Journal of Neuroscience Methods 235 (2014) 277–284

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Computational Neuroscience

SCOPRISM: A new algorithm for automatic sleep scoring in mice

Stefano Bastianini[a], Chiara Berteotti[a], Alessandro Gabrielli[c], Flavia Del Vecchio[b], Roberto Amici[b], Chloe Alexandre[d], Thomas E. Scammell[d], Mary Gazea[e], Mayumi Kimura[e], Viviana Lo Martire[a], Alessandro Silvani[a], Giovanna Zoccoli[a,*]

[a] PRISM Lab, Alma Mater Studiorum – University of Bologna, Bologna, Italy
[b] Physiological Regulation in Wake-Sleep Cycle Lab, Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum – University of Bologna, Bologna, Italy
[c] Department of Physics and Astronomy, Alma Mater Studiorum – University of Bologna, Bologna, Italy
[d] Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA, USA
[e] Max Planck Institute of Psychiatry, Munich, Germany

HIGHLIGHTS

- We tested a new open-source sleep-scoring algorithm (SCOPRISM) on 92 mice.
- We successfully validated SCOPRISM in wild-type mice and mouse models of obesity and narcolepsy.
- We cross-validated SCOPRISM on mice and rats recorded and analyzed in other labs.
- We developed a quick and easy visual flow-chart for the correct use of SCOPRISM.

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 23 July 2014
Accepted 24 July 2014
Available online 1 August 2014

Keywords:
Sleep
Scoring
Validation
Mice
Rat
Narcolepsy

ABSTRACT

Background: Scoring of wake–sleep states by trained investigators is a time-consuming task in many sleep experiments. We aimed to validate SCOPRISM, a new open-source algorithm for sleep scoring based on automatic graphical clustering of epoch distribution.
Methods: We recorded sleep and blood pressure signals of 36 orexin-deficient, 7 leptin knock-out, and 43 wild-type control mice in the PRISM laboratory. Additional groups of mice ($n = 14$) and rats ($n = 6$) recorded in independent labs were used to validate the algorithm across laboratories.
Results: The overall accuracy, specificity and sensitivity values of SCOPRISM (97%, 95%, and 94%, respectively) on PRISM lab data were similar to those calculated between human scorers (98%, 98%, and 94%, respectively). Using SCOPRISM, we replicated the main sleep and sleep-dependent cardiovascular findings of our previous studies. Finally, the cross-laboratory analyses showed that the SCOPRISM algorithm performed well on mouse and rat data.
Comparison with existing methods: SCOPRISM performed similarly or even better than recently reported algorithms. SCOPRISM is a very simple algorithm, extensively (cross)validated and with the possibility to evaluate its efficacy following a quick and easy visual flow chart.
Conclusions: We validated SCOPRISM, a new, automated and open-source algorithm for sleep scoring on a large population of mice, including different mutant strains and on subgroups of mice and rats recorded by independent labs. This algorithm should help accelerate basic research on sleep and integrative physiology in rodents.

Abbreviations: BP, blood pressure; EEG, electroencephalographic; EMG, electromyographic; HTG, orexin-ataxin3 narcoleptic transgenic mice with hybrid genetic background; KO, orexin knock-out narcoleptic mice; NREMS, non-rapid-eye movement sleep; Ob/ob, leptin knock-out obese mice; REMS, rapid-eye movement sleep; TG, orexin-ataxin3 narcoleptic transgenic mice with pure genetic background; W, wakefulness; WT, wild-type mice.

* Corresponding author at: Dipartimento di Scienze Biomediche e Neuromotorie, Alma Mater Studiorum – Università di Bologna, Piazza di Porta San Donato 2, 40126 Bologna, Italy. Tel.: +39 051 2091726; fax: +39 051 2091737.
E-mail address: giovanna.zoccoli@unibo.it (G. Zoccoli).

http://dx.doi.org/10.1016/j.jneumeth.2014.07.018
0165-0270/

1. Introduction

Scoring wake–sleep states based on electroencephalographic (EEG) and electromyographic (EMG) recordings is a time-consuming process, yet reliable scoring is critical in sleep research. Pre-clinical sleep laboratories increasingly investigate mouse models because of the power of genetic tools applicable to this species. Most of these laboratories base their studies on manual sleep scoring by trained investigators (El Helou et al., 2013; Gondard et al., 2013; Kantor et al., 2013). However, this often becomes the experimental bottleneck and a potential source of subjectiveness affecting research outcomes. To overcome these difficulties, different commercial or open-source algorithms for automatic sleep scoring have been proposed in the last few years (Brankack et al., 2010; Rytkonen et al., 2011; Sunagawa et al., 2013; Veasey et al., 2000). Some of these algorithms are computationally intensive (Sunagawa et al., 2013), or have not been tested on independent datasets (i.e. cross-laboratory validation) (Brankack et al., 2010; Rytkonen et al., 2011; Sunagawa et al., 2013; Veasey et al., 2000). Subjectiveness still represents a problem for those algorithms that require a pre-stage of manual scoring on a subset of recording data (Rytkonen et al., 2011). Finally, validation of these algorithms has been performed either on a limited number of mice ($n = 6$–9) (Brankack et al., 2010; Rytkonen et al., 2011; Sunagawa et al., 2013) or only on non-mutant mice only (Brankack et al., 2010; Rytkonen et al., 2011; Veasey et al., 2000). Importantly, a sleep-scoring algorithm have not been validated in mouse models of narcolepsy (Chemelli et al., 1999; Hara et al., 2001), one of the most intensively studied human sleep disorders. On the other hand, recent technical tools, such as telemetric devices, allow researchers to measure cardiovascular and respiratory variables simultaneously with EEG and EMG (Bastianini et al., 2011; Lo Martire et al., 2012; Silvani et al., 2009). The development of an automatic sleep-scoring algorithm tested on mutant mice with multiple physiological recordings would thus accelerate integrative physiology as well as behavioral studies.

In the past few years, we have investigated sleep structure and sleep-dependent cardiovascular control in different strains of mutant mice, including leptin-deficient mice with genetic obesity and hypocretin (orexin) deficient mice as a model of narcolepsy (Bastianini et al., 2011; Lo Martire et al., 2012; Silvani et al., 2009). All these studies involved manual sleep scoring at a high (4 s) temporal resolution by trained investigators. To speed up the procedure, manual sleep scoring consisted of using raw EEG and EMG recordings to correct or confirm a suggestion provided by an automatic sleep-scoring algorithm, which we had developed for this purpose.

Here, we aimed to retrospectively validate the performance of our automatic sleep-scoring algorithm (SCOPRISM) in correctly discriminating wake–sleep states in different mouse strains. In particular, our validation procedure consisted of 3 main steps: (a) we evaluated the algorithm performance in terms of accuracy, specificity, and sensitivity of wake–sleep state discrimination. We paid particular attention to algorithm sensitivity, which is usually a point of weakness of automatic algorithms (Rytkonen et al., 2011; Sunagawa et al., 2013), especially in terms of rapid-eye-movement sleep (REMS) discrimination. (b) We tested whether differences between groups of mutant mice, which we previously found and published in terms of sleep structure and sleep-dependent cardiovascular control employing manual sleep scoring (Bastianini et al., 2011; Lo Martire et al., 2012; Silvani et al., 2009), would still have been significant had we relied on automatic sleep scoring only. (c) We performed a cross-laboratory validation evaluating the robustness of the SCOPRISM algorithm on mouse and rat data recorded and analyzed by independent research teams.

2. Materials and methods

The study protocols were approved by the Bologna University ethics committee on animal experimentation and complied with the National Institutes of Health guide for the care and use of laboratory animals.

2.1. Mouse strains

The study involved a total of 86 male mice recorded in the PRISM lab and already described in other publications (Bastianini et al., 2011; Lo Martire et al., 2012; Silvani et al., 2009). In particular, groups consisted of: (a) hypocretin-ataxin3 transgenic (TG) narcoleptic mice (Hara et al., 2001) with genetic ablation of hypocretin neurons and with pure (TG, $n = 12$) or hybrid (Hara et al., 2005) (HTG, $n = 16$) C57BL/6J genetic background; (b) hypocretin gene knock-out mice (Chemelli et al., 1999) (KO, $n = 8$); (c) leptin-deficient mice (ob/ob, $n = 7$, Harlan Laboratories, Holland); (d) merged group of all WT controls ($n = 43$) including mice with pure ($n = 26$) or hybrid ($n = 17$) C57BL/6 genetic background. For cross-lab SCOPRISM validation, we also analyzed two additional groups of WT mice recorded and scored at Beth Israel Deaconess Medical Center (T.E.S. and C.A; $n = 8$; mice were purchased from Jackson Lab, Bar Harbor, ME, USA) and at the Max Planck Institute of Psychiatry (M.K. and M.G.; $n = 6$; C57BL/6N mice were bred in the facility of Max Planck Institute of Biochemistry, Martinsried, Germany). Finally, for cross-species validation of SCOPRISM, we analyzed a group of WT (Sprague-Dawley) rats recorded and analyzed in the lab of physiological regulation in wake–sleep cycle, Department of Biomedical and Neuromotor Sciences in Bologna, Italy (R.A. and F.D.V; $n = 6$; rats were purchased from Charles River Italy).

2.2. Surgery, sleep recordings, and data acquisition

All the mice recorded in the PRISM lab were implanted with a pair of stainless-steel miniature screws (00-96 × 3/32, Plastics One, Roanoke, 6 VA, USA) put in contact with the dura mater to obtain an ipsilateral fronto-parietal EEG signal (differential derivation). The frontal screw was placed 1 mm anterior and 1 mm lateral to bregma. The parietal screw was placed 1 mm anterior and 1 mm lateral to lambda. A pair of Teflon-coated stainless steel wires (Cooner Wire, Chatsworth, CA, USA) was inserted in the posterior neck muscles to record the EMG signal. The EEG and EMG signals were transmitted with a cable connected to a rotating swivel (SL2 + 2C/SB, Plastics One, Roanoke, 6 VA, USA). Mice were also implanted with a telemetric blood pressure (BP) transducer (TA11PA-C10, DSI, Tilburg, the Netherlands) connected to a catheter inserted into the abdominal aorta. Simultaneous recordings of EEG, EMG and BP were performed for at least 44 h on mice freely behaving in their own cages. Ambient temperature during recordings was always set at 25 °C except for 10 HTG and 8 WT mice, which were recorded at 30 °C (Lo Martire et al., 2012). The EEG and EMG signals were amplified, filtered (EEG: 0.3–100 Hz; EMG: 100–1000 Hz; 7P511J amplifiers, Grass, West Warwick, RI, USA), sampled at 1024 Hz, and down-sampled at 128 Hz for data storage. The EEG and EMG amplifier gains were adjusted for each mouse to avoid signal saturation. The BP signal was sampled at 1024 Hz. For mouse recordings in the T.E.S. lab, epidural stainless steel screws electrodes (Plastic Ones) were implanted for ipsilateral frontoparietal EEG recordings (differential derivation; 1.5 mm lateral and 1 mm anterior to bregma; 1.5 mm lateral and 1 mm anterior to lambda). EMG electrodes were made from fine, multi-stranded stainless steel wire (AS131; Cooner Wire), and were inserted into the neck extensor muscles. All electrodes were attached to a micro-strip connector affixed to the animal's head with dental cement. EEG/EMG signals were acquired