Supplementary Online Material: "Developmental Changes in Ultradian Sleep Cycles across Early Childhood: Preliminary Insights"

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SUPPLEMENTAL METHODS

We analyzed the scored data using a range of statistical techniques to assess changes in sleep architecture with age and with time since lights off. As described in the Methods, durations of total sleep, NREM sleep, and REM sleep, and mean durations of cycles, NREM sleep episodes, and REM sleep episodes were computed for each subject at each age. For each type of duration, two-way ANOVA and paired t-tests were performed to test the null hypothesis that the mean durations are the same for each pair of ages. Skipped REM sleep episodes were not included in the calculation of REM sleep episode duration. 95% confidence intervals for the means are presented.

Kaplan-Meier survival curves for age-related changes in cycle, NREM, and REM sleep episode duration: We computed Kaplan-Meier survival curves by plotting the proportion of sleep episodes of duration greater than or equal to t for each duration t. We used the log-rank statistic to test whether the survival curves differed significantly. Because data for the three curves were taken from the same subjects, the curves are not independent. We therefore used the bootstrap approach to compute the p-value {Neuhaus, 1993 #447}. Specifically, for each subject we created a set of simulated durations at 2Y by sampling at random with replacement from the three sets of durations at 2Y, 3Y, and 5Y. We created samples of simulated durations at 3Y and 5Y in the same way. We generated 10,000 simulated data sets in this manner. The bootstrap p-value is the proportion of those data sets for which the value of the log-rank statistics exceeded the value observed from the actual data set.

Cox proportional hazards models for changes in cycle, NREM, and REM sleep episode duration: Cox proportional hazards models were used to model cycle duration, NREM duration, and REM duration. The hazard describes the rate that a cycle, NREM sleep episode, or REM sleep episode of a given duration will fail (terminate) as a proportion of those not yet failed. Age was included as a categorical covariate, and time since lights off was included as a continuous covariate. Absolute clock time was confounded with between-subject variation since, based on the protocol, the scheduled lights off time for each child depended on his/her awakening time the previous day. Therefore, we used time since lights off, rather than clock time, in order to account for the dependence between the duration of an event and the duration of subsequent events while avoiding this confound. To adjust for differences between subjects, an indicator for subject was also included. The assumption of proportional hazards was tested for each covariate, and the hypothesis of proportional hazards was not rejected. The hazard coefficient is the estimated difference in the natural logarithms of the hazards.

Linear models for changes in NREM/REM sleep episode duration with time since lights off: We plotted the line of best fit for NREM and REM sleep episode durations versus time since lights off, adjusting for subject and age. For NREM sleep episode durations, the intercepts of the lines of best fit differed significantly with age, but slopes did not. We therefore computed separate intercepts for each age group and plotted best-fit lines with a common slope for each intercept. For REM sleep episode durations neither intercepts nor slopes differed significantly with age, so we plotted the single line providing the best fit to all the data.

Percentage of NREM sleep per cycle: To determine the relationship between the percentage of NREM sleep per cycle and cycle number (and indicator correlated with time since lights off), we considered consecutive pairs of cycles. For each consecutive pair of cycles, paired t-tests were performed on the mean difference between the percentage of the cycle spent in NREM sleep in the n^{th} and $(n+1)^{st}$ cycle. Only subjects with both an n^{th} and $(n+1)^{st}$ cycle were considered for these analyses. Thus, fewer and fewer subjects contributed to the later cycles.

Age-related change in frequency of cycles and skipped REM sleep episodes: To assess a change in the frequency of cycles between 2Y and 5Y, we counted the number of subjects with each cycle at each age. We tested the null hypothesis that a 9^{th} cycle was equally likely to occur in each of the age groups versus the alternative that the probability of occurrence of a 9^{th} cycle was lower at 5Y. Under the null hypothesis, for a subject who has a 9^{th} cycle at exactly one of three ages, the probability that there is no 9^{th} cycle at 5Y is 2/3. For a subject who has a 9^{th} cycle at exactly two of three ages, the probability that there is no 9^{th} cycle at 5Y is 1/3. Subjects who never had a 9^{th} cycle or who had 9^{th} cycles at all three ages contribute no information about the null hypothesis. Therefore, the probability, under the null hypothesis, of observing no 9^{th} cycles at 5Y, conditional on the occurrence of *m* subjects with one 9^{th} cycle and *n* subjects with two 9^{th} cycles, is $p= (1/3)^m(2/3)^n$. The test of the corresponding null hypothesis for the 8^{th} cycle was similar. Since some children had isolated NREM sleep episodes (rather than complete cycles) at the end of the sleep period, we also tested the null hypothesis that a 9^{th} NREM sleep episode is equally likely to occur in each of the age groups versus the alternative that the probability of occurrence of a 9^{th} cycle was lower at 5Y.

In addition, we identified cycles in which skipped REM occurred, and we examined the occurrence of skipped REM sleep for possible age-dependence.