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ORIGINAL ARTICLE

Sleep spindle characteristics and arousability from nighttime transportation noise exposure in healthy young and older individuals

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Abstract

Study Objectives: Nighttime transportation noise elicits awakenings, sleep-stage changes, and electroencephalographic (EEG) arousals. Here, we investigated the potential sleep-protective role of sleep spindles on noise-induced sleep alterations.

Methods: Twenty-six young (19–33 years, 12 women) and 18 older (52–70 years, 9 women) healthy volunteers underwent a repeated measures polysomnographic 6-day laboratory study. Participants spent one noise-free baseline night, followed by four transportation noise-exposure nights (road traffic or railway noise; continuous or intermittent: average sound levels of 45 dB, maximum sound levels of 50–62 dB), and one noise-free recovery night. Sleep stages were scored manually and fast sleep spindle characteristics were quantified automatically using an individual band-pass filtering approach.

Results: Nighttime exposure to transportation noise significantly increased sleep EEG arousal indices. Sleep structure and continuity were not differentially affected by noise exposure in individuals with a low versus a high spindle rate. Spindle rates showed an age-related decline along with more noise-induced sleep alterations. All-night spindle rates did not predict EEG arousal or awakening probability from single railway noise events. Spindle characteristics were affected in noise-exposure nights compared to noise-free nights: we observed a reduction of the spindle amplitude in both age groups and of the spindle rate in the older group.

Conclusions: We have evidence that spindle rate is more likely to represent a trait phenomenon, which does not seem to play a sleep-protective role in nighttime transportation noise-induced sleep disruptions. However, the marked reduction in spindle amplitude is most likely a sensitive index for noise-induced sleep alterations.

Statement of Significance

Growing epidemiological evidence suggests various adverse health effects of long-term nighttime transportation noise exposure. In the EU, 7.9 million adults are estimated to experience sleep disturbances due to nighttime transportation noise exposure with additional community noise sources such as neighbor noise not even considered. Individuals differ greatly in sleep-related noise sensitivity. A better understanding of the mechanisms that underlie these differences could help to identify targets for intervention. This study in young and older healthy individuals indicated that sleep spindles, discussed in the literature for their potential sleep-protective function, do not significantly modify noise-induced sleep alterations after controlling for age and may therefore not serve as a physiological marker of sleep-related noise sensitivity.

Key words: road traffic noise; railway noise; arousal; awakening; EEG; automatic detection; intermittency ratio

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Introduction

Why are some individuals' brains more effective in canceling out noise during sleep than others? Sleep-related noise sensitivity exhibits marked inter-individual variability [1]: some are difficult to arouse, while others are repeatedly disturbed by external stimuli such as nighttime transportation noise that elicits additional awakenings, sleep stage changes, or electroencephalographic (EEG) arousals [2–5]. EEG arousals are defined as abrupt shifts in EEG frequency towards higher frequencies [6] and differ from awakenings in their transitory nature and concomitant changes in heart rate dynamics [3]. Sleep spindles—sleep-related EEG oscillations, that occur spontaneously during non-rapid eye movement (NREM) sleep—showed a sleep-protective function [7]. Spindles are identified by their frequency (approx. 12–15 Hz for fast spindles), duration (typically between 0.5 and 2 s [8, 9]), and characteristic shape from cortical EEG recordings and their density has high inter-individual variation [9]. Individuals with higher all-night sleep spindle rates had higher EEG arousal thresholds for a variety of commonly experienced noise types presented during NREM sleep than individuals with lower all-night sleep spindle rates [7].

Neurons in the thalamic reticular nucleus (TRN) play a pacemaking role for spindle oscillations, but the spindle event itself is network-generated within a corticothalamocortical circuitry [10, 11]. TRN neurons potentiate thalamocortical (TC) projection cells whose rhythmic inhibitory post-synaptic potentials result in excitatory postsynaptic potentials in the cortex [11]. It has been hypothesized that TC cell firing during spindles gates afferent signaling to the cortex [10] in order to isolate the cortex from environmental throughput and thus facilitate off-line memory consolidation or brain plasticity processes [12, 13]. Indeed, differential information processing of auditory stimuli—usually very brief, only several ms lasting stimuli—during spindle presence relative to absence was demonstrated in humans using event-related potential studies [14–16] and combined event-related electroencephalographic/ functional magnetic resonance imaging (EEG/fMRI) studies [16, 17]. However, there is less consensus on the sleep-protective role of spindles in the presence of noise stimuli with higher ecologic validity (i.e. non-artificial and longer lasting noise stimuli).

Additionally, age plays an important role for noise-induced sleep disruptions: with aging the neural network exhibits marked transformations such as a deterioration in grey and white matter [18–20] that might impact on sleep structure and EEG oscillations [21, 22]. A decrease in sleep efficiency and slow wave sleep (SWS) or an increase in the number of spontaneous EEG arousals are typical age-related changes in sleep macro- and microstructure [23–25]. Spindle characteristics also exhibit age-related alterations: when comparing to younger adults or adolescents, a reduction in spindle rate [8, 9, 21, 23, 26–29], duration [9, 23, 26–28, 30], and amplitude [8, 9, 26, 27, 30] are typically reported. Age comparisons for spindle frequency, however, indicate a small [23, 28], but a largely inconsistent [8, 9, 26, 27] increase with aging. For example, reduced white matter integrity in the relevant spindle circuitry (i.e. corpus callosum or thalamic radiation) was associated with a reduced spindle rate in aging [21]. As a result of marked age-related sleep changes, noise sensitivity, a trait-like evaluative and perceptive predisposition towards environmental noise in general, might increase with aging [31, 32], which in turn can influence the self-reported evaluation of sleep [33] or nighttime noise annoyance [34].

This polysomnographic (PSG) study explores the potential sleep-protective role of sleep spindles in healthy young and older adult volunteers exposed to nighttime transportation noise. As real-world nighttime noise from road traffic and railways may include both intermittent periods as well as rather continuous noise, the used noise scenarios reflected both continuous (two nights) and more eventful (two nights) noise-exposure situations to ensure high ecologic validity. In a first step, all-night transportation noise effects on sleep outcome variables (i.e. sleep structure and continuity) and spindle characteristics (i.e. rate, duration, frequency, and amplitude) were evaluated. In a second step, all-night spindle rate was related to sleep outcome variables: if spindles have sleep-protective features, sleep structure and continuity in individuals with a high all-night spindle rate should be less affected by noise exposure than in individuals with a low all-night spindle rate; the same should apply to young individuals who have higher all-night spindle rates than older individuals. In a last step, we carried out an event-related analysis and included all-night spindle rate among other sleep-related and acoustical parameters [2, 3, 5] with the aim to predict EEG arousal and awakening probability from single railway noise events (RNE). In addition, we evaluated spindle characteristics during exposure and non-exposure periods to test reactive spindle activity [35, 36] and relations to acoustical characteristics of the RNE [37].

Methods

Participants

Forty-four healthy volunteers of two age groups (26 young: 19–33 years, 12 women; 18 older: 52–70 years, 9 women) were selected for the study. 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. All participants slept habitually 8 ± 1 hour, showed good self-reported sleep quality (Pittsburgh Sleep Quality Index [38], PSQI ≤ 5), normal general daytime sleepiness (Epworth Sleepiness Scale [39], ESS ≤ 10), and had no signs of sleep disorders, such as sleep-related movement and breathing disorders (confirmed via PSG during one screening/adaptation night prior to study admission). They were free from depressive symptoms (Beck Depression Inventory, BDI-II < 9) and 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 [40] at the frequencies 250, 500, 1000, 2000, 3000, and 4000 Hz) tested manually with an audiometer (Bosch ST-10, Stuttgart, Germany). Further exclusion criteria comprised smoking, night shift work within 3 months or transmeridian travel within 1 month prior to study start, extreme circadian preference (Munich Chronotype Questionnaire [MCTQ] [41], MCTQ Mid sleep on free days corrected for sleep duration [MSF_{sc}] < 2 or MCTQ MSF_{sc} ≥ 7), or drug misuse. Participants were not selected upon habitual noise exposure or sensitivity to noise, but self-reported noise sensitivity varied considerably as measured by the short version of the German *Lärmempfindlichkeitsfragebogen* (LEF-K) [42] and the Noise Sensitivity Questionnaire (NoiSeQ) [43] (Table 1).

Two young participants did not finish the experiment (both quitted on the fifth day for personal reasons) and were

substituted in order to maintain the balancing but data were nevertheless included for analysis. Two participants of the older group dropped out of the experiment due to medical reasons (one female: severe back pain that required pain medication; one male: general discomfort and headaches). They were not substituted and their entire data were excluded. In total, data of 42 participants were considered for the analysis.

The study protocol, screening questionnaires, and consent forms were approved by the local ethics committee (Ethikkommission Nordwest- und Zentralschweiz, Switzerland, #2014–121) and confirmed 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 under continuous PSG recording. Participants were exposed to different transportation noise scenarios during four nights and spent two noise-free nights (Figure 1). Noise-free nights were always the first (baseline night: BL) and the last night (recovery night: RC). The transportation noise scenarios were applied in an incompletely counterbalanced sequence: scenarios A and B (more continuous characteristic) alternated with scenarios C and D (more intermittent characteristic). The sequence was balanced within age and sex groups (Figure 1). 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. The scheduled sleep episode at habitual bedtime was 8 hours in duration for every participant. The final awakening was either experimenter-induced (after the end of the 8-hour sleep episode) or spontaneous (i.e. ≥ 3 minutes before the end of the 8-hour sleep episode; 23.17% of nights). Noise scenario playback started immediately after lights off and was without knowledge of the sleep stage. The reproduced sound in the bedroom was recorded continuously using a microphone and logged with the EEG recording device for time synchronization and verification. Participants spent days and nights in single windowless, soundproof, and temperature regulated bedrooms under constant ambient lighting levels (lux levels at the participant's eye during waking periods between 50 lux when sitting in bed and 150 lux when sitting at the table). Global self-reported sleep quality was assessed every morning ("Taken everything together, how well did you sleep?"; scale 0–100). Noise annoyance for every single night was assessed en bloc in the morning of the last night ("How annoyed have you been during the respective night (1–6) by the noise?"; scale 0–100).

Prior to the study start, participants kept a regular sleep-wake cycle with self-selected habitual bedtimes and wake times for 1 week (nighttime sleep duration 8 hours ± 30 minutes, no naptaking) as verified by accelerometers worn at the non-dominant wrist (Actiwatch AW4; Cambridge Neurotechnologies, Cambridge, United Kingdom) and self-reported sleep-logs. Additionally, participants were asked to restrict consumption of alcohol, caffeinated beverages, and chocolate to moderation during 1 week prior to the study in order to level out effects of these substances on sleep and waking functions.

Table 1. Demographic data and questionnaire scores (*M* and *SD*) of the sample split by age

Sample characteristics	Young	Older
N (F, M)	26 (12, 14)	16 (8, 8)
Age (year)	24.58 (3.51)	60.83 (5.90)*
BMI (kg/m^2)	22.21 (2.10)	22.02 (2.13)
ESS	4.85 (2.84)	5.75 (2.96)
PSQI	2.19 (1.10)	2.88 (1.63)
PSQI sleep duration	7.88 (0.63)	7.81 (0.36)
MCTQ sleep duration work	8.13 (0.87)	8.10 (0.87)
MCTQ sleep duration free	8.40 (0.97)	8.29 (0.89)
MCTQ MSF_{sc}	4.27 (0.67)	3.33 (1.11)*
STAI-trait anxiety	26.64 (7.40)	28.31 (6.47)
LEF-K	10.96 (4.04)	14.44 (3.56)*
NoiSeQ Global	1.23 (0.43)	1.59 (0.34)*
NoiSeQ Sleep	1.12 (0.64)	1.38 (0.52)

F refers to female; M: male; BMI: body mass index; NoiSeQ Global: Noise Sensitivity Questionnaire-global score; NoiSeQ Sleep: Noise Sensitivity Questionnaire-subscale "Sleep".
*Significant difference between age groups ($p < 0.05$, Welch's two-sample *t*-test that is somewhat invariant to unequal sample sizes and variances).

Noise scenarios

Five prerecorded real-world inspired acoustical scenarios were played back in the bedroom during the night: one essentially noise-free (NF) and four transportation noise scenarios (Road A–C, Rail D; see Table 2). Throughout the paper, all reported acoustical metrics are based on A-weighted sound pressure levels (SPL). Scenario NF, played back during BL and RC nights, was designed to yield a constant hourly $L_{Aeq,1h}$ of 30 dB at the ear of the sleeper. It mimicked a rather tranquil real-world bedroom situation with a tilted window and very low transportation noise exposure. It consisted of sounds of crickets and of distant traffic. The four noise scenarios differed with respect to noise source (different road traffic situations and railway noise) and along a new acoustic exposure descriptor termed Intermittency Ratio (IR) [44], which characterizes the "eventfulness" of noise exposure situations. They were played back with a constant hourly equivalent continuous SPL, $L_{Aeq,1h}$, of 45 dB at the sleeper's ear. This approximately corresponds to an average outdoor façade level of 60 dB for a tilted window. Road scenario A represented a four-lane highway (speed limit of 120 km/hour) with approximately 1.000 vehicles per hour at a distance of 400 m. Road scenario B represented a distance of 50 m from a two-lane country road (speed limit of 80 km/hour) with approximately 250 vehicles per hour. Road scenario C represented a one-lane urban road (50 km/hour) at a 15 m distance with approximately 100 vehicles per hour. Rail scenario D represented a railway noise situation with 10 non-overlapping train pass-by events per hour.

The sound stimuli were created by sound sampling, where recordings from single vehicle pass-bys were modified and mixed. Monophonic, calibrated sound recordings were taken outdoors under free-field conditions. The spectral effect of sound transmission through a tilted window was accounted for by using a digital filter that attenuated the high-frequency content. For the road scenarios (A–C), realistic traffic was simulated using measured traffic flow statistics. For the rail scenario (D), five independent train pass-bys were recorded, four freight trains at 250 m and one commuter train 30 m from the track, and were played back with a pseudorandom equidistant spacing of 300 s.

The transportation noise scenarios were designed to achieve the predefined level requirement in realistic and relevant exposure situations. Therefore, apart from transportation noise, they also contained the identical ambient noise from scenario NF.

The audio files were played back from portable audio devices (702T digital recorder, Sound Devices, Reedsburg, WI) through one active monitor loudspeaker (Focal CMS 50, Focal-JMlab, La Talaudière, France) at a distance of 2 m to the sleeper's head. Prior to the study start, the bedrooms were acoustically measured and calibrated using a sound level meter and by adjusting the playback volume.

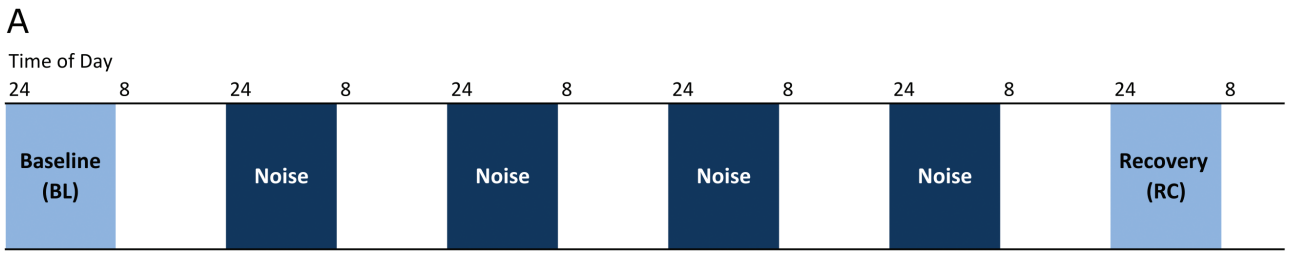
To acoustically characterize the sound stimuli, acoustical metrics were calculated from the audio data in a post hoc analysis. To that aim, the sound signals were first convolved with a measured room impulse response of the loudspeaker and the laboratory to consider the effects of the loudspeaker and the room acoustics on the reproduced sound at the sleeper's head. From these sound pressure signals, the A-weighted SPL history was calculated, from which several other metrics were derived (Tables 2 and 3). The five RNE differed with respect to event duration, maximum SPL (L_{AFmax}), sound exposure level (L_{AE}), and the maximum slope of the SPL (maxSPLslope; Table 3). A RNE started when the SPL exceeded a given threshold (here: 35 dB) and ended when the SPL fell below this threshold. The parameter maxSPLslope was determined based on regression lines fitted to the SPL (maximum slope of single regression lines fitted to 15 dB spreads of the SPL of a single RNE). In addition, the equivalent continuous SPL over 10 s ($L_{Aeq,10s,max}$) was calculated to assure compatibility of stimulus intensity with the literature [7, 45].

Sleep recording and outcome variables

The PSG comprised electroencephalogram (EEG), electromyogram (EMG), electrooculogram (EOG), and electrocardiogram (ECG) and was collected using a 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 10–20-electrode system referenced against averaged mastoids). The 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. Submental EMG was recorded bipolarly. 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).

The PSG recordings were identified according to standard criteria by four experienced raters in our laboratory blind to the respective noise condition; inter-rater accordance was assured >85%. One scorer analyzed all six nights of one participant and the number of scored files was balanced according to the participant's sex and age. Scorers had regular scoring sessions to discuss questionable epochs and align local scoring procedures. Artifacts were rejected by visual inspection. Noisy or flat channels (on average more than 5 minutes of bad signal quality in total per night) were excluded from the analysis. In total, 246 nights were used: two nights were excluded due to technical problems. Signals were additionally offline-filtered between 0.5 and 32 Hz for visual scoring of sleep stages and EEG arousals. For sleep staging, the recommendations of the American Academy of Sleep Medicine (v2.3) [46] were applied. In addition, SWS was further subdivided into NREM3 and NREM4: ≥50% of slow wave activity per epoch according to Rechtschaffen and Kales [47]. For EEG arousal scoring, the recommendations of the American Sleep Disorders Association [6] were adopted; EEG arousal on- and offsets were determined. Time of awakenings—a sleep stage change from any sleep stage to wake—was pinpointed visually as re-occurrence of alpha or faster rhythms.

The following variables were included as outcome measures for sleep structure: total sleep time (TST), sleep efficiency (SE),



B

Night 1	Night 2	Night 3	Night 4	Night 5	Night 6
NF	A	C	B	D	NF
NF	D	A	C	B	NF
NF	B	D	A	C	NF
NF	C	B	D	A	NF
NF	A	D	B	C	NF
NF	C	A	D	B	NF

Figure 1. Experimental protocol. (A) Schematic overview of the experimental protocol. Each nighttime episode was scheduled at the individual habitual bedtime (here, for illustrative purposes 24-8). Noise free baseline and recovery nights (light blue; hourly $L_{Aeq,1h}$ of 30 dB) always preceded resp. followed the different noise nights (dark blue; hourly $L_{Aeq,1h}$ of 45 dB). (B) The sequence of the different noise scenarios was incompletely counterbalanced and was designed according to the following rule: scenarios A and B (more continuous characteristic; shaded dark blue) alternated with scenarios C and D (more intermittent characteristic; dark blue). This sequence was balanced within age, sex, and genotype (for the young) groups.

Table 2. Characteristics of the acoustical scenarios

Scenario	Source	L_{Aeq} (dB)	L_{AFmax} (dB)	L_{A5} (dB)	L_{A10} (dB)	IR
A	Road	45	53	49	48	0.3
B	Road	45	60	52	48	0.7
C	Road	45	62	52	48	0.8
D	Rail	45	62	53	46	0.9
NF	Ambient/background	30	39	35	34	0.3

L_{Aeq}: equivalent SPL; L_{AFmax}: maximum SPL; L_{A5}: SPL exceeded 5% of the time; L_{A10}: SPL exceeded 10% of the time; IR: Intermittency Ratio [35].

onset latencies to NREM1, to NREM2, to SWS (i.e. first occurrence of respective sleep stage after lights off), and to REM (first occurrence of REM after NREM2 onset), minutes spent in NREM1, NREM2, NREM3, NREM4, REM, and wake after sleep onset (i.e. time spent between sleep onset NREM1 and the final awakening in the morning), and the number of complete NREM-REM sleep cycles.

Sleep cycle definition based on a slight modification of the initial criteria proposed by Feinberg and Floyd [48]. NREM parts of a cycle (minimum duration of 20 minutes) comprised the time interval between NREM1 and successive REM onset (minimum duration of 5 minutes). However, for the first cycle different criteria were adopted: the first cycle started with NREM2 and the first REM part was allowed to be shorter than 5 minutes in duration. To account for omitted REM during the first cycle, the following criteria adopted from Jenni and Carskadon [49] were introduced: for all episodes with first NREM part cycle duration > 120 minutes, the first NREM part was divided into two, if SWS was interrupted by > 12 minutes (i.e. every other sleep stage than SWS). Consequently, the first cycle ended with the last epoch of this interruption and the second started with the subsequent SWS onset. For cycle-related analyses, only completed cycles were included (i.e. end with REM sleep that was followed by at least 5 minutes of NREM sleep or wakefulness), that differed between participants and nights: N = 32 with three cycles, N = 135 with four cycles, N = 68 with five cycles, and N = 11 with less than three or more than five completed cycles.

Sleep continuity was assessed using the number and average duration of EEG arousals per hour of TST, the number and average duration of awakenings per hour of TST (final awakening excluded), the number of reciprocal NREM-REM transitions (NR: NREM-to-REM, RN: REM-to-NREM), and the total number of sleep stage changes per hour of TST.

Sleep spindle detection and outcome variables

Spindle detection followed a sequential two-step process: frequency peak identification by eye in the relevant spindle frequency range of all-night NREM power spectra (NREM2+SWS) with 9–12 Hz for slow spindles at averaged frontal derivations and 12–15 Hz for fast spindles at averaged centro–parietal derivations according to expected topographical distribution for slow and fast spindles [50] and spindle event detection. Individual spindle frequency peaks were used to account for profound inter-individual differences in spindle spectra [51, 52]. Power maxima in the fast spindle range were averaged over two nights (screening/adaptation night without any acoustical playback and the noise-free BL night) (fast spindle peak: young: 13.25 ± 0.48 Hz; older: 13.56 ± 0.74 Hz). Power maxima in the slow spindle range were identified in the noise-free BL night, but were not readily identifiable in nine participants [52, 53] so the analysis was limited to fast spindles. Spindles were detected in artifact and arousal-free EEG segments during NREM sleep stages 2–4 (NREM2+SWS) using an automatic algorithm that adopted methodology proposed by Mölle et al [54]. The SpiSOP toolbox is free, copyrighted software and is distributed and documented under www.spisop.org. In short, the root mean square (RMS) of each filtered EEG signal (band-pass filtered with ± 1.5 Hz around the individual fast frequency peak; –3 dB cutoff) was determined (window size of 0.2 s) and smoothed with a moving average (window size of 0.2 s). Spindles were detected by amplitude thresholding the RMS signal (> 1.5 times the standard deviation of the filtered signal of the respective channel for 0.5–3 s).

Spindles were detected for all central and parietal derivations. The main outcome variable was the all-night spindle rate at EEG channel C3 during the noise-free BL night as this was originally used to relate spindle activity to arousal thresholds [7]. Due to technical problems, for one participant C3 signal of one recording was bad and replaced by C4 as spindle rates did not differ significantly between hemispheres. Additional analyses also included parietal derivations as a topographically specific spindle impairment was reported in the older [26, 29]. The all-night spindle rate was calculated as number of detected spindle events per minute of NREM2+SWS. Additional spindle characteristics were determined: average duration (i.e. the time between threshold crossing in seconds), average oscillatory frequency (in hertz), and maximum amplitude (peak-to-peak difference in microvolt) during NREM2+SWS. All outcomes

Table 3. Acoustical characteristics of the single RNE (scenario D)

Number	Duration (s)	L_{AFmax} (dB)	L_{AE} (dB)	L_{Aeq} (dB)	$L_{Aeq,10s,max}$ (dB)	MaxSPLslope (dB/s)
RNE1	52.1	50.1	62.4	45.2	48.4	1.0
RNE2	16.9	60.8	67.0	54.7	54.8	5.2
RNE3	63.6	60.9	71.2	53.2	56.1	1.7
RNE4	64.9	54.0	66.8	48.7	52.7	1.0
RNE5	113.7	61.7	75.0	54.5	60.6	0.7

RNE refers to railway noise event; duration: time when SPL is above threshold of 35 dB; L_{AFmax}: maximum SPL; L_{AE}: sound exposure level; L_{Aeq}: equivalent SPL; $L_{Aeq,10,max}$: equivalent SPL over 10 s (maximum value); maxSPLslope: maximum slope during event (see text for calculation).

were also calculated for each NREM sleep cycle to account for age-dependent differences in spindle activity over consecutive NREM sleep cycles [9, 21, 26, 30] and during NREM2 and SWS only to test differences between sleep stages.

Event-related cortical activations: arousability from RNEs

Due to its highly intermittent characteristic, for the railway noise scenario (scenario D), cortical activations could be related to distinct, well-defined pass-by events. For the other three scenarios, the event-related analysis was not possible due to a more continuous temporal variation of the SPL. Cortical activation probabilities (i.e. awakening probability and EEG arousal probability) were calculated as ratios between the number of noise associated awakenings/EEG arousals and the number of adequate noise events. A cortical activation was considered noise associated if it occurred within the time span of the particular RNE. Noise events were considered inadequate if the respective onset met one of the following three criteria: occurrence prior to the first sleep onset of NREM2, occurrence during intra-sleep wakefulness, awakening in 30 s or EEG arousal in 10 s prior to noise onset [1, 5]. The longer the scanned window (here, event duration), the higher the probability that a spontaneous, non-noise-associated EEG arousal is attributed to this window so that the spontaneous arousal probability might not be comparable between the different noise events whose duration differed greatly. Thus, in addition to cortical activation probabilities, cortical activation rates were calculated as ratio between the number of noise associated awakenings/EEG arousals and the duration of adequate noise events. In sum, 3360 RNEs were applied; 2840 events contributed to the analysis (15.48% were excluded based on the aforementioned exclusion criteria; mean exposure time per participant: 70.86 minutes; 35.69 minutes non-arousal associated RNEs during NREM2+SWS).

"Virtual" events (i.e. periods during the two noise-free nights with the same duration and distribution across the night as during the RNE scenario subjected to the same aforementioned exclusion criteria) [1–3] were used for two purposes: to determine spontaneous cortical activation probabilities and to test the effect of noise exposure [36, 37] on spindle characteristics with comparing exposure (during RNE duration) to non-exposure periods (during "virtual" event duration). Exposure versus non-exposure comparisons were restricted to NREM events and to non-EEG arousal/awakening associated events to not confuse effects of noise and EEG arousal/awakening on spindle characteristics. In total, 3280/3120 "virtual" events (BL/RC) were considered; 2769/2590 events were used for analysis (15.58/16.99% were excluded; mean "exposure" time per participant: 70.91/69.32 minutes; 39.05/37.07 minutes non-arousal associated events during NREM2+SWS).

Statistical analyses

All self-reported sleep quality, sleep, and spindle outcomes were analyzed using linear mixed-effects models with a random intercept for the participant, the within-participant factor noise scenario (1 + 4 + 1 different noise nights), the between-participant factor age group (young and older), and the interaction between the two factors. Planned orthogonal contrasts were used to test the difference between the pooled two noise-free nights and pooled four noise-exposure nights, the pooled noise-free nights and the individual noise-exposure nights, and finally, the first and the last night to test the effect of the time in the experiment on all outcome variables; each contrast testing was done in separate for both age groups. Stratified analysis were performed for individuals with a low and a high spindle rate (based on the median all-night spindle rate during NREM2+SWS of individual means over all centro-parietal derivations during the noise-free nights) to test whether noise exposure modified sleep structure and continuity differently in these two spindle groups. The spindle rate (means over all centro–parietal derivations during the noise-free nights) was also correlated with other person self-report measures that might play a role in sleep quality: self-reported noise sensitivity (LEF-K, NoiSeQ subscale sleep), trait anxiety (STAI), and self-reported sleep quality (PSQI). Correlations were derived using Pearson correlation coefficients. Additional factors (apart from noise and the age group) were included in the model for a detailed analysis of the effects of the spindle rate, the NREM sleep cycle, or acoustical characteristics of single RNEs: for the sake of clarity, these factors will be described in the respective result paragraphs.

For the event-related analyses, logistic regression models with a participant-specific random intercept were used to test the effect of the C3 BL all-night spindle rate (as well as separate models for all other derivations) on EEG arousal and awakening probability from single RNEs. Acoustical (maximum SPL, maxSPLslope), sleep-related (sleep stage prior to threshold exceedance of the SPL: NREM1 versus NREM2, SWS versus NREM2, and REM versus NREM2, sleep cycle since sleep onset, study night), and subject variables (age group: young versus older, sex: female versus male) were included. To test whether the effect of spindles, that showed marked reduction with aging [8, 9, 21, 23, 26–29], is independent of the age group, three separate models were fitted: one model including only the BL spindle rate, a second model that included both the BL spindle rate and the age group, and a third model that only included the age group. If the effect of the BL spindle rate is significant in a model controlled for age (Model 2), this might be indicative of an indirect (mediating) effect of the BL spindle rate for the relationship between age and EEG arousal probability [55]. In addition, model fit comparisons were used to select the better predictor (age group or BL spindle rate) for EEG arousal probability using the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with the lowest value indicative of the superior model. To account for the divergent temporal dynamics of spindle activity and cortical activation probabilities across successive NREM cycles, cortical activation probabilities and spindle activity were correlated within each NREM cycle, separately for both age groups. For this, the analysis was restricted to NREM cortical activations as spindles are characteristic for NREM sleep.

All analyses were performed in R [56]. Mixed models were fitted with lme4::lmer via the *afex* package (v0.18-0) [57]. Denominator degrees of freedoms for all effects were approximated using the Kenward-Rogers procedure. Type 3 sums of squares were used. Post hoc tests and planned contrasts were run using the *lsmeans* package (v2.26-3) [58]: *p*-values were adjusted using an approximation of the Dunnett or the Tukey adjustment, depending on the type of comparison. Logistic regression models were fitted using lme4::glmer (v1.1–13) [59] and model non-convergence issues were solved by centering continuous predictor variables. The alpha level was set to $p < 0.05$.

Results

Sleep structure and continuity

Noise effects and interaction with age

Noise exposure increased the number of arousals(especially during NREM sleep) and the number of total sleep stage changes (planned contrasts of the pooled two noise-free nights with the pooled four noise-exposure nights; all $p < 0.05$, Dunnett's test; Supplementary Tables S1 and S2). In the older individuals, sleep was more fragmented under noise exposure than in noise-free nights as indicated by an increase in the number of NREM EEG arousals, the number of awakenings from NREM sleep, the amount of NREM1, and the number of total sleep stage changes. According to planned contrasts between the noise-free nights and the individual noise nights, the noise effects were mainly driven by the road scenarios B and C. In the young subgroup, however, pooled noise exposure decreased the latency to REM and increased amounts of REM sleep without any clear indication of differences for the single noise nights. But, these effects can only partially be attributed to the noise as they also demonstrated time-in-study effects (see following paragraphs).

Age effects

Minutes in intra-sleep wake and NREM stages 1–2, total number of arousals per hour TST (during NREM and REM), awakenings per hour TST (particularly from NREM), total number of sleep stage changes, the number of reciprocal NREM-REM transitions, and the latency to SWS were significantly higher in the older compared to the young group, while total sleep time, sleep efficiency, and minutes in SWS were significantly lower (Supplementary Tables S1 and S2).

Time-in-study effects

REM-related variables showed a time-in-study effect with a decrease in latency, an increase in the number of NREM-REM transitions and the duration of REM arousals throughout the protocol for both age groups. Additionally, the young subgroup showed an increase in REM sleep, in the duration of NREM arousals, and latency to NREM2 over the course of the protocol (planned contrasts between the first and the last experimental night; all $p < 0.05$, Dunnett's test; results are shown in the last column of Supplementary Tables S1 and S2).

Sleep spindles

Sleep spindle characteristics (during NREM2+SWS), noise effects, and interaction with age

When controlling for EEG derivation, all-night *spindle rate* was stable across noise nights for the young individuals (all planned contrasts not significant with $p > 0.05$, Dunnett's test; Figure 2 for EEG derivations C3 and P3; analyses based on all three derivations of central and parietal positions), but decreased during noise nights compared to the noise-free nights for older individuals what was present in all scenarios but road noise scenario B ($p < 0.05$, Dunnett's test). *Spindle duration* decreased upon noise exposure in both age groups and was significantly reduced in the young individuals upon noise exposure in all scenarios but train scenario D ($p < 0.05$, Dunnett's test). *Spindle amplitude* showed consistently significant differences between the pooled noise-free and the pooled noise nights in both age groups: maximum spindle amplitude was significantly reduced during exposure of road scenarios A and B ($p < 0.05$, Dunnett's test). *Spindle frequency* was stable across noise nights for both age groups ($p < 0.05$, Dunnett's test). All interactions with the EEG derivation were nonsignificant.

The additional within-participant factor cycle (up to five factor levels for the number of sleep cycles) was included in the model. For *spindle rate* per minute NREM2+SWS at C3, post hoc tests for the significant interaction between age and cycle [$F(4,882.56) = 64.00$, $p < 0.001$] revealed that the rate increased progressively across sleep cycles until cycle 4 in the young subgroup (best fitted by a simple linear trend) and was fairly stable in the older subgroup (pair-wise post hoc comparisons largely insignificant, except for the first cycle that demonstrated a higher spindle rate; P3 had the same results; Figure 3). Spindle rate differences between age groups increased across the night and were maximal during the fourth NREM cycle.

Sleep spindle characteristics, age effects, and interaction with topography

We observed a reduction in *spindle rate* in older as compared to young individuals what was particularly present at central when compared to parietal derivations (interaction between topography (central and parietal) and age [$F(1,444) = 61.63$, $p < 0.001$]). *Spindle duration* was significantly reduced in older when compared to young individuals [$F(1,40.12) = 36.47$, $p < 0.001$] and both groups had longer spindles at parietal as compared to central derivations [$F(1,444) = 86.31$, $p < 0.001$]. *Spindle amplitude* was lower at central but not parietal derivations in the older compared to the young and spindle amplitude was higher at central than at parietal derivations in the young but not the older (post hoc testing of the significant interaction between age and topography [$F(1,444) = 111.11$, $p < 0.001$]. The *spindle frequency*, however, was not significantly different between age groups but was higher at parietal than central derivation in both age groups what was more pronounced in the older (post hoc testing of the significant interaction between age and topography [$F(1,444) = 34.13$, $p < 0.001$]).

Sleep spindle rate and sleep structure and continuity

Planned contrasts within the two spindle groups (low versus high based on the median all-night spindle rate) on all sleep structure and continuity outcomes failed to reveal any consistent differences between spindle groups, except for an increase in latency to NREM1 in the low spindle rate group of the older individuals during road scenario A. Across all nights, irrespective of the noise, higher spindle rates during NREM2+SWS were associated with longer NREM2 duration ($p < 0.001$, 18.30 minutes increase per unit in spindle rate) and fewer number of awakenings per hour TST ($p = 0.048$, 0.28 n/hour TST decrease per unit spindle rate) in mixed models with the additional continuous variable *spindle rate* (all-night NREM2+SWS spindle rate during the respective night averaged over all centro-parietal derivations). In addition, higher spindle rates coincided with fewer number of arousals ($p = 0.009$, 4.17 n/hour TST decrease per unit spindle rate) in the older and shorter NREM4 duration ($p < 0.001$, 17.23 minutes decrease per unit spindle rate) in the young individuals.

Sleep spindle rate and self-reported variables

Noise reduced global self-reported quality of sleep [$F(5,167.21) = 3.70$, $p = 0.003$], irrespective of age: this was mirrored in the planned contrasts of the noise-free nights for the pooled noise exposure and for all road noise-exposure nights on the individual noise contrast level ($p < 0.05$, Dunnett's test). Noise exposure was perceived as annoying [$F(5,190.17) = 20.48$, $p < 0.001$], irrespective of age: annoyance was significantly higher for all noise-exposure nights (for pooled exposure as well as on the individual noise contrast level) compared to the noise-free nights. Spindle rates (averaged over all centro-parietal derivations) during noise-free nights were not correlated with change in self-reported sleep quality (between combined noise and noise-free nights: $r = -0.03$, $p = 0.868$) or with change in noise annoyance (between combined noise and noise-free nights: $r = -0.12$, $p = 0.468$). Lastly, the spindle rates during the noise-free nights were not significantly correlated to any tested person characteristic: self-reported noise sensitivity (LEF-K: $r = -0.08$, $p = 0.634$; NoiSeQ subscale Sleep: $r = -0.05$, $p = 0.752$); trait anxiety (STAI: $r = -0.20$, $p = 0.210$); or self-reported sleep quality (PSQI: $r = -0.24$, $p = 0.133$).

Sleep spindle characteristics during noise exposure

Spindle rates during exposure compared to non-exposure periods were not significantly different. The additional model factor single RNE (five different noise events), however, revealed that the spindle rates were related to acoustical characteristics of the single RNEs. There was a significant interaction between the factor noise (yes/no) and single RNE [$F(4,358.06) = 4.69$, $p = 0.001$]: post hoc tests revealed that this effect was driven by noise event RNE2 (among the loudest with a maximum SPL of 60.8 dB and with the highest maxSPLslope of 5.2 dB/s; Table 3 for event characteristics), that caused a significant reduction in spindle rates on all tested centro–parietal derivations, similarly for both age groups (Figure 4A). Corroborating our earlier reported all-night findings, *spindle amplitude* was significantly reduced during exposure compared to non-exposure periods in both age groups (present in all derivations but more pronounced at central derivations). Exposure versus non-exposure spindle amplitude differences, however, were not related to any acoustical characteristic of the event.

Event-related cortical activations: arousability from RNEs

NREM cortical activation probabilities from single RNE varied quite considerably between individuals and ranged from 1.89 up to 53.66% for EEG arousal (mean ± SD probability: 26.55 ± 12.81%) and between 0 and 12.50% for awakening (mean ± SD probability: 4.05 ± 3.61%). After adjusting for a range of relevant contributing parameters [2, 3, 5], awakening probability from single RNE was not significantly related to all-night BL spindle activity (Table 4; same results for all other centro–parietal derivations). Awakening probability increased with maximum SPL, sleep

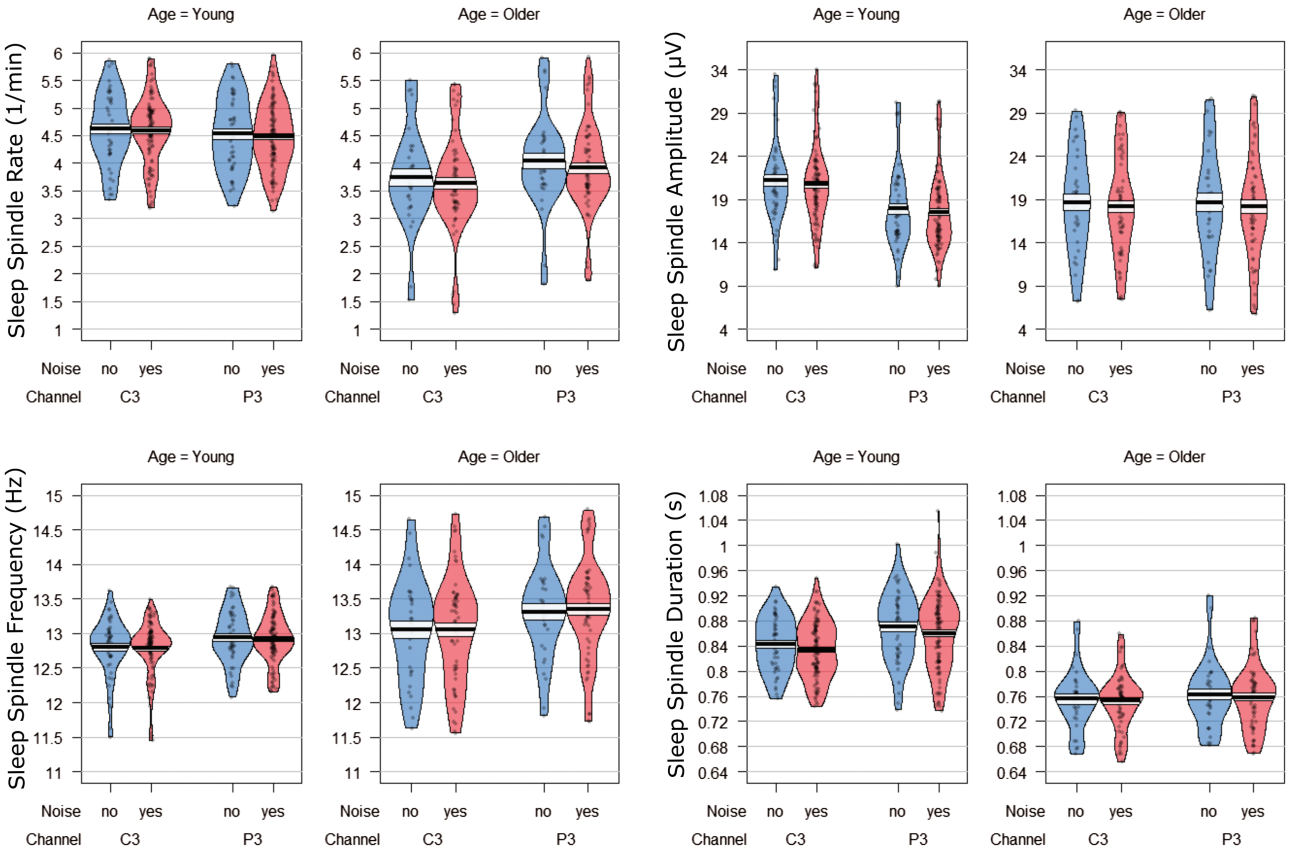


Figure 2. Sleep spindle characteristics (NREM2+SWS). (A–D) Sleep spindle characteristics (NREM2+SWS) for the different noise conditions. Noise "no" denotes noise-free nights (pooled for baseline and recovery night) and Noise "yes" denotes noise nights (pooled for all four different noise nights). Characteristics are plotted as a RDI plot (Raw, Description, and Inference): the raw data were jittered horizontally, the bean indicates the underlying distribution, the superimposed line denotes the mean, and the rectangle denotes the standard error.

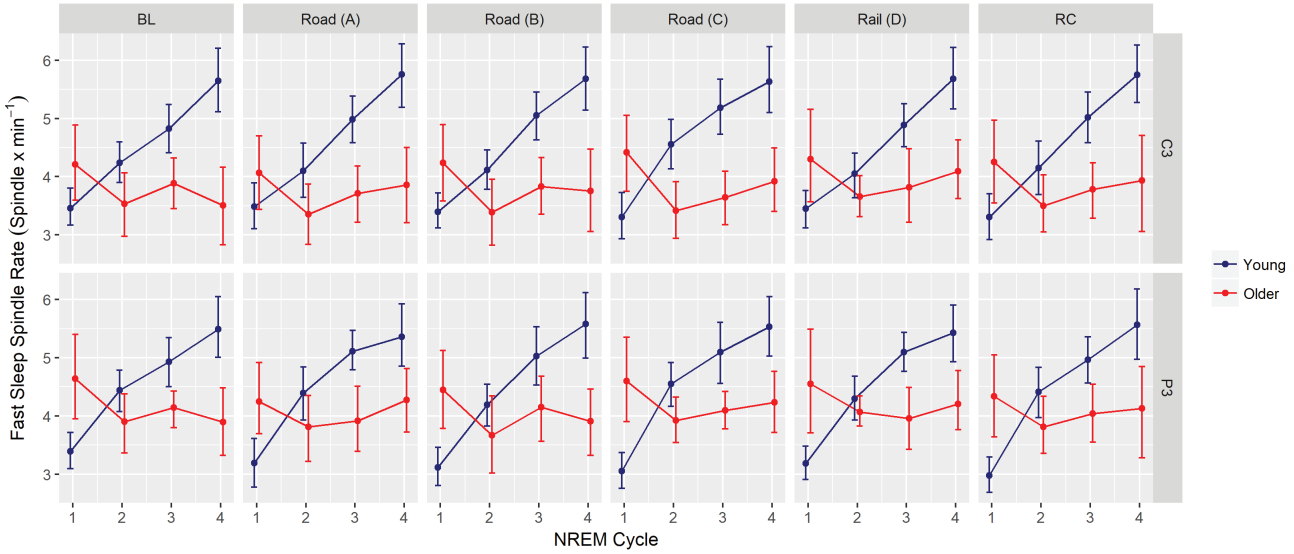


Figure 3. Sleep spindles across successive NREM cycles. Sleep spindle rates for derivations C3 and P3 during NREM2+SWS are shown across successive four NREM cycles according to noise exposure for young (*N* = 26) and older participants (*N* = 16): mean ± 95 % confidence intervals. Individual nights were excluded if the number of completed cycles was different than 3, 4, or 5 (*N* = 11). Individual cycles were only included if they were completed (i.e. end with REM sleep that was followed by at least 5 minutes of NREM sleep or wakefulness) to account for within-cycle variation of the spindle rate.

cycle, and with prior sleep stage NREM1 when compared to NREM2. For EEG arousal probability from single RNEs, all-night BL spindle activity only contributed significantly to the model when it was not adjusted for age: as soon as the age predictor was included, all-night BL spindle activity lost predictive value (Table 4; same results for all other centro–parietal derivations). Consequently, there was no indication of an indirect effect of the BL spindle rate on the relationship between age and EEG arousal probability. Both used performance metrics (AIC and BIC) indicated that Model 3 was the superior model, including only the age group. EEG arousal probability increased with maximum SPL, maximum SPL slope, sleep cycle, older age and was significantly higher from NREM1 and significantly lower from SWS when compared to NREM2.

As there was no effect of the all-night, trait-like spindle rate on EEG arousal probability from RNEs, we further explored state effects of the spindle rate taking inter- and intra-individual differences in the spindle rate across sleep cycles into account. The correlations (within-cycle, within-age group) between the same-night spindle rates and NREM EEG arousal probabilities were largely nonsignificant (the two significant correlations indicated two positive relationships in the young individuals); thus, inter-individual differences in the spindle rate were not inversely related to NREM EEG arousal probabilities, not only on the all-night but also on the level of the sleep cycle (Figure 5). In the aforementioned logistic model (excluding the sleep cycle as a factor), the sleep cycle-specific spindle rate did not significantly influence EEG arousal probability (both with and without the age factor; for all EEG derivations; $p > 0.05$; data not shown).

The further exploration of the significant acoustical factors revealed a significant interaction between noise (yes/no) and single RNE (five different events) for the cortical activation rate (EEG arousal and awakening rate combined for REM and NREM events) [$F(4,360) = 80.47$, $p < 0.001$]. Post hoc tests revealed that this effect was driven by noise event RNE2 (among the loudest with a maximum SPL of 60.8 dB and with the highest maxSPLslope of 5.2 dB/s; Table 3 for event characteristics), that had significantly higher noise-associated cortical activation rates than spontaneous cortical activation rates (for "virtual" events; Figure 4B). The significant interaction between single RNE and age [$F(4,360) = 3.68$, $p = 0.006$] indicated that older individuals had a higher increase in cortical activation rates for the noise events RNE2 and RNE3 (both among the loudest) than young individuals.

Discussion

The present analyses sought to investigate the sleep-protective role of sleep spindles under different nighttime transportation noise exposures. While sleep structure was largely unaffected by noise exposure, sleep continuity was disrupted in an age- and noise scenario-dependent manner. Older individuals, whose sleep is generally more fragmented, had an increased frequency of NREM EEG arousals, both spontaneous and event-related, and awakenings from NREM. They had more total sleep stage changes and spent more time in NREM1, especially in nights under road noise exposure (scenarios B and C). Contrary to our hypotheses, spindle activity was neither related to differences in sleep structure or continuity in noise-exposure nights nor was it a significant predictor for cortical activation probabilities from single RNEs. Moreover, cortical activation probability that increased throughout the night was not related to naturally occurring variation in spindle rate over successive NREM sleep cycles. Spindle amplitude, on the other hand, was consistently decreased during noise compared to noise-free nights across all EEG derivations and age groups, both in the all-night analyses and during selected intervals of noise exposure compared to non-exposure in the event-related analysis.

At first glance, our results seem to contradict Dang-Vu et al [7]., who demonstrated that arousal thresholds were related to all-night baseline spindle rates in a sample of young adults with a mean age of 26.3 years. However, arousal thresholds differ from arousal probabilities such that the former describes the sound intensity needed to elicit an arousal, whereas the latter

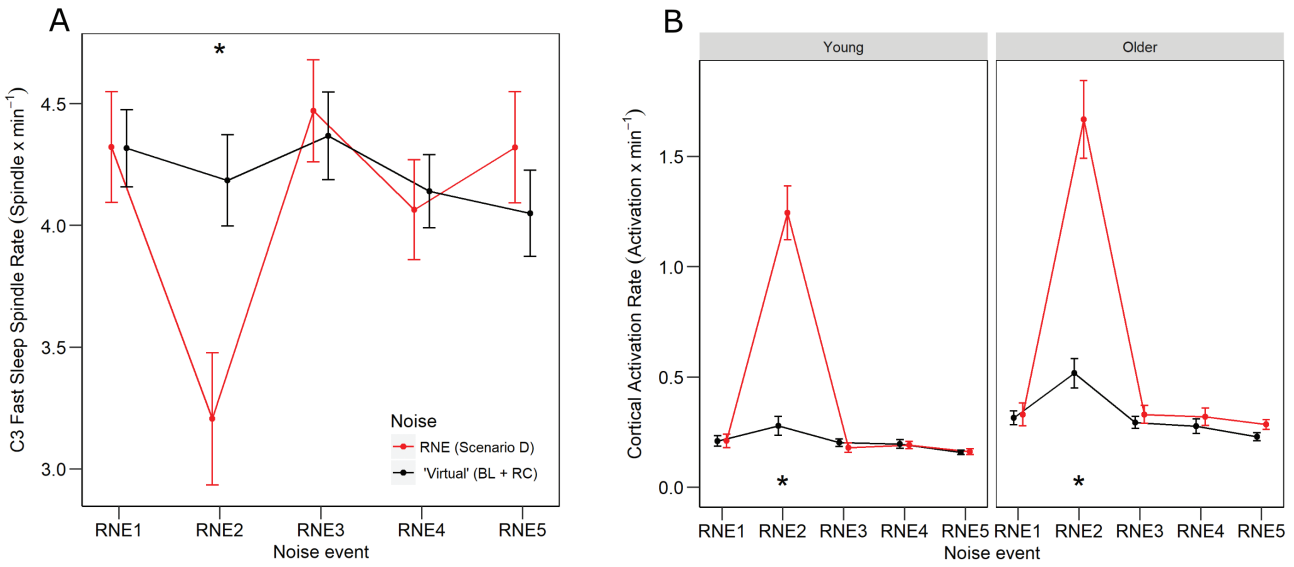


Figure 4. Effects of single RNE on sleep spindle rates and cortical activation rates. (A) C3 spindle rate during exposure (N = 1414 RNE; red) and non-exposure (N = 3022 "virtual" events; black) periods during NREM2+SWS. Events have different acoustical characteristics (Table 3). Selected intervals of "virtual" events during the noise-free nights (baseline: BL, recovery: RC) had the same duration and distribution as single RNEs in the railway night (scenario D). Here, events were only included if not associated with an awakening or an EEG arousal. (B) Cortical activation rates (EEG arousal and awakening rates combined) for all (i.e. NREM and REM events) railway (N = 2840 events; red) and "virtual" (N = 5359; black) noise events.*Significant with $p < 0.05$, Tukey's test.

describes the percentage of arousal-associated noise events. Arousal thresholds in Dang-Vu et al [7]. were determined using a series of 10-s events with increasing intensity (5-dB increments starting at 40 dB) until an arousal occurred or the maximum intensity of 70 dB was reached. Our experimental procedure did not include on-line stimulation adjustments as predefined real-world inspired noise scenarios were played back during the night with a limited maximum SPL range between 50.1 and 61.7 dB (all starting from a background level of 30 dB). Depending on the applied acoustical metric, the maximum intensity in our stimuli was about 10 dB (for the equivalent continuous metric $L_{Aeq,10s,max}$ and approx. 17 dB for the maximum SPL) lower than the maximum intensity used by Dang-Vu et al [7]., but was well within the range of their mean arousal threshold per individual (40–60 dB) and within the range people are exposed to under real-life conditions during the nighttime [60]. The slope of rise of the SPL and the duration of single noise events influence cortical activation probabilities [2, 5, 61]. These are acoustical parameters that varied in our experiment but were held constant in Dang-Vu et al [7]. Moreover, it was demonstrated that the arousal probability depends on the type of the presented sound: electronic sounds, such as phone ringing, consistently exceeded arousal

Table 4. Results of logistic regression models for event-related awakening and EEG arousal from single RNE (scenario D)

	Awakening		EEG arousal		
Variable	Model 1	Model 2	Model 1	Model 2	Model 3
Intercept	−3.4490 (0.2105)***	−3.3807 (0.2395)***	−1.2491 (0.1436)***	−1.5484 (0.1578)***	−1.5376 (0.1471)***
L_{AFmax} (dB)	0.0859 (0.0267)**	0.0862 (0.0268)**	0.0470 (0.0113)***	0.0469 (0.0113)***	0.0469 (0.0113)***
Maximum slope (dB/s)	0.0195 (0.0564)	0.0193 (0.0565)	0.0853 (0.0287)**	0.0855 (0.0288)**	0.0855 (0.0287)**
Prior sleep stage NREM1 (versus NRME2)	1.1299 (0.2598)***	1.1374 (0.2603)***	1.5243 (0.1574)***	1.5184 (0.1574)***	1.5173 (0.1573)***
Prior sleep stage SWS (versus NREM2)	−0.2350 (0.3829)	−0.2524 (0.3841)	−1.0364 (0.1852)***	−1.0075 (0.1853)***	−1.0073 (0.1853)***
Prior sleep stage REM (versus NREM2)	0.1613 (0.2492)	0.1565 (0.2494)	0.1942 (0.1091)	0.2018 (0.1091)	0.2017 (0.1091)
Sleep cycle	0.1874 (0.0783)*	0.1847 (0.0785)*	0.0679 (0.0379)	0.0714 (0.0379)*	0.0717 (0.0378)*
Study night (day)	0.0037 (0.061)	0.0031 (0.0606)	0.0171 (0.0489)	0.0197 (0.0428)	0.0197 (0.0428)
Male sex (1 = yes, 0 = no)	−0.2472 (0.2408)	−0.2639 (0.241)	0.2208 (0.1890)	0.2873 (0.1669)	0.2825 (0.1650)
Spindle rate (n/minute NREM2+SWS)	−0.0812 (0.1488)	−0.1251 (0.1675)	−0.2346 (0.1131)*	0.0236 (0.1240)	
Older age (1 = yes, 0 = no)		−0.1559 (0.2779)		0.7245 (0.2113)***	0.7003 (0.1688)***
Variance random subject (*SD*)	0.1598 (0.3998)	0.1531 (0.3913)	0.2742 (0.5236)	0.1886 (0.4343)	0.1887 (0.4344)

Regression coefficients with standard errors in parenthesis. The dependent variable in these analyses is awakening/EEG arousal probability that is coded with 0 = no awakening/EEG arousal and 1 = awakening/EEG arousal. To address model non-convergence issues, all continuous predictor variables were centered at their respective mean: L_{AFmax}: 57.58 dB; MaxSPLslope: 1.90 dB/s; Sleep cycle: 2.84; Study night: 2.44 nights; Spindle rate (at C3 during the noise-free baseline night): 4.28 n/minute NREM2+SWS. Model fit for model 1 (AIC = 2907.39; BIC = 2972.40), model 2 (AIC = 2898.98; BIC = 2969.89), and model 3 (AIC = 2897.01; BIC = 2962.02).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

probability from transportation noise by far for all tested SPLs with a constant slope of rise of the SPL [45]. We do not question the role of sleep spindles for differential information processing of very brief stimuli that was repeatedly shown [14–17] but argue that mechanisms other than sleep spindle activity alone may play a role under real-life exposure conditions when arousability also depends on the meaning and significance of stimuli [62, 63]. For our single RNEs, cortical activation probability depended on the maximum SPL and the maximum slope of the SPL. Steep rising SPL of single noise events are indicative of a fast approaching noise source and therefore signal a potential threat to the sleeping individual. Indeed, it was demonstrated that activity in the amygdala—functioning as a detector of biologically relevant stimuli [64]—was increased for rising SPL stimuli as compared to falling SPL stimuli during wakefulness [65]. Consequently, other markers of the sympathetic tone during sleep denote the importance of the slope of rise of the SPL: the steeper the slope of rise of a single noise event, the greater the heart rate elevation [3], systolic and diastolic blood pressure increase [66] or motility as measured with high-resolution actigraphy [61].

In addition to the evaluation of all-night inter-individual differences in spindle rates, within-individual and within-night differences can be used to predict arousability. Spindle rates vary across successive NREM cycles in an age-dependent manner: while fast spindle rate increases linearly across cycles 2–4 in the young, it is rather stable in the older; except for the first cycle that has higher spindle rates in both age groups [9, 67]. If spindles are sleep-protective, cycle-specific intra-night variability in spindle rates should affect arousal probabilities, both event-related and spontaneous. But, the linear increase in spindle rates across successive NREM cycles in the young was accompanied by an increase rather than a decrease in arousal probability from single RNEs. The increase of event-related arousal probability reflects a sleep-homeostatic reduction of sleep consolidation consistent with the literature [2, 3, 5]. Interestingly, in the older, the fairly stable spindle rate across cycles was also accompanied by an increase in arousal probability across the night. In the same vein, Pivik et al [37]. demonstrated that within-night differences in the spindle rate on an even finer temporal scale, 2-minute pre-exposure, were not consistently predictive for awakening probabilities or awakening thresholds (i.e. stimulus intensity needed to elicit awakening). In mice, on the other hand, phase differences in a 0.02-Hz oscillation in sigma power (frequency range of 10–15 Hz), were associated with awakening from sleep in response to 20 s auditory stimuli: awakening occurred when the sigma power was in the descending phase during noise exposure as compared to non-awakening when sigma power was in the ascending phase [68].

We observed a stimulus intensity-dependent decrease of spindle rates during noise exposure in the absence of overt cortical activations: 61 dB sound stimuli with a high maximum slope of the SPL reduced spindle rates by approx. 20% when compared to “virtual” events. In the same vein, Kawada et al [69]. showed that truck pass-bys also resulted in a decrease of spindle rates as compared to pre-exposure spindle rates, which recovered as a function of the stimulus intensity: spindle rates upon

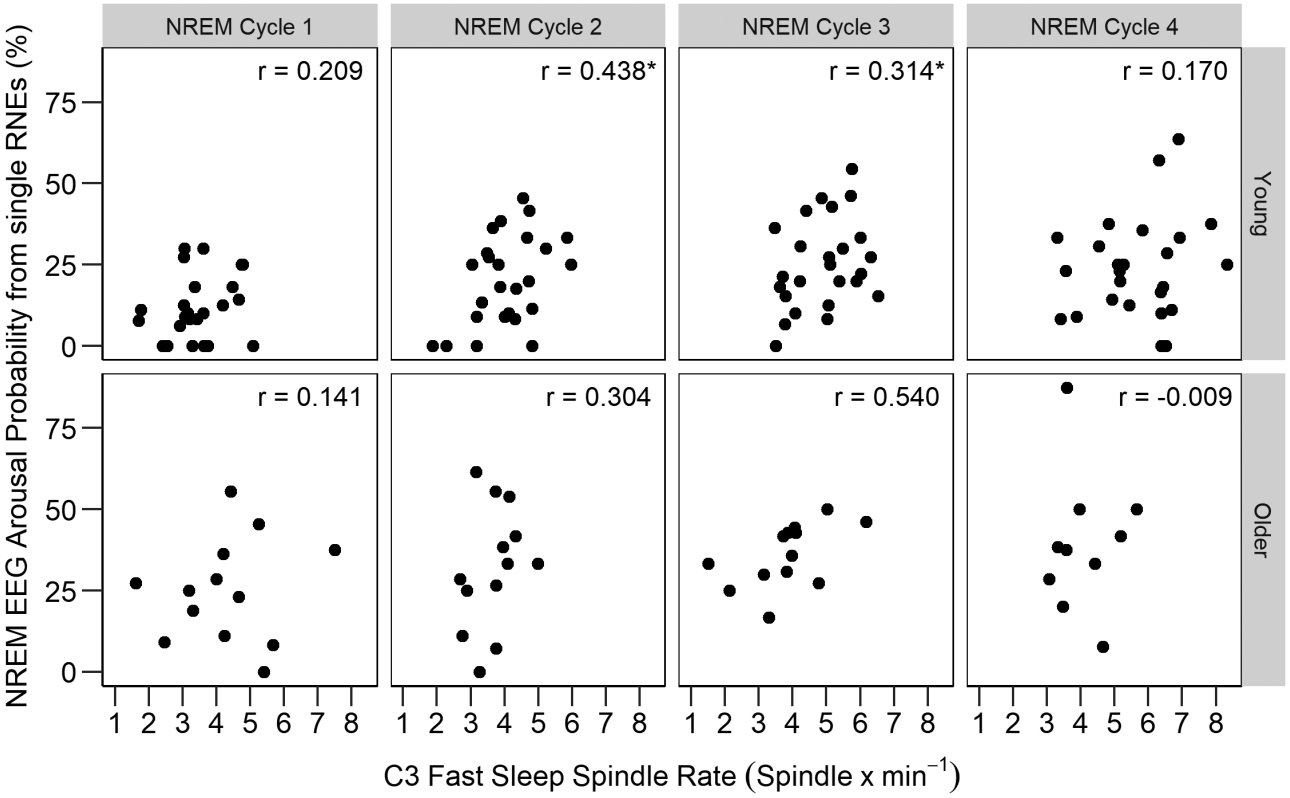


Figure 5. Sleep spindle rates and arousal probabilities from single RNE across successive NREM cycles. Within-cycle correlations between the NREM EEG arousal probability from single RNE and the same-night C3 spindle rate are shown. The young participants (N = 25) are displayed in the upper and the older participants (N = 13) in the lower panels. Individual nights were excluded if the number of completed cycles was different than 3, 4, or 5 (N = 4) and individual cycles were only included if completed (i.e. end with REM sleep that was followed by at least 5 minutes of NREM sleep or wakefulness) to account for within-cycle variation of the spindle rate. *Significant with $p < 0.05$.

55 dB exposure recovered faster than 60 dB and 65 dB exposure that recovered to pre-exposure levels only after 3 minutes. Pivik et al [37]. showed that repetitive stimulation with artificial 3 s sounds was accompanied by a significant decrease of spindle rates during noise exposure as compared to pre-exposure rates what was also more pronounced with increasing stimulus intensity. In other studies, auditory stimulation did not affect spindle rates [70] or even triggered spindle generation: upon white noise stimulation in the spindle frequency range [35] or during selected intervals of auditory stimulation compared to NREM2 periods without auditory stimulation during an afternoon nap [36]. In the latter, however, stimulus intensities of the used auditory stimuli were at the individual's awake perception threshold, therefore much lower than in our experiment.

The observed all-night and event-related decrease in spindle amplitude might be interpreted as a disruption of synchronization of TC oscillation and was also demonstrated by others where auditory stimulation sequences of 50 ms click sounds resulted in a reduction of spindle power when compared to sham stimulation [71]. It has long been noticed that sensory stimulation can elicit K-complexes (KCs) [72], single slow oscillations that occur predominately during NREM2 sleep, even in the absence of overt cortical activations. Auditory evoked KCs (either isolated or followed by a spindle or a burst of additional KCs or slow waves) were followed by a 50% reduction of EEG power in the 13–14 Hz frequency band (a surrogate for spindle activity that is even stronger correlated with the mean spindle amplitude than the spindle rate) [9] what was interpreted as an inhibition of spindle generation [73]. The observed all-night reduction in spindle amplitude might be due to a cumulative evoked KC or EEG arousal effect what needs to be demonstrated in future analyses.

Overall, we did not find an independent effect of spindles on a variety of sleep structure and continuity markers of noise disturbed sleep (using average sound levels of 45 dB and maximum sound levels of 50–62 dB) after controlling for age. Spindle rates were lower and sleep was more fragmented in the older compared to the young individuals. The overall and topographically specific spindle reduction in the older is consistent with the literature [8, 9, 21, 23, 26–29] and was related to differences in white matter integrity of the underlying spindle generating networks [21]. Sleep spindles are trait-like transitory EEG oscillations, which may reflect stable sleep but do not necessarily protect the sleeper against external stimuli such as nighttime transportation noise. Arousal thresholds are lower during SWS compared to NREM2 sleep [2, 5, 74, 75] and whether marked age-related differences in slow-wave activity or characteristics of slow oscillations modify noise effects on sleep will be demonstrated in future analyses. The reduction in spindle amplitude, however, might serve as a sensitive marker for noise-induced sleep disturbances. Meanwhile, biologically relevant acoustical characteristics of single noise events, such as the slope of rise of the SPL, may play an important role in modifying information processing even during intact spindle rhythmicity.

Limitations

Our automatic spindle detection algorithm potentially suffers from the well-described caveats for automatic detection, such as a lower performance in older individuals compared to the gold-standard, human visual detection [8]. To address this issue, amplitude thresholds were adjusted individually as EEG power densities in the frequency ranges of slow-waves, theta and sigma are lower in older than in young individuals [76, 77]. On the other hand, reduced spindle rates with advancing age is a robust finding demonstrated using both visual [26] and automatic [8, 9, 23, 27, 28] detection.

Two types of sleep spindles were described from cortical EEG recordings with differences in frequency and topographical distribution, that suggest distinct functional roles [78]. We only analyzed fast spindles as slow spindle peaks were not readily identifiable in the majority of our participants. Slow spindles could have modified sleep differently, though it is not very likely as demonstrated by Dang-Vu et al [7].

Supplementary material

Supplementary material is available at *SLEEP* online.

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Notes

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