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ORIGINAL ARTICLE

Ultradian modulation of cortical arousals during sleep: effects of age and exposure to nighttime transportation noise

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Abstract

Study Objectives: The present study aimed at assessing the temporal non-rapid eye movement (NREM) EEG arousal distribution within and across sleep cycles and its modifications with aging and nighttime transportation noise exposure, factors that typically increase the incidence of EEG arousals.

Methods: Twenty-six young (19–33 years, 12 women) and 16 older (52–70 years, 8 women) healthy volunteers underwent a 6-day polysomnographic laboratory study. Participants spent two noise-free nights and four transportation noise exposure nights, two with continuous and two characterized by eventful noise (average sound levels of 45 dB, maximum sound levels between 50 and 62 dB for eventful noise). Generalized mixed models were used to model the time course of EEG arousal rates during NREM sleep and included cycle, age, and noise as independent variables.

Results: Arousal rate variation within NREM sleep cycles was best described by a u-shaped course with variations across cycles. Older participants had higher overall arousal rates than the younger individuals with differences for the first and the fourth cycle depending on the age group. During eventful noise nights, overall arousal rates were increased compared to noise-free nights. Additional analyses suggested that the arousal rate time course was partially mediated by slow wave sleep (SWS).

Conclusions: The characteristic u-shaped arousal rate time course indicates phases of reduced physiological sleep stability both at the beginning and end of NREM cycles. Small effects on the overall arousal rate by eventful noise exposure suggest a preserved physiological within- and across-cycle arousal evolution with noise exposure, while aging affected the shape depending on the cycle.

Statement of Significance

Sleep is a dynamic process and frequent activation phases physiologically disrupt the continuity of sleep. Here, we observed that cortical arousal rates had an u-shaped time course suggesting that both the beginning and end of non-rapid eye movement (NREM) sleep cycles are phases of reduced physiological sleep stability. Aging and eventful noise exposure differentially affected the shape of the physiological arousal rate evolution. While aging affected both the overall time course level and the shape, eventful transportation noise exposure increased the level without changing its shape suggesting that external stimuli fragment sleep along the physiological texture of the EEG arousal time course. When evaluating the effects of aging and nighttime noise exposure on sleep fragmentation, the physiological microstructural evolution needs to be considered.

Key words: sleep stability; sleep fragmentation; GLMM; sleep cycle

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Introduction

Transient activation phases during sleep (i.e. autonomic arousals, sleep stage changes, cortical arousals, or awakenings) are generally considered to fragment sleep and, as a result, negatively impact the recuperative value of sleep [1–5]. Transient activation phases can occur in response to external stimuli, such as transportation noise [6–11] or high-intensity white noise [12–14]. They also increase with aging without external stimuli and are part of the normal aging process in humans [15–19]. However, besides their potential negative effect on sleep continuity, transient activation phases are also an integral and essential characteristic in the ultradian time course of physiological sleep [15, 20, 21].

Cortical arousals, as one class of transient activation phases during sleep, are not distributed randomly, but tend to cluster around certain time points during sleep [21–23]. Typically, sleep is organized in 4–5 ultradian sleep cycles of 90–110 min each, which comprise an episode of non-rapid eye movement (NREM) sleep followed by an episode of rapid eye movement (REM) sleep [24]. Temporal variations of cortical arousals can therefore be examined on the level of the sleep cycle (within-cycle) as well as over the course of an entire night sleep period (across-cycle). So far, these variations were almost exclusively investigated within the framework of the cyclic alternating pattern (CAP), a marker of sleep instability [23, 25] but insufficiently for the most established marker for cortical arousal: EEG arousal defined according to the rules of the American Academy of Sleep Medicine (AASM) [26].

CAP is a rhythmic NREM EEG pattern characterized by sequences of A and B phases: EEG activity during A phases is either synchronized (subtype A1), desynchronized (subtype A2), or mixed (subtype A3), with the latter two overlapping with EEG arousals; B phases are composed of EEG background activity [23, 25]. Regarding the time course, it was observed that subtypes A2 and A3 occur more frequently during the ascending (i.e. when sleep is more superficial and progressing toward REM) than the descending (i.e. the first part of the ultradian sleep cycle when sleep progresses from lighter to deeper sleep) part of a sleep cycle [21–23]. Intra-night variations of spontaneous number of EEG arousals per hour have primarily been examined across sleep cycles while detailed time course analyses within sleep cycles have not yet been investigated. Results suggest no variation of EEG arousals across cycles [27] or time elapsed since sleep onset [28]. Spontaneous EEG arousals occur more frequently from both stage 1 NREM (N1) and REM sleep compared to stage 2 NREM sleep (N2) and slow wave sleep (SWS); EEG arousal rates are generally lowest during SWS [27, 28]. Within-cycle evolution of slow-wave activity (SWA) follows an inverted u-shaped pattern with a gradual buildup, a plateau phase, and a rapid decline toward the transition to REM sleep depending on time asleep during the night [29–31] suggesting that the underlying arousal and SWS-generating mechanisms might be antagonistic. Across the night sleep period, however, the gradual decrease of SWA across cycles [30, 32, 33] does not mirror the across-cycle stability of EEG arousal [27] suggesting that EEG arousals are not a good marker for sleep homeostasis. Taking the two perspectives into consideration, namely transient activation phases as a marker for sleep fragmentation and as an integral and essential characteristic of physiological sleep, we were interested in modeling the EEG arousal time course and its modifications with aging and nighttime transportation noise exposure.

We analyzed the microstructural architecture of NREM sleep using EEG arousals as a marker for transient activation phases. Data were acquired in a sample of healthy young and older individuals that underwent a 6-day polysomnographic (PSG) laboratory study and had two noise-free and four noise exposure nights (four different noise exposure situations: two with continuous and two characterized by eventful noise). To model the time course of EEG arousals, we normalized the NREM episodes of a cycle by subdividing it into 10 parts of equal length (within-cycle effect) and did this for the first four cycles across the night sleep period (across-cycle effect). The aim of the paper was threefold. First, we were mainly interested in modeling the time course of EEG arousals both within and across sleep cycles, which we expected to vary in a u-shaped pattern within cycles but not across cycles. Second, we investigated age-related modifications of temporal EEG arousal distributions. All-night number of EEG arousals per hour increase with aging [15, 16], but it is unclear how within- and across-cycle dynamics differ between age groups. Third, building on the notion that transportation noise increases the number of all-night EEG arousals [6, 8], we were interested whether EEG arousals during noise exposure nights had a similar temporal distribution pattern than during undisturbed nights. It is currently unknown whether the additional EEG arousals during noise nights occur at the same or other, additional time points than those of the physiological EEG arousal time course.

Methods

Participants

Data of 42 healthy volunteers in two age groups (26 young: 24.6 ± 3.5 years, 19–33 years, 12 women; 16 older: 60.8 ± 5.9 years, 52–70 years, 8 women) were included for analyses; two participants of the older group dropped out of the experiment due to medical reasons (data excluded), and two participants of the young group dropped out after four nights due to personal reasons (data included). All participants were free from any acute or chronic illness and current medication (as assessed by means of clinical history, physical examination by a study physician, and routine blood and toxicological urine testing; young women without hormonal contraceptive use) and had good sleep (habitual sleep duration per night 8 ± 1 h; normal subjective sleep quality PSQI ≤ 5 (Pittsburgh Sleep Quality Index) [34]; normal general daytime sleepiness ESS ≤ 10 (Epworth Sleepiness Scale) [35]; and no signs of sleep disorders, such as sleep-related movement and breathing disorders as confirmed via PSG during one screening/adaptation night prior to study admission. All had normal sex- and age-appropriate hearing thresholds (maximum hearing loss of the better ear no greater than the 10th percentile of an otologically normal population [36] at the frequencies 250, 500, 1000, 2000, 3000, and 4000 Hz) tested manually with an audiometer (Bosch ST-10, Stuttgart, Germany).

The study protocol, screening questionnaires, and consent forms were approved by the local ethics committee (Ethikkommission Nordwest- und Zentralschweiz, Switzerland, #2014-121) and conformed to the tenets of the Declaration of Helsinki. All participants gave written informed consent prior to study participation and received financial compensation for participation. Data acquisition took place between October 2014 and June 2016.

Protocol and procedure

The protocol comprised six consecutive nights and days in the sleep laboratory. All participants were exposed to four different transportation noise scenarios that were played back during four nights with an incompletely counterbalanced sequence: scenarios with a more continuous noise characteristic (Road A–B) were alternated with scenarios with a more eventful noise characteristic (Road C, Rail D). Participants spent two noise-free nights that were always the first (baseline night: BL) and the last night (recovery night: RC). Participants were informed about the initial and the last noise-free nights but had no knowledge about the dynamics of the different transportation noise scenarios. Time in bed was scheduled at individuals' habitual bedtime and lasted 8 h for every participant. Noise scenario playback started immediately after lights off. Days and nights were spent in single windowless, soundproof, and temperature-regulated bedrooms (22°C) under constant ambient lighting levels during waking periods (between 50 and 150 lux at the participant's eye).

Participants were asked to keep a regular sleep–wake cycle with self-selected habitual bed and wake times for 1 week prior to the study start (nighttime sleep duration 8 ± 0.5 h, no nap taking). Compliance was verified by accelerometers worn on the nondominant wrist (Actiwatch AW4; Cambridge Neurotechnologies, Cambridge, United Kingdom) and self-reported sleep-logs. During 1 week prior to the study start, they were also asked to restrict consumption of alcohol, caffeinated beverages, and chocolate to moderation to level out effects of these substances on sleep and waking functions. Young women were generally tested during the early follicular phase of their menstrual cycle (one woman was tested during the late luteal phase and progressed to the follicular phase over the course of the experiment; another woman was during the luteal phase for the whole experiment).

Noise scenarios

We used five pre-recorded real-world inspired acoustical scenarios for playback in the bedroom during the night: one essentially noise-free (NF) and four transportation noise scenarios (Road A–C, Rail D) that differed with respect to noise source (different road traffic situations and railway noise) and noise exposure situation (more continuous, more eventful). Scenario NF ($L_{Aeq,1\,h}$ of 30 dB at the ear of the sleeper; represents a rather tranquil real-world bedroom situation with a tilted window and a layer of steady road traffic sound from a remote road network plus natural sounds) was played back during BL and RC nights. Transportation noise scenarios ($L_{Aeq,1\,h}$ of 45 dB at the ear of the sleeper; corresponds to an average outdoor façade level of 60 dB for a tilted window) were designed to represent relevant exposure situations (Table 1). Road A represented a four-lane highway (speed limit of 120 km/h) with approximately 1,000 vehicles per hour at a distance of 400 m. Road B represented a distance of 50 m from a two-lane country road (speed limit of 80 km/h) with approximately 250 vehicles per hour. Road C represented a one-lane urban road (50 km/h) at a 15 m distance with approximately 100 vehicles per hour. Rail D represented a railway noise situation with 10 nonoverlapping freight and passenger train pass-by events per hour. Creation of acoustical scenarios has been previously described in detail [11].

The noise scenarios were classified as three types with each factor representing two nights: noise-free (BL and RC night), continuous noise exposure (Road A and B), and eventful noise exposure (Road C and Rail D). Road C included 400 single road noise events that differed according to duration (16.6–58.8 s), maximum sound pressure level (SPL) (52.6–62.4 dB), and maximum slope of the SPL (2.4–6.4 dB/s). Rail D included 80 single railway noise events that differed according to duration (16.9–113.7 s), maximum SPL (50.1–61.7 dB), and maximum slope of the SPL (0.7–5.2 dB/s). Noise events were distributed equally across the night (see Supplement 1.1.2).

The audio files were played back from portable audio devices (702T digital recorder, Sound Devices, Reedsburg, WI) through an active monitor loudspeaker (Focal CMS 50, Focal-JMlab, La Talaudière, France) at a 2 m distance to the sleeper's head. The sound reproduction chain was calibrated with a sound level meter (Nor-121, Norsonic, Norway).

All reported acoustical metrics are based on A-weighted SPL.

Sleep recording

The PSG was recorded on Vitaport-3 digital recorder (TEMEC Instruments B.V., Kerkrade, The Netherlands) with a sampling rate of 256 Hz (storage rate 128 Hz, 1.024 Hz for ECG signals). The EEG was recorded at 12 scalp sites (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, O2 according to the 10- to 20-electrode system referenced against averaged mastoids). The electrooculogram (EOG) was recorded from two electrodes that were placed at the outer canthi of both eyes with one electrode above and one below the horizontal. The submental electromyogram (EMG) was recorded bipolarly. The electrocardiogram (ECG) was recorded with two electrodes placed at the center of the sternum and the left rib bone. Signals were filtered during recording (EEG, EOG, and ECG between 0.159 and 30 Hz; EMG between 1 and 70 Hz). Sleep staging and EEG arousal scoring followed the standard criteria of the AASM (v2.3 [26]) and was conducted by four experienced raters, blind to the specific noise exposure. One scorer analyzed all six nights of the same participant and the number of scored

Table 1. Characteristics of the acoustical scenarios

Scenario	Noise source	Noise type	$L_{Aeq,1\,h}$ (dB)	L_{AFmax} (dB)	L_{A5} (dB)	L_{A10} (dB)
A	Road	Continuous	45	53	49	48
B	Road	Continuous	45	60	52	48
C	Road	Eventful	45	62	52	48
D	Rail	Eventful	45	62	53	46
NF	Ambient/background	Noise-free	30	39	35	34

SPL refers to sound pressure level; $L_{Aeq,1\,h}$: hourly A-weighted equivalent SPL; L_{AFmax}: maximum SPL with time weighting FAST; L_{A5}: SPL exceeded 5% of the time; L_{A10}: SPL exceeded 10% of the time.

participants was balanced between scorers according to the participant's sex and age. Inter-rater accordance was assured to be >85% by regular meetings to discuss questionable epochs and align local scoring procedures.

Arousal scoring

Arousals were visually identified by experienced scorers according to the standard criteria of the AASM (v2.3 [26]) as "an abrupt shift of EEG frequency including alpha, theta and/or frequencies greater than 16 Hz (but not spindles) that lasts at least 3 seconds" [26, p. 45].

Sleep cycles

Definition of sleep cycles was largely based on standard criteria [24] with NREM episodes of a cycle (minimum duration: 20 min) being the time between the first epoch of N1 and the subsequent REM onset (≥5 min). In the first cycle, the minimum REM duration was allowed to be shorter than 5 min. Occasionally, a skipping of the first REM episode is observed: where REM is expected (i.e. after a consolidated period of SWS), only a lightening of the sleep process (i.e. a sleep stage transition to N1 or a brief awakening) occurs, especially during the first night in a new environment [37] or in younger individuals [38]. A NREM episode of a cycle was divided into two episodes if it was >120 min in duration (wakefulness excluded) and SWS was interrupted by >12 min by any other sleep stage than SWS [38, 39]. Consequently, the second cycle started with the subsequent SWS onset. We included only the first four cycles [27] that were complete, i.e. where REM sleep was followed by at least 5 min of NREM or wakefulness and only nights with at least three and not more than five completed cycles (see Figure 1). Consequently, sleep epochs after the last REM part and before the final awakening were neglected as were episodes after the fourth cycle. In most cases, the final awakening was experimenter-induced (after the end of the 8-h sleep period) and not spontaneous (≥3 min before the end of the 8-h sleep period; 23.17% of nights). In total, 234 nights were analyzed (33 nights with three, 134 with four, and 67 nights with five completed cycles), while 12 nights needed to be excluded (for 11 nights, the number of completed cycles was less than three or more than five; in one night, REM latency was less than 15 min). From a total of 903 cycles, 48 (5.31%) were excluded: 4 (0.44%) because the REM episode was <5 min for cycles 2–4 and 44 (4.87%) because the cycle duration was outlying short or long, based on a cycle duration shorter or longer than the upper/lower quartile ± 1.5 times the interquartile range of the cycle duration (see Supplement 1.1.3). The median NREM episode length of the 855 sleep cycles was 70 min.

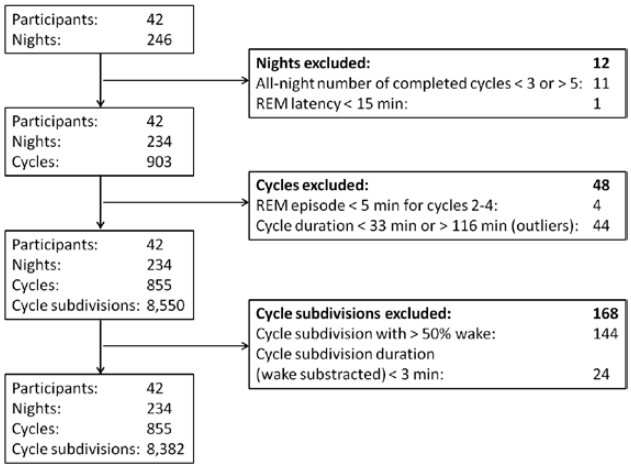

Figure 1. Flow diagram of the selection of nights, cycles, and cycle subdivisions.

Outcome variables

Analysis of sleep macro- and microstructure variables was restricted to the first four sleep cycles. Sleep macrostructure variables included total sleep time (TST), sleep efficiency (SE), onset latencies to N1, N2, SWS (i.e. first occurrence of respective sleep stage after lights off), and REM (first occurrence of REM after N2 onset), percentage of TST spent in N1, N2, SWS, and REM (i.e. all for the episodes between N1 onset and the final awakening in the morning) as well as % intra-sleep wake of SPT (sleep period time). Sleep microstructure variables included EEG arousal rates (n/h TST, n/h NREM, and n/h REM) as well as awakening rates (n/h TST, n/h NREM, and n/h REM).

As differences between NREM and REM sleep EEG arousals are to be expected [40], we limited the analyses to NREM sleep and only modeled within- and across-cycle effects during NREM episodes. Each NREM episode of a sleep cycle was divided in 10 parts of equal length (based on the scoring window duration of 0.5 min; if the quotient was uneven, duration of this cycle subdivision [CSD] was 1/10 NREM part + 0.5 min which was assigned randomly). The dependent variable was the EEG arousal rate during each CSD, modeled by the number of EEG arousals per CSD and an offset (the logarithm of CSD length). In addition, SWS was calculated as percentage of time spent in SWS per CSD duration (% of CSD duration). From a total of 8,550 CSDs, 168 (1.96%) were excluded (144 [1.68%] because of >50% wake in CSD; 24 [0.28%] because CSD length corrected for wakefulness was shorter than 1/10 of the minimum cycle duration of all individuals).

Statistical analyses

For statistical analyses of standard sleep variables, linear mixed models were used, which included random subject effects to account for the repeated measurements within participants. We included factors for noise type (noise-free vs. eventful noise vs. continuous noise), age group (young vs. older), and the interaction between the two.

We used generalized linear mixed models (GLMM) to fit the time course of arousal rates per CSD. The distribution of arousal rates was highly skewed to the right due to the absence of any EEG arousal in 45.38% of the CSDs (see Supplement 1.1.3). The distribution did not suggest any transformation to achieve normality and did not comply with a Poisson distribution. Thus, we opted for a negative binomial distribution, an alternative to the Poisson distribution used to model data that contain many zeros. Details of the statistical modeling and all intermediate steps are documented in the Supplement. We started with a simple model with only the main effects, the offset, and a random subject effect and explored residual variances with respect to different discrete distributions. The main effects and only factors considered were age group (young vs. older), noise

type (noise-free vs. eventful noise vs. continuous noise), cycle (1 to 4), and CSD (1 to 10). Next, we evaluated orthogonal polynomial time trends regarding their ability to represent the 10-level within-cycle effect with fewer parameters. After this, we explored all possible two-way interactions and added them to the model, whenever likelihood-ratio tests indicated significance of the included two-way interaction. In the resulting model with all fixed effects specified, we first addressed possible collinearity between the fixed effects, as well as residual variance heterogeneity and time-related error structures. In a final step, we explored whether the resulting model could be simplified.

To address the first aim, the modeling of the within- and across-cycle time course of EEG arousal rates, we evaluated the two main effects of CSD and cycle, as well as their interaction. To address our second aim, the modification with age, we evaluated the main effect of age as well as the possible interaction of age with CSD and cycle. A significant interaction between age and the polynomial time trends or the cycle factor would suggest that the within- and across-cycle arousal rate time course differs between young and older participants, while the lack of such an interaction would suggest that the within- and across-cycle arousal time course is the same for both age groups. For the third aim, the modification with noise, the procedure was similar, evaluating main effects and significant two-way interactions to test whether the possible noise effect is uniform or nonuniform across the tested variables age, polynomial time trends, and cycle. In the case of nonsignificant interactions between age or noise and the polynomial CSD effects, we also tested the interaction between age or noise and CSD as a 10-level factor, to ensure that the lack of effect was not due to the specific parametrization of CSD.

Because of the similarity of the time course of arousal rates and that of SWS, which is more evident for the within- than the across-cycle time course, we added a separate analysis that explored whether the variations in the percentage of SWS could partially or fully explain the variations of arousal rates within and across cycles. The use of a mediation analysis, the preferred statistical approach to address this type of question, is not yet implemented in the context of GLMM with a negative binomial distribution (see Supplement 2), so that we chose a very simplified approach to evaluate the effect of SWS. We compared the coefficients of determination (R^2) of the following five models: main effects (age, noise, polynomial time trends for CSD, and cycle) only (Model 1; M1), main effects and interactions (i.e. the final model [M2]), SWS only (M3), main effects and SWS (M4), and finally main effects, interactions, and SWS (M5). Additionally, we compared the significance of the main effects between M2 and M5.

All analyses were performed in R [41]. All models were fitted using the *glmmTMB* library (v0.2.2.0) [42]. We used the *car* package (v3.0-0) [43] to evaluate significance of fixed effects using Wald chi-square tests. For post hoc testing (marginal effects, interactions, and pairwise comparisons), we used the *emmeans* package (v1.2.3) [44] and adjusted *p*-values for multiple comparisons (Tukey method). Residuals for the GLMMs were simulated using the *DHARMa* package (v0.2.0) [45]. R^2 was calculated using the sjstats package (v0.17.0) [46].

Results

Sleep variables

Sleep macro- and microstructure variables for the selected intervals of the first four sleep cycles as well as the effects of noise and age are depicted in Table 2. Older participants had higher percentages N1 and N2 sleep and lower percentage of SWS than young participants; latencies to N1, N2, and REM sleep as well as awakening rates from REM sleep did not differ between age groups. NREM EEG arousal rates were significantly higher in older than in young individuals ($\chi^2 = 14.79$, $p < 0.001$) and did not differ between noise types ($\chi^2 = 1.01$, $p = 0.602$). Differences between the selection of the first four

Table 2. Sleep structure and continuity during selected intervals according to respective noise type and age group

	Young Participants (*N* = 26)			Older Participants (*N* = 16)				
	Noise-free	Eventful	Continuous	Noise-free	Eventful	Continuous		
	48 nights	49 nights	52 nights	30 nights	25 nights	30 nights	Age	Noise
TST (min)	359.2 (71.0)	372.4 (64.4)	373.8 (66.2)	335.3 (75.6)	355.7 (51.6)	323.5 (85.8)	Young > older	
SE (%)	95.0 (3.0)	95.3 (3.2)	95.5 (2.8)	90.5 (4.4)	92.8 (3.6)	90.7 (8.5)	Young > older	
Sleep latency N1 (min)	10.7 (8.5)	9.9 (8.2)	9.9 (7.0)	11.1 (6.1)	9.9 (6.2)	12.0 (11.3)		
Sleep latency N2 (min)	18.2 (10.3)	18.1 (10.3)	15.5 (8.2)	16.5 (7.7)	15.7 (6.9)	18.0 (12.1)		
SWS latency (min)	32.1 (14.9)	30.6 (10.8)	26.9 (8.2)	43.6 (29.9)	45.1 (38.3)	44.5 (24.4)	Young < older	
REM latency (min)	76.3 (29.3)	64.9 (18.5)	67.0 (25.3)	74.7 (27.0)	69.2 (24.7)	64.5 (19.7)		Noise-free > continuous noise
Intra-sleep wake (% of SPT)	1.9 (2.0)	1.8 (1.4)	1.6 (1.4)	5.7 (3.9)	4.6 (2.8)	4.6 (2.7)	Young < older	
N1 (% of TST)	11.8 (3.7)	11.6 (3.4)	11.3 (3.4)	17.3 (6.7)	16.1 (4.0)	17.5 (6.7)	Young < older	
N2 (% of TST)	45.8 (6.8)	45.0 (7.3)	45.4 (7.6)	48.6 (10.4)	53.5 (6.3)	50.9 (9.1)	Young < older	
SWS (% of TST)	20.3 (7.6)	19.1 (6.9)	19.4 (7.9)	10.7 (11.5)	9.3 (6.3)	9.8 (9.1)	Young > older	
REM (% of TST)	22.1 (5.7)	24.3 (5.7)	23.9 (5.1)	23.4 (5.3)	21.2 (4.4)	21.8 (5.8)		
EEG arousal rate (n/h TST)	10.0 (3.8)	10.6 (3.0)	10.6 (3.8)	17.6 (9.2)	16.2 (5.1)	18.2 (9.1)	Young < older	
EEG arousal rate (n/h NREM)	9.4 (4.3)	10.0 (3.3)	9.9 (4.2)	16.3 (9.2)	14.9 (5.2)	17.1 (9.9)	Young < older	
EEG arousal rate (n/h REM)	12.5 (5.7)	12.8 (6.0)	13.0 (6.4)	22.8 (15.5)	21.6 (9.8)	22.9 (13.8)	Young < older	
Awakening rate (n/h TST)	1.3 (0.9)	1.3 (0.8)	1.2 (0.7)	2.0 (0.9)	1.9 (0.8)	1.8 (0.7)	Young < older	
Awakening rate (n/h NREM)	1.3 (0.9)	1.5 (1.0)	1.3 (0.8)	2.2 (1.1)	2.2 (0.9)	2.1 (1.0)	Young < older	
Awakening rate (n/h REM)	2.7 (2.5)	2.4 (1.9)	2.4 (2.0)	2.5 (2.0)	2.4 (1.6)	2.8 (2.3)		

Means (Standard deviations). TST refers to total sleep time; SE: sleep efficiency = TST/TIB; TIB: time in bed; TIB here: lights off until the end of cycle 4; SPT: sleep period time. The last two columns indicate the direction of significant post hoc tests for the factors age and noise type (all $p < 0.05$, Tukey).

sleep cycles and the all-night sleep period for all sleep variables are described elsewhere (see Supplement 1.1.1) and showed that the selected sleep period was characterized by better sleep with higher sleep efficiency, increased SWS, and lower arousal and awakening rates compared to the all-night sleep period.

Distribution of EEG arousal rates

Figure 2 displays the within- and across-cycle time course of number of EEG arousals (upper panels) and percentage time spent in SWS per CSD (lower panel). In the preliminary model, all main effects, i.e. the factors for age group, noise type, cycle, and CSD contributed significantly to the model (see Supplement for detailed results and intermediate steps). The 10-level within-cycle effect could be further reduced using fourth order polynomials, which included the following terms: a positive linear, a positive quadratic, a negative cubic, and a positive quartic component with the quadratic component being by far the most prominent. Five significant two-way interactions between factors were included in the model (see also Table 3), which are described in detail in the following. In addition, we included a first order autoregressive error structure (likelihood-ratio test, $\chi^2 = 95.10$, $p < 0.001$) to account for the correlation of residuals within cycles across CSDs.

We observed a characteristic within-cycle distribution that was largely determined by a u-shaped pattern, as readily observable in the effects display of the predicted marginal means in Figure 3A. However, the within-cycle time course was not independent of time of the night as indicated by the significant two-way interactions between cycle and the CSD linear, quadratic, and quartic trends, respectively. The *CSD linear trend*, describing the overall change in arousal rates from the beginning to the end of the cycle, was significantly different between cycle 1 and 2 and cycle 1 and 3 (Tukey post hoc, $p < 0.007$ for all comparisons). The *CSD quadratic component* was responsible for the prominent u-shape of the distribution and the larger its coefficient, the narrower the u-shape. The quadratic coefficient was largest for the first cycle, smaller for the fourth cycle, and even smaller for the second and third cycle, reflected in the broader shapes in cycles 2 and 3. However, only the differences between cycle 1 and 2 and cycle 1 and 3 were statistically significant ($p < 0.001$ for all comparisons). The *third order polynomial trend* was not involved in any interaction and had a significant but minor effect compared to the other polynomial CSD effects. Its effect on the overall shape of

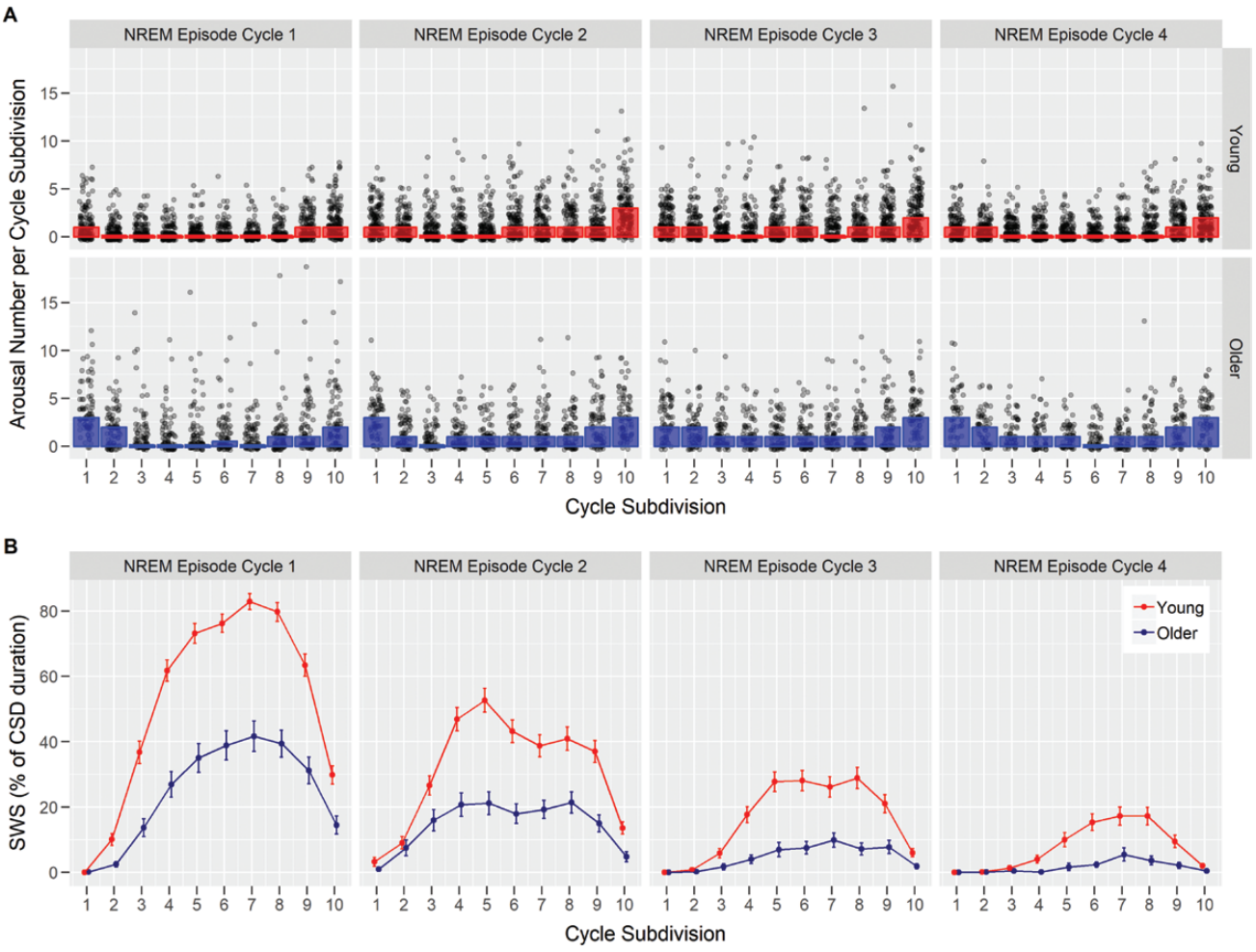

Figure 2. (A) Number of arousals (raw data) per cycle subdivision (CSD) during the first four normalized NREM sleep cycles in young (in red, upper panel) and older (in blue, lower panel) individuals. Each dot depicts the number of EEG arousals during one night at the respective point in time; bars depict the median. (B) Slow wave sleep (SWS) as the average percentage of time spent in SWS per CSD duration during the first four normalized NREM sleep cycles in young (red) and older (blue) individuals. Error bars represent ± SEM. CSD is depicted with a standardized length of 6.9 min, the overall average CSD length.

the time course was to broaden the middle part of the cycle, changing the time course from a u-shape in the direction of a bathtub-shape. The effect of the *CSD quartic component* was to broaden the middle part of an already u-shaped time course by increasing the value in the middle (at 50%) and decreasing the values in the middle of the first (25%) and second half (75%), thereby leveling the time course in this part of the cycle. The quartic coefficient was largest for cycle 2 and progressively smaller for cycles 3, 1, and 4. Post hoc tests indicated that the quartic trend in cycle 4 was significantly smaller than all other cycles, and had an opposite sign compared to all other cycles ($p < 0.05$).

Table 3. Wald chi-square tests of the final model (M2)

Effect	χ^2	df	P
Age	14.00	1	<0.001
Noise	6.76	2	0.034
Cycle	83.54	3	<0.001
CSD linear trend	19.40	1	<0.001
CSD quadratic trend	367.18	1	<0.001
CSD cubic trend	4.45	1	0.035
CSD quartic trend	20.02	1	<0.001
Age × cycle	83.67	3	<0.001
Age × CSD linear trend	18.32	1	<0.001
Cycle × CSD linear trend	18.86	3	<0.001
Cycle × CSD quadratic trend	28.64	3	<0.001
Cycle × CSD quartic trend	19.73	3	<0.001

CSD refers to cycle subdivision.

Modification with age

Because of the presence of significant interactions, we interpreted the significant main effect for age with caution; nevertheless, it is obvious that participants in the older age group had a higher overall arousal rate than younger participants (Figure 3A and C). In addition, the significant interaction between cycle and age indicated that arousal rates were significantly lower during the first as compared to all other cycles in the young (Tukey post hoc, $p < 0.001$ for all comparisons), while in the older, cycle 4 had significantly higher arousal rates than cycles 1 and 2 ($p < 0.005$ for all comparisons; Figure 3C). Post hoc tests of the significant interaction between the CSD linear trend and age indicated that in the young age group, arousal rates at the end of the cycle were consistently higher than at the beginning of the cycle (CSD linear trend with a positive coefficient), while in the older age group, arousal rates tended to be higher at the beginning of the cycle compared to its end (CSD linear trend with a negative coefficient).

Modification with noise

Arousal rates were higher during the eventful (scenarios C and D) compared to the noise-free exposure scenario (Tukey post hoc, $p = 0.03$; Figure 3B). All tested two-way interactions were nonsignificant, which included the interaction between noise and the age group ($\chi^2 = 1.46$, $p = 0.48$), noise and cycle ($\chi^2 = 12.10$, $p = 0.06$), and noise and the within-cycle effects, both using polynomial time trends (CSD linear trend: $\chi^2 = 1.61$, $p = 0.45$; CSD quadratic trend: $\chi^2 = 0.30$, $p = 0.86$; CSD cubic trend: $\chi^2 = 3.19$, $p = 0.20$; CSD quartic trend: $\chi^2 = 1.32$, $p = 0.52$), or CSD treated as a factor ($\chi^2 = 19.86$, $p = 0.34$).

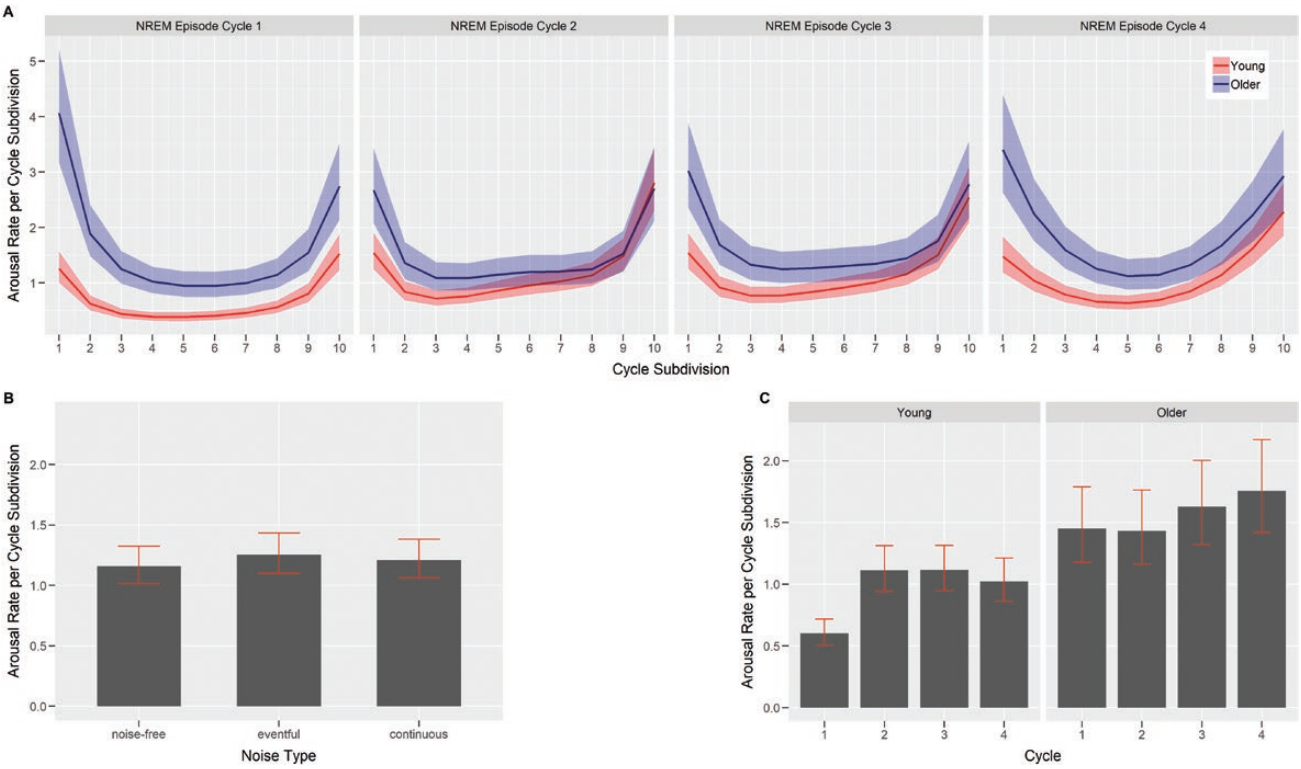

Figure 3. (A) Estimated marginal means for arousal rates based on the final model in young (red) and older (blue) individuals. (B) Estimated marginal means over the three different noise types. (C) Estimated marginal means over the NREM cycle for the two age groups. The EEG arousal rate displayed here is the number of arousals per cycle subdivision (CSD) with a standardized length of 6.9 min, the overall average CSD length. Mean ± 95% confidence intervals.

Table 4. Coefficients of determination (R^2) for the different tested models

Model	R^2
M1 Main effects only (age, noise, polynomial time trends, and cycle)	0.27
M2 Main effects and interactions (final model)	0.35
M3 SWS only	0.38
M4 Main effects and SWS	0.44
M5 Main effects, interactions, and SWS	0.52

Effect of SWS

Table 4 gives the coefficients of determination for five different models: main effects only (M1), main effects and interactions (M2, our final model), SWS only (M3), main effects and SWS (M4), and main effects, interactions, and SWS (M5). Model 5 had the highest coefficient of determination among all tested models indicating that the inclusion of SWS further improved our final model. Comparing M2 and M3 revealed that our final model had a lower goodness of fit than the SWS-only model indicating that SWS alone might be as good as the 12-term model (M2) in predicting the time course of EEG arousal rates. Nonetheless, the comparison between M3 and M4/M5 suggests that, independently of SWS, within- and across-cycle effects further improved the goodness of fit. Furthermore, in both comparisons (M1 vs. M2 and M4 vs. M5), the inclusion of significant two-way interactions in addition to the main effects improved the model fit. While both the noise condition and the interaction between cycle and CSD linear trend no longer contributed significantly to the model when SWS was included, all other predictors, main effects and interactions, still contributed significantly in both models (M2 and M5). More detailed results and a discussion of the shortcomings of our statistical approach are provided in the Supplement.

Discussion

The present analyses sought to examine the temporal distribution of EEG arousals both within and across sleep cycles and whether this distribution was modified by age and transportation noise exposure. The main results were: (1) arousal rates varied considerably within sleep cycles in a u-shaped or more bathtub-shaped course depending on the time of the night. (2) Older participants showed higher overall EEG arousal rates, being higher at the beginning than at the end of each NREM sleep episode in contrast to an opposite pattern in young participants. (3) EEG arousal rates increased during eventful noise exposure when compared to noise-free nights in both age groups across all NREM sleep episodes; although this was not consistent across analyses.

EEG arousal rates had a characteristic temporal distribution that was best described by a u-shaped curve with the highest number of arousals both at the beginning and end of a normalized NREM sleep episode. This characteristic distribution might be indicative of phases of decreased physiological sleep stability at the beginning and end of cycles. The results are in accordance with the distribution of CAP subtypes 2 and 3 which, per definition, share characteristics used for scoring EEG arousals [21–23, 47]. In general, the observed EEG arousal distribution is compatible with the typical NREM sleep architecture, which can be subdivided into three consecutive phases: initially, sleep stability is low and gradually EEG synchrony increases; next is a plateau phase of relative stability; and finally EEG synchrony rapidly declines toward REM sleep when sleep stability is again relatively low [23, 31]. The increase of EEG arousals preceding the onset of REM sleep in both age groups suggests a relationship with REM sleep and conditions that promote this state [23, 47, 48]. We chose EEG arousal as a marker for ultradian variation of activation during sleep, but other activation indices also vary along the ultradian cycle. For the temporal distribution of body movements [49], heart rate dynamics [33], transient changes in EMG muscular tone [50] as well as EEG beta power fluctuations during sleep [51] a time course similar to that of EEG arousals has been described with activity peaks at the beginning and toward the end of NREM cycles. Finally, several heart rate variability indices also showed a marked u-shaped pattern within NREM cycles, though only in young but not in older individuals [52].

Generally, EEG arousal rates increased across the night, but were only statistically different between the first and all other cycles in the young and between the fourth and the first two cycles in the older. The across-cycle fluctuations are not in accordance with the literature where no variations were reported across cycles [27] or time elapsed since sleep onset [28] and might be explained by differences in methodological approaches and the EEG arousal definitions. De Gennaro *et al.* [28] calculated arousal rates per hour of elapsed sleep time thereby not considering sleep cycles or any differences between NREM and REM sleep and Sforza *et al.* [27] calculated arousal rates per sleep cycle, but pooled NREM and REM arousals. In addition, we adopted the standard 3-s minimum duration criterion [26], while Sforza *et al.* [27] and De Gennaro *et al.* [28] additionally included events with a duration between 1.5 and 3 s. On the other hand, this might only be a minor concern as apparently only 1.9% of all arousal events were below 3 s in duration [27].

The observed overall increase of EEG arousal rates in the older participants is consistent with the literature [15, 16, 19]. Increased sleep fragmentation is a frequently reported age-related change of sleep architecture [53] and might be due to the decreased ability to maintain consistent and stable sleep states with aging [17, 54]. In the present study, age-related modifications of the EEG arousal time course were examined using interactions. There was a significant interaction with the CSD linear trend: while the linear trend was positive in the young, indicating lower EEG arousal rates at the beginning compared to the end of a NREM episode, this coefficient was negative in the older. It might be speculated that increased EEG arousal rates at the beginning of a cycle reflect an impaired sleep deepening process when sleep states switch to NREM sleep, either from wakefulness during the sleep onset period or from REM sleep for subsequent sleep cycles. However, the other tested interactions with polynomial time trends lacked significance suggesting that the basic physiological texture of arousal distribution during sleep cycles is largely unaffected by age.

EEG arousal rates were increased during nights with eventful noise exposure compared to noise-free nights. The absence of significant interactions with noise exposure allows for the conclusion that EEG arousals during noise exposure nights occurred at similar points in time than during physiological, noise-free conditions, therefore increasing the overall level but not changing the shape of the EEG arousal time course. This result was not

consistent with results based on the all-night NREM EEG arousal rates (as depicted in the Supplement Table S1) which were not affected by noise exposure likely due to methodological reasons. Occasionally, the REM episode of a cycle is interspersed with phases of NREM sleep—as long as this NREM sleep is shorter than a predefined time interval, per definition, this NREM sleep occurs during the REM episode [24]. For the all-night analysis, all EEG arousals occurring during NREM sleep were considered, while for the time course modeling only EEG arousals were considered that occurred during continuous NREM sleep episodes without any intervening REM sleep. Consequently, it is likely that the all-night analysis included more EEG arousals, namely those occurring during brief NREM intrusions during continuous episodes of REM sleep. Compared to the other effects in the final model, the noise effect was rather weak and the described small differences in considered NREM sleep might explain the significance of the noise factor in one but not the other analysis. During noise exposure nights, typically some EEG arousals are noise event-related (i.e. occur in temporal proximity to a noise event), while the other occur spontaneously (i.e. without an overt eliciting noise event). Indeed, many event-related EEG arousals replace spontaneous EEG arousals and only partly add to them [6]. However, it is not possible to observe whether a recorded arousal is a replacement or a true event-related arousal, which is why we decided to model the time course using all EEG arousals and not only a subset of event-related EEG arousals. Focusing on event-related EEG arousals only, would also have limited analyses to two nights per individual as the other noise exposure nights included more continuous noise, which per design had few distinct noise events to relate EEG arousal to. Consequently, the strength of our experimental design is the inclusion of these two very different noise exposure situations and results suggest that only eventful noise exposure had an effect on the microstructural architecture of NREM sleep when compared to noise-free conditions despite both eventful and continuous noise exposure nights having the same constant hourly $L_{Aeq,1\,h}$ of 45 dB.

Within cycles, the well-known evolution of SWS [29, 30] progresses inversely to the evolution of arousal rates so that we also tested whether the variations in the percentage of SWS could partially or fully explain the variations in arousal rates within and across sleep cycles. Regrettably, we could not use mediation analyses as these were not yet implemented in the context of GLMM with a negative binomial distribution. We therefore compared coefficients of determination of different models as an approximation. However, computing coefficients of determination in the context of GLMMs is also challenging due to the treatment of the random effects as well as the underlying distribution, so that the respective results need to be interpreted with caution. Our results suggest that a considerable part of the observed effects could be mediated by SWS, but that even with SWS in the model, there were additional SWS-independent within- and across-cycle effects that contributed further to the model. Similarly, Terzano *et al.* [47] reported that the time course of CAP subtypes A2 and A3, normalized in a similar way over the first five sleep cycles, was largely but not exclusively determined by SWA.

Our dependent variable, EEG arousal rate, contained a high number of zeros, a situation not uncommon in biology [55], which poses a particular challenge to any modeling. An excess number of zeros can have different reasons that significantly influence model choices (see Supplement 1.2.1). In the present analysis, we found that the distribution was consistent with a negative binomial distribution when all main effects were included in the model. Other model options, however, might also have been adequate. Of particular interest, are the so-called zero-altered models, which assume that there are actually two processes that produce the observed distribution with excess zeros. The first process determines whether or not there is any arousal, while the second process determines the number of arousals once the first process has overcome a critical threshold or hurdle. A tempting scenario is that the first process is represented by SWS while age, noise, and the other effects represent the second process. The models presented in this paper can therefore be seen as a starting point and an intermediate representation rather than a final model. Another model choice that deserves discussion is the orthogonal polynomial time trends used to represent the within-cycle CSD effect. Our main concern was physiological interpretability of the effect which is not given when regarding CSD as a factor with 10 levels. Orthogonal polynomials are an alternative because they model a time course rather than single time points which improves interpretability, particularly when considering interactions, e.g. between group differences in the time course. However, this is only realistic for a limited number of polynomials. As detailed in the Supplement (1.2.2), a model with six polynomial trends fits the data as well as CSD as a factor but we decided to go with only four polynomial trends because we felt that six were no longer interpretable. Another option includes generalized additive mixed models (GAMMs) that use flexible smoothing functions. However, choice of smoothing function and parameters would have added considerable complexity which in turn impedes interpretability. Nevertheless, this underlines that our presented model is only one of several possible and that there remain several promising avenues for future studies.

In conclusion, EEG arousal rates varied considerably within NREM sleep cycles in a u-shaped or more bathtub-shaped course depending on the time of the night suggesting that both the beginning and the end of cycles are phases of reduced physiological sleep stability. Older participants had higher overall arousal rates and a different distribution of arousals at the beginning and the end of the cycle. Eventful transportation noise exposure increased the overall level of EEG arousals but did not change the shape of the time course suggesting that eventful noise exposure leads to an unspecific increase of EEG arousals, which was embedded within the physiological structure of sleep stability during the night.

Limitations

Our analyses are based on a few limiting assumptions. The number of completed sleep cycles can vary across nights both within and between individuals [24, 31]. Here, we restricted the analysis to nights where the number of completed cycles was between three and five cycles assuming that fewer or more completed cycles represent outliers. Similarly, the duration of the NREM episode of a cycle was restricted to a duration between 33 and 116 min. Consequently, this procedure limits the generalizability of our results for nights with more, less, shorter, or longer cycles. Furthermore, we pooled data of individual nights with three, four, or five completed cycles assuming that within- and across-cycle effects on the arousal

rate do not vary with the number of completed cycles per night. To the best of our knowledge, differences in homeostatic regulation, spontaneous arousal rates, or arousal thresholds as a function of number of completed cycles have not yet been addressed in the literature, so that it remains open whether this assumption is valid.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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