Age-related Changes in Sleep in the Rat

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Summary: Human sleep in old age is characterized by a number of changes, including reductions in sleep efficiency, amounts of visually scored slow-wave and REM sleep, and amplitude of the diurnal sleep/wake rhythm. In older rats, some, but not all, of these traits have been reported, including a decrease in the mean duration of sleep bouts, an increase in the number of sleep bouts, and a modest reduction of REM sleep. Studies of the diurnal rhythm of total sleep have had varied results. There are, however, virtually no data indicating at what point across the rat's lifetime the changes seen in old age begin to occur. In order to more fully characterize sleep in older rats, and to develop data on when they first appear, we have examined sleep in young adult (3 months), middle-aged (12 months), and older (24 months) rats during 24 hours under constant dim light. Analyses of variance revealed no age-related changes in total sleep, NREM or REM sleep, wake time after sleep onset, or three different measures of the amplitude of the sleep/wake circadian rhythm. There were, however, significant age-related reductions in high-voltage NREM sleep ("HS2"), the mean length of sleep bouts, and REM-onset duration. These were seen in the 1-year-old rats, indicating that the changes seen in the older animals were evident by midlife.

Key words: Sleep; REM sleep; circadian rhythms; aging; temperature

The complaint of poor sleep and the resultant use of sleeping pills increase with advancing age in humans.[1] These changes in sleep are reflected in polygraphic evidence of deficits in total sleep and sleep efficiency, as well as increased sleep latency and awakenings during the night. In addition, there is an age-related reduction in specific sleep stages, including a decline in time spent in visually scored slow-wave sleep and REM sleep,[2-4] although the latter may be less marked when expressed as a percentage of total sleep.[5] In the aging rat many—though not all—of these processes seem to occur; under entrained conditions there is, for instance, a decrease in the length and an increase in the number of sleep bouts.[6]

The decline in total sleep time after middle age in humans is small in magnitude.[7] The classic study of Feinberg et al[8] indicated that, across the human lifetime, total sleep time decreases in the shape of a cubic curve, while the number of awakenings after sleep onset increases linearly. One review found four studies which showed a decrease in total sleep, two which found no change, and one which reported an increase.[5] Similarly, studies of rats have had mixed results. Total 24-hour sleep has been reported to not change significantly,[9,10] decline in the light but not the dark phase under 12:12 LD conditions,[11,12] or decline slightly.[6]

A more consistent finding in humans has been the decrease in the amplitude of the day-night rhythm of sleep and waking.[5,13] Most[6,9,11,12] but not all[10] studies of older rats under entrained conditions have noted a similar decline. There is some evidence that this can be reversed by increased light intensity,[9] but not by enriching the environment.[12] The decrease in amplitude has also not been seen in a study of rats in constant dim red light.[14]

It is not yet known at what point across a rat's lifetime these changes begin to appear. Aside from a single longitudinal study,[10] most work in this area has compared young

Accepted for publication December, 1998

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adult (about 4-8 months) and very old (about 22-30 months) animals.[6,9,11,12] Thus it is uncertain when these various changes seen in very old rats are first manifested. The goal of the present study is to more fully characterize age-related alterations in sleep in the rat, and to determine the timing of onset, ie, whether some or all of these processes are evident in middle-aged animals.

METHODS

Studies were performed on three groups of male Fisher rats, including those which were: "young": age 3 months (n=20); "middle-aged": age 12 months (n=14); and "old": age 24 months (n=15)

Rats were kept in a 12:12 light:dark cycle, with lights turned on at 1000 hours, from the time of their arrival in the laboratory until the beginning of the experiment. Surgical implantation of recording electrodes and related procedures prior to the study have been presented in detail by Bergmann et al.[15] In summary, rats were anesthetized with ketamine hydrochloride and xylazine; two sets of stainless-steel screws were then installed laterally on the skull to record the cortical electroencephalogram (EEG) with minimal theta EEG activity, and two were implanted more medially to obtain EEG richer in theta. Stainless steel wire electrodes were implanted in the temporalis muscle to record the electromyogram (EMG). The skull was coated with P-10 Resin Bonded Ceramic (3M Dental Products, Catalog No. 9301S), and the recording plug was cemented to it with dental acrylic (Minit Weld, Teledyne Getz). A temperature-sensitive transmitter (MiniMitter Co., Sunriver, Ore.) was implanted in the peritoneal cavity for measurement of core temperature.

After 1 week of recovery, rats were placed in the disk-over-water sleep-deprivation apparatus,[15] which served as the recording chamber, for 3 days of adjustment, under continuous dim light conditions. On the fourth day, starting at 1000 hours, the 24-hour sleep recording period—which comprised the data presented in this paper—was begun. During this period, the sleep-deprivation procedure was not being performed, so that functionally the rats were being housed in a normal cage in which the floor area (1800 cm^2) was approximately four times that of their home cages. The room containing the disk-over-water apparatus was kept in constant dim light, at an ambient temperature of 21° τo 24°C.

As described previously,[15,16] the PASS automated scoring system, which has been validated against visual and behavioral scoring, generated an interpretation of waking or various sleep stages for each 30-second epoch according to the following algorithm:

Waking: Low-amplitude EEG, low-theta EEG, high EMG.

Paradoxical sleep (PS), or REM sleep: low-amplitude EEG and EMG and high theta activity.

Non-rapid eye movement sleep (NREM): all sleep that is not PS.

The NREM sleep was in turn divided into:

Low-voltage sleep: NREM sleep containing low-amplitude EEG, EMG and theta.

High-voltage sleep (HS): NREM sleep with high EEG amplitude. This in turn is subdivided into:

HS1: The fraction of HS with EEG amplitude less than the modal EEG amplitude.

HS2: The fraction of HS with EEG amplitude greater than the modal EEG.

Definitions of various sleep measures, all based on the use of 30-second epochs, include:

Sleep episode (bout): At least three consecutive epochs of nonwaking scores (sleep onset), continuing until the occurrence of a waking episode.

Waking episode: At least three epochs of waking, separated by no more than two epochs with sleep scores. This is superseded by the rule for REM episodes—ie, if criteria for both are met, the REM episode continues.

Arousal episode: Epochs of waking inside sleep episodes which do not meet the criteria for waking episodes.

REM sleep episode: At least two epochs with REM scores. REM epochs may be separated by no more than two epochs of another stage.

REM-onset duration: The time from the onset of a sleep episode to the onset of a REM episode.

REM-to-REM cycle length: Time from the beginning of one REM episode to the beginning of the next REM episode.

Intermittent wakefulness (wakefulness after sleep onset): Number of waking epochs within sleep episodes.

Statistical analyses of possible age-related changes in sleep stages or other measures (eg, sleep bout length) were performed by use of one-way analyses of variance (ANOVAs), in which the independent variable was age and the dependent variable was the measure in question. In those cases in which the ANOVA showed a significant effect of age, post hoc analyses were performed by use of the Least Significant Difference (LSD) test. In order to determine whether any age-related changes in sleep differed in the time of their occurrence across the 24 hours, we also performed two-way ANOVAs, in which the independent variables were age and time period (sequential 6 hour segments of the 24-hour recording), and in which the dependent variables were the various sleep measures.

For purposes of determining whether there were age-related changes in the amplitude of the circadian rhythm of sleep and waking, three complementary analyses were performed for total sleep, NREM and REM sleep: (1) the mean "day/night ratios" (ratio of the total amount in the first to the second 12 hours of the recording) for each of

these measures were determined—this approach is limited as it does not allow for any possible phase shifting which might take place; (2) a least-squares cosine fit was performed for total sleep in 20-minute blocks, thus producing an amplitude and an acrophase measure. The amplitude measure is the 24-hour sinusoidal component of the fraction of time spent in sleep; and (3) an "A" statistic, which has previously been used to determine amplitude of circadian rhythms of sleep in the rat[11,17] was applied to the 20-minute blocks. Unlike the cosine fit procedure, the "A" statistic does not make assumptions about the type of curve involved. It is based on the average amplitude of a rhythm, and is expressed as a number between 0 and 100, in which 100 would be found if all the epochs of a sleep stage were in a single 12-hour time block, and 0 would indicate there was the same amount of the sleep stage during each block.

All of these measures assumed a 24-hour free-running period, as the recording time of 24 hours was too short to estimate the period more exactly. These measures then served as dependent variables in one-way analyses of variance in which the independent variable was age.

Possible age-related changes in the circadian rhythm of core temperature were examined by use of the cosine fit procedure and a modification of the "A" statistic,[11,17] such that amplitude was expressed as mean trough-to-trough (deg. C). In both cases, the time until maximum values was reported from a starting point of 1000 hours (which was the beginning of the 12-hour lights-on period prior to entering the animal into the continuous dim light conditions of the study). Statistical significance was determined by ANOVA, the Tukey HSD test (using the mean square error from the ANOVA), or by regression analysis using age group as a linear variable.

RESULTS

Total Sleep and Sleep Stages

As seen in Fig. 1, the ANOVA revealed no significant effects of age on total sleep time, wake time, REM or NREM sleep. Inside NREM sleep, however, there was a significant progressive decline in the percentage of time spent in high-voltage NREM (HS2) in the young, middle-aged, and older rats (23.34±1.01%, 18.54±1.60%, and 15.25±1.04% respectively; df=2,388; F=4.144, $p<0.05$). Post hoc testing revealed that the older and middle-aged rats differed significantly from the young animals ($p<0.01$ and $p<0.05$ respectively). There were significant effects of time (ie, the four sequential 6-hour time blocks) on all variables, reflecting the lower amounts of sleep in the second 12 hours of the recording (2200-1000), particularly 2200-0400 (the third 6-hour period). Thus an analysis of HS2 sleep indicated lowest values were evident in the third 6-hour period (Fig. 2). There was a corresponding increase in

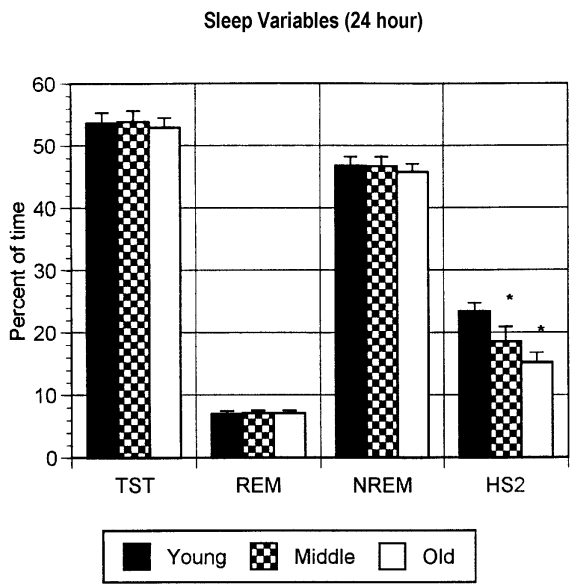

Figure 1.—Sleep variables in young, middle-aged and old rats across 24 hours. Data in this and all subsequent graphs are expressed as a percentage of recording time, error bars reflect standard error of the mean (SEM), and asterisks indicate significant differences ($p<0.05$) from young animals tested at the same time period, as determined by post-hoc LSD tests. Abbreviations in all figures: young = 3 months of age; middle = 1 year of age; old = 24 months of age.

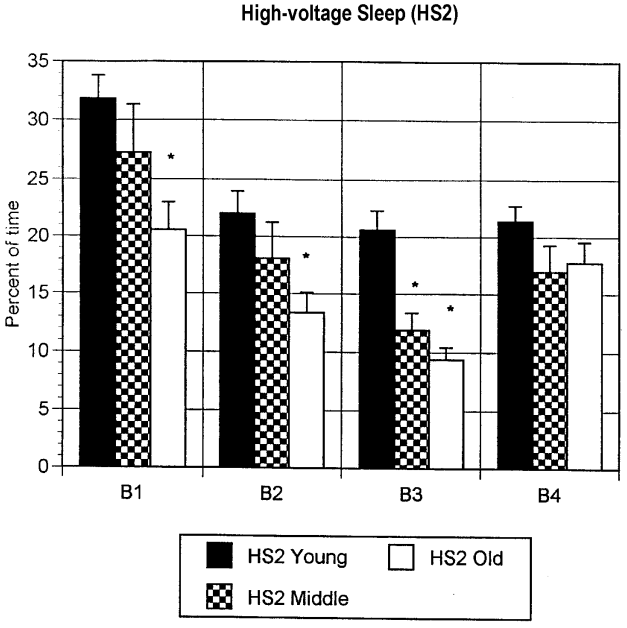

Figure 2.—High-voltage NREM sleep (HS2) as seen in sequential 6-hour time blocks during the 24-hour recording. An ANOVA revealed significant effects of age ($p<0.0001$) and time period ($p<0.0001$), but no significant interaction between the two.

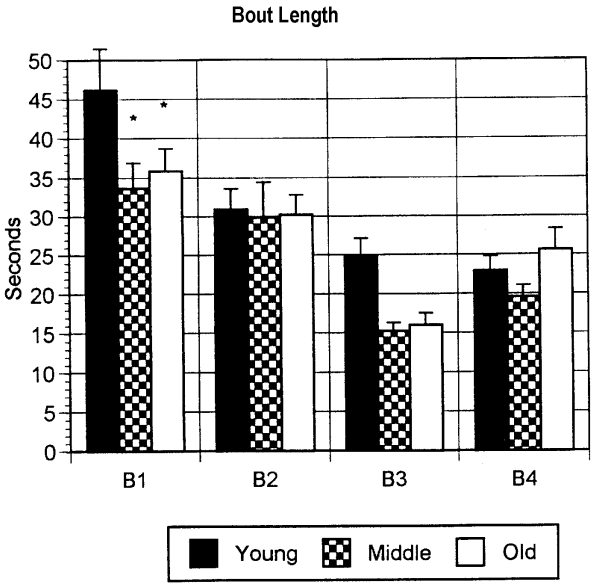

Figure 3.—Length of sleep bouts (episodes) in young, middle-aged and old rats. A two-way ANOVA revealed significant effects for age ($p < 0.01$) and time period ($p<0.0001$), but no significant interaction between the two. In addition to the significant changes in middle-aged and old rats compared to the young animals in the first 6-hour period, there was a trend ($p < 0.06$) for old rats to have shorter sleep bouts during the third period.

low (LS) and midlevel voltage (HS1) NREM with progressive age, explaining why total NREM sleep was unaffected by age. Although, as already mentioned, there was not a significant age effect on REM sleep, there was a significant age × time period interaction, primarily reflecting very low amounts of REM sleep in the third 6-hour period. REM onset duration declined from the younger to the middle-aged and older rats (19.41±0.64, 15.96±0.77 and 17.79±0.79 epochs respectively, df=2,388, F=6.472, $p<0.01$). Post hoc testing revealed that both the middle-aged and older rats differed significantly from the younger rats ($p<0.01$ in both cases).

Measures of Sleep Quality

The number of sleep bouts, the number and mean length of arousal episodes and waking episodes, the total amount of waking time after sleep onset, and the number of REM-NREM cycles were not significantly affected by age. The mean length of sleep bouts was significantly shortened in the middle-aged and older rats (31.22±1.93, 24.59±1.75 and 26.94±1.53 epochs respectively; df=2,389,F=3.965, $p<0.05$); this was most evident in the first 6-hour period (Fig. 3). For the 24 hours taken as a whole, the mean duration of the REM-NREM cycles (time from the beginning of one REM period to the beginning of the next) significantly differed among the young, middle-aged, and older rats at 29.02±0.71, 27.30± 0.85, and 24.44±0.68 epochs respectively (df 2,46; F=3.972, $p<0.05$), and post hoc testing revealed that the difference in the young and old rats was significant at the $p<0.01$ level.

Table 1.—Day/night ratios of sleep variables

	Young		Middle age		Old	
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
TST	1.393	0.082	1.559	0.114	1.435	0.065
NREM	1.315	0.075	1.434	0.082	1.367	0.057
REM	2.22	0.394	3.82	0.932	2.1	0.209

Analyses of variance revealed no significant differences for age for any of these variables. Abbreviations: TST = total sleep time; NREM = non-rapid eye movement sleep; REM= rapid eye movement sleep (paradoxical sleep); young = 3 months of age; middle-age: 1 year of age; old= 2 years of age. Values are expressed as percentages of total recording time (24 hours).

Table 2.—Core temperature data

	Cosine Fit		"A" Statistic	
	Amplitude (deg. C)	Acrophase (Hr)[1]	Maximum amplitude (deg. C)	Maximum amplitude occurs at (Hr)[1]
Young[2] (n = 17)				
Minimum	0.176	-11.261	0.340	-10.667
Maximum	0.921	-3.494	1.820	-3.000
Mean	0.428	-7.511	0.827	-7.588
Std. error	0.041	0.474	0.080	0.527
Middle aged (n = 12)				
Minimum	0.247	-10.292	0.399	-9.667
Maximum	0.846	-4.571	1.407	-5.000
Mean	0.485	-7.498	0.861	-7.111
Std. error	0.055	0.548	0.095	0.518
Old (n = 14)				
Minimum	0.245	-10.102	0.433	-9.333
Maximum	0.831	-5.887	1.601	-4.667
Mean	0.508	-8.337	0.957	-8.095
Std. error	0.056	0.292	0.108	0.295

(1) Time (in hours) from 1000 hours, which was the beginning of the 12-hour lights-on period prior to entering the animal into the continuous dim light conditions of the study (see Methods section).
(2) There were no significant differences between age groups, either by ANOVA, Tukey HSD test, or by regression analysis using age group as a linear variable.

Amplitude of Circadian Variation of Sleep Variables

The ratios of the amounts of total, NREM and REM sleep during the first 12 hours (1000-2200) to the second 12 hours (2200 to 1000) of the recording were calculated as measures of the amplitude of the circadian rhythm of sleep.

No significant age-related changes were detected (Table 1). Similarly, ANOVAs indicated no significant effect of age on the amplitude measures of sleep epochs in the cosine fit or "A" statistic procedures.

Amplitude of Circadian Variation of Core Temperature

As seen in Table 2, there were no statistically significant changes in any measure, including amplitude or duration until peak amplitude using either cosine fit or modified "A" statistic procedures.

CONCLUSIONS

In summary, this study demonstrated the presence of clear alterations of sleep quality, but not duration, which are already present in midlife. The observed absence of an age-related change in 24-hour total sleep adds to the weight of previous studies in rats,[9,10] and suggests perhaps that any changes with age are more subtle than to be reflected in the total amount of sleep. Human studies of REM sleep have tended to show an age-related decline in absolute amounts, although results are more mixed when expressed as a percentage of total sleep time.[5] Previous studies of rats have indicated modest[6,10] or not-so-modest[11] declines in REM. We found no evidence of age-related changes in total amounts of REM sleep. Aside from differences in strain of rat, the only other obvious experimental difference was that the former studies were performed in 12:12 light:dark conditions, whereas the present study was performed in constant dim light.

One measure of sleep quality, wake time after initial sleep onset, was unaltered in older rats, differing from the human literature. The mean length of sleep bouts, however, declined in the old rats, as reported in previous studies.[6,10] Inside NREM sleep, there was indeed an age-related decline in high-amplitude sleep (HS2). We know of no comparable data with which to compare this point. One rat study reported no age-related difference in theta-to-delta amplitude ratio,[11] and two studies found no difference in delta amplitude.[6,10] The present data suggest that quantification of high-amplitude NREM (HS2) in the rat, which is based on a measure of amplitude across the entire EEG spectrum of 1.5-20 Hz,[15] provides a clearer temporal parallel to the decline in slow-wave sleep across the human lifetime than do measures of delta amplitude or power in the rat. We also found a decline in REM-onset duration, as also seen with aging in humans.[18,19]

One of the more consistent findings in the human literature has been a decline in the amplitude of the day-night rhythms of sleep and waking.[13] Our study, like that of van Gool, Witting and Mirmiran,[14] found no evidence for this under the experimental conditions of constant dim light. This result is also similar to that of Zepelin, Whitehead, and Rechtschaffen,[10] who examined old rats under a 12:12 light-dark cycle. A decrease in the circadian amplitude for NREM and REM sleep was reported by Rosenberg, Zepelin, and Rechtschaffen[6] in rats under a 12:12 LD condition. One study suggests that this age-related change can be reversed by increased light intensity during the light phase of a 12:12 light-dark cycle,[9] and it possible the conditions of our study (constant dim light) accounts for this difference in results.

One of the purposes of this study was to examine the point at which the changes in sleep during aging begin to be manifest. We observed that the characteristic findings of old age in terms of the length of sleep bouts, the amount of HS2 NREM sleep, and REM-onset duration, were already present in the middle-aged animals. Whether these alterations in sleep, which have been evident for at least a year by the time a rat reaches 2 years of age, are related to alterations in functioning will be the subject of future research.

ACKNOWLEDGMENT

The authors acknowledge the excellent technical assistance of Ms. Ericka Laufenberg, Mr. Jeffrey Limjuco, Ms. Thythy Pham, and Mr. Kevin Rosi. This study was partially supported by NIH grants 2PO1 AG 11412 and 1KO7HL03640.

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