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

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ABSTRACT

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.

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 toxicology 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 ictal 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

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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 patters 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 (frequently 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; Nidermeyer & 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 Reiher and Lebel (1977). They resemble the Greek letter “mu” and are often seen in drowsiness or light sleep. Wickets may be misdiagnozed 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, 2003). Increased synchrony (Fig. 5) is common during sleep stage transitions and hypnagogic and hypnopompic hypersynchrony are considered normal variants of drowsiness that may be misdiagnozed 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

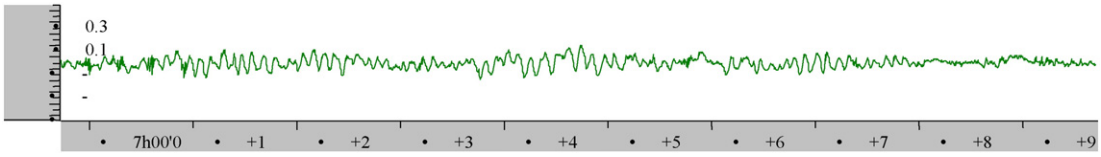

Fig. 1. A single channel of EEG, showing the rich mix of frequencies and amplitudes that comprises the normal EEG, in this example, from a Beagle dog. EEG consists not only of the second-to-second mix of amplitudes and frequencies, but also shows larger rhythmic oscillations characterized by slower frequencies. Physiologically, the underlying mechanism of these large oscillations is partially due to the interaction between the thalamus and cortex. It is also based on the intrinsic rhythmic capacity of the large neuronal networks in the cortex.