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Survival analysis indicates that age-related decline in sleep continuity occurs exclusively during NREM sleep

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Abstract

A common complaint of older persons is disturbed sleep, typically characterized as an inability to return to sleep after waking. As every sleep episode (i.e., time in bed) includes multiple transitions between wakefulness and sleep [which can be subdivided into rapid eye movement (REM) sleep and non-REM (NREM) sleep], we applied survival analysis to sleep data to determine whether changes in the “hazard” (duration-dependent probability) of awakening from sleep and/or returning to sleep underlie age-related sleep disturbances. The hazard of awakening from sleep – specifically NREM sleep – was much greater in older than in young adults. We found, however, that once an individual had spontaneously awakened, the probability of falling back asleep was not greater in young persons. Independent of bout length, the number of transitions between NREM and REM sleep stages relative to number of transitions to Wake was approximately six times higher in young than older persons, highlighting the difficulty in maintaining sleep in older persons. Interventions to improve age-related sleep complaints should thus target this change in awakenings.

Keywords

Sleep; aging; insomnia; survival analyses

1. Introduction

Subjective complaints of disturbed or un-refreshing sleep are frequent in the U.S. population, especially among older persons (Ancoli-Israel, 2005, National Sleep Foundation, 2002, National Sleep Foundation, 2003). Insomnia affects ~20 million Americans yearly, with an estimated treatment and lost-work cost of \$100 billion (Daley, et al., 2008, Kessler, et al., 2010, Roth, 2007). Accurate assessment or definition of the exact phenotype(s) of poor sleep quality is crucial for designing and assessing treatment. Most methods for assessing sleep quality focus on the total number of minutes of various sleep stages across the night, but seldom quantify the dynamic processes that occur within a sleep episode. These sleep dynamics are hypothesized to contribute to subjective sleep quality, yet until recently they have been difficult to quantify. Two statistical approaches for assessing sleep dynamics are the rate of transitions between states (e.g., between sleep and wakefulness) and bout duration analyses. Although the two approaches are related, they describe different aspects of sleep dynamics. An additional barrier to quantifying changes in sleep with aging or pathology is that some measures, such as sleep and wake bout durations (length of time within each state), may be correlated within an individual and do not follow a statistically normal distribution. Therefore, statistics such as mean and standard deviation, and tests based on normal distributions, are not appropriate for describing these data or for comparing between conditions or subject populations. In addition, they do not take advantage of the wealth of information within the collected data, such as transition rates, bout durations and other measures of sleep structure and dynamics.

Within a sleep episode, transitions between Sleep, which can be subdivided into the physiologically different sub-states of NREM and REM sleep, and Wake occur. We used survival-based analyses of sleep and wake bout lengths and transition analyses to quantify

age-related changes in sleep dynamics. This probabilistic method is based on the concept that a state (e.g., Sleep or Wake within a sleep episode) “survives” until there is a transition into another state. Survivor analyses can be used on data with non-normal statistical distributions and also allow use of “censored” data which, in the present analysis, occurs when the end time of a bout is unknown due to data loss from recording difficulties, scheduled termination of the sleep episode by laboratory personnel, or other reasons. These methods can also be used to determine if transition rates are similar at all bout durations, or if the transition rate for very short bouts is different from that of long bouts. The duration-dependent probability of transitioning out of the state, also known as the “hazard” rate, therefore supplies information about the stability of the state. Survival-based analyses allow the quantification of the relative distribution of bout lengths, and can provide information about the underlying physiology involved in initiating, maintaining, and terminating each sleep state. This method has been used in individuals with sleep apnea to quantify the differences in the hazard of awakening (Norman, et al., 2006, Penzel, et al., 2005) and falling back to sleep (Penzel, et al., 2005) compared with unaffected individuals.

We applied these analyses to data from healthy young and older persons in two types of protocols to quantify changes in sleep dynamics with healthy aging, which we have previously found to impair consolidation of NREM sleep (Dijk, et al. 2001). In a forced desynchrony protocol, the sleep/wake cycle length is not 24 hours in length and therefore sleep and wake can be studied at all circadian phases so that the influence of circadian rhythms as well as length of time awake or asleep can be investigated. In the other type of protocol, sleep occurred only at the habitual times for each individual, which is a restricted subset of all circadian phases.

2. Materials and Methods

2.1 Subjects

Subjects were healthy by medical history, physical exam, ECG, and clinical tests of blood and urine, and none were taking prescription or non-prescription medications. For at least one week before, and throughout the inpatient portion of the protocol, no caffeine, alcohol, or nicotine use was allowed. Subjects were psychologically healthy as determined by questionnaires and an interview with a clinical psychologist. Older subjects had no clinically significant sleep abnormalities as determined by screening questionnaires and diagnostic polysomnogram. The protocols were approved by Partners’ Healthcare IRB and all subjects gave informed consent.

2.2 Inpatient Protocols

Data were from four studies that utilized two types of protocols:

1. Forced desynchrony protocol (Dijk, et al., 1999): 13 older (64–74 years) and 11 young (21–30 years) subjects who participated in a month-long inpatient forced desynchrony protocol during which they were scheduled on a 28-hour activity/rest cycle, with polysomnographic recordings during each scheduled 9.33-hour sleep episode. This protocol was designed so that sleep episodes began at many different circadian phases across the 360° circadian cycle. There were a total of 229 sleep episodes from young subjects and 265 from older subjects.
2. Habitual sleep time protocols: (study *a*) data from the third baseline 8-hr sleep episode before the start of the forced desynchrony in the 13 (9 M; 4 F) older and 11 (all M) young subjects above (Dijk, et al., 1999); (study *b*) data from the third baseline 8-hr sleep episode in 14 (4 M; 10 F) older (65–75 years) and 5 (all M) young (18–25 years) subjects (Klerman, et al., 2001); (study *c*) data from the third

baseline 8-hr sleep episode in 12 (9 M; 3 F) older (65–76 years) and 26 (17 M; 9 F) young (18–29 years) (Duffy, et al., 2009)(study d) data from the first baseline 7–9 hr sleep episode (depending on the individual subject's habitual sleep duration) in 17 (11M, 6 F) older (60–76 years) and 18 (10 M, 8 F) young (18–27 years) (Klerman and Dijk, 2008).

All inpatient studies were conducted at the Brigham and Women's Hospital General Clinical Research Center Intensive Physiological Monitoring Unit or the Environmental Scheduling Facility. All events were scheduled relative to each individual's habitual sleep and wake times. Sleep was recorded and scored using standard criteria (Rechtschaffen and Kales, 1968); each 30-second epoch was classified as wake, non-rapid eye movement (NREM) sleep, or REM sleep. Standard summary sleep statistics are presented in the Supplementary Materials (Supplementary Table S1).

2.3 Survival Analyses

No data from before the first epoch of any stage of sleep within a scheduled sleep episode were included in the survival statistics. We defined a 'bout' as a series of consecutive 30-second epochs of the same state (wake, sleep, NREM sleep or REM sleep), and the bout (defined as Wake, Sleep, NREM Sleep or REM Sleep, respectively) lasted until a bout of another state began. To explore the effect of bout length, we tested minimum bout lengths of 1.0, 2.0, 3.5, 5.0, and 7.5 minutes in the forced desynchrony data set.

Cox proportional hazards regression models for multiple events data were applied to study the "survival" probability - the probability of a bout length greater than a specific value - using the software *R* (<https://www.r-project.org>) and SAS 9.2 (SAS Institute Inc., Cary, NC). To account for the fact that we were modeling multiple events—sleep bouts—for each subject and thus there might be correlation between the bouts within each subject and to fit a model which accounts for correlated observations within subjects, a robust sandwich estimate for the covariance matrix was used which resulted in a robust standard error for the parameter estimates. Hypotheses testing of the regression parameters were carried out based on the robust sandwich covariance matrix estimates and did not need the assumption of the independence of observations within a subject. Ninety-five percent confidence intervals were calculated for the estimated "survival" probability. Note that for this analysis, there must be another state between bouts, by definition. Competing risk approaches were also applied to study the "survival" probability of a bout that transitions to another stage using the stratified extension of the Cox proportional hazard models (Swihart, et al., 2008).

All analyses were performed for Wake and Sleep bout categories (without subdividing the type of sleep), and separately for NREM Sleep and REM Sleep bout categories. One, two and three exponential curves were fit to the survival curves using MatLab v7.8 Curve Fitting Toolbox v 2.0 (MathWorks, Natick MA), and best fit selection was by adjusted R-squared values.

3. Results

3.1 Effect of minimum bout length

The first step was to determine the minimum bout lengths to be used for further analyses; this requires balancing the analysis goals of capturing relevant sleep state changes while minimizing inter-scorer variability (Norman, et al., 2003). Shorter minimum bout lengths allow more detailed quantification of changes; however, they are more sensitive to how single epochs of the recording may be scored by different individuals. We therefore began by performing survival analyses using different minimum bout lengths of 1.0, 2.0, 3.5, 5.0, and 7.5 minutes in the forced desynchrony data set. The overall shapes of the survival curves

did not change among these different minimum bout lengths (Figure 1), although, as expected, there was a decrease in the very short bout lengths and an increase in longer bout lengths as the minimum bout duration was increased. For example, at a minimum bout length of one minute, the median Sleep bout length was 49 minutes in young adults but only 11 minutes in older adults; at a minimum bout length of 7.5 minutes, the median for young adults was 350 minutes and 118 minutes for older adults. For Wake bout lengths, at a minimum bout length of one minute, the median bout length was 2 minutes in both young and older adults; however, at a minimum bout length of 7.5 minutes, the young adults had a longer median bout length (66 minutes) than older adults (37 minutes), reflecting an increase in the probability of falling back to sleep in the older as compared with the young subjects. Since the patterns for each stage were similar across all minimum bout lengths, a minimum bout length of 2.0 minutes was chosen for subsequent analyses.

3.2 Bout distribution patterns

The distributions of Wake, Sleep, NREM Sleep, and REM Sleep bouts were non-normal. The shapes of the survivor curves were different for each state, but similar within state in young and older adults (Figure 2 for the forced desynchrony data set and Supplementary Figure S1 for the habitual sleep timing data sets). The different bout-length-dependent distribution patterns for Wake, Sleep, NREM Sleep, and REM Sleep suggest differential physiologic regulation of those different states (Figure 2 and Supplementary Figure S1). The REM Sleep survival curve was fit with a mono-exponential distribution (appearing linear on a log-linear plot), implying that the hazard of transitioning out of REM Sleep is constant (with invariant slope) and therefore independent of the length of time already spent in REM Sleep. The time constant (inverse of hazard) of that REM Sleep curve fit was 12 minutes for both young and older subjects. Sleep bouts (combined NREM and REM Sleep) were also fit with a mono-exponential distribution, with time constants of 43 minutes for older and 169 minutes for young subjects. Therefore, the hazard for Sleep was different in young and older subjects, unlike REM Sleep, in which no age related difference in hazard was found.

In contrast, for Wake and NREM Sleep bouts, the slope of the survival curves depended on the bout length (Figure 2 for the forced desynchrony data set and Supplementary Figure S1 for the habitual sleep timing data sets): there was a higher hazard (steeper negative slope in figures) of transitioning to another state for both Wake and NREM Sleep bouts of less than ~10 minutes than for longer bouts. This higher hazard implies initial instability in both of these states for the first 2–10 minutes: this initial instability included ~80% of all the Wake bouts but only ~50% of the NREM Sleep bouts in older and 20% in young. In other words, both young and older participants fell back to sleep within 10 minutes following 4 out of 5 nocturnal awakenings. In contrast, once they fell asleep, 4 out of 5 sleep bouts in young participants were longer than 10 minutes whereas only 50% of the sleep bouts in older participants were 10 minutes or longer. Because of the higher initial hazard, Wake bouts were fit with two exponentials. The time constants for older subjects were 4 and 82 minutes, while for young subjects they were 4 and 116 minutes; these time constants are similar in the age groups. Note that for Wake bout lengths greater than ~20 minutes and for NREM sleep bout lengths greater than ~30 minutes, the hazard of transitioning (slope of linear portion in log-linear plot) from Wake to another state was approximately constant for both young and older subjects; therefore the overall age-related difference in transition rate primarily occurred in the short (less than ~20 minute) Wake and NREM sleep bouts. For NREM Sleep bouts, in addition to the initially high hazard for short bout lengths, there was a second precipitous increase in the hazard (appearing as a steeper negative slope in plots) of transitioning out of this state at ~50 minutes, suggestive of the NREM-REM sleep cycle, in which REM sleep typically commences after about an hour of NREM sleep (Feinberg and Floyd, 1979). NREM sleep bouts could not be well fit with one, two, or three exponentials.

We examined whether bout survival depended on the state prior to or following the current state. Bout survival depended on the next state for NREM Sleep and for REM Sleep (Figure 3A). The NREM Sleep-to-Wake and NREM Sleep-to-REM sleep survival curves have different shapes. For NREM Sleep-to-REM Sleep transition, after an high hazard rate for bouts 2~10 minutes in length, the hazard was very low (flat slope in Figure 3) for bouts between ~10 and ~40 minutes duration, indicating a physiologic constraint on those transitions and yielding information on the strength of the dynamics underlying the NREM Sleep - REM Sleep cycle. The NREM Sleep-to-REM Sleep transition then had a precipitous increase in hazard at ~50 minutes for this subset of all NREM episodes, resembling the survival curve for all NREM Sleep bouts. The NREM Sleep-to-Wake survival curve is closer to linear (in a log-linear plot) than NREM Sleep-to-REM Sleep. The hazards for NREM Sleep-to-Wake and for NREM Sleep-to REM Sleep at durations >50 minutes are approximately the same. REM Sleep bouts transitioning to NREM Sleep had longer survival than the REM Sleep bouts transitioning to Wake, especially in the older subjects (Figure 3), but the general shape of the curves are similar. In contrast, the preceding state did not affect bout survival characteristics for NREM Sleep (from Wake or REM Sleep) or for Wake (from NREM Sleep or REM Sleep) (data not shown). There were too few Wake-to-REM Sleep transitions to perform analyses involving transitions from Wake to REM Sleep. When we applied the competing risk approach, the general shape of the transition curves and the relative values for older and young subjects remained the same, though the absolute probability estimates were slightly different.

3.3 Age related differences when sleep episodes occurred at all circadian phases

When age-related differences were examined across all circadian phases (Figure 2), all minimum bout lengths had significant age-related differences for Sleep and NREM Sleep but no significant age-related differences were observed at any of the minimum bout lengths for REM Sleep ($p=0.79$). Older adults had significantly shorter “survival” in Sleep ($p<0.0001$) than young adults due to their significantly shorter survival in NREM Sleep ($p<0.0001$). For Wake bouts, there were statistical differences between the age groups only at some minimum bout lengths: the difference was not significant for minimum bout lengths of 1.0 minutes ($p=0.92$), but was significantly longer for young subjects for minimum bout lengths of 2.0 minutes ($p=0.044$) or longer (3.5 minutes: $p=0.0056$; 5 minutes: $p=0.0033$; 7.5 minutes: $p=0.014$). The Cox hazard ratios with 95% confidence ratios for the 2.0 minute minimum bout length data were for the two age groups were: Wake 0.78 [0.61,0.99] ($p=0.044$); Sleep 0.26 [0.18, 0.37] ($p<0.0001$); NREM sleep 0.54 [0.46, 0.63] ($p<0.0001$) and REM sleep 1.03 [0.82, 1.30] ($p=0.79$).

This differential survival in Sleep for young and older subjects resulted in approximately five times more Sleep and Wake bouts in older than young subjects, and therefore many more Sleep and Wake bouts per sleep episode when those sleep episodes were scheduled at all circadian phases (Table 1). Despite the slightly increased hazard, and therefore slightly shorter Wake bout survival (Figures 1 and 2), in older subjects there is a vast increase in the total number of bouts, causing an increased number of long Wake bouts (a prominent complaint about sleep quality reported by many older individuals), even though the hazard for long Wake bouts (as shown in Figures 1 and 2) is the same in both age groups.

When relative transition probabilities independent of bout length were considered (Table 2, Figure 3B), dramatic age-related differences resulting in increased awakenings also were observed. While young individuals were ~7 times more likely to transition from NREM Sleep to REM Sleep (remaining asleep) than to Wake (awakening), older individuals had approximately the same probability of transitioning from NREM Sleep to either REM Sleep or Wake. Therefore, older individuals had a much larger probability of awakening from NREM sleep rather than remaining asleep by transitioning into REM Sleep. Similarly,

young individuals were ~6 times more likely to transition from REM Sleep to NREM Sleep than to Wake, but older individuals were ~1.5 times more likely to transition from REM Sleep to NREM Sleep than to Wake. Therefore, older subjects were much less likely to remain asleep (by transitioning into NREM sleep) than younger subjects. These increased probabilities of transition from NREM Sleep or REM Sleep to Wake instead of to another sleep state (REM Sleep or NREM Sleep) in the older individuals contributed to the increased number of Sleep and Wake bouts observed in the older group.

3.4 Age related differences when sleep episodes occurred only at circadian phases of habitual sleep

Because there are prominent circadian rhythms in Wake and REM sleep, but only weak circadian rhythms in NREM sleep (Dijk, et al., 1999), we also performed survival analyses on data from sleep episodes that occurred only at circadian phases of habitual sleep times. Due to minor differences in the data sets (see **2. Materials and Methods**), the four data sets were not combined for this analysis. The overall shapes of the survival curves (Figure S1) were the same as for the forced desynchrony data set. In these four data sets, as in the forced desynchrony data set, older adults had significantly shorter survival in Sleep ($p < 0.0001$ in all four data sets) and significantly shorter survival in NREM Sleep ($p < 0.0001$ in all four data sets) than young adults. However, there was no difference between the two age groups in Wake survival ($p > 0.25$ in all data sets). The two smallest data sets (**a**, **b**) of the four had no significant difference in REM sleep survival ($p = 0.94$, $p = 0.40$, respectively); the other two data sets (**c**, **d**) did show significant differences ($p = 0.008$, $p = 0.039$, respectively). A possible explanation for this difference might be the lack of statistical power to detect any difference in REM survival in data sets **a** and **b** at the circadian phase of habitual sleep.

Alternatively, the age-related Wake bout survival difference may only be observed when sleep occurs at non-habitual circadian phases (as observed in the forced desynchrony data set), while the age-related REM sleep bout survival difference may only be observed when sleep occurs at habitual circadian phases. Using Markov-based analyses on the same forced desynchrony data set, we previously reported age effects in Wake bouts at only some circadian phases but age effects in Sleep (NREM Sleep and REM Sleep combined) bouts at all circadian phases (Klerman, et al., 2004).

4. Discussion

Our findings of age-related changes in the statistical distribution and transitioning of sleep state bouts confirm that a primary cause of sleep maintenance problems in aging is a decreased ability to remain in NREM sleep. The increased amount of wake within scheduled sleep episodes in older persons is due to more frequent awakenings rather than to a decreased ability to fall back to sleep. These results using survival analysis are consistent with and help account for our previous more limited reports based on a subset of these data, in which we analyzed the frequency and duration of awakenings relative to recent history of NREM and REM sleep (Dijk, et al., 2001) and inter-state transition rates using Markov transition analyses (Klerman, et al., 2004). In our previous analyses (Dijk, et al., 2001) using one of the datasets in this report, we reported an increased frequency of awakening in older persons, especially from NREM sleep; however, that report analyzed the data relative to the percentage of sleep stages or wake prior to awakening and rather than by examining the dynamics of transitions between stages. The current survival-based method, however, adds additional information about the bout-length dependent distribution of transitions to/from Wake, Sleep, NREM sleep and REM sleep stages, as well as the differences in transition probabilities depending on the state from and to which the transition is occurring. The

advantage of survival analyses is that they allow more thorough, bout-dependent and non-normal distribution based analyses of these very complex data.

Since sleep timing and content is regulated by both circadian and homeostatic influences (Saper, et al., 2005), the changes in sleep with aging may be due to changes in circadian rhythms, sleep homeostasis and their interactions with aging. This new analysis method may be potentially used to address the relative importance of circadian phase and/or sleep homeostasis with aging. However, the appropriate metric for whether sleep homeostatic pressure at sleep onset changes with aging is the build-up rate, not decay rate; therefore application of this method to sleep dynamics within a sleep episode is not appropriate for that question. Instead, additional experiments, including dose-response curves of wake duration from relatively short (e.g., with a nap mid-day) to long (after a sleep deprivation), are required to determine whether homeostatic influences on sleep change with healthy aging.

There are reports from studies in humans and other animals on bout duration changes with aging (Arnardottir, et al., 2010, Blumberg, et al., 2005, Mendelson and Bergmann, 1999, Rosenberg, et al., 1979, Zepelin, et al., 1972). However, those reports have used mean and standard deviation and other statistics that are both inappropriate for the distribution of the data and do not take advantage of the wealth of information within bout-length or transition analyses. Recently McShane and colleagues analyzed mice data using the assumption of two components within the data: short and longer bouts (McShane, et al., 2010). While this is an improvement over simpler statistics, our analyses of human data suggest that the data are more complex, and require continuous bout-duration dependent measures. Lo et al (Lo, et al., 2002) fit different curves, including exponential and power law, to the cumulative distributions of Sleep and Wake data. However (i) they did not account statistically for multiple observations from each individual; (ii) they only studied Sleep and Wake and not the individual states of NREM sleep and REM sleep; and (iii) a recent report demonstrated the statistical difficulties of differentiating the appropriateness of power law versus multi-exponential fits in similar data (Chu-Shore, et al., 2010). Our survival methods also do not assume a specific distribution; such a priori assumptions may affect the results obtained, and also are able to account for the multiple observations from each individual. Median statistics while they do not assume a particular statistical distribution, summarize the data with a single number rather than including the bout length-dependent changes quantified by survival analyses.

Our findings suggest that the most effective therapies for reducing the sleep disruptions associated with healthy aging should target the continuity of NREM sleep bouts, especially those of short duration. The types of analyses used here will also be useful in understanding the physiological basis of sleep problems in other patient groups, such as individuals with insomnia and narcolepsy; such analyses will provide insight into how sleep maintenance is affected in those conditions. The participants in this study were healthy, on no medications and without sleep disorders and therefore did not have causes of sleep disturbances frequently observed in older people, including pain, sleep disordered breathing or apnea with arousals, periodic leg movements, or even a bed partner with these conditions. The sleep dynamics of older persons with sleep disorders or other medical conditions may have even more differences than the sleep dynamics of younger individuals; each medical condition will have to be studied separately to determine its impact on sleep. Survival analyses can also be used to better understand the effects of medications (e.g., hypnotics or stimulants), other substances (e.g., caffeine or melatonin), or other interventions on sleep.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effect of different minimum bout lengths on survival graphs of Wake and Sleep for young and older subjects in the forced desynchrony protocol. For each log-linear plot, the x-axis represents the bout durations in minutes and the y-axis represents the percentage of bouts remaining in that state for that bout length or longer. Dotted horizontal lines indicate the 50th (median) and 90th percentiles. Data are shown for minimum bout durations of 1.0 minutes (black circles), 2.0 minutes (grey circles), 3.5 minutes (black triangles), 5.0 minutes (grey triangles) and 7.5 minutes (grey diamonds).

Figure 2. Survival curves with 95% confidence intervals for Wake, Sleep, NREM Sleep, and REM sleep bouts in young (grey symbols) and older (black symbols) subjects in the forced desynchrony protocol for a two-minute minimum bout length. The format is the same as in Figure 1.

A.

B.

Figure 3.

A. Survival curves with 95% confidence intervals for transitions from NREM Sleep to Wake, NREM Sleep to REM Sleep, REM Sleep to Wake, and REM Sleep to NREM Sleep in young (triangles) and older (circles) subjects in the forced desynchrony protocol for a two-minute minimum bout length. The format is the same as in Figure 2. Note that the survival curves do not represent the hazard for the entire NREM sleep or REM sleep population, but only for the specific subset of bouts indicated. **B.** For all bout lengths, the ratio of transitions within sleep-to-transitions to wake by sleep state for the two age groups. Both the ratio of transitions from NREM sleep-to-REM sleep/NREM-sleep-to Wake (left

panel) and the ratio of transitions from REM sleep-to-NREM sleep/REM-sleep-to Wake (right panel) are shown.

Table 1

Median and Range of Number of Bouts per Sleep Episode per Subject for the Forced Desynchrony Protocol Data.

	Young		Older	
	Median	Range	Median	Range
Wake	2.2	1.4–5.1	9.2	2.9–15.2
Sleep (NREM + REM)	2.9	2.2–5.6	9.7	3.3–15.5
NREM Sleep	10.1	8.6–11.1	12.3	7.5–15.9
REM Sleep	8.2	7.1–9.9	7.0	4.4–10.5

The median and range (minimum-to-maximum) of the average number of bouts per sleep episode per subject across all sleep episodes in the forced desynchrony protocol. Note that only data from sleep episodes with <5% missing data are included in this table; data from all sleep episodes are included in the survival analyses. One older subject and seven young subjects had at least one sleep episode with zero Wake bouts; overall, 0.4% of all older subject sleep episodes and 10.2% of all young subject sleep episodes had zero Wake bouts. Because the data are not normally distributed, we have not reported the mean, standard deviation, or t-test probabilities of differences.

Table 2
Relative Percentages of all Inter-state Transitions for the Forced Desynchrony Protocol Data.

Current stage	Next stage	Young Mean (S.D.)	Young Range	Older Mean (S.D.)	Older Range	T-test significance for age difference
NREM Sleep	REM Sleep	42 (5)	33–47	24 (6)	14–37	P<0.0001
NREM Sleep	Wake	6 (4)	2–13	21 (6)	10–29	P<0.0001
REM Sleep	NREM Sleep	35(6)	24–40	16 (6)	6–29	P<0.0001
REM Sleep	Wake	6 (3)	3–12	10 (4)	1–16	P=0.0212
Wake	NREM Sleep	10 (5)	6–18	27 (6)	13–34	P<0.0001
Wake	REM Sleep	1 (1)	0–2	2 (2)	0–5	P=0.0347

The mean (standard deviation) and range (minimum-to-maximum) of the average inter-state transition probabilities per subject across all sleep episodes in the forced desynchrony protocol. Note that only data from sleep episodes with <5% missing data are included in this table; data from all sleep episodes are included in the survival analyses.