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Short communication

Reductions in paradoxical sleep time in adult rats treated neonatally with low dose domoic acid

D.A. Gill[a], J.F. Bastlund[b], N.J. Anderson[b], R.A. Tasker[a,*]

[a] Department of Biomedical Sciences, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, Canada C1A 4P3
[b] H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Copenhagen, Denmark

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ABSTRACT

One hallmark of neurological dysfunction is a reduction in paradoxical sleep (PS) time. To determine if adult rats treated neonatally with low dose domoic acid have altered sleep patterns, a home cage analysis of electroencephalogram (EEG) waveforms was performed using radio telemetry. Domoate treated rats spent significantly less time in PS than controls during daytime hours even though they spent the same total amount of time sleeping, and showed no difference in stage shifts into the PS stage.

In the rat brain, the first few postnatal weeks are a critical period for the development of appropriate neuronal connectivity, and a disruption to this process may result in far-reaching alterations that persist well into adulthood. It has been previously demonstrated that exposing rat pups to very low, sub-convulsant doses (20 μg/kg) of domoic acid (DOM) during the second postnatal week of life results in long-term changes in both behaviour and hippocampal cytoarchitecture and neurochemistry that are consistent with many current animal models of temporal lobe epilepsy [9,12,15,16,26] as well as other forms of neurological disease [1,2,10,11]. The changes in this new model (herein referred to as novelty induced seizure-like; NIS-L) appear to be progressive, eventually culminating in cell loss at later time points [12]. Additionally, decreases in both generalized and focal seizure threshold using chemical and electrical paradigms [15,16] have also been reported in NIS-L rats, further linking early DOM exposure with modified excitatory function in adulthood.

A naturally occurring marine toxin that is structurally similar to kainic acid, DOM is believed to exert its excitatory effects primarily through an agonist action at the kainate sub-family of glutamate receptors, although it can also activate all members of the glutamate receptor group at varying concentrations (for review, see [25]). Therefore, in addition to the importance of understanding the possible consequences of DOM ingestion from a clinical perspective, the compound is also a valuable tool for investigation into neuronal alterations that can result from disruptions to the glutamate system either during adulthood, or at various time points during central nervous system (CNS) development.

The connection between sleep and seizure has been well documented in epilepsy literature [33,7,21,19], and many studies have further defined specific correlations between the duration of particular sleep stages and seizure incidence [23,31,18,22]. In most reports, two main sleep stages are evaluated; slow-wave sleep (SWS), sometimes referred to as non-REM (NREM) sleep, that consists mainly of waveforms in the lower frequency range with no appreciable electromyogram (EMG) activity, and rapid eye movement (REM) sleep, defined as sleep consisting of higher frequency (e.g. beta) EEG, again with no appreciable accompanying EMG. This latter form of sleep is also known as paradoxical sleep (PS) due to the striking similarity of its waveforms to those of an awake brain, and it is this terminology that will be employed in the current study. Altered sleep patterns have long been recognized as symptomatic of many forms of neurological dysfunction including epilepsy [7], stroke [4], demyelinating disease [3] and schizophrenia [24]. In this respect, changes in PS can be an important indication for the relevance of an animal model to specific pathological disorders.

In the last few years, the study of both seizure activity and sleep patterns has been greatly enhanced through the use of techniques such as radiotelemetry, which allows EEG waveforms to be recorded from freely moving rats [5,6,34]. This approach provides a

* Corresponding author. Tel.: +1 902 566 0662; fax: +1 902 566 0832.
E-mail address: tasker@upei.ca (R.A. Tasker).

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flexible method for the investigation of brain wave activity without requiring tethering, or other similar on-going experimenter manipulations that could interfere with the animal's normal, day-to-day activities. Additionally, the procedure allows for even more versatility through the simultaneous recording of an EMG lead, providing valuable information for studies such as those involving sleep, which rely on a moment by moment analysis of activity levels for conducting accurate assessments.

In the current study, PS time was evaluated in both treated and control rats using home cage analysis of cortical EEG and EMG waveforms from adult animals implanted with radio telemetry instrumentation. Since the NIS-L model is considered to be characteristic of temporal lobe epilepsy (TLE), it was hypothesized that treated rats would experience decreased PS sleep, consistent with both current animal models of epilepsy, as well as the human condition.

Animals in this study were part of a larger investigation looking at EEG alterations in DOM-treated rats following watermaze exposure, and consisted of male offspring born in-house from untimed Sprague–Dawley rats (Charles River, Germany) that were culled within 24 h of birth to a maximum of 10 pups per litter. From postnatal day (PND) 8–14, pups were weighed and given a single daily injection (10 ml/kg, s.c.) of either 20 μg/kg domoic acid (DOM) (BioVectra dcl, Canada) or saline (SAL), as described previously [12]. On PND22, rats were weaned and group housed (2–4 per cage) under a 12 h light–dark cycle (lights on at 06:00 h) in plastic cages (43 cm × 27 cm × 18.5 cm), with a plastic tunnel (14 cm × 9 cm × 8.5 cm) and a wooden block (5 cm × 1 cm × 1 cm) as environmental enrichment and were left undisturbed, except for normal maintenance, until the commencement of surgeries. Room temperature was maintained at 21 ± 2 °C and relative humidity was 55 ± 5%. Standard rodent food (SDS, UK) and tap water was available *ad libitum*. Following surgery, rats were individually housed for the duration of testing. All surgeries and testing were performed between 09:00 and 18:00 h, and were carried out under a license from the Danish Ministry of Justice, and in accordance with the EC Directive 86/609/EEC and the Danish law regulating experiments on animals.

At approximately 90 days of age, rats were anaesthetized with a 0.2 ml/100 g (s.c.) injection of one part Hypnorm (0.315 mg/ml fentanyl + 10 mg/ml fluanisone, Jassen Inc., USA), and one part Dormicom (5 mg/ml midazolam, F. Hoffmann-La Roche AG, Switzerland) in two parts sterilized water. Radiotelemetry transmitters (TL10M3-F50-EEE or TL10M3-F50-EET, Data Sciences International, St. Paul, MN, USA) were surgically implanted in a subcutaneous pocket formed on the animal's left flank for continuous cortical (n = 20; 10-DOM, 10-SAL) EEG/EMG recording as described previously [6]. Cortical placements consisted of two leads positioned each side of midline and ~2 mm anterior to bregma and two leads ~2 mm anterior to lambda to generate a total of two cortical bio-potentials, as well as two leads implanted into the *musculus cervicoauricularis* providing one EMG feed. All rats were treated prior to surgery and for 7 days post-surgery with Rimadyl vet. (1 ml/kg s.c., 1:10 dilution, Pfizer, Denmark) and Baytril vet. (2 ml/kg s.c., 1:10 dilution, Bayer Health Care, Denmark) and allowed 3 full weeks to recover. Further pain relief was given immediately following surgery through an s.c. injection of Temgesic (0.3 mg/ml buprenofin, Schering-Plough, USA). Local irritation was treated with 5% Xylocaine crème (lidocainechloride, AstraZeneca, Norway).

The telemetry system used to record EEG/EMG from animals was composed of TL-10M3-F50-EEE (3 bio-potential) and TL-10M3-F50-EET (2 bio-potential) magnetic activated transmitters, RPC-1 receiver plates, data exchange matrices, and computers installed with Dataquest A.R.T. 2.2 (Data Sciences International, USA). The sampling frequency was set at 250 Hz for all recordings. The data for the current study represent one discrete 24 h continuous

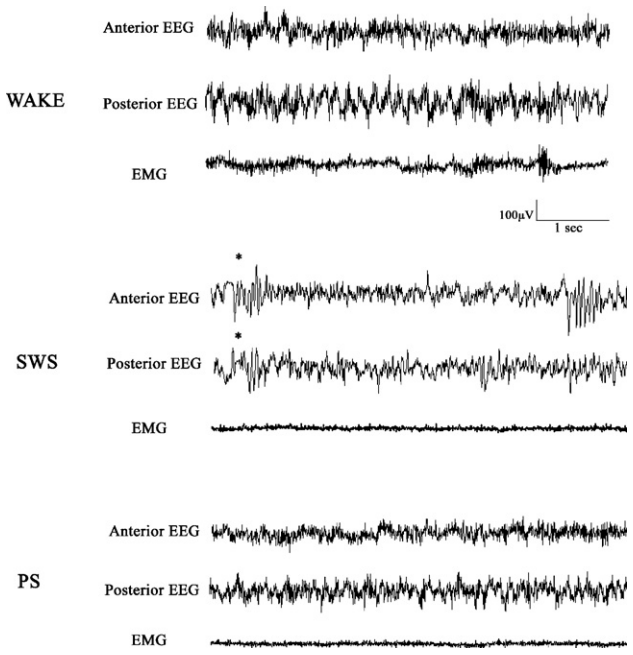

Fig. 1. EEG/EMG tracings showing a representation from the same rat for each of the three defined stages used for analysis: wake, slow-wave sleep (SWS), and paradoxical sleep (PS). Note the rhythmic EEG activity and characteristic sleep spindles in both anterior and posterior leads (indicated by asterisks) and lack of EMG activity during slow-wave sleep, while EEG patterns in paradoxical sleep revert back to waveform similar to that of the awake animal, and the EMG remains quiet.

EEG monitoring of home cage activity for all animals with care taken to minimize and/or eliminate any possible environmental confounds.

All traces generated by radiotelemetry EEG (both anterior and posterior leads) and EMG bio-potentials were imported in Somnologica 3.1.0.1171 software (Flaga Medical Devices, Iceland) for visualization and analysis through the Animal Sleep Report Rodent Scoring Module (v3.3.1). Measurements included duration and number of episodes for the following stages: (1) wake (moderate to high frequency EEG with EMG activity); (2) SWS (predominant waveforms in the lower frequency range and no EMG activity) and (3) PS (higher frequency (e.g. beta) EEG with no EMG activity) (Fig. 1).

The presence of artifacts in EEG and/or EMG signals can lead to faulty scoring of affected epochs when using automated scoring algorithms. To ensure that only robustly scored recordings were included in the final analysis, an operator experienced in manual EEG/EMG analysis discarded traces in which artifact levels would produce unreliable scoring. In total, 8 out of 18 recordings were discarded in this manner, such that N = 10 (5-DOM, 5-SAL). Between groups differences were analyzed using independent t-tests (one-tailed), and results are expressed as mean ± SEM. Analyses were performed using SAS (v.6.11), with α = 0.05.

This study was conducted to explore possible alterations in sleep duration in the NIS-L rat model. The first parameter measured was total sleep time. All rats slept an equivalent total amount during the 24 h analyzed, with treated rats sleeping an average of 841.7 min (SEM ±59.9), and controls experiencing 804.4 min (SEM ±40.8) of total sleep time. This similarity in total sleep remained when the time period was divided into two 12 h sessions representing daylight (when nocturnal animals such as rats would normally sleep), and night (when the majority of time would be spent awake) (data not shown).

To determine whether postnatal DOM treatment produced long-term effects on the amount of time spent in each sleep stage,