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Research Report

EEG gamma frequency and sleep–wake scoring in mice: Comparing two types of supervised classifiers

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ABSTRACT

There is growing interest in sleep research and increasing demand for screening of circadian rhythms in genetically modified animals. This requires reliable sleep stage scoring programs. Present solutions suffer, however, from the lack of flexible adaptation to experimental conditions and unreliable selection of stage-discriminating variables. EEG was recorded in freely moving C57BL/6 mice and different sets of frequency variables were used for analysis. Parameters included conventional power spectral density functions as well as period-amplitude analysis. Manual staging was compared with the performance of two different supervised classifiers, linear discriminant analysis (LDA) and Classification Tree. Gamma activity was particularly high during REM (rapid eye movements) sleep and waking. Four out of 73 variables were most effective for sleep–wake stage separation: amplitudes of upper gamma-, delta- and upper theta-frequency bands and neck muscle EMG. Using small sets of training data, LDA produced better results than Classification Tree or a conventional threshold formula. Changing epoch duration (4 to 10 s) had only minor effects on performance with 8 to 10 s yielding the best results. Gamma and upper theta activity during REM sleep is particularly useful for sleep–wake stage separation. Linear discriminant analysis performs best in supervised automatic staging procedures. Reliable semi-automatic sleep scoring with LDA substantially reduces analysis time.

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1. Introduction

Sleep research in human subjects and animals is of increasing importance, mainly due to the assumed role of sleep in memory consolidation (for recent reviews see: Axmacher et al. (2009); Brankačk et al. (2009) and Walker (2009)) and to the expanding need for sleep phenotyping of genetically modified mice (Pang et al., 2009). The main two sleep stages NREM (non rapid eye movements) and REM (rapid eye movements) can be easily distinguished from each other and from waking by visual inspection of the EEG and EMG. In rodents, reliable staging can be achieved from epidural recordings of cortex

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Abbreviations: ANOVA, analysis of variance; EEG, electroencephalography; EMG, electromyography; FFT, Fast Fourier transform; LDA, linear discriminant analysis; NPV, negative predictive value; N, NREM, non rapid eye movements; R, REM, rapid eye movements; SEM, standard error of the means; PAA, period-amplitude analysis; PCA, principal component analysis; PPV, positive predictive value; W, waking

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areas overlaying the dorsal hippocampus (allowing for the detection of theta rhythms) together with EMG activity from neck muscles (Timo-Iara et al., 1970; Brankačk and Buzsáki, 1986; see Fig. 1A and for details see Experimental procedures section). Manual sleep scoring by visual inspection, however, is extremely time-consuming. As a rule of thumb, analysis time almost equals recording time. Therefore, during the past three decades numerous attempts of automatic or semi-automatic sleep scoring have been made, using a large variety of methods (reviews in Robert et al. (1999); in mice: Veasey et al. (2000); recently: Crisler et al. (2008)). Despite of these enormous efforts, the accuracy of most commercially available sleep scoring programs is dissatisfying. Besides being costly, these programs are fixed and have been often designed for a particular experimental condition performing poorly under differing conditions.

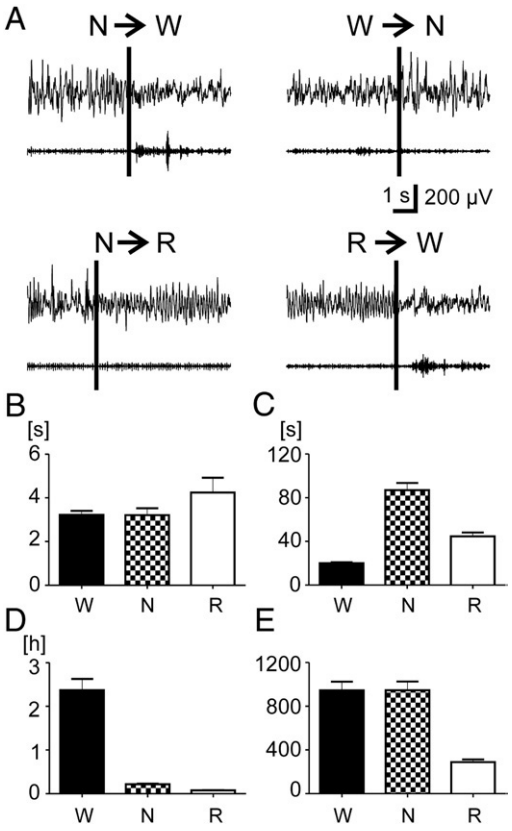

Fig. 1 – A: Examples of EEG and EMG recordings during representative transitions (transitions points: vertical markers) of sleep–wake stages: from NREM sleep to waking (N→W); from waking to NREM sleep (W→N); from NREM to REM sleep (N→R) and from REM sleep to waking (R→W). The upper traces show EEG recordings from the lateral parietal cortex overlying hippocampus, the lower traces are recordings of neck muscle EMG. Time and amplitude scales are identical for all traces. Note the increase in EMG activity and a drop in EEG amplitude during awakening (N→W, R→W). Please note also the high-amplitude slow wave activity during NREM, the lower amplitude irregular activity in quiet waking (W→N shortly before N) and the regular theta (7–8.5 Hz) rhythm during REM sleep (R). These changes in EEG and EMG activity were used as criteria for the manual sleep–wake staging. B–E: Means and S.E. (N=10 animals) of minima (B), of individual medians (C), of maxima (D) and of the number (E) of manually classified stage durations for waking (W), NREM (N) and REM (R).

We have developed a custom-made program for reliable and flexible sleep–wake stage scoring with supervised learning algorithms. We compared three different sleep scoring methods with results of visual inspection: i) a conventional threshold formula as often implemented in commercially available programs (Mochizuki et al., 2004); ii) linear discriminant analysis (LDA); and iii) Classification Tree (Tree). The latter two solutions are supervised classifiers (MacLachlan, 1992; Fielding, 2006), allowing for flexible adaptation to different experimental conditions and parameters. After processing small sets of training data, the program reliably differentiated waking, NREM and REM, using EMG, delta, upper theta and upper gamma amplitudes as discriminating parameters.

2. Results

2.1. *Manual sleep–wake scoring by visual inspection*

Continuous EEG recordings of at least 72 h were recorded from ten mice and were manually scored into three stages: wakefulness (W), NREM (N) and REM (R) according to the criteria described in Experimental procedures. Fig. 1A shows representative transitions between the three stages indicated with vertical markers (vertical lines in Fig. 1A). The term "transition" is used in its direct meaning, i.e. the end of one stage corresponding to the beginning of the next stage and not in the meaning that one stage may be separated from the next by a transitional period not belonging to either of the two neighbouring stages. High-amplitude EMG activity indicated wakefulness when combined with low-amplitude irregular EEG (quiet waking) or with regular theta rhythm (active waking; see "W" in Fig. 1A). Lower EMG activity with high-amplitude delta (1–4 Hz) waves was classified as NREM (see "N" in Fig. 1A) and low EMG activity with regular theta (4.5–8.5 Hz) rhythm corresponded to REM sleep (see "R" in Fig. 1A). The criteria of placing transitional markers are demonstrated in Fig. 1A: W→N: first large-amplitude slow wave; N→W: beginning of EEG desynchronisation; N→R: beginning of regular theta rhythm, regardless of its amplitude; R→W: end of regular theta rhythm, regardless of EMG. The duration of manually scored stages varied between 1.6 s and 218 min. Averaged (mean±SEM) over ten animals, minimal stage durations (Fig. 1B) corresponded to 3.2±0.2 s in waking, 3.2±0.3 s in NREM and 4.2±0.7 s in REM sleep. The means of individual medians (W: 20.1±0.8 s, N: 87.0±6.6 s, R: 44.6±3.5 s) are shown in Fig. 1C and the mean maximal stage durations (W: 8539.0±925.0 s, N: 778.5±41.3 s, R: 272.6±9.2 s) are shown in Fig. 1D. Fig. 1E illustrates the mean numbers of stages (W: 946±80, N: 946±80, R: 289±24). No stage duration differences were found between female (N=7) and male (N=3) mice.

We then averaged the percentages of different states for the two recorded dark/light periods in each animal. No difference was found between the seven female and three

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