EEG in non-clinical drug safety assessments: Current and emerging considerations

Simon Authiera,⁎, Marcus S. Delattee, Mary-Jeanne Kallmanc, Joanne Stevensd, Carrie Markgrafeb

a CitoxLAB North America, 445 Armand Frappier, Laval, QC H7V 4B3, Canada
b Division of Anesthesia, Analgesia and Addiction Products (DAAAP), CDER, U.S. Food & Drug Administration, Silver Spring, MD, USA
c Kallman Preclinical Consulting, Greenfield, IN 46140, USA
d Department of Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA
e Safety Assessment, Merck Research Laboratories, Kenilworth, NJ 07033, USA

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A B S T R A C T
Electroencephalogram (EEG) data in nonclinical species can play a critical role in the successful evaluation of a compound during drug development, particularly in the evaluation of seizure potential and for monitoring changes in sleep. Yet, while non-invasive electrocardiogram (ECG) monitoring is commonly included in preclinical safety studies, pre-dose or post-dose EEG assessments are not. Industry practices as they relate to preclinical seizure liability and sleep assessments are not well characterized and the extent of preclinical EEG testing varies between organizations. In the current paper, we discuss the various aspects of preclinical EEG to characterize drug-induced seizure risk and sleep disturbances, as well as describe the use of these data in a regulatory context. An overview of EEG technology—its correct application and its limitations, as well as best practices for setting up the animal models is presented. Sleep and seizure detection are discussed in detail. A regulatory perspective on the use of EEG data is provided and, tying together the previous topics is a discussion of the translational aspects of EEG.

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1. Introduction

A recent survey indicated that most drugs approved in Japan between 1999 and 2013 with reported adverse drug reactions (ADRs) classified as seizures/convulsions in patients were not identified to have a seizure liability during preclinical development (Nagayama, 2015). When considering seizure/convulsion observed at any dose, only 25 out of 105 (23.8%) approved drugs showed concordance of preclinical and clinical data for seizurogenic effects based on ADRs. When observed in preclinical studies, seizures/convulsions were identified in repeat toxicity studies (64%), proconvulsion safety pharmacology studies (40%) or in other safety pharmacology studies (28%). Proconvulsion safety pharmacology studies typically include models aimed to characterize the risk of drug-induced seizures such as EEG studies to monitor for ictal activity and seizure threshold tests. Other safety pharmacology studies include a wide range of pharmacology models (e.g. cardiovascular, respiratory, gastrointestinal and even other neurological models) which are defined under the ICH S7A guideline (U.S. Food and Drug Administration, 2001). Industry practices as they relate to preclinical seizure liability assessments are not well characterized and the extent of preclinical seizure liability testing varies between organizations (Authier et al., 2016). Spontaneous seizures are reported in various species including rats (Nunn & Macpherson, 1995; Satomoto et al., 2012) and dogs (Bielfelt, Redman, & McClellan, 1971) and it is crucial to differential spontaneous seizures from drug-induced ical activity. Susceptibility to drug-induced seizures differs between species (Bassett et al., 2014) but also between age groups (Himmel, 2008) within the same species rendering translation of preclinical results to humans challenging. Irrespective of the limitations when using animal models in drug development, preclinical seizure liability testing strategies aim to succeed at risk identification and support clinical trial risk management.

In a recent survey on preclinical neurotoxicology investigations, a minority of participants reported using pre-dose electroencephalography (EEG) (Authier et al., 2016) to confirm suitability of the animals for inclusion on study. As technology advances have increased the availability of non-invasive EEG monitoring and analysis (Pouliot et al., 2015), typical safety testing paradigms may need to be challenged.

Tremors and other behavioral effects such as ataxia, myoclonus or emesis are often observed in early toxicology investigations such as
maximum tolerated dose (MTD) studies. MTD studies are conducted during drug development as part of the toxicology investigations as defined under the ICH guideline M3(R2) (U.S. Food and Drug Administration, 2010). Once the MTD is identified, the drug dose levels that induce significant adverse effects may never be used again in the organized sequence of preclinical drug safety testing studies. A common concern when tremors are present is the presence of underlying abnormal EEG activity. Surface ECG monitoring is commonly included in preclinical toxicology studies but EEG assessments are classically introduced only once a neurological concern is identified. Monitoring EEG during MTD or repeat dose toxicology studies may represent an opportunity for early identification of a CNS risk. With older patient populations recognized to have an increased seizure incidence (Vélez & Selwa, 2003), this concern may be of increased clinical relevance given the life-threatening consequences of status epilepticus. Beyond seizurogenic risks, a number of drugs in development may alter sleep architecture (Rachalski et al., 2014) with potential negative impacts on the patient population. Selective serotonin reuptake inhibitors (SSRIs) are widely prescribed but are also associated with sleep disturbance (Ferguson, 2001) such as delayed REM sleep onset, increase awakenings and reduce REM sleep. Here we discuss the various aspects of preclinical EEG assessments to characterize seizure risk and also investigate potential drug-induced sleep disturbances.

2. Fundamentals of EEG

To fully appreciate the role of EEG in nonclinical safety evaluation, an understanding of the fundamentals of the technology is important. The fundamentals of EEG will detail what underlies the generation of EEG waves, both from an anatomical and an instrumentation perspective and will review descriptive versus interpretation of EEG waveform patterns as well as describing typical normal EEG patterns.

2.1. What is EEG?

EEG is the recording of electrical activity from the brain's cortical surface. Neuronal output is in μV, unlike the mV electrical signals from recording an ECG, and needs to be amplified by 10^6 to be displayed. Most of the EEG’s electrical signal arises from neuronal post-synaptic potentials (PSP). Action potentials are too small and too short to record. PSPs can be excitatory (EPSP), causing the post-synaptic neuron to fire, or can be inhibitory (IPSP), causing the post-synaptic neuron not to fire. The combination of EPSPs and IPSPs induce current flow around neurons, which is recorded as EEG. The complex neuronal activity from millions of cortical neurons generates the irregular EEG signal that translates into seemingly random and changing waveforms (Fig. 1). By contrast an evoked potential is an integrated signal that is synchronized by a precipitating stimulus such as a noise or flashing light.

2.2. EEG instrumentation

While a wide range of EEG electrode types can be used (Galanopoulou et al., 2013), the most common use for nonclinical EEG is from the cortical surface. This recording can be accomplished using scalp electrodes in a restrained subject, or by using telemetry, consisting of surgically implanted electrodes that send signals to a remote receiver. Specialized applications may use depth electrodes surgically implanted into the parenchyma of the brain (frequency into the hippocampus or thalamus). However obtained, the signal is amplified, filtered, displayed and recorded for analysis. Human EEG uses a system of standard placement of scalp electrodes, the 10–20 system (Jasper, 1958). Digital recording from this array allows the data to be displayed in different montages, which helps in defining abnormal waveforms and in localizing the source of the abnormality. EEG in nonclinical species such as rodents, a standard electrode placement is not essential while a standard placement is typically beneficial in larger species (e.g. non-human primates).

2.3. Interpretation of EEG patterns

For clinical and nonclinical applications, reading and understanding EEG waveforms is based on a systematic and organized process to recognize abnormal from normal patterns. Interpretation of a typical 10 second strip of 3-channel EEG from a non-human primate (Fig. 2) will require an exhaustive investigation of the context in which this activity was recorded (Table 1). For pre-seizure detection, the typical pattern is the spike. Spike morphology is generally electro-negative (deflects up first), the rise is faster than the fall, it is paroxysmal, is 20–80 msec in duration and is of high voltage: 200–300 μV (Fig. 3). A precise description is essential when identifying an EEG pattern as normal or pathological. For example, a 3 Hz spike and wave pattern is classic finding in absence seizure (Panayiotopoulos, 1999); 2 Hz spike and wave typical for a seizure disorder while 6 Hz spike and wave is a normal EEG variant identified as “14 and 6 positive spikes” or “ctenoids” (Bassett et al., 2014; Niedermeyer & Croft, 1961).

A number of normal EEG variants can be mistaken for seizures. Wickets (Fig. 4) are sharply contoured waves with a rhythmic frequency at 7–11 Hz that were first described by Reiber and Lebel (1977). They resemble the Greek letter “nu” and are often seen in drowsiness or light sleep. Wickets may be misdiagnosed as epilepsy (Krauss, Abdallah, Lesser, Thompson, & Niedermeyer, 2005). Other common EEG morphologies mimicking epileptiform discharges include hyperventilation-induced slowing, phantom spike-and-wave, hypnagogic and hypnopompic hypersynchrony (Azzam & Bhatt, 2014; Benbadis & Tatum, 2005). Increased synchrony (Fig. 5) is common during sleep stage transitions and hypnagogic and hypnopompic hypersynchrony are considered normal variants of drowsiness that may be misdiagnosed as seizure activity. The morphology of rhythmic mid-temporal discharges (RMTD; previously called psychomotor variant) shows patterns that are notched and flat-topped, lasting 1–10 s (Fig. 6).

Artifacts are also a major consideration during EEG interpretation. It is important to distinguish patterns generated from the brain from artifacts created by factors outside the CNS. Movement is a frequently seen artifact, as muscles generate larger voltage signals than do neurons. Movement artifact is not only from whole body movement but can be caused by tongue or eye movements. Tongue movements cause the baseline to undulate. Use of an ocular electrode placed above the eye can help detect and localize eye movements. Usually, movement artifacts affect scalp electrodes more often than implanted telemetry system electrodes. One exception in nonclinical species is chewing: chewing, particularly in monkeys or dogs (Fig. 7A and B), is frequently seen with EEG telemetry as the animals are free to move around the
cage and engage in typical daily activities. Chewing activity can be differentiated from EEG by the presence of high frequency and high amplitude electromyographic (EMG) transients with typical crescendo/descrescendo profiles owing to muscular contractions. EMG is not exhaustively discussed in the current publication but may be used to assess convulsion, activity level or sleep stages. A wide range of muscle groups can be monitored in laboratory animals including the temporal muscles, neck muscles or limbs. The use of temporal muscles can be useful in the interpretation of EEG traces from an electrode montage using the 10–20 system as the EMG activity can be compared to the matching EEG traces to confirm artifacts. Neck muscles are typically used in sleep studies (Rachalski et al., 2014). Video monitoring can also be considered for interpretation of EEG (Authier et al., 2009). Video-EEG is considered the gold standard in clinical neurology and similar methodologies can be applied to safety pharmacology studies.

3. EEG analysis

3.1. qEEG and spectral analysis

Spectral analysis is recognized as a common clinical (Fisher, Scharfman, & deCurtis, 2014) and preclinical (Bassett et al., 2014) tool for data analysis for sleep and seizure assessments. EEG signals was advanced in the 1970s with the advent of qEEG (or quantitative EEG), giving added clarity and information of EEG signals by analyzing its patterns using computer and mathematical methods. Fast Fourier Transform (FFT) can be used to quantify the EEG power across a range of frequencies (Sterman, 1981). FFT converts the EEG signal to a representation of the power (cumulative amplitude) across the range of frequencies (Fig. 8A–D). An increase in higher frequencies can be observed prior to seizure with various positive control drugs (Engel, Bragin, Staba, & Mody, 2009; Zijlmans, Jacobs, Zelmann, Dubesau, & Gotman, 2009).

Conversely, an increase in qEEG powers may also be observed with drugs that are not seizurogenic such as diazepam (Authier et al., 2014), highlighting the diversity of qEEG profiles which may be observed.

qEEG changes may differ between brain regions adding to the interpretation considerations. The use of frontal, parietal and/or occipital EEG derivation(s) needs to be weighted in the selection of the preclinical EEG model given the limitations generally caused by hardware specifications but also cranial anatomy in some species. EEG derivations that are located in the midline sagittal plane are often preferred to conduct qEEG investigations in all laboratory species due to the limited cranial muscles present at this level therefore minimizing the interferences caused by electromyographic (EMG) activity.

Social interactions and circadian cycle are key considerations in the study design of qEEG studies. Comparison of time-matched qEEG data from control and treated periods from the same animal can be used to evaluate potential drug effects. Interactions with congeners are highly beneficial to the animals but will also alter qEEG parameters. In this context, a drug inducing significant effects on the behavior of an animal in the room may affect the qEEG profile of all animals in the same room as communication between individuals occurs. To avoid bias caused by pharmacologically modified behavioral interactions, a qEEG study design would typically involve dosing on different days or in different rooms on the same day.

Like most physiological parameters, qEEG is highly influenced by the circadian cycle and proper interpretation usually relies on comparison of data captured precisely at the same time of the day in the same animals (Authier et al., 2012). Owing to circadian cycle effects, a qEEG cross-over design will often control the dosing time but husbandry and feeding activities should also be completed precisely at the same time of the day for all treatment sessions. As for most other telemetry studies, the presence of the technical personnel in the room should be avoided when planning qEEG analysis. In large animal species, maintaining the same technical personnel throughout the study will also increase data stability given the potential stress associated with a new caretaker. Despite a wide range of factors that need to be controlled in preclinical qEEG, spectral analysis remains one of the most sensitive endpoint with direct applicability in safety pharmacology studies and safety assessments with the ability to identify low amplitude effects with a limited number of individuals.

3.2. Automated or manual EEG analysis in the assessment of seizurogenic effects

Software for automated seizure detection on pre-clinical EEG traces are typically designed to identify spikes trains (Authier et al., 2009) while epilepsy detection algorithms available for patient EEG allow for detection of ictal and interictal traces (Acharya, Sree, Swapna, Martin,
in all species, including humans, and many of its features are compliance with long term treatment. Sleep architecture is well studied disruptions due to a new medication would almost certainly decrease with the aging population, polypharmacy is more common and sleep economic burden of sleep loss ($100 billion US; Stoller, 1994) but well as a diagnostic for sleep disorders. Assessing sleep early on in novel therapies for insomnia (Winrow et al., 2011) and epilepsy as tion, the evaluation of sleep has been used as an endpoint to discover as detect adverse events early on in the drug discovery process. In addi-
cacy but can also demonstrate engagement of its intended target as well as a diagnostic for sleep disorders. Assessing sleep early on in drug discovery has become important not only when considering the economic burden of sleep loss ($100 billion US; Stoller, 1994) but with the aging population, polypharmacy is more common and sleep disruptions due to a new medication would almost certainly decrease compliance with long term treatment. Sleep architecture is well studied in all species, including humans, and many of its features are remarkably similar from mouse to man, making EEG one of only a hand-
ful of highly translatable central nervous system biomarkers (Ivarsson, 2009; Paterson, Nutt, & Wilson, 2011; Veasey et al., 2000).

The assessment of sleep stages is referred to as polysomnography, which requires the electrophysiological collection of up to three core measures. EEG measures the spontaneous electrical activity over single or multiple brain regions while electromyogram (EMG) measures muscle electrical activity, and electrooculogram (EOG) assesses eye move-
ments. Biopotential activity of these core measures can be detected in animals with surgically implanted electrodes implanted over the cortex (known as ECoG or electrocorticogram) for chronic sleep stage assess-
ments and for frequency changes (see below). This method offers 24-
hr/7-day EEG data collection, including temperature, locomotor activity and heart rate, all while the animal is free to roam in its home cage without any tethering limiting movement. The combination of these mea-
ures is used to define individual sleep stages during individual analysis epochs, usually not greater than 30 s. A trained human EEG scorer or validated computer algorithm assigns a score of the predomi-
nant sleep pattern within each epoch, using gold standard scoring rules established by Rechtschaffen & Kales in 1968 (Rechtschaffen & Kales, 1968) and recently updated by the American Academy of Sleep Medi-
cine (AASM) in 2007 (Iber, Ancoli-Israel, Chesson, & Quan, 2007). The overall sleep architecture can then be determined from the collection of individual scoring epochs over the duration of the recording or during a period of interest.

Wake patterns are easily recognized by the trained eye by the pres-
eence of desynchronized, high frequency, low amplitude EEG, increased muscle tone and rapid eye movements. Sleep patterns are broken down into three distinct non-REM stages (termed N1, N2 and N3) and REM sleep (rapid eye movement), each marked with distinct features that help define them. N1 is a transitional stage into falling asleep, marked by low voltage EEG and the beginning of slow eye movements. N2 has moderate voltage EEG with the introduction of sleep spindles and K-complexes. As the individual progresses into N3, increased synchrony occurs in EEG as delta waves appear, voltage is high and as such this stage is defined as “slow wave or delta sleep”. REM sleep is characterized by EEG that is desynchronized (as in wake but with a pre-
dominance of theta power) while muscle tone measured by EMG regist-
ers atonia, and EOG measures the appearance of distinct bursts of characteristic eye movements (Iber et al., 2007). Consolidated, diurnal sleepers such as non-human primates and humans, normally cycle throughout the various non-REM stages during their sleep phase before ultimately reaching REM sleep, EEG/EMG/activity are assessed in poly-
phasic rodents (transition rapidly through sleep stages) typically by assigning sleep stages to either active wake, quiet sleep, deep sleep or REM sleep (Toth & Bhargava, 2013). EOG activity is typically not

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4. Non-clinical sleep assessments

Preclinical sleep assessment is gaining rising importance in the ad-
vancement of a pharmaceutical candidate as drug companies recognize the value of translatable biomarkers that not only are predictive of effi-
cacy but can also demonstrate engagement of its intended target as well as detect adverse events early on in the drug discovery process. In addition, the evaluation of sleep has been used as an endpoint to discover novel therapies for insomnia (Winrow et al., 2011) and epilepsy as well as a diagnostic for sleep disorders. Assessing sleep early on in drug discovery has become important not only when considering the economic burden of sleep loss ($100 billion US; Stoller, 1994) but with the aging population, polypharmacy is more common and sleep disruptions due to a new medication would almost certainly decrease compliance with long term treatment. Sleep architecture is well studied in all species, including humans, and many of its features are remarkably similar from mouse to man, making EEG one of only a hand-
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& Suri, 2013). Normal and ictal EEG morphologies are species and indi-
vidual specific and also vary depending on the electrode montage used. To account for these variations, it is generally useful to adapt the detection algorithm to the experimental conditions that are used. While spike train detection is an important aspect of EEG analysis, a plethora of other changes may be indicative of a seizure risk and manual review of EEG traces by an electroencephalographer is usually an important part of the analysis. Abnormal EEG activity may occur with concomitant clinical signs and systematic review of EEG traces at times when clinical signs were noted is often appropriate. For seizure activity mediated via neuronal receptors or other concentration dependent mechanisms, the abnormal EEG activity may exhibit an incidence distribution correlated with higher plasma and CNS tissue exposure. Pre-defined manual re-
view of EEG traces distributed over the monitoring period based on the expected pharmacokinetics (PK) of the compound can be relevant and increase sensitivity to detect abnormal EEG activity or biomarkers of increased seizure risk.

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Fig. 5. Increased synchrony in EEG from a freely moving cynomolgus monkey using implanted telemetry.

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Fig. 6. Example of human EEG showing rhythmic mid-temporal discharges (RMTD) (R: right side, L: Left side).
assessed in rodents, as these are difficult electrodes to implant and maintain long term in this species.

qEEG has been shown to be a powerful and dynamic tool, as bursts (or power) of sinusoidal waves have been linked to certain mental states such as sedation or stimulation and have even been validated as a biomarker for disorders such as ADHD (increased theta-beta power ratio) (Leiser, Dunlop, Bowlby, & Devilbiss, 2011). Recent innovative drug discovery approaches have compared frequency changes relative to vehicle within certain sleep stages, allowing for better comparisons to standard of care treatments for sleep disorders such as insomnia (Fox et al., 2013). EEG patterns consist of five basic frequencies, alternating between states of increased and decreased synchrony within the signal, dependent on the sleep pattern the animal is in at the time of measurement. At its slowest frequency and most synchronized state, delta activity is detected and ranges between 0.5–4 Hz and is associated with deep sleep. With increased frequency and desynchrony, delta is terminated and transitions into theta activity (4–8 Hz). Theta activity is often associated with REM sleep. Alpha activity (8–12 Hz) is readily seen in relaxed or quiet wakefulness. Sigma activity (12–16 Hz) is typically present in the awake state. Frequencies greater than 16 Hz are referred to as beta (16–30 Hz) and gamma (>30 Hz) and are detected during active mental activity (Jobert et al., 2012). As a general rule, the cumulative absolute amplitude as calculated by FFT at lower frequencies (e.g. delta) will be lower than values for higher frequencies (e.g. beta or gamma). Hence, pharmacological evaluations typically rely on relative changes within a given power band (Bassett et al., 2014).

Typical study designs for the assessment of sleep architecture/frequency changes in animals involve evaluating the core measures (qEEG only requires EEG) as mentioned above with the potential exclusion of periods with high EMG activities. Chronic dosing studies preferably involve pre-screening animals in order to power studies with animals that have high quality EEG/EMG/EOG signals, good baseline circadian patterns and are void of paroxysmal EEG activity. After baseline activity has been assessed, animals are assigned to a chronic, multiple day dosing regimen with either a parallel or cross-over design (randomly assigned to drug first or vehicle first treatment and then crossed over to the opposite regime). Cross-over studies are preferable, as each animal can act as their own control when a manipulation (drug or some other treatment) is compared to a negative control (either vehicle or sham). The use of genetically manipulated mice and rats or knockouts can add value to pharmacological experiments as they can demonstrate the dependence of the drug’s effects on a given target or gene product in animals with a targeted genetic disruption. Knockouts serve as their

Fig. 7. A. Chewing artifact at EEG recorded with telemetry in a freely moving jacketed Beagle dog. B. Chewing artifact at EEG recorded with telemetry in a freely moving cynomolgus monkey.

Fig. 8. A. Fast Fourier Transform (FFT) on EEG (Cz–Oz) from a telemetered cynomolgus monkey during generalized seizure with a peak amplitude at 6 Hz (Graph amplitude axis from 0 to 200 mV). B. Fast Fourier Transform (FFT) on EEG (Cz–Oz) from a cynomolgus monkey during the post-ictal period with significant attenuation of all frequencies (Graph amplitude axis from 0 to 70 mV). C. Fast Fourier Transform (FFT) on EEG (Cz–Oz) from a cynomolgus monkey during deep sleep (stage N3) with an increase in lower frequencies (Graph amplitude axis from 0 to 70 mV). D. Fast Fourier Transform (FFT) on EEG (Cz–Oz) from a cynomolgus monkey during active wake with an increase in higher frequencies (Graph amplitude axis from 0 to 70 mV).
A) Fast Fourier Transform (FFT) on EEG (Cz-Oz) from a telemetered cynomolgus monkey during generalized seizure with a peak amplitude at 6 Hz (Graph amplitude axis from 0-200 mV).

B) Fast Fourier Transform (FFT) on EEG (Cz-Oz) from a cynomolgus monkey during the post-ictal period with significant attenuation of all frequencies (Graph amplitude axis from 0-70 mV).

C) Fast Fourier Transform (FFT) on EEG (Cz-Oz) from a cynomolgus monkey during deep sleep (stage N3) with an increase in lower frequencies (Graph amplitude axis from 0-70 mV).

D) Fast Fourier Transform (FFT) on EEG (Cz-Oz) from a cynomolgus monkey during active wake with an increase in higher frequencies (Graph amplitude axis from 0-70 mV).
own controls and comparisons can be made against wild-types
individuals.

Other than drug exposure, several factors can impact sleep architecture. Factors such as increased stress, illness, and changes in social/physical environment(s) have been shown to be disruptive to normal physiological circadian patterns (Bruse et al., 2012; Irvine, White, & Chan, 1997). EEG studies in animals should aim to keep extraneous, uncontrolled factors at a minimum. Normal and uncontrollable changes during sleep include changes in respiratory drive which may induce changes to sleep patterns. With the increased incidence of diagnosis of sleep apnea and an aging population enduring polypharmacy, sleep studies undertaken early in drug development can protect patients down the line who are already at risk for sleep issues. EEG studies done preclinically can also inform on efficacy, qualify normal EEG activity and identify potential adverse events of a drug (and ultimately affect prescription rate down the line), as well as outline risks associated with sleep loss and changes in sleep, making it an extremely powerful tool to assess brain activity. While some methodological & analytical differences do exist when assessing EEG in animals vs. humans, the sleep/wake systems in the brain are remarkably similar, allowing for translatable, pharmacodynamic assessment of pharmaceutical candidates early on in the drug discovery process.

5. Species differences and interpretation of EEG studies

Differences have been identified between animal species for the susceptibility to drug-induced seizure (Bassett et al., 2014). The rank order between species for seizure susceptibility can differ between compounds but the Beagle dog is often the most susceptible (Elander, 2013) possibly owing to genetic predisposition to idiopathic epilepsy (Edmonds et al., 1979). Cases for which the Beagle dog was less susceptible than other species were also reported (Authier, unpublished data) and careful and conservative interpretation of the preclinical data is always warranted. Within an animal model, inter-individual differences can be observed for anticonvulsant (Bankstahl, Bankstahl, & Löscher, 2012; Brandt, Volk, & Löscher, 2004; Klein, Bankstahl, & Löscher, 2015) and proconvulsant effects (Bassett et al., 2014; Himmel, 2008). The route of administration can also account for part of the variability observed in EEG studies with oral dosing typically associated with greater variations in exposures than parenteral dosing and consequently increased CNS effect variability.

Decisions on the safety of drugs may be based on a broad range of considerations including in silico modeling, receptor affinity, drug exposure, histopathology and/or seizure characteristics (e.g. precursor EEG changes, precursor clinical signs, seizure onset triggers, duration/self-limiting, success of emergency treatments in animals, recovery, kindling effects, inter-individual variability and incidence, species differences, etc. (Markgraf et al., 2014). In particular, clinical signs in animals may be correlated with the exhibition of seizures and/or abnormal brain activity following drug administration (see Section 8 for further discussion). However, skepticism exists that the exhibition of these signs in animals does not correlate with signs that are predictive of seizure and/or brain activity in humans. Therefore, a caveat remains that the sequence and drug exposure concentrations at which these precursor signs of seizure are observed in animal studies may differ from the human response. Precursor clinical signs of CNS toxicity are often non-specific (e.g. tremors, nystagmus, emesis, ataxia) and the mechanisms involved may also differ between species. While differences can be observed, animal models remain a cornerstone of drug safety testing prior to first in human administration. Similar precursor sign profiles are observed across species with pentylenetetrazol (PTZ), a commonly used positive control (Bassett et al., 2014) but also for a broader range of pharmaceutical agents (Easter et al., 2009). Seizure liability studies often aim to determine the EEG NOEL (No Observed Effect Level) or NOAEL (No Observed Adverse Effect Level) in the test system to later estimate safe doses for clinical investigations. There is agreement among the authors that plasma concentrations at doses that produce seizures and/or abnormal brain activity in animals are likely correlated to those that may produce the same effect in humans. Therefore, plasma concentrations at the dose estimated as a NOAEL in animals may be used to establish safety margins, which are useful when developing a clinical dose escalation plan and PK dose-stopping criteria (see Section 8 for further discussion).

6. Seizure risk assessment: Data to consider when assessing seizure risks

Seizure risk assessment may be based on various findings such as PK data on the parent drug (and biologically active metabolites), clinical and anatomical pathology findings, physiological alterations, and behavioral observations. The PK profile(s) should include values for time to peak concentration (i.e., T_max), peak concentration (C_max), overall systematic exposure (AUC), and elimination half-life (t1/2). Values for T_max and t1/2 should be considered when evaluating the time course for EEG findings and premonitory signs of convulsions. This consideration may be helpful in determining which chemical is responsible for inducing seizures and other related toxicities based on the plasma levels of each chemical at the time these effects occur. Seizures are known to be induced following alterations in clinical pathology endpoints that include sodium, calcium, and magnesium. Also, anatomical changes in the brain may induce convulsions and seizures (e.g., head injury). With regard to behavior, premonitory signs of convulsions in animals include head and body tremors, incoordination, and ataxia (Bassett et al., 2014; Smythe, Ryan, & Pappas, 1988). Although these signs may be related to convulsions, there are instances in which each may be due to other pharmacological effects produced by the product. For example, behaviors such as tremors and shaking may be due to hypothermia. Note that there is evidence that hyperthermia and hypothermia can induce seizures in animals (Smythe et al., 1988; van Gassen et al., 2008; Kallman 2008). EEG can provide a definitive confirmation of the presence of seizure based on the observation of ictal activity (i.e. EEG spike train). Clinically, there is no minimum time to define a seizure and EEG discharges accompanied by clinical signs of seizure qualify as an electrographic seizure irrespective of their duration (Fisher et al., 2014). Overall, the seizure risk assessment should consist of various types of data to determine if a seizure occurred and, if so, the exposure level of the chemical that induced this effect.

7. Safety margins: Factors to consider when estimating margins

Although there is an official FDA policy on how to determine safety margins for the starting dose of initial clinical trials in adult healthy volunteers, the guidance that provides this information does not provide insight on how to determine safety margins for dose-escalation treatment schedules (see the FDA guidance for industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf). The safety evaluation for each drug must take into consideration the risks and benefits to the intended patient population. In general, a safety margin of ≥ 10-fold is usually acceptable for products that produce irreversible and unmonitorable adverse effects (e.g. unexplained death, severe anatomical pathology findings such as liver necrosis, seizures). A few key factors to consider when establishing safety margins include patient population, route of administration, and drug interactions. Patient populations with increased vulnerability to convulsions include those with a history of seizures and/or head injury (Kim et al., 2006; Schierhout and Roberts, 2012). Also, patient populations with a history of chronic use of alcohol and other drugs may decrease the seizure threshold and increase vulnerability to convulsions (Enevoldson, 2004; Hillbom, Pieninkeroine, & Leone,
The age of the study population should also be taken into account since seizure vulnerability may be increased in younger patients due to the underdevelopment of detoxification systems and in older patients given the inefficiency of the same systems. In rodents, findings demonstrate that spontaneous seizures (e.g., spike wave seizures) increase in frequency as animals age, which provides further evidence that the age of subjects is a critical variable in evaluating seizures (Ellens et al., 2009; Kelly, 2010; Sgro, Benagh, Modlin, & Kallman, 2006; Sitnikova, Hramov, Grubov, & Koronovsky, 2014). The route of administration is considered since the absorption of the product may vary depending on the route employed. For example, drugs administered intravenously (IV) are entirely bioavailable (i.e., 100%), whereas those that are administered via other routes may not be. The absorption of drugs via these other routes may be highly variable across patients, which adds uncertainty to the margin needed to ensure safety. In regard to potential drug interactions, safety margins greater than 10-fold may need to be appropriate when the test product is co-administered with a proconvulsant or convulsant agent.

8. Examples when nonclinical EEG studies can help determine safe clinical exposure levels

Drugs effects may represent biological responses that occur spontaneously in organisms and that overlap temporally. Also, the potency in which a drug (and its metabolites) produces an effect may vary across species, resulting in the observation of different levels of sensitivity to the drug. These issues complicate the interpretation of findings across animal species in which convulsions and abnormal brain activity (includes seizures) are observed. Therefore, the scenarios discussed below illustrate the use of EEG techniques to determine whether clinical signs and convulsions were due to abnormal brain activity that is drug-related and to establish the most sensitive and/or relevant species. The goal of any toxicology study is to define doses that are appropriate to study in humans and to avoid unwanted side effects. This is completed by both defining a NOEL/NOAEL and the exposure levels at which unacceptable adverse effects occur. The scenarios below illustrate the use of exposure levels in the most sensitive and/or relevant species to establish PK dose-stopping criteria for clinical studies. In the scenarios discussed, EEG techniques are used to establish exposure levels at which abnormal brain activity (includes seizures) is not observed at either the NOEL or NOAEL estimated in animals. These levels are used to establish PK dose-stopping criteria that set the top clinical exposure levels (i.e., for Cmax and/or AUC) at 1/10 that measured at either the NOEL or NOAEL estimated in the most sensitive and/or relevant animal species. This approach is used given the nature of the toxicity of concern (abnormal brain activity) and to help ensure that the toxicity is not observed in patients, especially when the benefits of treatment do not outweigh the risks associated with it. Note that the scenarios do not represent findings from an actual study report. These scenarios were written to illustrate challenges that may arise when evaluating potential therapeutics that may be seizurogenic agents that do not significantly alter clinical pathology endpoints. The conclusions provided are from the perspective of the authors and represent one of many approaches to interpreting the findings provided.

As mentioned above, convulsions may occur spontaneously in animals. EEG studies offer a definitive means to determine if clinical signs such as convulsions or other premonitory signs are due to abnormal brain activity or not. This can be complicated by the background incidence of convulsions in the species tested. For example, Drug A (0, 20, 100, or 500 mg/kg) is administered IV to dogs in a single-dose toxicology study. Drug A is known to produce hypothermia and sedation in multiple species. In the current study, dogs exhibit hypoactivity at ≥100 mg/kg; tremors across all doses; and a convulsion at 500 mg/kg (high dose). The convulsion is exhibited in a dog that exhibited tremors. Given the observation of tremors in this animal it is considered a potential premonitory sign of convulsions in the low and mid dose groups.

Therefore, a NOAEL cannot be established since a convulsion was observed at the high dose and tremors are exhibited across the doses tested. This scenario is challenging since the incidence of convulsions was low and in a species known to exhibit spontaneous convulsions (Bielfelt et al., 1971) and the tremors observed may have simply been secondary to hypothermia. This information suggests that the convulsion may not be drug related and warrants further EEG testing. In a separate study, drug naïve dogs exhibit tremors, in the absence of abnormal EEG activity at the doses previously tested. The convulsion is not reproduced in a second study and EEG data suggest no evidence of abnormal brain activity in the presence of premonitory signs. These data support the conclusion that the convulsion observed in the initial study may have been spontaneous given its low incidence and the lack of abnormal EEG activity in treated dogs.

In scenarios in which abnormal brain activity is detected via EEG techniques, PK dose-stopping criteria can be established for the clinic based on plasma levels of drug in the most sensitive species. For example, Drug B is orally administered to rats (0, 1, 10, or 17 mg/kg) and dogs (0, 0.5, 2.5, or 5 mg/kg). Based on PK data, the oral bioavailability of Drug B ranges from 10 to 70% and the excretion of unchanged drug in urine samples appears complete (100%) across species. Both species exhibit convulsions at the highest dose tested. Given the observation of shaking and uncoordinated behavior at the high dose group both signs were considered premonitory signs of convulsion in animals from the low- and mid-dose groups that exhibit them. A NOAEL cannot be estimated in either species since convulsions are observed at the high dose and premonitory signs are exhibited across the doses tested. The highly variable oral bioavailability confounds the determination of the most sensitive species based on body surface area comparisons. As a follow-up, Drug B (same doses) is evaluated in rats and dogs using EEG techniques in order to correlate the findings with exposure data. In rats, abnormal EEG activity is observed at the high dose and characterized by the appearance of high frequency gamma activity, repetitive bursts of sharp waves, and increased sharp waves. In dogs, abnormal EEG activity is observed at the mid- and high-dose and is characterized by the appearance of high frequency gamma activity at the high dose and repetitive bursts of sharp waves and increased sharp waves at the mid and high dose. Across species, abnormal EEG activity is observed at 1 h following treatment, which correlates with the averaged Tmax value for Drug B. There is no evidence of frank seizure in either species at the doses tested. A NOEL can be estimated in rats and dogs, respectively, at 10 mg/kg and 0.5 mg/kg based on the lack of abnormal EEG activity at these doses. The use of EEG techniques provides a definitive evaluation of alterations in CNS activity, which in turn allows for a more informed decision as to whether or not the premonitory signs exhibited are associated with abnormal brain activity. Overall, the dog is deemed the most sensitive species given that the Cmax value at its NOEL (333 ng/mL) was lower than that in rats (555 ng/mL).

PK dose-stopping criteria can be established based on the NOEL in dogs, given the nature of the toxicities that define it. The PK dose-stopping criteria establish the top Cmax value allowed in the clinic. The top value (33.3 ng/mL) is set 10-fold lower than the exposure level at the NOEL in the dog given the marked variability in the oral absorption of the drug and the nature of the toxicity of concern (i.e., abnormal brain activity). This scenario illustrates that premonitory signs such as shaking and uncoordinated activity may be indicative of abnormal EEG activity in animals; however, these signs do not always correlate with EEG abnormalities. Also, findings in the dog demonstrate that abnormal EEG activity occurs at exposure levels of drug that are lower than those at which frank convulsions occur.

EEG data can also be useful in evaluating drugs with a different metabolic profile in the test species compared to humans, a scenario that is not always straightforward in terms of how to appropriately establish safety margins to inform clinical dose-escalation limitations. As such, being able to correlate the appearance of abnormal EEG activity with the Tmax of a parent or major metabolite can provide valuable data to
assist in the design of the proposed clinical study. For example, Drug C is orally administered to monkeys, dogs, and rats. Convulsions are observed in these species following the administration of Drug C, which is known to form a biologically active metabolite M123. Comparison of the drug/metabolite ratio in the nonclinical test species to humans may help determine which animal species may be the most relevant. In this example, the metabolite was formed in dogs and monkeys, but not rats and the metabolic profile in the monkey most closely resembles that of humans. As all species exhibited convulsions, the parent is clearly contributing to the convulsions, however, the role of the metabolite, if any, in the convulsions noted is still not clear.

As a follow-up, Drug C and the isolated M123 metabolite is evaluated in monkeys using EEG techniques to determine if abnormal EEG activity is induced by one or both compounds. Evidence of frank seizure and abnormal EEG activity is observed in monkeys at the mid and high dose of Drug C, confirming that the observed convulsions are indeed seizures. Frank seizure and abnormal EEG activity are also observed following treatment with the M123 alone, demonstrating that both compounds must be considered seizurogenic. A NOEL for Drug C is estimated at 2 mg/kg (Cmax = 22 ng/mL) based on evidence of abnormal EEG activity and frank seizure at higher doses. A NOEL could not be established for M123 given the abnormal EEG activity and frank seizure observed at the dose tested. However, these data provide reasonable justification that the monkey can be used to establish real-time PK dose-stopping criteria based on the parent exposure levels alone, given the comparability of the metabolic profile with humans. Therefore, the top critical Cmax was established at 2.2 ng/mL for the parent drug as this plasma level takes into account both the parent and metabolite levels. If the metabolic profile was not comparable to humans, stopping criteria would have to take into consideration both the parent and the metabolite.

This scenario illustrated that the most relevant animal species may be determined based on the metabolite profile in the species tested, the isolated metabolite should be tested alone to evaluate its effects on EEG activity, and the PK stopping criteria should include top exposure levels for the parent drug and metabolites demonstrated to produce abnormal EEG activity and other adverse effects. A risk assessment should be determined for both the parent drug and its biologically active metabolites. This assessment should be based on findings from studies that evaluate the parent drug and its biologically active metabolites alone and in combination. Doses of the metabolite should be selected to ensure exposure levels comparable to that observed following treatment with the doses of parent drug tested in order to ensure the evaluation of relevant exposure levels. The Tmax values for both the parent and its metabolites should be compared to the time points in which adverse effects such as abnormal EEG activity are observed in order to understand the potential role of these chemical entities in the effects measured following treatment. The interaction of the parent drug and its metabolite may be complex and result in different pharmacological and toxicological profiles. Therefore, these results must be carefully interpreted, especially when used to establish PK dose-stopping criteria.

9. Translation of non-clinical EEG to the clinic

Translation of EEG findings to the clinical environment makes the critical impact on the regulatory environment. Other measures of convulsive alterations such as preconvulsive assessments, where the novel new drug is combined with a known convulsant like picrotoxin, kainic acid, electroshock, pentylentetrazol, or prior electrical kindling to determine changes in threshold are useful preclinical tools that lack direct translation to the clinic. An indirect ranking of the convulsive liability of compounds may be possible but these same paradigms are not conducted with human subjects since all of them depend on establishing convulsion prior to adding the new drug to the paradigm. Essentially, how can these preclinical assessments support human safety and the design of clinical evaluations of new pharmaceuticals? The primary preclinical assessment tool that can be monitored in humans is EEG. Several factors must be considered in the quality of preclinical EEG for human translation and safety evaluations. The major issues that must be considered are species differences, age differences in EEG, EEG assessment tools, and the limitations of predictability from preclinical assessment to the clinical environment.

The first approach in understanding translation is making a distinction between reported observations of convulsion from seizures or the alteration in the electrical activity patterns distinguished from EEG recordings. Typically the goal would be to avoid convulsion by establishment of a same dose or identification of premonitory effects that would aid in the avoidance of convulsive doses of the new compound. For a premonitory event to be useful in managing a clinical drug trial the event must occur at lower doses that do not produce convulsions/seizures or be separated from the convulsive/seizure event by a long period of time. Potential premonitory events cannot be an aura to the seizure which indicates that the seizure is eminent since these aura events do not provide sufficient time to abate the seizure. Fig. 9 illustrates how clinical signs, other toxicity indicators, and plasma drug level can be used to put a seizure effect into perspective. Timeframe can be an important variable for monitoring delayed onset seizures with repeated dosing and the data generation approach to monitor delayed onset convulsions and Cmax driven convulsions would be quite different. See Table 2 for a summary of differences between acute and chronic EEG monitoring methods. For the delayed onset events, EEG in the non-precipitated situation may change overtime prior to the onset of seizure events (Carfagna, Sgro, Areezo, & Kallman, 2010; Kallman, 2006). Alterations in muscular endpoints including myoclonus (Kojovic, Cordivari, & Bhatia, 2011) can be easily defined as convolution by observers. Incorrect labeling of these muscular events as convolution is frequent and can only be corroborated by EEG monitoring. The distinction between muscular changes and actual EEG spike activity dramatically affect the clinical understanding of these effects. Similarly, drug induced catalysis may be incorrectly defined as convolution (Bricker, Sampson, & Ablordepey, 2014; Heitz & Bence, 2013; Sanberg, Bunsey, Giordano, & Norman, 1988; Winters, Ferrar-Allado, Guzman-Flores, & Alcaraz, 1972), again with EEG serving as a key biomarker for proper identification of the drug induced effect.

Once a true seizure profile has been identified the finding should be put into perspective to improve clinical safety. Many drugs used therapeutically have seizure profiles at therapeutic doses (Alper, Schwartz, Kolts, & Khan, 2007). Typically the seizure profile for clinical compounds is observed at the higher doses, with long term use of the drug, and in patients with a history or predisposition to seizure prior to drug administration. The most critical factors to understand for translation is the margin of the seizurogenic effect to the therapeutic dose, establishing a NOEL/NOAEL understanding the pre-seizure events that would be reliably monitored, the therapeutic endpoint, and whether the effect is Cmax driven or has a delayed onset. All of these preclinical variables would impact the design of clinical testing and the probability of drug development success in the clinic.

The consistency of the observation of convulsions/seizures should be evaluated across species. Sometimes a compound has a unique
metabolite profile (Sullivan, Hanasono, Miller, & Wood, 1987) that could be associated with convulsive behavior. Most toxicology programs include a rodent and large animal species which is usually the dog. As one ascends the phylogenetic scale there are more sophisticated mechanisms to inhibit seizures as a result of increased cortical inhibition and enhanced reduction in the spread of electrical excitation from hemisphere to hemisphere characteristic of convulsive profiles. An observation of convulsion in the rat may not be corroborated in the dog or primate (Kallman, Sgro, Markgraf, & Ballering, 2014). The primate brain, organization of the cortex, corpus callosum and descending regulation of CNS stimulation is most like the human (Katz, Lasek, & Silver, 1983) but many times not the species of choice for more detailed toxicology evaluations where pharmacokinetics, metabolite profile, and other toxicological effects are well described in GLP studies. As previously discussed, the dog offers some special considerations since this species is frequently selected for characterizing toxicity but is recognized to present a higher rate of seizure profiles prior to drug treatment (Bieffelt et al., 1971). Some laboratories have resorted to prescreening dogs prior to the start of toxicology studies where there is an indication that convulsion/seizure is a potential safety issue. These are conducted on restrained dogs by a short (e.g. 2–10 min) acute EEG recording with surface needle electrodes to determine if seizure activity or abnormal EEG morphologies is present. Table 3 lists the major pros and cons of prestudy screening applied to non-clinical studies. The application to the toxicology study, i.e. pre-FHD or pre-Phase 3 clinical testing, may impact the decision to apply prescreening.

A final consideration is to contrast data from preclinical to clinical EEG evaluations. Table 4 lists the technical differences between the animal and human approaches for recording. The two approaches can provide different levels of sensitivity for detection with the human approach generally considered as more sophisticated and often of longer duration since a continuous recording approach is commonly used. When the focus is on seizure activity and not qEEG characterizing the human and animal data are more consistent and less variable than when qEEG characterization is of interest.

### 10. Discussion

EEG appears to be underutilized in non-clinical drug safety testing. Epidemiological considerations weight for an increasing importance of CNS safety issues owing to the aging population and growing prevalence of polypharmacy. Seizure and sleep liabilities are manifest applications of EEG to safety testing of drug candidates but challenges remain in the use of this methodology including data interpretation, translation from animals to the clinic and drug safety profile variants. Interpretation challenges originate from the abundance of data but also from the inherent diversity of EEG morphologies that can be obtained. Mechanisms to explain drug induced adverse effects are often poorly characterized which adds to the uncertainty during seizure risk assessment using animal data. As discussed, thorough characterization of pharmacokinetics can inform species comparisons with the goal of predicting the human response. Some agents induce adverse CNS effects after acute administration while others require chronic exposure.

### Table 2
Comparison of preclinical acute and chronic EEG recordings.

<table>
<thead>
<tr>
<th>Acute EEG</th>
<th>Chronic EEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>No surgical implantation</td>
<td>No surgical implantation/surface or deep electrodes</td>
</tr>
<tr>
<td>No mapping of sites</td>
<td>Can map sites</td>
</tr>
<tr>
<td>Restrained animal</td>
<td>No restraint</td>
</tr>
<tr>
<td>Short duration recording</td>
<td>24/7 up to 3 months or more</td>
</tr>
<tr>
<td>Identification of seizure activity/spiking no qEEG or staging</td>
<td>Identification of seizure activity/spiking, pharaco-EEG, qEEG, staging</td>
</tr>
<tr>
<td>More diagnostic rather than time expansive</td>
<td>Focus on time expansive recordings</td>
</tr>
</tbody>
</table>

### Table 3
Pros and cons of using screening EEGs on large animal studies.

<table>
<thead>
<tr>
<th>Advantages of EEG Prescreens</th>
<th>Disadvantages of EEG Prescreens</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Dogs may have a high rate of convolution at the time of receipt from suppliers – Reported as 4% (Arezzo 2005) and as 0.02% based on the vendor database</td>
<td>• Not as useful in rodents where the incidence is lower (less than 1%) and rodent strain is an issue</td>
</tr>
<tr>
<td>• Can be incorporated into an ongoing toxicology study</td>
<td>• May have to conduct additional studies to understand special population sensitivity</td>
</tr>
<tr>
<td>• Few Ns in large animal toxicology studies (typically 3–6/treatment group)</td>
<td>• Although humans are typically not prescreened they could be established to establish a clinical safety margin</td>
</tr>
<tr>
<td>• Special population preclinical data can be obtained later in drug development, if required, in a separate study</td>
<td>• May have to understand later issues related to special sensitivity of therapeutic population since humans on later clinical trials may be less homogeneous than animal population</td>
</tr>
<tr>
<td>• Early goal is to understand threshold or NOAEL under consistent conditions</td>
<td>• Additional cost to toxicology studies – may require additional animals for exclusion</td>
</tr>
<tr>
<td>• Consistency of population for making decisions for early healthy subject safety trials</td>
<td>• Must develop exclusion criteria</td>
</tr>
<tr>
<td>• Prescreens for other endpoints on toxicology studies is based on the idea of healthy animals concept</td>
<td>• Not a good prescreen requirement for all toxicology studies but rather for cause or expected profile</td>
</tr>
<tr>
<td>• Can distinguish changes due to drug exposure in non-epileptic animals</td>
<td>• Cannot detect more subtle qEEG changes but seizure activity only in prescreens</td>
</tr>
<tr>
<td>• Initial Phase 1 clinical studies are focused on normal or healthy clinical population</td>
<td>• Might be incorrectly applied when interested in EEG effects other than threshold determinations</td>
</tr>
<tr>
<td>• Supportive of studies designed to determine if observational convulsions are seizures or due to motoric changes. Baseline EEG must be normal to make this determination</td>
<td>• Requires high quality expertise</td>
</tr>
</tbody>
</table>

Tachyphylaxis, a decrease in the response to a drug, is commonly observed with CNS active drugs and may impact the design of EEG studies. During toxicology studies, tremors (involuntary symmetrical oscillations of a body part), myoclonus (brief, involuntary muscle jerk caused by abrupt muscle contraction) and convulsions (abnormal, violent and involuntary contraction or series or contractions of the muscles) may be observed. Each of these observations may be associated with EEG biomarkers of increased seizure risk or frank ictal activity. EEG monitoring is essential for proper characterization of the clinical events that are observed. As a well-established diagnostic methodology, EEG monitoring has the potential to offer a high translational value but a complex matrix of scientific and regulatory considerations is involved during decision-making leading to the inclusion of this neurological monitoring endpoint in preclinical studies. From design to interpretation, the

### Table 4
Differences between preclinical and clinical EEG recordings.

<table>
<thead>
<tr>
<th>Preclinical</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Acute recordings in restrained animals</td>
<td>• Holter monitoring and acute scalp electrodes in resting state</td>
</tr>
<tr>
<td>• Telemetry recordings in surgically prepared animals</td>
<td>• Multiple sites</td>
</tr>
<tr>
<td>• Usually 1–2 general sites on brain surface</td>
<td>• Limited to therapeutic doses for evaluation of surface</td>
</tr>
<tr>
<td>• Deep electrode for evaluation of hippocampal theta in sleep evaluation</td>
<td>• Video-EEG considered as gold standard</td>
</tr>
<tr>
<td>• Can evaluate dose higher than therapeutic doses</td>
<td>• Coincidental EMG for signs not possible</td>
</tr>
<tr>
<td>• Can record simultaneous video and EMG</td>
<td>• Confidence in identification of seizure/pre-seizure activity but less confidence in qEEG.</td>
</tr>
</tbody>
</table>
drug development team is faced with multiple aspects to integrate as they exercise the art of safety pharmacology.

Conflict of interest

None of the authors have any conflicts of interest, other than their employment in commercial pharmaceutical companies, academic institutions or contract research organizations. No information is presented in this paper that advocates for or promotes commercial products from any of our organizations.

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