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Analyzing large data sets acquired through telemetry from rats exposed to organophosphorous compounds: An EEG study

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ABSTRACT

The organophosphorous compound soman is an acetylcholinesterase inhibitor that causes damage to the brain. Exposure to soman causes neuropathology as a result of prolonged and recurrent seizures. In the present study, long-term recordings of cortical EEG were used to develop an unbiased means to quantify measures of seizure activity in a large data set while excluding other signal types. Rats were implanted with telemetry transmitters and exposed to soman followed by treatment with therapeutics similar to those administered in the field after nerve agent exposure. EEG, activity and temperature were recorded continuously for a minimum of 2 days pre-exposure and 15 days post-exposure. A set of automatic MATLAB algorithms have been developed to remove artifacts and measure the characteristics of long-term EEG recordings. The algorithms use short-time Fourier transforms to compute the power spectrum of the signal for 2-s intervals. The spectrum is then divided into the delta, theta, alpha, and beta frequency bands. A linear fit to the power spectrum is used to distinguish normal EEG activity from artifacts and high amplitude spike wave activity. Changes in time spent in seizure over a prolonged period are a powerful indicator of the effects of novel therapeutics against seizures. A graphical user interface has been created that simultaneously plots the raw EEG in the time domain, the power spectrum, and the wavelet transform. Motor activity and temperature are associated with EEG changes. The accuracy of this algorithm is also verified against visual inspection of video recordings up to 3 days after exposure.

1. Introduction

Organophosphorous (OP) compounds are known to induce cardiac and respiratory distress and prolonged seizures through inhibition of acetylcholinesterase. Cholinesterase inhibition and acetylcholine increases generate secondary changes in dopamine and neuroepinephrine concentrations (McDonough and Shih, 1997; Shih et al., 2003). These secondary changes lead to glutamatergic hyperexcitation, excessive calcium influx, and status epilepticus (SE), a condition where seizures are prolonged and continuous (Sloviter, 1999). If not treated, SE can result in severe brain damage, the primary consequence of OP-induced seizures. In SE patients, brain damage is induced by neuroexcitotoxicity (Wasterlain and Shirasaka, 1994; Shih et al., 2003; De Lorenzo et al., 2005) as a result of acetylcholine increases, excitatory amino acids, and excessive calcium influx.

Oscillatory brain patterns can be both normal and pathological. Normal brain oscillatory synchronization is strongly correlated with cognitive function and behavioral state (Singer, 1999; Engel et al., 2001; Gross et al., 2004; Pareti and Palma, 2004; Schnitzler and Gross, 2005; Cantero and Atienza, 2005), and abnormal brain oscillations have been linked to pathologies characterized by dysfunction of the cholinergic system and epilepsy (Timofeev and Steriade, 1997; Traub, 2003; Schnitzler and Gross, 2005). Among abnormal brain oscillations, spike/wave activity present during epileptic seizures is well defined. This activity is the result of hypersynchronous firing in the epileptic focus (Engel, 1993). The most commonly affected areas during SE are limbic regions, including the hippocampus, amygdala, pyriform cortex, and cortex (Turski et al., 1983; Scremin et al., 1997; Carpentier et al., 1990; Petras, 1994; Shih et al., 2003). Our laboratory has been assessing the neuroprotective effects of novel therapeutics against seizures induced by soman exposure in rodents, a model similar to pilocarpine-induced seizures (Turski et al., 1983). These studies encompass electrophysiological and behavioral seizure occurrence and duration, basic

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behavioral outcomes, and neuropathology. The determination of SE duration is fundamental for the evaluation of potential neuroprotectants in this setting.

The use of telemetry to capture continuous recordings has resulted in a large accumulation of both EEG waveform and parameter (activity and temperature) data. This large volume of data presents difficulties for analysis and interpretation. Several studies have addressed the need for analytical tools capable of optimally performing spectral analyses (Rossetti et al., 2006; Romcy-Pereira et al., 2008; Lehmkuhle et al., 2009) seizure estimation, and spike detection (Saab and Gotman, 2005; White et al., 2006; Casson et al., 2007; Hopfengärtner et al., 2007; Jacquin et al., 2007). Artificial neural networks have proven to be the most statistically effective tool (Gabor et al., 1996; Gabor, 1998; Kiymik et al., 2004; Nigam and Graupe, 2004; Srinivasan et al., 2007; Tzallas et al., 2007; Patnaik and Manyam, 2008), but lack time efficiency for the analysis of large data sets. Third-party software tools designed for seizure detection are adjusted for specific parameters found in human subjects, such as sleep stages and spike and wave activity. The use of such tools for the evaluation of large data sets resulting from long-term pharmacological studies is not reliable, since it is both time consuming computationally and tailored to a specific subject group. Several tools have been created to allow users without programming experience to run complex EEG analysis algorithms (Chronux Analysis Software, http://chronux.org; Delorme and Makeig, 2004; Mørup et al., 2007; Romcy-Pereira et al., 2008). These tools are extremely reliable, but are not usually adjusted for large data sets with multiple parameters, as those acquired in long-term pharmacology studies. In this work, we propose the use of a simple methodology to evaluate long-term recordings of motor activity, temperature and electrocorticogram acquired through telemetry in Sprague–Dawley rats. The main objective of this paper is to present a time efficient method for the evaluation of large amounts of telemetry data and estimation of SE duration. Most pharmacological studies sample data in fixed windows not resulting in a large accumulation of data. In our case, groups of animals are monitored with telemetry up to 18 days. Reducing the sampling rate and/or the number of samples is not an option since each animal may respond differently to exposure and treatment. Events like seizures do not occur in pre-determined periods. As a result, the need for time efficient tools for analysis of large data sets led us to elaborate a simple and non-time consuming method for evaluation of EEG, activity, and temperature data collected through telemetry. Biological parameters such as temperature and activity must be analyzed and correlated with neurophysiologic measurements in a timely manner such that group statistics can be examined. A series of algorithms are presented here that were prepared in MATLAB R2008a (The MathWorks, Inc., Novi, MI) to accomplish these tasks using a universal file format (European Data Format; EDF; Kemp et al., 1992; Kemp and Olivan, 2003). The algorithms reduce the presence of artifacts in the recorded data by removing high amplitude artifacts and performing spectral decomposition using fast Fourier and wavelet transforms (Shao et al., 2002; Khan and Gotman, 2003; Drongelen, 2006), while also preserving spectral and temporal characteristics of the signal. Large data sets are batch processed minimizing user intervention and allowing more expansive, unbiased, and complete interpretation of results. A graphical user interface (GUI) was created to allow easy access to the analysis tools.

2. Materials and methods

2.1. Surgical preparation of subjects

Male Sprague–Dawley rats ($n = 77$) were surgically implanted with a subcutaneous wireless transmitter (Data Systems International - DSI, Arden Hills, MN), to record bi-hemispheric cortical EEG waveform activity as well as body temperature and motor activity (3 days pre-exposure and 14–15 days post-exposure). The electrodes are made of stainless steel (cortical screws) and placed in the frontal and parietal cortices at 5 mm mediolateral to the sagital suture, 5 mm anteroposterior to Bregma (4 total screws). For the first 3–5 days following surgery, animals were continuously monitored for signs of infection or other factors that might interfere with pre- or post-exposure assessments. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NRC Publication, 1996 edition. Throughout the present study, rats were allowed free access to food and water and were kept in a reverse, 12/12-h light–dark cycle.

2.2. Telemetry recording

Rats were placed in individual cages, each positioned on AM radio receiving pads (RPC-1; Data Systems International - DSI, Arden Hills, MN) that detect the signals from the transmitter and send them to an input exchange matrix. Each analog output for an animal (two channels) was connected to the analog input matrix, capable of receiving input from up to four receivers. Data was digitized at 250 Hz using PCI-card model number CQ2240 (Data Systems International - DSI, Arden Hills, MN) that receives input from the exchange matrix. The signal was then sent to a computer and telemetry data was recorded through Dataquest ART 4.1 (Acquisition software; Data Systems International - DSI, Arden Hills, MN). Two channels of cortical EEG data, one channel monitoring temperature and one channel collecting activity measures, were recorded in 16 rats simultaneously. The DSI transmitter uses a voltage-controlled oscillator which converts the biopotential difference into a frequency signal. The biopotential channels are encoded in pulse-to-pulse intervals that are transmitted by the F40-EET as RF waves. The relationship between the transmitted interval in microseconds and the input signal in millivolts is described by the calibration entered into the Dataquest ART 4.1 in units of microseconds per millivolts. Attenuation of the signal is very low due to the close proximity of the transmitter to the receiver. The filtering at the device level for the system (implant and acquisition system) is described as less that 3 dB attenuation at 1 Hz and 50 Hz in the case of the F40-EET. The filtering within the implanted transmitter is nominally 0.6 Hz (−3 dB) for the high-pass filter and 60 Hz (−3 dB) for the low-pass filter. It is generated by one-pole of high-pass filtering and one-pole of low-pass filtering.

The activity of each animal is derived from the strength of the signal and the signal strength is sampled at 16 Hz. When the signal strength changes by a set amount, the data exchange matrix generates an activity count. The number of counts is proportional to both distance and speed of movement. However, the activity is a relative measure, not the distance traveled. Approximately 0.2 gigabytes (GB) of data were accumulated per rat per day, resulting in about 4 GB per rat over the course of the entire experiment.

2.3. Exposure

Following a 7-day post-surgical stabilization and baseline EEG recording period, rats were transported to the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) for exposure to soman. On the day of exposure, the oxime scavenger HI-6, a cholinesterase reactivator (125 mg/kg, i.p.) was given 30 min prior to 1 LD_{50} of soman (110 μg/kg, s.c.). One minute after soman exposure, atropine sulfate, a competitive cholinergic inhibitor (2 mg/kg, i.m.) was administered to mimic available field treatment regimens. Additionally, diazepam (DZP; 10 mg/kg, s.c.), a GABA complex