Journal of Neuroscience Methods 259 (2016) 90–100

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Computational neuroscience

Noninvasive dissection of mouse sleep using a piezoelectric motion sensor

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HIGHLIGHTS

- A piezoelectric sensor can accurately differentiate sleep from wake and sense breathing in mice.
- Piezoelectric signal features were clustered into multiple states using a hidden Markov model.
- Sleep states that differed in breathing regularity were strongly correlated with REM/NREM.
- This technology will permit high-throughput screening of sleep traits for genetic or drug studies.

ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form 1 October 2015
Accepted 4 November 2015
Available online 12 November 2015

Keywords:
Noninvasive sleep scoring
Mouse
High-throughput screening
Genetics
Sleep
REM
NREM
Piezoelectric
EEG
Supervised
Unsupervised
Hidden Markov model

ABSTRACT

Background: Changes in autonomic control cause regular breathing during *NREM* sleep to fluctuate during *REM*. Piezoelectric cage-floor sensors have been used to successfully discriminate sleep and wake states in mice based on signal features related to respiration and other movements. This study presents a classifier for noninvasively classifying *REM* and *NREM* using a piezoelectric sensor.
New method: Vigilance state was scored manually in 4-s epochs for 24-h EEG/EMG recordings in 20 mice. An unsupervised classifier clustered piezoelectric signal features quantifying movement and respiration into three states: one active; and two inactive with regular and irregular breathing, respectively. These states were hypothesized to correspond to *Wake*, *NREM*, and *REM*, respectively. States predicted by the classifier were compared against manual EEG/EMG scores to test this hypothesis.
Results: Using only piezoelectric signal features, an unsupervised classifier distinguished *Wake* with high (89% sensitivity, 96% specificity) and *REM* with moderate (73% sensitivity, 75% specificity) accuracy, but *NREM* with poor sensitivity (51%) and high specificity (96%). The classifier sometimes confused light *NREM* sleep – characterized by irregular breathing and moderate delta EEG power – with *REM*. A supervised classifier improved sensitivities to 90, 81, and 67% and all specificities to over 90% for *Wake*, *NREM*, and *REM*, respectively.
Comparison with existing methods: Unlike most actigraphic techniques, which only differentiate sleep from wake, the proposed piezoelectric method further dissects sleep based on breathing regularity into states strongly correlated with *REM* and *NREM*.
Conclusions: This approach could facilitate large-sample screening for genes influencing different sleep traits, besides drug studies or other manipulations.

1. Introduction

Our understanding of sleep is advancing rapidly; yet every milestone achieved in probing the structure and function of this intriguing phenomenon reveals a new layer of complexity. Many sleep-related peculiarities are, at least in part, heritable (O'Hara and Mignot, 2000; Franken and Tafti, 2003). Genetic dissection using animal phenotypes is therefore expected to provide fundamental insights into sleep and wakefulness. Among mammals, mice have the best genetic and genomic resources for finding the genes that contribute to each sleep trait and they are increasingly being used to characterize behavior for genetic and drug studies. For instance, quantitative trait locus (QTL) analysis (Hunter and Crawford, 2008) is one technique that has been used for identifying genome regions

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http://dx.doi.org/10.1016/j.jneumeth.2015.11.004
0165-0270/

associated with polygenic traits that can be quantified on a continuous scale, such as the mean duration of sleep bouts. However, discovery of sleep-related genes involves screening large cohorts to correlate observed behavior with genetic profile, which is time-consuming.

The gold standard method for sleep analysis in mammals is polysomnography, a panel of simultaneous physiological measurements that comprises an electroencephalogram (EEG) and electromyogram (EMG) (Steriade, 2000) at a minimum. Three major vigilance states are defined in mice based on EEG/EMG appearance: (1) Wakefulness (*Wake*), characterized by low amplitude, broadband EEG and high-powered, variable EMG; (2) Paradoxical or rapid eye movement sleep (*REM*), characterized by a theta EEG rhythm (6–9 Hz) and suppressed EMG (except for occasional muscle twitches); and (3) Non-REM sleep (*NREM*), sometimes termed slow wave sleep, characterized by low frequency, large amplitude delta EEG oscillations (0.5–4 Hz) and low, tonic EMG. EEG/EMG measurement in rodents is an invasive and resource-intensive process, a critical barrier to the discovery of sleep-related genes. While the only acceptable way to accurately discriminate vigilance state is through manual or automated scoring of the EEG/EMG signals, the required effort (surgery, recovery, etc.) limits the use of EEG/EMG in the large-scale experiments needed for genetic analysis of rodent behavior. Besides, a tether is often required for signal acquisition, which may restrict natural behavior and make simultaneous screening of multiple animals difficult. Non-tethered telemetric systems exist but still require the invasive implantation of electrodes, battery pack, preamplifier, and transmitter that may again alter behavior and hinder movement, especially in small animals like the mouse.

Many noninvasive behavioral phenotyping systems have been devised for small animals that use video tracking (Publicover et al., 2009), wheel running (Wisor et al., 2009), light beam breaking (Nairizi et al., 2009), and accelerometry (Venkatraman et al., 2010; Brodkin et al., 2014) to distinguish gross and subtle awake behaviors such as grooming, feeding, locomotion, rearing, circling, and even quiet wakefulness from sleep. Although these technologies have potential for high-throughput use, they mainly perform actigraphy: none of them reliably separate sleep into its components, namely *REM* and *NREM*, and none have been used effectively in high-throughput studies. A completely noninvasive system that discriminates between *REM* and *NREM* in mice as reliably as EEG/EMG would greatly facilitate high-throughput screening for the discovery of genes relevant to sleep and sleep-related disorders.

Physiological differences in *REM* regulation help us distinguish it from *NREM*. Skeletal muscle tone, already low in sleep, is further inhibited during *REM* leading to a visibly flaccid posture when compared to *NREM*. The feasibility of detecting this change in aspect during *REM* using videographic image analysis has recently been investigated (McShane et al., 2012) and appears promising, but was not deemed feasible in other video studies (Fisher et al., 2012) and is harder to perform with high throughput. Skeletal muscle paralysis is not the only peculiarity associated with *REM* though: mentation and irregular autonomic activity can produce ballistic eye movements (hence the name *REM*), variable heart rate (Calasso and Parmeggiani, 2008), irregular breathing (Friedman et al., 2004), phasic muscle twitches (Geisler et al., 1987), and even middle ear muscle activity (Benson and Zarcone, 1979). The observation that the regular breathing associated with *NREM* becomes irregular in *REM* (Friedman et al., 2004) suggests that a contact sensor that responds to ventilatory movement might be useful for telling them apart. In fact, a piezoelectric sensor placed on the mouse cage floor is known to detect pressure variations associated with respiratory effort when the animal is relatively still (Flores et al., 2007). The resulting quasirhythmic "piezo" signal differentiates sleep from quiet or active wakefulness with accuracy comparable to a human observer (Donohue et al., 2008). Mang et al. (2014) found that the decision statistic used by the same classifier to distinguish sleep from wakefulness (Donohue et al., 2008) appears to change in value following *REM–NREM* transitions as well. Sato et al. (2010) used a piezoelectric transducer to monitor mice and documented rapid increases in breathing rate during sleep with atonic posture, presumably in *REM*. They subsequently used this piezoelectric system to differentiate *REM* from *NREM* and *Wake*, but on the basis of immobility and perceived heart rate signals, in a small sample of wild type mice (Sato et al., 2014).

The literature cited above strongly suggests that the piezo could detect episodes of *REM* in mice based on signal changes associated with the irregular respiratory rhythm, but it does not tell us whether these measured respiratory changes occur in *REM* alone. The purpose of the present investigation is to determine how well behavioral states that are separable in terms of muscle tone and respiratory rhythm (as quantified by the piezo signal) correspond to electrophysiologically distinct vigilance states: namely *Wake*, *NREM*, and *REM*. To answer this question, first piezo signal features indicative of breathing regularity and muscle tone are extracted from 24-h recordings in each of 20 mice in sequential epochs. Each time series of piezo signal features is automatically segmented using an unsupervised hidden Markov model (HMM) classifier into states that form natural clusters in the feature space in terms of breathing regularity and muscle tone. The states identified by the HMM are compared with the true vigilance states (*Wake*, *NREM*, and *REM*) determined by manual scoring of simultaneously acquired EEG/EMG measurements. The concordance between piezo-derived state scores and manual scores is assessed and analyzed for potential sources of error. Finally, conclusions are drawn regarding the feasibility of using the piezo sensor to noninvasively stage sleep in mice, which is expected to reduce the need for EEG/EMG analysis in large-sample screening of sleep phenotypes.

2. Methods

2.1. Overview

EEG, EMG, and piezo signals were acquired from mice along with video for a 24-h period. Two human raters blinded to the piezo signal independently labeled vigilance state in sequential 4-s epochs as *NREM*, *REM*, or *Wake* by inspecting the EEG, EMG and video recordings and using conventional criteria. Features designed to quantify subtle movements and breathing from the piezo signal were estimated for each epoch of data. Natural clusters that separated the data into three distinct states were identified in the feature space: a relatively high activity state with variable breathing patterns; a low activity state characterized by a regular breathing rhythm; and another quiescent state but with relatively irregular breathing. Epochs of the piezo feature time series were mapped onto these three behavioral states using an unsupervised probabilistic classifier – a hidden Markov model (HMM) – with the general expectation that the states would correspond to *Wake*, *NREM*, and *REM*, respectively. Finally, the model-predicted states were compared against consensus human scores to assess whether this expectation was reasonable and enabled noninvasive classification of vigilance state and estimation of commonly used sleep metrics.

2.2. Animal procedures

All procedures used in the study were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky. Twenty adult male mice (C57BL/6J, Jackson Labs, Bar Harbor, Maine, USA), each 8–10 weeks old and weighing 24–29 g, were