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Journal of Neuroscience Methods 148 (2005) 43–48

JOURNAL OF NEUROSCIENCE METHODS

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A visual aid for computer-based analysis of sleep–wake state in rats

Viara R. Mileva-Seitz, Rhain P. Louis, Richard Stephenson *

Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ont., Canada M5S 3G5

Received 12 August 2004; received in revised form 21 October 2004; accepted 5 April 2005

Abstract

Computer-based sleep scoring systems are often calibrated by reference to a conventional visual analysis of electroencephalographic (EEG) and electromyographic (EMG) traces. However, these types of data place high demands on digital storage capacity which may limit the duration or feasibility of some studies. The present paper describes an approach to visual analysis that involves reconstruction of a waveform (termed a "pseudopolygram" (PPG)) from conditioned data derived from the EEG and EMG. The PPG is the sum of three sine waves, each of which has a distinct frequency (non-REM sleep (NREM), 3 Hz; rapid eye movement sleep (REM), 7 Hz and wakefulness (WAKE), 60 Hz) and amplitude proportional to the value of a state-specific scoring variable. Thus, in NREM sleep the wave depicting the NREM quantifier has high amplitude and produces a PPG with dominant 3 Hz frequency. In REM sleep, the wave depicting the REM quantifier has high amplitude and produces a PPG with a dominant 7 Hz frequency, and in WAKE the PPG is dominated by 60 Hz. Thus, the PPG provides a means for visual discrimination of the three behavioural states. Validation studies found an overall reliability of 94% compared with conventional visual analysis of EEG and EMG. The PPG was also found to remain accurate in rats after 24 h of sleep deprivation.

Keywords: Wistar rats; Sleep; Electroencephalogram; Electromyogram; Pseudopolygram

1. Introduction

The assignment of behavioural state is conventionally based on the visual interpretation of a "polysomnogram" (PSG), which consists minimally of electroencephalographic (EEG) and electromyographic (EMG) records, sometimes in combination with other physiological and behavioural variables (Robert et al., 1999). PSG recordings from rats are usually divided into epochs of 5–30 s in duration and each epoch is usually assigned one of three behavioural states; wakefulness (WAKE), rapid eye movement sleep (REM), or non-REM sleep (NREM), although additional states or stages are sometimes defined (Gottesmann, 1992). The monitoring and recording of sleep–wake states in mammals is labour-intensive and can be error-prone because assessment of the PSG is not entirely objective, involving a process of pattern recognition guided by rules based on established correlations between behaviour and electrophysiology (Costa-Miserachs et al., 2003; van Luijtelaar and Coenen, 1984). Many epochs are difficult to score with certainty because, for many reasons, the EEG and EMG waveforms may not appear characteristic of any one state, or they may contain transitions between states. Satisfactory resolution of this problem requires a substantial amount of training and experience on the part of the rater. It also requires sustained concentration in a task that is inherently tedious, leading inevitably to fatigue and increased risk of error over time. Thus, in studies where large quantities of data are to be acquired, and especially where analysis is required in real-time, a computer-based sleep scoring system is a practical necessity.

Numerous computer algorithms have been developed for on-line real-time sleep scoring in human and animal studies (Robert et al., 1999). Many of these have been shown in validation studies to be as accurate as human raters (Louis et al., 2004; Neckelmann et al., 1994). The majority of these computer systems are semi-automated, in that they compare incoming data, on an epoch by epoch basis, with predetermined threshold values, and assign state according to whether the data fall above or below the thresholds. The threshold

* Corresponding author. Tel.: +1 416 978 3491; fax: +1 416 978 8532.
E-mail address: rstephsn@zoo.utoronto.ca (R. Stephenson).

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doi:10.1016/j.jneumeth.2005.04.004

values themselves are derived from preliminary recordings that have been subjected to conventional visual analysis. Use of these computer-based sleep scoring systems rests on the assumption that the reliability of the computer analysis remains high over time, an assumption that only holds as long as the threshold values remain valid. Thus, the veracity of the thresholds must be checked periodically, a process that involves recording or observing EEG and EMG traces, subjecting them to visual analysis and comparing this with the computer output.

Recently, Louis et al. (2004) developed and validated a computer-based sleep scoring system (CBS^3) for use in rats. This system is semi-automated in the sense described above, requiring the input of three threshold values derived from visual analysis of the PSG. In our ongoing research we encountered a situation where, for technical reasons, we were unable to record raw EEG and EMG signals and were therefore unable to conduct periodic verification of the threshold values. This prompted us to develop an alternative approach in which a visual display (which we have termed a "pseudopolygram" (PPG)) is reconstructed from the CBS^3-conditioned PSG data. The present study describes this method and validates its use for defining threshold values and for post hoc analysis of conditioned data acquired using the CBS^3 system.

2. Methods

2.1. Subjects

Data from seven rats (three tethered and four with implanted radiotransmitters) used in a previous study (Louis et al., 2004) were subjected to further analysis in the present study. Four additional male Wistar rats (Charles River Laboratories, Saint-Constant, PQ, Canada) were used to investigate the effects of 24 h sleep deprivation (SD) on CBS^3 and PPG performance. They weighed (mean ± S.D.) 385.0 ± 157.9 g at the time of surgery. Animals were housed individually and maintained on a 12-h light:12-h dark cycle. Standard rat chow (Lab Diet, PMI Nutrition International, Brentwood, MO) and water were available ad libitum. All procedures were performed in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the animal care committee at the University of Toronto.

2.2. Surgery

Four rats were implanted under general anaesthesia with radio transmitters (model TL10M3-F50-EET, Data Sciences International, St. Paul, MN). All cranial implants contained bipolar EEG electrodes and a reference electrode, which were screwed into the skull (size 080 stainless steel) and sealed with cranioplastic cement. Bipolar EMG electrodes were implanted into the dorsal neck muscle. The electrodes were tunneled subcutaneously to the abdomen, and the transmitter was sutured to the wall of the peritoneal cavity. All animals were allowed at least one week for recovery after surgery.

2.3. Recordings

During all PSG recordings, EEG and EMG analog signals were simultaneously passed through a Pentium 4 PC running the CBS^3 program written in LabVIEW 7.0 format (National Instruments Corp., Austin TX) for online analysis (sampling at 400 Hz), and a Maclab/8 (ADInstruments, Inc., Grand Junction, CO) for conventional visual analysis (sampling at 100 Hz). Briefly, for each 5 s epoch, the EEG signal was filtered (Chebyshev IIR digital bandpass filters) into five frequency bands: delta (δ; 1.5–6 Hz), theta (θ; 6.5–10 Hz), alpha (α; 10.5–15 Hz), beta (β; 22–30 Hz), and gamma (γ; 35–45 Hz) and average EEG amplitudes (μVrms) were calculated for each band. Average EMG amplitude (μVrms, Chebyshev bandpass filter 10–100 Hz with 60 Hz notch filter) was also recorded for each epoch. The CBS^3 scoring variables were calculated as described previously, and the three-step CBS^3 algorithm (EMG-first version) was used (Louis et al., 2004).

Each epoch was assigned a state (WAKE; REM; NREM) by two approaches to visual scoring (see below) and by the semi-automated CBS^3. Any epochs that could not be scored visually were labeled either "undefined" or "transitional". Each epoch was also defined as "unequivocal", in cases of high rater confidence or "equivocal", in cases of low rater confidence. An overall confidence index (CI) was assigned for each recording:

CI (%) = (number of unequivocal epochs/total number of epochs) × 100. Only unequivocal epochs were used for further analysis in this study.

2.4. Protocol

A single rater (V.R.M.S.) conducted all sleep scoring procedures after preliminary training on separate data. In comparison with an experienced rater (R.P.L.), inter-rater agreement was 96% over 4095 epochs from seven animals using conventional visual analysis of PSG traces.

Reliability of the PPG was assessed by comparison with conventional visual analysis of raw PSG. More than 1000 sequential epochs were analyzed in each recording. For each recording, raw EEG and EMG traces were scored by conventional visual analysis and for all epochs designated as "unequivocal", the optimal threshold values (i.e. values that yielded greatest discrimination between the three states) for the CBS^3 algorithm were defined as described previously (Louis et al., 2004). The same recordings were also subjected to visual analysis using the PPG display (see below), and for those epochs designated as "unequivocal" in this analysis, the optimal threshold values were again defined. To avoid bias, half of the recordings (chosen at random) were subjected to conventional PSG visual scoring before PPG visual scoring, and the others were subjected to PPG visual scoring first. The

second analysis was conducted without reference to the results of the first analysis and the two analyses were separated by over a month. Paired *t*-tests (two-tailed) were used to determine whether the confidence indices and CBS^3 threshold values derived from the two methods of visual analysis were statistically different. CI values, expressed as ratios, were arcsine transformed before the paired *t*-test was applied.

Additional new PSG recordings were made to examine the reliability of the CBS^3 and the PPG in four rats before and after 24 h total sleep deprivation. SD was accomplished using a modified "flowerpot" method (Cohen and Dement, 1965). A Plexiglas cage (45 cm × 50 cm × 41 cm) was filled with water to a depth of 8 cm. Three rubber pedestals (5 cm diameter, protruding 2 cm above the water surface) were used as platforms for the rat to stand on. They were arranged 10 cm apart in a triangle formation, which allowed the rat to walk between them but not to sleep across them. A water bottle and food container were attached to one side of the cage. Efficacy of the SD method was confirmed by time lapse photography using a Sony HandiCam Vision Hi8 video camera (CCD-TR517 NTSC). Images were taken once every 2 min and saved to a computer in JPEG format for later examination.

As before, the first (pre-SD) PSG recording was subjected to two types of visual analysis (conventional scoring of raw PSG and scoring of PPG display) and optimal CBS^3 threshold values were derived from each. The second (post-SD) PSG recording was subjected to conventional visual analysis, and to CBS^3 analysis using each of the two sets of threshold values derived from the analyses of pre-SD data.

In all of the above analyses, "reliability" of the PPG-derived and CBS^3-derived scores was quantified for each behavioural state as:

$$\text{Reliability} = \frac{n_{\text{test}}}{n_{\text{std}}} \times 100$$

where n_{std} = number of epochs scored as a specific state using the standard method (i.e. conventional visual analysis of raw PSG) and n_{test} = number of epochs scored as the same state using the test method (i.e. PPG or CBS^3). Overall reliability (%) was calculated as the average of the three state-specific percentage reliability values.

2.5. Pseudopolygram

The main objective of this study was to use conditioned EEG and EMG data (in this case data conditioned by the CBS^3 program, although in principle other similar systems can be used instead) to recreate a visual waveform that could be used in place of the raw PSG to distinguish WAKE, NREM and REM states. CBS^3 output consists of six values per epoch (EMG amplitude plus 5 EEG amplitude values). The EEG amplitude values are combined into ratios in order to eliminate problems associated with changes in overall signal strength over time [NREM ratio = $(\delta \times \alpha)/(\beta \times \gamma)$; REM ratio = $\theta^2/(\delta \times \alpha)$]. In preliminary studies, it was found that a similar problem also applied to EMG signals in that EMG baseline differed between rats, which caused significant variation in the visual appearance of the PPG. For this reason, in the PPG analyses EMG amplitude was modified by zero suppression, using the minimum EMG amplitude value within a given recording as the zero reference. EMG baseline was found to be relatively stable for any given rat. This procedure yielded an increase of 6% in the reliability of the PPG-defined REM epochs and was used in all PPG-based visual analyses.

A PPG waveform was created on an epoch-by-epoch basis using LabVIEW (Fig. 1). Three sine waves were generated from the three CBS^3 scoring variables (NREM ratio, REM ratio and EMG amplitude with zero suppression). The sine waves were assigned visibly distinct frequencies, arbitrarily chosen to correspond to the "dominant" frequencies in the PSG for each behavioural state: NREM ratio, 3 Hz; REM ratio, 7 Hz; EMG amplitude, 60 Hz. The amplitudes of the generated sine waves were proportional to the recorded values of the respective CBS^3 scoring variables in each epoch (Fig. 1B). The three waves were then combined using simple wave summation to yield a single waveform, the PPG (Fig. 1C). The program was designed to display three sequential epochs which could be scrolled backwards and forwards to allow the use of temporal context as an aid in the designation of state for any given epoch, a process that was also employed in conventional visual scoring of the raw PSG.

Data are expressed as mean ± standard deviation and statistically significant differences were inferred when $P < 0.05$.

3. Results

3.1. PPG-derived confidence index and thresholds

The confidence index was significantly lower ($P = 0.017$, paired *t*-test on arcsine transformed data, d.f. = 10) in PPG visual analysis (CI = 70.1 ± 8.9%) than in conventional raw PSG visual analysis (CI = 79.4 ± 9.5%).

Threshold values for CBS^3 scoring variables (EMG amplitude, NREM ratio and REM ratio) were highly variable between rats and no systematic differences were found between rats fitted with tethers or transmitters. This applied to thresholds derived from both conventional visual scoring and PPG scoring. For example, inter-animal coefficients of variation (standard devation/mean × 100%) varied from 38 to 98%, which underscores the need to derive empirical thresholds in each animal (i.e. a standard value cannot be defined). The threshold values derived from conventional visual analysis of raw PSG were not statistically different from those derived from visual analysis of the corresponding PPG display (paired *t*-tests, d.f. = 10; EMG threshold, $P = 0.201$; NREM ratio threshold, $P = 0.180$; REM ratio threshold, 0.394).

Table 1 details the concordance between sleep–wake scores derived from conventional visual analysis and the corresponding scores derived from visual interpretation of the

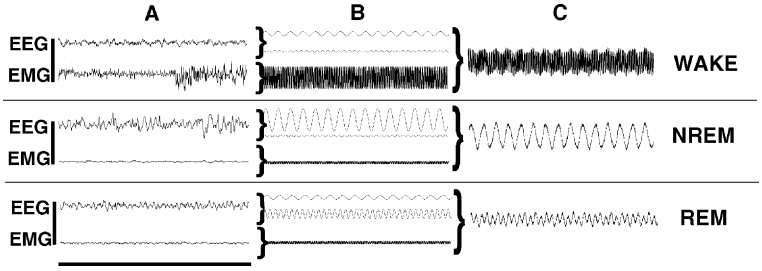

Fig. 1. Representative polysomnograms (PSG; A) and corresponding pseudopolygrams (PPG; C) from a single rat. Upper panel illustrates an epoch of unequivocal wakefulness (WAKE), middle panel indicates an epoch of unequivocal NREM sleep and lower panel illustrates an epoch of unequivocal REM sleep. In the PSG traces (A), vertical bars at left indicate calibration scales (EEG, 200 μV; EMG, 400 μV). Horizontal bar indicates 5 s time scale. The three component sine waves of the PPG are shown in (B). Each EEG trace is analyzed by the CBS^3 program and quantified as two amplitude ratios; NREM ratio and REM ratio (see text for details). A sine wave is generated for each of these quantities, and the amplitude of the sine wave is proportional to the value of the respective ratio. NREM ratio is represented by a 3 Hz wave (upper EEG-derived sine wave) and the REM ratio is represented by a 7 Hz wave (lower EEG-derived sine wave). EMG amplitude is represented in (B) by a 60 Hz sine wave. The PPG in (C) is obtained by summation of the three component sine waves in (B).

Table 1
Matrix illustrating concordance between 4701 epochs that were scored unequivocally using two methods of visual analysis; conventional scoring using standard polysomnogram (PSG), and visual analysis of the PPG display

	PPG visual score				Reliability (%)
	WAKE	NREM	REM	Total	
Conventional visual score (PSG)					
WAKE	1355	25	6	1386	98
NREM	111	2780	143	3034	92
REM	11	10	260	281	93
Total	1477	2815	409	4701	94

Data from seven rats.

PPG display. Reliability was high for each of WAKE, NREM and REM, and the overall reliability was 94%.

3.2. Effect of sleep deprivation on the performance of CBS^3 and PPG

Time-lapse photography for each of the four rats revealed that they did not obtain significant amounts of sleep during the 24 h SD protocol. Rats were never in the same position for more than two consecutive images, indicating that they moved at least once every 4 min. In addition, during the 1.5 h recording period immediately following SD, the rats exhibited prolonged periods of REM sleep, as well as long NREM–REM transitions, and very few spontaneous arousals, further indication that they were significantly sleep deprived during the 24 h spent in the flowerpot apparatus.

Table 2
Performance of CBS^3 in scoring post-SD recordings from four rats using threshold values derived from conventional visual analysis of the pre-SD recording. Matrix illustrating concordance between 3312 post-SD epochs that were scored unequivocally by conventional visual analysis and corresponding scores derived using CBS^3

	CBS^3 (PSG thresholds)				Reliability (%)
	WAKE	NREM	REM	Total	
Conventional visual score (PSG)					
WAKE	608	9	66	683	89
NREM	309	1445	75	1829	79
REM	22	71	707	800	88
Total	939	1525	848	3312	85

Table 3
Performance of CBS^3 in scoring post-SD recordings from four rats using threshold values derived from visual analysis of the PPG display of the pre-SD recording. Matrix illustrating concordance between 3312 post-SD epochs that were scored unequivocally by conventional visual analysis and corresponding scores derived using CBS^3

	CBS^3 (PPG thresholds)				Reliability (%)
	WAKE	NREM	REM	Total	
Conventional visual score (PSG)					
WAKE	662	17	4	683	97
NREM	345	1423	61	1829	78
REM	32	118	650	800	81
Total	1039	1558	715	3312	85

Tables 2 and 3 detail the reliability of the CBS^3 in scoring data from four sleep deprived rats using threshold values that were derived from prior recordings made when the rats were not sleep deprived. Table 2 illustrates CBS^3 performance when using threshold values derived from conventional visual analysis of the raw PSG, and Table 3 illustrates CBS^3 performance when using threshold values derived from visual analysis of the PPG display. Overall reliability of scoring was 85% in both cases. However, there were minor differences in state-specific reliability, notably a small increase in WAKE score reliability and decrease in REM score reliability using the PPG display.

4. Discussion

This study has confirmed that the PPG can be used in place of conventional raw PSG traces for visual scoring of sleep–wake state in rats. It was found that the CBS^3 threshold values derived from epochs that had been scored using the PPG were not significantly different from the corresponding CBS^3 threshold values obtained by conventional visual

analysis of the PSG. This conclusion was reinforced by the finding that overall reliability of CBS[3] scoring was the same whether PPG-derived thresholds or conventional PSG-derived thresholds were used.

Although conventional visual analysis of the raw PSG is generally considered to be the "gold standard" by which other methods are judged (as it is in the present study), it should be borne in mind that even this method is imperfect. The reason for this is that electrophysiological variables are not totally distinct between sleep–wake states and there is quantitative overlap (Benington and Heller, 1994; Bergmann et al., 1987; Bjorvatn et al., 1998; Costa-Miserachs et al., 2003; Hamrahi et al., 2001; Karasinski et al., 1994; Ruigt et al., 1989) that makes it difficult to judge the appropriate classification of some epochs. While most published descriptions of sleep scoring systems acknowledge this by restricting their validation analyses to unequivocally scored epochs (Costa-Miserachs et al., 2003; Hamrahi et al., 2001; Louis et al., 2004), very few quantify the proportion of the record that is deemed reliable (Ruigt et al., 1989). In our experience, CI varies considerably between raters and is not correlated with rater experience, a finding that underscores the subjectivity that is inherent in this process. The present study found that CI was 10% lower using the PPG than it was using conventional PSG, suggesting that approximately 10–20% more data are needed to yield the same number of unequivocal epochs when the PPG is to be used for threshold analysis, a minor potential disadvantage of the PPG-based analysis.

Inter-rater reliability is approximately 95% in this laboratory using conventional visual analysis of PSG, which therefore represents the maximum level of concordance that can be expected when comparing two different methods. In the current study, overall reliability of the PPG was 94% (Table 1). Concordance between PPG and PSG was very high for WAKE (98%) but lower for NREM (92%) and REM (93%). In Table 1, the largest apparent error arose because 143 (4.7%) of the 3034 epochs scored as NREM by PSG were designated as REM by PPG analysis. This indicates that there is a tendency for PPG scoring to yield false–positive REM scores, owing to some NREM epochs being associated with very low muscle tone and relatively low delta wave amplitude in some (but not all) animals. This error, however, had no significant effect on the overall usefulness of the PPG in defining threshold values for CBS[3] because NREM and REM threshold values derived from the PPG analysis were not statistically different from those derived from the PSG analysis. It does, however, indicate a need for very strict criteria in the visual identification of REM episodes using the PPG, with a highly conservative approach to the designation of putative REM epochs as "unequivocal". One potential solution could be to apply a "minimum consecutive epochs" criterion so that only epochs within clearly identified REM episodes are included in the unequivocal category. On the basis of the present data, however, it must be concluded that in its current form the PPG is not appropriate for use in studies where a highly accurate identification of REM and REM attempts is required.

Other methods, such as statistical analysis of the CBS[3] output, could conceivably be employed to define threshold values in circumstances where PSG is not available. However, this approach was rejected because it was found to be unreliable for the reasons described above; quantitative overlap in the electrophysiological characteristics of the three behavioural states and in epochs containing transitions between states or movement and other artifacts. We developed the PPG instead because it allowed the data to be "filtered" by identification and rejection of equivocal epochs. This was greatly facilitated by using contextual information that was not available in simple statistical analyses. Specifically, the sequential order of epochs was found to be very helpful in assigning state to each epoch in the PPG display, a factor that is also an integral part of conventional sleep scoring. Thus, the PPG-based visual analysis is identical in principle to conventional analysis of the raw PSG. It involves subjective judgments and relies on visibly distinct waveforms for each of the states to be categorized. Fig. 1 illustrates a typical PPG for unequivocally scored WAKE, NREM and REM epochs. The waveform appears distinctly different between states. This was accomplished by using distinctive frequencies for each of the three CBS[3] scoring variables. The choice of frequencies was arbitrary and for purely aesthetic reasons we decided to use frequencies that are characteristic of the traditional PSG in the three behavioural states; delta waves (3 Hz) for NREM, theta waves (7 Hz) for REM and fast waves (60 Hz) representative of EMG activity to indicate WAKE.

The present study has also extended previous work (Louis et al., 2004) by showing that the CBS[3] algorithm can be used with 85% reliability even after 24 h of total sleep deprivation, a procedure that is known to cause significant changes in EEG characteristics (Franken et al., 1991; Schwierin et al., 1999). In the previous study (Louis et al., 2004), reliability of scoring 24 h after setting the CBS[3] threshold values was 90.1% in non-SD rats which, in the context of the present data, implies that SD does induce changes that necessitate at least a daily check on the veracity of the threshold values. Importantly, SD had no effect on the reliability of PPG in visual scoring of sleep–wake state. In four rats, overall reliability of PPG scores (compared with PSG scores) was $89.5 \pm 9.5\%$ for pre-SD recordings and $95.3 \pm 4.3\%$ for post-SD recordings (paired t-test on arcsine transformed data, $P = 0.360$, d.f. = 3).

The PPG is a visual representation of the PSG after manipulation of the data by the CBS[3] program. The PPG does not add any information that is not present in the original PSG traces, but simply presents it in a modified form. The main disadvantage of this approach is that dynamic information, such as transient events that occur within the space of a single epoch, is lost. Furthermore, there is a need for each rater to gain experience with the PPG before it can be used confidently without reference to the conventional PSG. This, however, is no different from the training process required for conventional scoring, and the principle of correlating

waveform patterns to behavioural state is the same for both PSG and PPG.

The PPG offers advantages over the PSG. Recording the PSG is expensive in terms of digital memory requirements. For example, at 100 Hz per channel, a basic 2-channel PSG recording requires storage of 1000 samples in each 5 s epoch, a factor that might be limiting when long-term experiments (days or weeks) are planned, despite the increasing commercial availability of high capacity devices. For example, the feasibility of field studies using miniature implantable sleep loggers is currently severely restricted by memory capacity. The PPG would substantially alleviate this problem by reducing the stored data to six values per epoch. Another advantage is that the PPG allows post hoc analysis of CBS[3] scoring at any time in the recording. Thus, it is not necessary to restrict threshold checks to pre-determined intervals, as would be the case with periodic recording of PSG.

In conclusion, we have devised and validated a new method for the visual display of combined EEG and EMG waveforms for use in scoring of sleep–wake state in rats. This method was found to be as reliable as scoring with the raw EEG and EMG traces. When used in combination with the CBS[3] system, it provides a simple, reliable method for long-term analysis of behavioural state. This general approach of using conditioned (i.e. filtered, averaged etc.) data to reconstruct a visual trace, the appearance of which is distinct between the three behavioural states, is in principle applicable to any similar semi-automated sleep scoring system.

Acknowledgement

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

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