An Automated System for Recording and Analysis of Sleep in Mice

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Abstract: Significant differences in many aspects of sleep/wake activity among inbred strains of mice suggest genetic influences on the control of sleep. A number of genetic techniques, including transgenesis, random and targeted mutagenesis, and analysis of quantitative trait loci may be used to identify genetic loci. To take full advantage of these genetic approaches in mice, a comprehensive and robust description of behavioral states has been developed. An existing automated sleep scoring algorithm, designed for sleep analysis in rats, has been examined for acceptability in the analysis of baseline sleep structure and the response to sleep deprivation in mice. This algorithm was validated in three inbred strains (C57BL/6J, C3HeB/FeJ, 129X1/SvJ) and one hybrid line (C57BL/6J X C3HeB/FeJ). Overall accuracy rates for behavioral state detection (mean±SE) using this system in mice were: waking, 98.8±0.4; NREM sleep, 97.1±0.5; and REM sleep, 89.7±1.4. Characterization of sleep has been extended to include measurements of sleep consolidation and fragmentation, REM sleep latency, and delta density decline with sleep. An experimental protocol is suggested for acquiring baseline sleep data for genetic studies. This sleep recording protocol, scoring, and analysis system is designed to facilitate the understanding of genetic basis of sleep structure.

Key words: Sleep; mice; electroencephalography; genetics; sleep scoring; arousal index; sleep bouts; phenotype

INTRODUCTION

THE AVAILABILITY OF MOUSE STRAINS HARBORING VARIATIONS IN GENES INVOLVED IN SLEEP CONTROL WILL BE HELPFUL IN UNDERSTANDING the complexity of sleep at the molecular and biochemical level. Genetic, physiological and anatomical studies are providing candidate genes that may influence the control of sleep. The roles of these genes may be ascertained by the analysis of aberrant phenotypes of mice with null mutations generated by targeted mutagenesis in embryonic stem cells.1,2 Alternatively, to discover novel genes, techniques of forward genetics may be employed. Forward genetics approaches involve the identification of mouse strains with unique phenotypic traits, followed by genetic mapping of the loci controlling the traits. The phenotype-based approaches include analysis of quantitative trait loci (QTL) in inbred strains of mice3 and random mutagenesis screens for single gene mutations.4,5 All of these techniques may be used to understand the genetic control of sleep/wake behavior.

Sleep states are defined by specific electroencephalographic and electromyographic signals in mice as in other mammalian species. Rapid-eye-movement sleep (REM sleep) is characterized by postural muscle atonia, rapid-eye-movements and predominant theta wave activity in the hippocampal electroencephalogram (EEG), whereas non-rapid-eye-movement sleep (NREM sleep) is characterized primarily by lower frequency, higher amplitude waveforms in the cortical EEG.6,7 Sleep progresses from faster wave NREM sleep with spindle waves to slower wave NREM sleep, in which delta waves predominate. After a period of NREM sleep, REM sleep ensues.6 Unlike in humans, sleep activity in rodents is more evenly distributed across the 24-hour cycle,6 with a preponderance of both NREM sleep and REM sleep in the period of the 24-hour day with greater ambient light.7 Moreover, sleep cycle lengths of NREM sleep and REM sleep are shorter in laboratory rodents.8 While these differences exist, homeostatic9 and circadian10 control of sleep, as well as neurochemical modulation of sleep,11-18 appear consistent across species. The mouse provides a good genetic model for the study of sleep state control and timing, and the study of sleep states in mice may ultimately provide insight into the control of sleep in
A number of studies of sleep in different strains of mice suggest genetic influences on many aspects of behavioral state control. A high degree of variance between inbred strains of mice has been shown for many objective parameters of sleep and sleep control. These include differences in (1) total sleep time within a 24-hour light-dark cycle, (2) time spent in NREM sleep, (3) time spent in REM sleep, (4) the diurnal ratio of wakefulness, (5) REM sleep as a percentage of total sleep time, (6) sleep state ontogenesis, (7) the ratio of NREM sleep to REM sleep with aging, (8) effects of changes in ambient temperature, (9) responses to REM sleep deprivation, (10) effects of infectious challenges, (11) effects of immune modulation, and (12) EEG spectral profiles. Strains showing differences in one sleep parameter may not exhibit differences in other sleep parameters, suggesting that the genetic control of sleep will likely involve a multitude of genes.

A recent important study highlights the tremendous value of the mouse as a model organism for studies of genetics of sleep/wake control. A targeted null mutation of the prepeptide orexin was studied for its effect on sleep/wake behavior in the mouse. These mice exhibited behavioral and electrophysiological anomalies similar to narcolepsy in humans: increased sleep in the active period, shortened REM sleep latencies, and cataplexy-like behavior.

A complete phenotypic description of sleep in inbred strains of mice is necessary for the genetic analysis of sleep/wake control. The characterization of sleep/wake behavior in large numbers of mice can be performed more quickly using an automated sleep analysis system. Over the past three decades, numerous sleep recording and automated sleep analysis systems have been developed and modified for the study of sleep in laboratory rodents. The accuracy of each of the validated programs, relative to human scoring, is excellent. There are, however, potential limitations to the existing programs. Not all of the programs are readily available, and there are differences among the automated programs in both the parameters used to describe sleep and in the electrophysiological characteristics used to identify behavioral states. A phenotypic assay of sleep/wake activity should identify not only baseline behavioral state distribution, but also should measure parameters used in the analysis of human sleep: sleep fragmentation, REM sleep latency, sleep bout duration, the robustness of slow-wave sleep early in the sleep period, and sleep cycle durations.

To implement an automated sleep analysis system that can provide a complete phenotypic characterization of sleep structure in mice, we have adopted and modified an existing software system. This software, ACQ/SSSSS version 3.4© (ACQ 3.4), developed by Benington and Heller, was designed for the analysis of sleep in rats, and has been made freely available (with source code) to the sleep research community. We have validated the existing sleep analysis algorithm in mice and have added analyses to the program extending the characterization of sleep in mice. In this paper, we determine the accuracy of this program in sleep state detection in mice, provide a protocol for recording baseline sleep/wake activity, and we describe sleep structure in three inbred strains and one hybrid strain.

**METHODS**

**Mouse Strains**

Adult male mice, aged 13 and 19 weeks at the time of behavioral state recordings, were used for these studies. All mice were obtained from the Jackson Laboratory, Bar Harbor, ME. Mice weighed 25 to 30 grams on the day of surgical implantation. Three inbred strains of mice were implanted with EEG and EMG electrodes for chronic recordings (C3HeB/FeJ, n=17; C57BL/6J, n=15; 129X1/SvJ, n=10), as well as a hybrid strain (F2 C3HeB/FeJ X C57BL/6J, n=13). The Institutional Animal Care and Use Committee of the University of Pennsylvania approved the methods and study protocols in full.

**Surgical Implantation of Electrodes**

Recording electrodes were made as follows: Teflon-coated silver wire (Medwire, Mt. Vernon, NY) was used to make ball electrodes for the three electroencephalographic (EEG) electrodes. These were fashioned by shaving 4 mm of insulation from the distal tip of the electrode wire and then heating the exposed wire under flame until a 1 mm ball was formed. Initially, a stainless steel screw (size 00, Morris Precision) was used as the ground electrode, and in later animals, this was replaced by a fourth ball electrode. The silver wire was also used to fashion two neck electromyography (EMG) electrodes. For these, 8 mm at one end was stripped to create an electrically conductive loop 2-mm in diameter. All six electrodes were soldered onto a shielded multi-stranded cable (6-wire 30 gauge SH; Cooner Wire, Chatsworth, CA). The end of the cable proximal to recording equipment was soldered onto a 6-pin plug (363 plug, Plastics One, Roanoke, VA).

Prior to surgery, mice were anesthetized with 70-85 mg/kg ketamine and 9 mg/kg xylazine administered intraperitoneally. In all four strains, ketamine and xylazine provided 60 to 90 minutes of general anesthesia. Using aseptic techniques, the scalp hair was shaved, and a midline incision of 2 cm was made to expose the dorsal cranium and the neck musculature. The dorsal surface of the skull was completely cleaned of the periosteum. A trace amount of etching gel (P-10 gel, 3M Dental, Irvine, CA) was
placed over the cranium to increase the skull surface area. After five minutes, the skull was thoroughly rinsed with sterile saline and allowed to dry. Using a 21-gauge needle, four holes through the cranium were made for EEG and ground electrodes at 2 mm lateral to Bregma and 1 mm rostral (for two rostral EEG electrodes) and 2 mm lateral to Bregma/3 mm caudal to Bregma (for the ground and a caudal EEG electrode). The caudal electrode position was found optimal for the detection of hippocampal theta, after testing both 1 mm rostral and 1-2 mm caudal to this site. EEG electrodes and the ground electrode were implanted 1 mm below the skull’s surface (on the dura) and secured first with dental paste (Resin Bonded Ceramic, 3M) and then with dental acrylic (P-10 Acrylic, 3M). The neck EMG loops were sutured to dorsal neck musculature with 5-0 silk ties. Cranial electrodes were secured. The incision was then closed with a suture. Each mouse received 0.005 mg/kg gentamycin intramuscularly and was placed in a clean cage in the recording chamber. The cable was then connected to a fully rotating commutator (SLC 6, Plastics One, Roanoke, VA) supported by a counterweight. Three days after surgery, animals received a second injection of gentamycin (same dose).

Recording Procedures

Four individual mouse cages were housed inside a sound-attenuated, shielded and well-ventilated chamber (EPC-010, BRS/LVE, Laurel MD), with a 12-hour lights-on (07:00 h to 19:00 h) and 12 h lights-off schedule. The ambient lighting was 75 lux for the lights-on period and <1 lux for lights-out. The temperature in the recording chambers was held constant at 25°C±2°C, as measured daily during recordings.

EEG signals were filtered at 0.3 and 35 Hz (1/2 max, 6dB/octave), and EMG signals were filtered at 1 and 100 Hz, and both signals were amplified (12A5, AC amp, Astro-Med, West Warwick, RI). Various combinations of the rostral-caudal EEG electrodes were viewed polygraphically (Grass 12-32-35S, Neurodata Acquisition, Astro-Med) using an electrode selector board (12 PB_36 Electrode Selector, Astro-Med) to find optimal EEG combinations for detecting active waking, theta activity in the EEG, and slow wave sleep. Either caudal electrode could function as the ground or EEG electrode. Signals were sent to an A/D board (Converter 4801A, ADAC, Woburn, MA) by way of a mini-phone plug (CAB-20333, Astro-Med) connected to a male BNC cable. Prior to beginning recordings, the EEG signals for each mouse were calibrated using a 100 mV calibration signal from the polygraph.

The behavioral state acquisition and analysis program used for these studies was ACQ 3.4 from Dr. Joel Benington. This recording and analysis system was originally developed for behavioral state study in rats.38 The optimal EEG and EMG signals for four mice, per computer, were each digitized at 100 Hz. EEG was recorded as raw signals. The program performs Fourier analysis on 10-second epochs of the EEG signals and then sums spectral power for three frequency bands: delta (0.5-4.0 Hz), sigma (10-14 Hz) and theta (6-9 Hz). The program rectifies the EMG signal and then records a moving average amplitude for each 10-second epoch.

Behavioral states are determined based on three variables: (1) the product of sigma density and theta density (sigma*theta), (2) delta density divided by theta density (delta/theta), and (3) the EMG amplitude. High EMG and low sigma*theta is characteristic of waking, while low EMG and high sigma*theta is characteristic of sleep. High delta/theta is characteristic of NREM sleep, while low delta/theta is characteristics of REM sleep.

![Figure 1](image-url) — Sleep state scoring algorithm. Plotted in the upper panel scattergram are values for every 10-second epoch for the product of sigma and theta densities (S*T) versus the amplitude of the integrated EMG (EMG) for a 24-hour period. Plotted epochs fall into two general clusters: waking and sleep. A line may be placed separating the two clusters. This line is distinguished by its x-intercept and its slope. Typically, epochs with significant movement artifact will have high EMG values, so that these epochs will be correctly scored as waking. The lower panel scattergram plots EMG vs. delta/theta densities. This generally results in three loose clusters, where the lower left hand cluster represents REM sleep, the upper cluster represents waking and the lower right represents NREM sleep. An X-intercept just beyond the REM sleep cluster is used to distinguish REM sleep from NREM sleep. Both intercepts and the slope may be adjusted to balance overcalls and undercalls for a given behavioral state.
thresholds for these variables are determined based on visual examination of scatter plots displaying sigma*theta vs. EMG (see Fig. 1, upper panel) and delta/theta vs. EMG (see Fig. 1, bottom panel). Waking was distinguished from sleep by defining slope and intercept values for the sigma*theta vs. EMG plot, and NREM sleep was distinguished from REM sleep by defining a delta/theta threshold.

**Recording Protocol**

After the seven to nine day post-operative recovery period, baseline sleep activity was recorded for five to seven days. Sleep deprivation was then performed continuously throughout one 12-hour lights-on period, by way of gentle handling (stroking the head, vibrissae and back with a feather duster). Animals were stroked whenever they were observed to be quiescent for more than 10 seconds, or if EEG activity showed higher amplitude or slower waveforms. EEG and EMG activity was recorded during all but the initial 30 minutes of the sleep deprivation period, when baseline signals were downloaded. During the period in which EEG signals could not be viewed, mice were stroked on faces and vibrissae when immobile. Two hours prior to the end of the 12-hour deprivation, water bottles were filled, the bedding was changed and a week’s supply of food was placed in the cage. Sleep deprivation continued until the lights were turned off at the usual time, and animals remained in the recording chambers for one week following sleep deprivation, during which sleep/wake activity was continuously recorded.

**Validation and Inclusion of Data**

Sleep-wake states were scored visually by two human scorers for a one-hour sample of raw EEG and integrated EMG signals. For human scoring, waking was defined as a predominance (within a 10-second epoch) of fast, desynchronized waves and high EMG amplitude. NREM sleep was defined as a predominance of higher amplitude EEG waves, consistent with either sigma or delta waveforms, and REM sleep was defined as a high frequency EEG with a significant predominance (>75%) of theta waves and a very low EMG amplitude. Figure 2 illustrates samples of the electrographic signals as they appear on the computer screen during off-line analysis of each 10-second epoch. If signals for a given mouse could not be used to visually determine sleep/wake activity because of noise, poor signal quality or movement artifact, the data for that mouse were not analyzed further.

For all mice with visually recognizable sleep states, human state scores were compared with the computer's interpretation of state, and the algorithm's intercepts and slope were adjusted to optimize the accuracy of the computer-determined behavioral states and to balance overcalls and undercalls for a given state (see Fig. 1). An accuracy value for each state for 360 consecutive epochs was calculated in each mouse. A second one-hour sample from a different day was then used for validation purposes. This was typically selected as an hour within the middle third of the light period in an effort to increase REM sleep epochs within the one hour sample. A third sample was studied for accuracy during a random hour of the dark period and compared with the mid-day light period hour in six mice for each strain. In cases where sleep deprivation was performed and recordings were acceptable after sleep deprivation, a third or fourth validation was performed to determine the stability of sleep analysis accuracy after sleep deprivation by gentle handling. With practice, this entire process required one person-hour per mouse to validate, determine the most accurate settings, and perform the analysis. Mice for which we were unable to obtain an accuracy for the computerized algorithm of at least 90% for waking and NREM sleep and at least 75% for REM sleep, were included in the validation analysis but rejected from sleep structure analysis.

**Parameters of Sleep and Their Definitions**

The measures of sleep timing analyzed were: (1) time spent asleep in minutes (overall, in the light period and in the dark period), (2) the diurnal ratio of wakefulness, (3) sleep efficiency (overall, in the light period, and in the dark period), (4) percentage of recording time spent in NREM sleep (overall, in the light period, and in the dark period), (5) mean EEG delta density in NREM sleep, (6) the magnitude of the decrease in delta density over the course of the light period, (7) mean EEG sigma density in NREM sleep, (8) percentage of recording time spent in REM sleep (overall, in the light period, and in the dark period), (9) mean EEG theta density in REM sleep, (10) the diurnal ratio of REM sleep, (11) mean bout lengths for sleep states (minutes per total sleep bout, minutes per bout of NREM sleep, and minutes per bout of REM sleep), (12) arousal index, and (13) REM latency. Detailed descriptions of how each of these variables was defined and calculated are below.
The time spent asleep was defined as the total time for all NREM sleep and REM sleep epochs. This was calculated for the 24-hour cycle, the 12-hour light period and the 12-hour dark period, and expressed as a mean±standard error (SE) for each strain. The diurnal ratio of wakefulness was calculated as the ratio of all time spent in wakefulness epochs in the light period relative to time spent in wakefulness epochs in the dark period. This also was expressed as the mean±SE for each strain. Sleep efficiency was measured as the time spent in both, NREM sleep or REM sleep epochs as a percentage of the total time (24-hour or 12-light or dark period).

The sleep percentage of NREM was calculated as the percentage of recording time during which epochs were scored as NREM sleep. Delta density for NREM sleep was calculated as the average delta density for all NREM sleep epochs per animal, expressed as the mean±SE for each strain. To characterize how slow-wave activity declines across the rest period, we measured, for each baseline day, the density for 0.5–4 Hz in comparison to the remaining frequencies studied (4–20 Hz), expressed each hour’s value as a percentage relative to the last hour of the light period, and determined the slope of the best fit line for delta density change across the light period (delta decline). Mean sigma density was calculated for all NREM sleep epochs in each animal and expressed as the mean±SE for each strain.

To characterize REM sleep, we measured the percentage of time spent in REM sleep (24-hour, 12-hour light and 12-hour dark periods). These data were expressed as mean±SE’s. Mean EEG theta density was calculated for all epochs scored as REM sleep and expressed as mean±SE for each strain. The diurnal ratio of REM sleep was calculated as the time spent in REM sleep during the light period relative to the dark period.

Analysis of sleep bout lengths, arousal index, and REM sleep latency required additional programming. Total sleep bouts were defined as periods of three or more consecutive epochs scored as either NREM or REM sleep. NREM sleep bouts were defined as periods of three or more consecutive epochs scored as NREM sleep, and REM sleep bouts were defined as periods or two or more epochs scored as REM sleep. The termination of a sleep bout was defined by the presence of three or more consecutive wake epochs. To measure average duration of sleep bout lengths, data were transferred from the Statout files of ACQ 3.4 onto an Excel® spreadsheet. A custom script was written in PERL to detect combinations of consecutive epochs meeting criteria for the various types of sleep bouts. Data were calculated for each animal as the average duration in minutes. The arousal index (the number of times per hour that sleep is disrupted by one or more waking epoch) was measured in the following manner. Data were transferred from the Statout files of ACQ 3.4 onto an Excel® spreadsheet, and a custom script was written in PERL to detect epochs scored as waking following combinations of three or more epochs scored as NREM sleep or two or more epochs of REM sleep. This was expressed as the frequency of occurrence per hour of sleep. Using a similar approach, REM sleep latency was quantified. This was determined as the duration from the onset of three or more consecutive epochs of NREM sleep to two consecutive epochs of REM sleep. If three or more consecutive waking epochs occurred before the onset and offset of REM sleep latency, REM sleep latency was not measured.

The homeostatic response to short-term sleep loss was characterized using parameters: the percentage change from baseline in times spent in NREM sleep and REM sleep for the first and second six hours of the recovery period, the percentage change from baseline in delta density for NREM sleep for the same time periods, the percentage change in delta energy (the product of delta density and NREM sleep time) for the same periods, the change in NREM sleep bout length relative to baseline, and the change in the arousal index from baseline.

**Statistical Analysis**

Components of sleep structure during baseline activity were analyzed for differences between strains using factorial Analysis of Variance (ANOVA), and where applicable, the Tukey post-hoc tests were applied. Sleep state comparisons within mice were drawn using repeated-measures ANOVA, with Scheffe’s F-test when applicable. Paired t-tests with Bonferroni correction for the number of parameters analyzed were used to analyze the response to sleep deprivation. Statistical significance was achieved for probabilities of the null hypothesis at p<0.05.

**RESULTS**

**Validation of the ACQ 3.4 Algorithm in Mice**

The sleep analysis algorithm was validated for the light and dark periods at baseline in 24 mice (6 in each strain) and after 12 hours of sleep deprivation in 23 mice. Analysis of baseline sleep parameters was performed on all mice with stable baseline recordings and accurate computerized scoring using the ACQ 3.4 algorithm. Following baseline recordings, mice were deprived of sleep for the 12-hour light period. Recovery sleep was recorded for 24 hours following sleep deprivation. Baseline sleep parameters were analyzed in 36 of the 55 implanted mice. Those not analyzed included six that died within the peri- or post-operative period; in eight mice behavioral states could not readily be determined by human scorers, and in five mice behavioral states were not steady over the baseline period. Over the course of this study, the success rates for stable, accurate recordings increased steadily.
The use of this algorithm was validated for mice by comparing algorithmic state scores with state scores determined by visual inspection of electrographic parameters for a) one hour of baseline in the light period (n=28), b) one hour in the dark period (n=24) and c) one hour of recovery sleep in the light period (n=23). Table 1 summarizes the accuracy and validation data for each mouse strain and for each state for the light period. Overall agreement between the two scoring methods was 95.2%±0.7, with a range by strain and state of 81.4% to 99.3%, during baseline recordings and a range of 75.2% to 99.6% after sleep deprivation. There was no main effect of strain on the validity of the program to accurately score any of the behavioral states, except that the accuracy with which the program detected REM sleep was higher in the C3H mice than in other strains (p<0.01 for each other strain in comparison). Overcalls and undercalls for each state were balanced in all strains. As a group, the accuracy of the program in determining REM sleep was lower than its accuracy in determining waking or NREM sleep (p<0.05).

For each state, there was no significant difference in accuracy between the first hour of scored epochs used to compare with human scoring and the sample used in the baseline validations procedure, as shown in Table 1. The accuracy of the program in determining REM sleep was also determined, and no significant difference was found between daytime and nighttime validations (Table 2). In addition, there was no difference between the baseline accuracy and the post-sleep deprivation accuracy for waking and NREM sleep. There was, however, a decline in the accuracy of scoring REM sleep after sleep-deprivation from 89.7%±1.4 for baseline to 85.3%±2.2 after sleep loss (p<0.05).

### Graphical Display of Sleep Parameters

We have developed two graphical displays of sleep states to examine on-going data obtained from individual animals and to identify technical problems or phenotypic outliers. Figure 3 illustrates the two ways to display data.
Figure 3—Graphical displays used to rapidly find outliers in genetic sleep studies. Two versions are presented. On the left is a display of hourly percentages of each behavioral state. This example shows a steady-state pattern for all three behavioral states and provides general information on ultradian cycle length, ultradian amplitudes, diurnal ratio amplitude, and estimates of NREM sleep time and REM sleep time. The graphical displays to the right are double-raster plots as used for wheel-running activity in screening for mutant circadian rhythms. Sensitivities may be adjusted to distinguish three bin-sizes for various percentages of time spent in each behavioral state. These raster plots reveal the higher levels of waking in the early dark period, and the increase in REM sleep time within the second portion of the lights-on period.

Figure 4—Individual hourly percentage distributions for each behavioral state are shown for two different mice. Fig 4a. shows behavioral state percentages for a C57BL/6J mouse. The upper panel displays NREM sleep hourly percentages, the middle panel displays REM sleep percentages, and the lower panel shows waking percentages. Notice the apparent decline in REM sleep midway through the baseline recordings. Review of raw signals revealed deteriorating electrical signals in this animal. Fig 4b. shows hourly percentages for a 129X1/SvJ mouse with blunted ultradian and circadian fluctuation, typical for that substrain of mice. Notice at 130 hours into recording baseline, there was an unexplained and significant increase in wakefulness lasting approximately 12 hours, followed by a sleep rebound. This was unexplained, and not seen in other mice within the same recording chamber.
Expression of each state can be displayed as a percentage of all 10 second epochs scored as a given behavioral state within each hour of total recording time across multiple days (Figure 3a). Alternatively, the diurnal distribution of each state can be displayed in a double raster plot, as is commonly used in studies of circadian rhythms (Fig. 3b).

Establishing steady-state baseline sleep

Prior to calculating baseline sleep/wake parameters in the mice, hourly percentage distributions were plotted for REM sleep, NREM sleep and waking for each hour of the baseline recordings (Figure 4). This display of the baseline sleep:wake states was monitored for steady-state pattern over the five to ten day period. Occasionally (in 2 of 41 mice), we detected a gradual increase or decrease in one or more of the behavioral states, particularly REM sleep (Fig. 4a), persisting for several days or longer, or an unexplained sleep disruption for several hours or more in an individual mouse (3 of 41 mice, as in Fig. 4b). From these hourly percentage graphs, a sample of three to five days of baseline recordings was selected for further analysis.

Baseline Sleep Parameters in Each of the Studied Strains

Total sleep: One-hour samples of EEG and EMG signals were reviewed for the 36 [C3HeB/FeJ (n=10), C57BL/6J (n=10), C3HxC57 (n=9) and 129X1/SvJ (n=7)] mice with steady-state baselines and validated computerized sleep state scoring. Baseline sleep state distribution timing data are presented in Table 3. There was an overall difference across strains of mice in time spent asleep across

<table>
<thead>
<tr>
<th></th>
<th>C3HeB/FeJ (n=10)</th>
<th>C57BL/6J (n=10)</th>
<th>C3HeB/FeJ* C57BL/6J (n=9)</th>
<th>129X1/SvJ (n=7)</th>
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<tr>
<td>Time Spent Asleep in 24 Hours (min)</td>
<td>T:613.6±24.7</td>
<td>T:665.11±20.7</td>
<td>T:562.7±25.2</td>
<td>T:857.1±33.7*</td>
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<tr>
<td></td>
<td>L:410.8±12.7</td>
<td>L:449.6±7.9</td>
<td>L:344.5±14.3*</td>
<td>L:440.2±13.6</td>
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<td></td>
<td>D:202.9±15.9</td>
<td>D:215.5±13.8</td>
<td>D:218.2±28.9</td>
<td>D:416.9±26.4*</td>
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<tr>
<td>Wake Diurnal ratio</td>
<td>0.60±0.02</td>
<td>0.54±0.01</td>
<td>0.78±0.07</td>
<td>1.01±0.08*</td>
</tr>
<tr>
<td>(Light:Dark)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep Efficiency</td>
<td>T:42.6±1.7</td>
<td>T:48.2%±2.4</td>
<td>T:39.1%±1.7</td>
<td>T:51.7%±4.8*</td>
</tr>
<tr>
<td>(% of 24 hour cycle asleep)</td>
<td>L:57.0%±1.8</td>
<td>L:62.7%±1.0</td>
<td>L:47.8%±2.0*</td>
<td>L:56.1%±3.7</td>
</tr>
<tr>
<td></td>
<td>D:28.2%±2.2</td>
<td>D:33.7%±4.1</td>
<td>D:30.3%±4.0</td>
<td>D:47.3%±6.0*</td>
</tr>
<tr>
<td>Average sleep cycle duration (min)</td>
<td>T:13.5±0.8</td>
<td>T:14.8±0.8</td>
<td>T:12.6±0.7</td>
<td>T:15.1±2.9</td>
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<tr>
<td></td>
<td>N:10.4±0.8</td>
<td>N:12.7±1.1</td>
<td>N:11.3±0.5</td>
<td>N:12.6±1.8</td>
</tr>
<tr>
<td></td>
<td>R:4.8±0.6</td>
<td>R:4.1±0.6</td>
<td>R:4.0±0.2</td>
<td>R:2.9±0.7*</td>
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<tr>
<td>Percentage of time spent in NREMS</td>
<td>T:37.7%±1.7</td>
<td>T:42.3%±1.4</td>
<td>T:35.6%±1.9</td>
<td>T:50.7%±4.3*</td>
</tr>
<tr>
<td></td>
<td>L:49.4%±1.8</td>
<td>L:56.9%±1.6</td>
<td>L:47.6%±1.7</td>
<td>L:54.4%±3.4</td>
</tr>
<tr>
<td></td>
<td>D:25.9%±2.1</td>
<td>D:27.6%±1.8</td>
<td>D:23.6%±2.8</td>
<td>D:47.0%±5.4*</td>
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<td>Delta density of NREMS</td>
<td>55.8±11.9</td>
<td>64.0±16.3</td>
<td>79.3±22.4</td>
<td>77.7±12.2</td>
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<tr>
<td>Delta density decline for light period</td>
<td>-5.1±0.7</td>
<td>-7.2±2.3</td>
<td>-4.8±0.7</td>
<td>-2.6±0.7*</td>
</tr>
<tr>
<td>Sigma density of NREMS</td>
<td>15.6±4.3</td>
<td>8.2±2.2*</td>
<td>15.2±6.2</td>
<td>13.0±3.3</td>
</tr>
<tr>
<td>Percentage of time spent in REMS</td>
<td>T:5.0%±0.2*</td>
<td>T:3.6%±0.5</td>
<td>T:3.7%±0.4</td>
<td>T:3.8%±0.5</td>
</tr>
<tr>
<td></td>
<td>L:7.7%±0.4</td>
<td>L:5.4%±0.8</td>
<td>L:5.6%±0.6</td>
<td>L:4.6%±0.5</td>
</tr>
<tr>
<td></td>
<td>D:2.3%±0.3</td>
<td>D:1.8%±0.3</td>
<td>D:1.7%±0.2</td>
<td>D:3.0%±0.5</td>
</tr>
<tr>
<td>Theta density of REMS</td>
<td>60.2±23.5</td>
<td>23.9±8.1*</td>
<td>38.9±15.6</td>
<td>36.6±12.9</td>
</tr>
<tr>
<td>REM sleep Latency (min)</td>
<td>7.5±0.7</td>
<td>9.6±1.6</td>
<td>8.0±0.7</td>
<td>9.5±1.1</td>
</tr>
<tr>
<td>REMS Diurnal Ratio</td>
<td>3.7±0.5</td>
<td>3.3±0.4</td>
<td>3.5±0.4</td>
<td>1.6±0.1*</td>
</tr>
<tr>
<td>Sleep Bout length (min)</td>
<td>T:12.9±0.9*</td>
<td>T:14.1±1.5</td>
<td>T:13.6±2.3</td>
<td>T:15.1±2.3</td>
</tr>
<tr>
<td></td>
<td>N:10.5±0.7</td>
<td>N:12.3±1.1</td>
<td>N:12.6±1.4</td>
<td>N:12.6±1.5</td>
</tr>
<tr>
<td></td>
<td>R:4.1±0.1</td>
<td>R:4.16±0.1</td>
<td>R:4.0±0.2</td>
<td>R:2.9±0.2</td>
</tr>
</tbody>
</table>
a 24-hour cycle (ANOVA, p<0.0001). Total sleep time
within the 24-hour cycle was similar for C57BL/6J,
C3HeB/FeJ and C3H X B6 mice (Scheffe’s post-hoc, N.S.).
In contrast, the 129X1/SvJ mice spent significantly more
time asleep than each of the other strains (Scheffe’s,
p<0.001). This increase in total time was directly related
to a two-fold increase (relative to each other of the strains) in
total sleep time during the dark period (p<0.0001).

Waking diurnal ratios: The diurnal ratios were higher
in C3H X B6 than C57BL/6J (p=0.005), and the diurnal
ratio was highest in the 129X1/SvJ’s (129X1/SvJ vs.
C3HeB/FeJ, p=0.0004; 129X1/SvJ vs. C57BL/6J, p<0.001;
129X1/SvJ vs. C3H X B6, p=0.008). In this substrain of
129 mice, the diurnal ratio of wakefulness time approached
1, and by repeated-measures ANOVA, there were no dif-
fferences in waking activity for the light vs. the dark periods
(p=0.74). In contrast, a diurnal rhythm in this substrain
was present for REM sleep activity (light:dark = 1.75,
p<0.001). Although this substrain is susceptible to retinal
degeneration,42 light entrainment appears normal as deter-
mined from graphical display records of individual mice
(Fig 4b).

NREM sleep: The differences between strains for
NREM sleep percentages paralleled the strain differences
observed in total sleep time. The 129X1/SvJ mice spent a
greater percentage of time in NREM sleep for the light period
than the other three strains (129X1/SvJ vs.
C3HeB/FeJ, p=0.008; 129X1/SvJ vs. C57BL/6J, p=0.05;
129X1/SvJ vs. C3H X B6, p=0.002). The 24-hour percent-
age of N-REM sleep time in C3H X B6 was similar to
C3HeB/FeJ mice. NREM sleep time was statistically less
for the hybrid strain than the percentage of time for
C57BL/6J mice (p=0.04). There were very small differ-
ences in NREM sleep percentage time in the light period
overall (p=0.008). Specifically, C57BL/6J mice spent a
slightly higher percentage of the light period in NREM
sleep than C3H X B6, p=0.02). Reflecting the pattern seen
with differences in total sleep times, the percentage of the

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Figure 5—Histogram displays for parameters of sleep architecture. Data are expressed as means±standard errors for each strain studied. Overall values for
C3HeB/FeJ and C57BL/6J mice are similar for most parameters, except REM sleep time for the light period, and are therefore reflected in the total 24-hour period as
well. The strain showing larger differences for most parameters was the 129X1/SvJ strain.
dark period spent in NREM sleep was two-fold higher in the 129X1/SvJ’s than in the other groups (129X1/SvJ vs. C3HeB/FeJ, p=0.0007; 129X1/SvJ vs. C57BL/6J, p=0.003; 129X1/SvJ vs. C3H X B6, p=0.0002).

REM sleep: The percentages of time spent in REM sleep were more variable within strains than percentages of time spent in NREM sleep. The between-group differences were of similar magnitude to the between-group differences for NREM sleep (a two-fold increase in the dark period for 129X1/SvJ mice, relative to other strains), but were not statistically significant overall. In contrast, REM sleep percentages during the light period were higher in the C3HeB/FeJ mice compared to 129X1/SvJ mice (p=0.01), while REM sleep percentages in the dark period were higher in the 129X1/SvJ mice compared to other groups (p<0.001). Despite differences in REM sleep times, the ratios of NREM sleep time to REM sleep time for the 24-hour period were similar for the four groups (p>0.6 in all cases).

Behavioral state distribution: Mean hourly distributions of NREM sleep and REM sleep (±SE) are shown in Figure 6 for the four strains tested. As previously reported,23 C57BL/6J mice have a biphasic sleep pattern with a second sleep-predominant time in the second half of the dark period. In contrast, C3HeB/FeJ mice show a mono-phasic sleep period with minimal sleep at lights-out, a gradual increase in sleep until through the first third of the lights-on period, followed by a decline in sleep in lights-out. The hybrid group shows an intermediate pattern. Hourly patterns also revealed less pronounced diurnal rhythms for both NREM and REM sleep in the 129X1/SvJ mice, although the rhythm was more appreciable for REM sleep.

REM sleep latency: REM sleep latencies during baseline sleep were similar for the three inbred strains and the one hybrid strain, with an overall mean latency from the onset of a NREM sleep bout to REM sleep of 9.5±1.1 minutes.

Sleep Fragmentation, Consolidation, and Delta Decline Across the Light Period

Sleep fragmentation (arousal index) did not differ among these four strains. Total sleep bout lengths were also similar in the four strains. REM sleep bouts, in contrast, were shorter in the 129X1/SvJ mice (p<0.05). The decline in delta density across the light period was most pronounced in the C57BL/6J mice (p<0.01) and was least pronounced in the 129X1/SvJ mice (the strain with more...
### Table 4—Response to 12-hour total sleep loss on recovery sleep

<table>
<thead>
<tr>
<th>Recovery Period Parameter</th>
<th>C3HeB/FeJ n=9</th>
<th>C57BL/6J n=6</th>
<th>129X1/SvJ N=n6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C3HeB/FeJ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ in Total Sleep (24 hours)</td>
<td>47.74 min ±43.94</td>
<td>190.50 min ±46.15*</td>
<td>-126.28 min ±165.59</td>
</tr>
<tr>
<td>Δ in NREM sleep (first dark period)</td>
<td>23.76 min ±25.45</td>
<td>141.45 min ±35.21*</td>
<td>-32.73 min ±81.46</td>
</tr>
<tr>
<td>Δ in NREM sleep (light period)</td>
<td>76.88 min ±25.83</td>
<td>25.75 min ±26.71</td>
<td>-43.78 min ±78.85</td>
</tr>
<tr>
<td>Δ in REM sleep (dark period)</td>
<td>-2.08 min ±2.31</td>
<td>21.32 min ±8.53*</td>
<td>5.78 min ±7.99</td>
</tr>
<tr>
<td>Δ in REM sleep (light period)</td>
<td>0.85 min ±2.97</td>
<td>9.73 min ±9.32</td>
<td>-5.50 min ±7.11</td>
</tr>
<tr>
<td>Δ Delta Density (dark period)</td>
<td>20.68±17.22</td>
<td>22.00±12.69</td>
<td>92.56±52.23</td>
</tr>
<tr>
<td>Δ Delta Density (light period)</td>
<td>-9.24±3.85</td>
<td>-37.67±15.68</td>
<td>68.92±49.31</td>
</tr>
<tr>
<td>Δ in NREM sleep bout length (24-hour recovery)</td>
<td>2.1min±0.3*</td>
<td>3.0min±6*</td>
<td>0.5min±3*</td>
</tr>
<tr>
<td>Δ in arousal index (arousals/hour)</td>
<td>-1.5±0.2*</td>
<td>-1.7±1.0</td>
<td>1.1±1.2</td>
</tr>
</tbody>
</table>

### Figure 8—Frequency distributions for sleep bout lengths (total sleep and REM sleep bouts) at baseline (upper panel) and after sleep deprivation (lower panels). Bout lengths are similar in the four groups C3HeB/FeJ (white), C57BL/6J (black), C3HeB/FeJ X C57BL/6J (gray), and 129X1/SvJ (speckle d). Total sleep bout lengths increased following sleep deprivation, particularly for bouts >12 minutes (asterisk, p<0.05).
Response to Sleep Deprivation Across the 12 Hour Light Period

Twelve hours of total sleep deprivation during the light period, ending at the onset of the dark period, produced highly variable recovery responses within strains, as well as across strains. Sleep deprivation was performed on just three C3HXB6 mice. These mice, therefore, are not included in the recovery sleep analysis. Comparisons were made between baseline sleep and the first 24 hours following the termination of sleep deprivation. C57BL/6J mice demonstrated an increase from baseline in both NREM sleep (141.70 minutes ± 33.20, p<0.05) and in REM sleep (21.32±8.53 minutes, p<0.01) during the first dark period post SD. Delta density, however, was below baseline, and these mice did not demonstrate an increase in delta energy. The 129X1/SvJ mice did not increase sleep times post SD, but significantly increased delta density and therefore, this group demonstrated an increase in the product of delta density and NREM sleep time: f (p<0.01). The most striking finding was a persistent increase in delta density for each hour of the 24-hour recovery period in the 129X1/SvJ mice, while the other two strains showed a persistent decline in delta density. C3HeB/FeJ mice showed highly variable changes in total sleep time in the 24 hours following sleep deprivation with no significant change (see Table 4). Similarly, there were no differences for the group in NREM sleep time or REM sleep time for either the first dark or light period. C3HeB/FeJ mice that did not show an increase in sleep times did have an increase in delta density for NREM sleep. Thus, delta energy in the recovery period was increased in all C3HeB/FeJ mice for the first dark period. Afterwards, delta density declined without a coinciding increase in NREM sleep time, such that delta energy for the first light period was different from baseline.

The impact of 12-hour sleep deprivation on REM sleep latency was evaluated by comparing REM sleep latencies for each mouse using average baseline values for the dark period and values obtained in the first dark period following sleep loss. The REM sleep latencies for the 129/SvJ mice were shortened from 8.95±1.0 minutes to 3.95±1.1 minutes (t=3.9 and p<0.03). In contrast, the C3HeB/FeJ and C57BL/6J mice showed no differences in REM sleep after sleep loss.

DISCUSSION

We tested the utility of an available sleep scoring program for developing a robust phenotypic assay for sleep structure in mice. Although the algorithm for scoring behavioral state was not changed, the characterization of sleep was expanded to include measures of sleep bout length, sleep fragmentation, the homeostatic response to sleep loss and REM sleep latency. In addition, we have presented in detail: (1) a protocol for recording behavioral states in mice, (2) descriptions of behavioral states and definitions of sleep parameters, and (3) reference baseline sleep parameters and sleep response patterns after 12 hour sleep deprivation in four strains of mice commonly used in genetics experiments.

Accuracy and Validation of the Program

The overall agreement between computerized scoring of sleep states by the ACQ 3.4 program and by human scorers was greater than 95% for sleep staging in mice. This degree of accuracy is comparable to the accuracy reported using the original Benington and Heller sleep analysis algorithm in rats, and is similar to the only other sleep analysis system validated in mice. The accuracy achieved in our study was enhanced by excluding data from mice in which behavioral sleep states were not identifiable by human scorers. However, rejection of electrographic data must be done with caution, particularly in the analysis of mutant lines of mice. Aberrant EEG and EMG signals may result from atypical physiological signals, which would be important to identify in mutant mice. It is necessary, therefore, to review a sample of raw EEG and EMG signals in all mice. That sample should include all behavioral states, and it is equally essential to examine the power spectral analysis for each mouse. A two-hour sample of the EEG and EMG data can be reviewed within five minutes to assess for abnormal waveforms, and spectral analysis can be performed in 10 minutes. Both of these measures should allow detection of aberrant electrographic waveforms or spikes that may be very important and otherwise missed if unscorable data were simply discarded.

We found no difference in the accuracy of the initial scored section and the validated section in either the light or dark periods. Therefore, determining the accuracy of computer scoring for any one-hour mid-light sample with all sleep states is adequate to measure accuracy and adjust the algorithm threshold to optimize computerized scoring in each mouse. In contrast, there was a slight decline in the accuracy of the program in detecting REM sleep after sleep deprivation by gentle handling. We believe that in studies involving sleep deprivation or extensive handling of the mice, a validation after the handling of mice is warranted to ensure continued accuracy. Accuracy in determining REM sleep after sleep deprivation can be improved typically by adjusting the EMG threshold for REM sleep.

The accuracy of the Benington and Heller algorithm in determining REM sleep was greater in the C3HeB/FeJ mice than in other strains. This particular strain demonstrated higher amplitude theta and more theta waveform activity in REM sleep. Further, this strain makes a rapid
transition from NREM sleep into REM sleep, while the other strains of mice have a longer NREM to REM sleep transition (20—40 seconds of slow waves interspersed with theta). The increased theta synchronization in this strain may provide a mouse model for studying the hypothesized REM sleep and theta activity mechanisms involved in hippocampal function.47

Limitations of This System

The Benington and Heller program was designed to analyze sleep off-line. Therefore, analysis with this system requires time to download EEG signal files, perform a Fourier transform, set thresholds, check accuracy and then perform the data analysis through an Excel® program. These steps can be tedious when dealing with records collected over several days for many mice. For this reason, data analyzed in the present study were typically collected for an entire week and then retrieved and analyzed. Downloading data requires cessation of recordings for at least one hour. Additional programming would be required to allow recording either directly onto a compact disc recorder or other data archival systems. The on-line signals were observed during collection to ensure adequate signal quality, prior to off-line analysis. Advantages of an off-line analysis system are that the experimenter may image raw signals on-line at any time. The accuracy may be determined independently of the algorithm at any time after the data are down-loaded.

A second limitation of the present program is that the code is somewhat dated, in that it does not reflect changes in computer architecture, or in programming languages. We are currently attempting an update, revising the file handling routines and providing a Windows® interface. This should reduce the time required for both the retrieval and analysis steps, permit on-line analysis of ongoing recordings, and make the programs easier to use.

Establishing a Recording Protocol

In the present study, we measured baseline sleep over five to seven days, following a seven to nine day recovery from surgery and acclimation to the recording cable and chamber. Stable day-to-day NREM sleep and REM sleep activity was present by day eight post-operatively in all mice that exhibited steady-state activity for three or more days. Steady-state sleep/wake behaviors were not observed in all mice tested. Therefore, we recommend obtaining a five-day baseline beginning eight days after surgical implantation of electrodes.

Measuring the response to sleep deprivation provides important information on the homeostatic control of sleep. In this study, we examined the response to 12 hours of total sleep loss throughout the lights-on period, terminating at lights-off. This protocol in rats provides greater percentage changes in both NREM sleep and REM sleep times. There are two concerns with the 12-hour duration of sleep deprivation. First, after six hours of sleep deprivation in mice, it is very difficult to maintain wakefulness, and later in the 12-hour period, stimulation is nearly constant and must be more vigorous. Second, in the present study, we observed large intra-strain differences in the response to sleep loss ending at lights-off. This large degree of variance within a genetically homogeneous group of mice is indeed interesting, but may result from environmental variance. Alternatively, this large variance may reflect larger magnitudes in the ultradian rhythms of behavioral states seen at this circadian time.46 Six hours of total sleep loss terminating within the lights-on period in mice results in much less intra-strain variance.24 Therefore, for genetic studies of sleep homeostasis, a protocol using six-hours of sleep loss for the first half of the lights-on period is preferred. Ideally, the homeostatic response should be characterized as a dose-response curve, and this will require further study.

Phenotypic Characterization of Sleep in Genetic Studies of Sleep in Mice

Over the past three decades, numerous parameters have been used to characterize sleep in mice. However, in rodents, these parameters have not been standardized. To provide an overall description of sleep, we have compiled a list of sleep parameters, including parameters commonly used to describe human sleep, such as the intensity of each behavioral state, the consolidation and the fragmentation of sleep, and the timing of NREM and REM sleep. We have provided detailed definitions and descriptions of the measurement for each sleep parameter in the Methods section. It is hoped that this list will generate discussion and ultimately some consensus concerning the phenotypic characterization of sleep in mice.

In humans, NREM sleep is scored as stages 1, 2 and 3/4, depending upon spindle and K complex frequency and the density of delta waves. We were not able to discern discrete spindle complexes and K complexes in mice. We, therefore, characterized NREM sleep with delta density, sigma density, bout length, and the arousal index, while REM sleep is characterized with theta density and bout length.

Characterization of Sleep in Mice Using this Sleep Analysis System

The overall hourly distribution patterns of behavioral states across the 24-hour light-dark cycle are remarkably similar to those published.8,10,22,24,26,27,39,40 The C57BL/6J mice in the present study demonstrated a second sleep-predominant period within the later portion of the dark period, as previously reported.24 However, there were differences...
between our observations of sleep state percentages for 24 hours and the light period, and those previously reported. While the percentage of time spent in waking for the 24-hour cycle and the percentage of time spent in NREM sleep were within the range of previously reported values (waking: 35% to 54% and NREM sleep: 35 to 57%), the average percentage of time spent in REM sleep in our study (3.8%) was less than reported values 4.8%, 24 5.4%, 44 5.6%, 8 5.8%, 14, 40 and 7.6%. We, therefore, instrumented and analyzed an additional four mice and re-validated all C57BL/6J mice in the present study. Accuracy for REM sleep scoring was >80% and with balanced false positives and negatives. Furthermore, the additional mice showed REM sleep percentages (4.2%) similar to those obtained in our initial group of mice. We believe the discrepancy between our percentages for REM sleep and those from other studies may be attributed to the differences in defining the onset of REM sleep. We required that theta waves comprise >75% of an epoch of REM sleep, which may be a more conservative definition. Clearly, a consensus on how to define REM sleep is needed among researchers studying sleep in mice.

REM sleep percentage in C3HeB/FeJ mice is similar to previously reported values, 29 while NREM sleep percentages in our animals was lower (34% in our study and 51% in the previous study). In studies validating sleep state interpretation in C57BL/6J mice and C3HeB/FeJ mice, the accuracy in scoring NREM sleep epochs are quite high (>95%), and the variance is quite low. 40 There are many reasons why NREM sleep percentages may differ, including not only definition of behavioral states, but ambient temperature and lighting, sex and age of the mice, food and water access, the duration of the recovery period from electrode implantation, and access to a running wheel or mobility allowed with the implant and cable. These confounding variables emphasize the need for very specific reference standards for scoring sleep in mice.

Response Patterns to 12 hour Sleep Deprivation

Sleep deprivation revealed patterns of responses unique to each strain. All C3HeB/FeJ mice showed an increase in delta energy for the dark period (or the first 12 hours of recovery). In most C3HeB/FeJ mice, this was attributable to an increase in NREM sleep time. In two of these mice, however, this increase in delta energy was due to a large increase in delta density in NREM sleep. The C57BL/6J mice all showed increased total sleep, NREM sleep and REM sleep times, each similar in magnitude to the amount of increased sleep reported in a recent study of C57BL/6J mice. 24 In the present study, C57BL/6J mice did not show an overall increase in delta energy. Whether the absence of a delta energy change in our study occurred because of a difference in the circadian time of sleep deprivation, the duration of deprivation, or because of changes in the amplitude of electrical signals after sleep deprivation, is unclear. Of interest, the differences in baseline sleep parameters across strains did not parallel the differences in recovery sleep parameters across strains. This suggests that genetic influences on homeostatic aspects of sleep control may differ significantly from the baseline genetic influences on sleep control in laboratory mice.

Strain Differences in Sleep Structure

There were few significant differences in baseline sleep times and behavioral state percentages between the C3HeB/FeJ and C57BL/6J substrains and their progeny, with the exception of an increase in REM sleep time for C3HeB/FeJ mice in the light period. This suggests that that C3HeB/FeJ and C57BL/6J may be excellent background strains for studies of single gene mutations that have to be analyzed on two backgrounds for the purpose of mapping. In contrast, the 129X1/SvJ mice demonstrated significantly higher amounts of NREM sleep and REM sleep in the dark period, thereby increasing sleep time and sleep efficiency, without affecting the NREM sleep:REM sleep ratio. Large differences in sleep parameters compared to other strains would pose a serious problem in selecting a background strain for breeding or a strain for comparison purposes. These data suggest that this particular substrain of 129/Sv mice is not well-suited for determining the impact of an altered gene on sleep.

CONCLUSIONS

In summary, we have evaluated an automated sleep scoring program for use in mice. Adequate agreement between the computer and human scoring can be obtained, provided care is taken in the implantation procedure to acquire and maintain high quality recording signals. We have modified the analysis to include parameters of sleep consolidation, fragmentation, bout lengths and REM sleep latencies, and these modifications will be made available to sleep researchers. The automated sleep scoring and comprehensive analysis program and the recording protocol have been designed to facilitate studies of the genetics of sleep in mice. This recording and analysis programs, with our modifications and source code, are available upon request. We have used this system to describe baseline sleep patterns in three commonly used inbred strains of mice. Standardization of electrophysiological parameters and measurement techniques, on a common set of inbred or mutant lines, will facilitate the comparison of sleep anomalies identified and/or reported by different laboratories.

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