the duration of the seizure-related incidents, and identify the post-ictal pattern associated with any frank or suspected seizure events. This is a formidable task and one that arguably has not been achieved in many pre-clinical studies of potential seizure risk.[1] A frank generalized seizure may go unobserved in a dog because of its brief duration and in a rodent because

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[1] Opinion and observation of Dr. J. Arezzo, who has participated in more than 30 industry sponsored pre-clinical studies of EEG and seizure.

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it occurs in the middle of the night. Further, while most researchers are trained to detect a frank tonic–clonic seizure, the identification of a partial seizure or a generalized non-convulsive absence seizure is a more difficult task.

Seizures are defined by abnormal synchronous electrical activity of groups of CNS neurons (Dunkan et al., 1995). Although behavioral and clinical features of seizure vary, their underlying trigger is always an escalating synchronization of neuronal activity (e.g., paroxysmal depolarizing shift) that can begin hours prior to a frank clinical event (Litt et al., 2001).

A seizure can remain focal (i.e., simple partial seizure), it can progress to a wider network (complex partial seizure), or it can be a generalized event at its onset involving both hemispheres and resulting in the loss of consciousness (grand mal seizures) (Niedermeyer & Lopes da Silva, 2005). A valuable classification of epileptic seizures based on etiology, symptoms and pathophysiologic mechanisms has been compiled and revised by the International League against Epilepsy (ILAE) (Fisher et al., 2005, 2014). Depending on its epicenter and nature, the manifestation of a seizure can range from subtle signs to severe convulsions, but it is critical to recognize that even a generalized seizure can occur in the absence of a convulsion. A critical and often stated tenet in the field is that "not all seizures result in behavioral convulsions and not all apparent convulsions are related to seizures."

The electroencephalogram (EEG) is a direct, quantitative measure of the electrical activity of the brain (Constant & Sabourdin, 2012) and it is the established and validated standard for exploring frank seizures or altered seizure thresholds in clinical populations (Niedermeyer & Lopes da Silva, 2005). When recorded from a reasonable montage of electrodes in an unanesthetized animal model, EEG can detect sub-clinical events that may predict seizures or altered seizure thresholds e.g., presence of organized, repetitive sharp waves, increased synchrony. Further, if seizures are present, EEG can document the onset, type, duration and spatial distribution of the event, as well as the change in brain activity that follows the seizure (i.e., post-ictal EEG patterns). Through a variety of technical developments, including improvements in telemetry, miniaturization of amplifiers, and the development of powerful analytic computer programs to score data, it is now both feasible and desirable to apply EEG in preclinical animal models assessing seizure risk.

Seizures noted at any stage in the drug pipeline are a serious risk to human health and may lead to withdrawal of an experimental compound. It is therefore surprising that the assessment of seizure liability is not clearly defined by regulatory guidelines. For instance, the ICH7A (ICH7A, 2001; Porsolt et al., 2002) mandates the evaluation of abnormal motor function and altered behavior as part of the "core battery" of safety pharmacology tests prior to the initiation of clinical trials, however it does not require specific tests for seizure detection. If identified only in the late stages of development, compound-induced seizures can be very costly. Therefore, a number of drug development programs have targeted seizure risk as a relatively early step in the evaluation of an experimental compound. Available procedures for this task include in silico computational models, in vitro pharmacological profiling and brain slice electrophysiology (Easter et al., 2009). Seizure risk has also been explored by pairing the compound in question with known chemoconvulsants (e.g., Pentylenetetrazol-PTZ) or electroshocks (Fisher, 1989; Hamdam et al., 2013; Potschka et al., 2000; Pitkanen et al., 2006). In addition to these approaches, the past decade has witnessed the direct application of EEG measures in unanesthetized mice, rats, dogs and monkeys, thus allowing well-established clinical criteria for increased seizure risk to be applied to pre-clinical models (Easter et al., 2009; Arezzo J., personal communication).

The no observed adverse effect level (NOAEL) of an experimental compound generally defines the safety margin for its initial clinical exposure. Without EEG, the NOAEL for drugs with seizure risk is usually set at the highest dose that is convulsion free, by observation. As discussed above, due to the limitations of observing and recognizing a frank seizure, this can be an insensitive and inaccurate level. The addition of pre-clinical EEG provides an alternative. The NOAEL can be reasonably set as the dose level that has not been associated with an observed frank seizure and one that does not generate EEG patterns known to be related to lowered seizure threshold. This is an emerging and still somewhat controversial area in the use of pre-clinical EEG that differs from the attempt to predict seizure in the clinical literature (Scaramelli et al., 2009; Stacey et al., 2011). Repetitive patterns of EEG that hit clearly established criteria (see Fig. 1), and that are often associated with behaviors such as myoclonic movements, can represent a compound-induced change in seizure thresholds. This is especially evident if the timing of pre-seizure EEG biomarkers such as the presence of organized sharp waves or increases synchrony are temporally related to aspects of dosing (i.e., occur at C_{max}). Despite the growing use of preclinical EEG, the criteria for clearly defining premonitory signs of seizure in animals remain a challenge across the industry.

A final aspect of the advantages of EEG in preclinical studies is the ability of this direct measure of neural activity to determine that some movements and behaviors previously thought to reflect aspects of seizures (e.g., tremor, ataxia, or a paucity of movement) can occur with normal patterns of EEG (King et al., 2014). Tremor, which is often cited as a precursor to seizures may in fact be related to fatigue, temperature, stress, or alterations of function in the basal ganglia, cerebellum or spinal cord. It is likely that, in addition to identifying increased seizure risk for some compounds, the correct application of EEG techniques will rule out seizures even in the face of altered behaviors for other compounds.

2. EEG rhythms and seizure detection

EEG signals are generated by large populations of neurons which can be recorded from both intact scalp and intracranial sites. The dominant generators include both inhibitory and excitatory postsynaptic responses from cortical pyramidal cells (Pitkanen et al., 2006). The EEG signal is often characterized by specific rhythms with defined frequency bands (i.e., delta 1–4 Hz, theta 4–8 Hz, alpha 8–13 Hz, beta 13–30 Hz and gamma 30–45 Hz). Lower frequencies are generally associated with sedation, while high frequencies are often coincident with arousal and this pattern appears consistent across mammalian species. EEG rhythms have been linked to sleep/wake cycles, state of alertness, type of activity, age, and pathology (Niedermayer & Lopes da Silva, 2005). The shift to high-frequency EEG signals (gamma or high-gamma activity) has been associated with seizures (Truccolo et al, 2014) and with increased seizure risk (Bragin et al., 1999; Staba, 2012). However, the link between gamma wave activity and seizure, should be made with caution, as the presence of EEG frequencies in the gamma range can also reflect multiple alternative adjustments in cortical processing.

A wide range of EEG patterns has been correlated with increased seizure risk and, in some cases, with specific seizure types (Abou-Khahil & Misulis, 2006). A thorough review of these patterns is beyond the scope of this text. However, several key biomarkers have emerged that are particularly applicable to pre-clinical models. The cardinal EEG biomarker for increased seizure risk in pre-clinical studies is a dramatic increase in synchronization that drives bursts of large amplitude, sharply contoured responses (often 5–10 fold increase in amplitude from

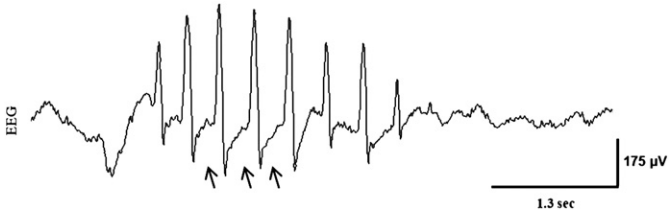

Fig. 1. Sharp waves escalating and decreasing in amplitude — subcutaneous recording in the rat. Note the consistency of the repeated wave shapes (see arrows).