Discrimination of behaviorally irrelevant auditory stimuli in stage II sleep

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In sleep, the brain responds to significant stimuli such as one's own name or loud tones. It is, however, not yet known whether in sleep, the brain's response can vary systematically with change in an irrelevant stimulus. Here, we varied the intensity of a 1000 Hz tone and recorded the neural response of the participants by using electroencephalography. The P200 component of the auditory-evoked potential increased linearly and significantly with intensity in wake and in stage II sleep. Pattern classification confirmed that there is information about tone intensity in the poststimulus period, especially in the period corresponding to the P200. The sleeping brain is capable of discriminating the fine aspects of a stimulus that is of no significance to the individual. ***NeuroReport***

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Introduction

Studies of awake, alert humans and animals have found that some part of the brain responds to and processes sensory stimuli even when the individual is unaware of them [1,2]. In these studies, the organism is alert, and unaware only to a subset of stimuli or some subset of attributes characterizing the stimulus. When the individual is asleep in contrast, he or she is unaware of the external environment *in toto*.

Although one is not aware of them in sleep, the brain still responds to stimuli that are behaviorally or biologically relevant to the individual. The P300 component, a late latency auditory-evoked potential (AEP) is often present during nonrapid eye movement (REM) sleep [3–7] and sleeping individuals generate an enhanced P300 in response to their own names than others' names or nonsense syllables [7]. Moreover, highly probable (repetitive) and rare (deviant) auditory stimuli are known to evoke different responses in sleep [8]. Cote and Campbell [6] varied the intensity of the sound stimulus and studied evoked electroencephalography (EEG) response in sleep. They found that a sound could generate a P300 component in the event-related potential (ERP) during REM sleep, provided it is sufficiently loud, and therefore biologically relevant. Even though they showed that other ERP components (e.g. N1 and P2) were affected by intensity of the sound, these components were not systematically analyzed. In sum, the sleeping brain maintains a substantial response to a subclass of stimuli that is of relevance to the individual. (To cite an extreme anecdotal but somewhat familiar example, parents of newborns often wake up at night in response to the newborn's cries but sleep through other sounds that are objectively louder).

What is still not known is whether the brain of a sleeping individual, like that of an awake one, is able to respond to a behaviorally irrelevant stimulus and even discriminate certain properties of it. We investigated this issue by probing for differences in the sleeping brain's evoked response to single frequency tones of low-to-moderate intensity that ranged from 53 to 63 dB.

Methods

Participants

Twelve healthy individuals (six females) participated in the study. None reported a history of hearing or neurological disorders. All participants gave informed consent before the experiment.

Stimuli

Sounds were 1000 Hz pure tones (60 ms duration, 10 ms rise and fall times). Software was scripted in MATLAB 7.0 (The MathWorks Inc., Massachusetts, USA) and a high-definition audio card (Realtek Inc., Hsinchu, Taiwan) was used to play the sounds. Stimuli were presented binaurally through two speakers placed 20 cm from either ear.

Setup

A trial consisted of a single tone of one of three randomly intermixed intensities (53, 58, and 63 dB sound pressure level) of equal probability ($P=1/3$). Intensities were calibrated by a sound pressure meter (UEi DSM101: Universal Enterprises Inc., Oregon, USA) placed in the

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middle of the bed. In our experience, sound intensities of 68 dB or above lead to arousals and even awakenings. Sound intensities were thus carefully chosen to minimize the probability that arousals would occur. Intertrial interval was 3000 ± 200 ms.

Electrophysiological recording

Polysomnography (EEG + electrooculography + electromyography) recording data were acquired using a 64+8 channel system (ActiveTwo BioSemi Inc., The Netherlands). Sampling frequency was 256 Hz. EEG signals were recorded with a band-pass filter setting of 0.16–100 Hz.

Task and procedure

Participants had to lay in supine position on a bed in a noise-reduced room for approximately 2.5 h on average. They typically remained awake for approximately 30 min, after which they fell asleep on their own, after about approximately 1.5 h of sleep on average, the observers woke up on their own, and then stayed awake for another 30 min, after which time the session was terminated. Polysomnography data were acquired throughout the session. Sessions were between 13 and 17 : 00 h during the day. Trials of varying tone intensity were randomly intermixed with equal probability. Participants passively listened to the tones throughout. No judgment regarding the sound was required.

Electroencephalography signal

We report AEPs from a single EEG electrode placed at the center of the scalp (Cz), namely the amplitude of the evoked potential response to sound in wake and stage II of non-REM sleep. We compared the amplitudes and latencies of four components of the auditory evoked response – P50, N100, P200, and N300. Arousals were scored using standard criteria [9]. Trials with arousals were excluded from further analysis. It is notable that there were only 4.4 ± 0.5 arousals and zero awakenings on average per participant (standard American Academy of Sleep Medicine criteria were used for the scoring of arousals). Trials in which response amplitude exceeded 75 μV at any instant, which typically characterize noise, were excluded as well.

Auditory-evoked potential analysis

Polysomnography data acquired were filtered off-line in MATLAB using Parks-McClellan FIR filters to minimize phase distortion. EEG data were band-pass filtered between 0.5 and 40 Hz. Electromyography data were filtered between 10 and 100 Hz. Electrooculography data were filtered between 0.5 and 100 Hz. Sleep staging was scored manually using standard Rechtschaffen and Kales criteria [10]. Data were reconstructed offline into discrete trials or sweeps. Trials were sorted and averaged on the basis of stimulus intensity (53/58/63 dB sound pressure level) and brain state (wake/stage II sleep). The mean AEP corresponding to each condition (three stimulus intensities × two brain states) was obtained by averaging 300 trials each, yielding a total of 1800 trials for analysis. On cases in which there were more than 300 trials of a particular condition, 300 were randomly selected. Data during slow wave sleep – stages III and IV – and REM sleep were not analyzed because of insufficient time spent in those states and, as a result, small number of trials of each. The amplitude of an individual component of the AEP was measured by using the standard baseline to peak method [11,12] within the time range corresponding to the given component (P50, 30–80 ms; N100, 80–150 ms; P200, 150–250 ms; N300, 250–350 ms). The 500-ms period just before sound onset for a given trial constituted the baseline. The amplitude of each AEP component was linearly fitted using the least squares criterion in MATLAB. Latency of a given component was defined as the occurrence of the peak response of the said component with respect to stimulus onset. Amplitude and latency of an AEP component thus obtained for each participant were averaged across all 12 participants.

Statistics

Repeated-measures analysis of variance was used to calculate statistical significance in all cases. The criterion for significance was a P value of less than 0.05. Statistical computations were done using SPSS v15.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Pattern classification

We trained classifiers to discriminate between the three sound intensities on the following four time periods following stimulus onset: (i) 30–80 ms, corresponding to the P50 component; (ii) 80–150 ms, corresponding to the N100 component; (iii) 150–250 ms, corresponding to the P200 component; and (iv) 250–350 ms, corresponding to the N300 component. We randomly selected and averaged 150 of 300 raw trials corresponding to a particular intensity and brain state for training, and used the average of the remaining 150 trials for test. In order to obtain more reliable measures, the entire selection procedure was repeated 1000 times for each AEP component and state of arousal (wake/sleep). Performance of a classifier was based on successful classification of test data alone. Classification test error was defined as the probability that the response to a sound of a particular intensity was classified as being that to a sound of different intensity. Given that there were three intensities of sound, an error probability less than $2/3=0.67$ is considered above-chance performance. In order that our results were not dependent on our choice of classifier, we used five different classifiers: Fisher's least square linear classifier, logistic linear classifier, nearest mean classifier, polynomial classifier, and the quadratic discriminant classifier. Software was scripted in MATLAB and used the prtools toolbox (*www.prtools.com*).

Results

Across our participant sample, total time awake averaged was 64.9 ± 3.4 min. Total sleep time averaged was 80.2 ± 6.7 min, of which 36.2 ± 6.7, 24.7 ± 4.7, 5.6 ± 1.8, and 10.7 ± 4.4 min were spent on average in stages I, II, III, and IV, respectively. Only one of 12 participants had any REM sleep.

Auditory-evoked potentials of wake and sleep

Figure 1 shows the grand mean AEPs in response to each of the three tone intensities examined in wake (Fig. 1, left) and in stage II sleep (Fig. 1, right). In accordance with [8,13], the amplitudes of AEP components P50, P200, and N300 were greater in light sleep than in wake (Fig. 1 right vs. left). This is because the negativity typical of the evoked response in wake disappears at sleep onset. In contrast, only the P50 component was delayed in light sleep as compared with wake; the later three AEP components were not significantly slower in sleep. Overall, the results suggest that the sleeping brain responds to pure tones of low-to-moderate intensity. Figure 1 further indicates that the sleeping brain not only detects, but also discriminates sounds: the amplitude of the various components varied with sound intensity in both wake and light sleep.

Modulation of auditory-evoked potentials amplitude by sound intensity

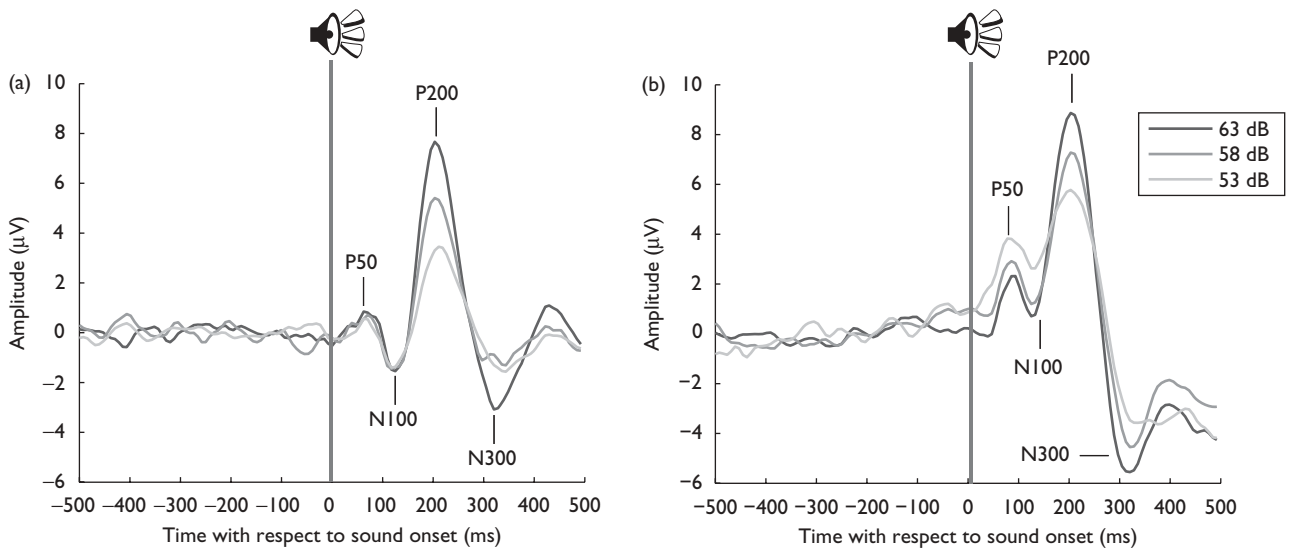
Further, we fitted mean amplitude versus tone intensity data with straight lines (Fig. 2a). As discussed earlier, the amplitudes of the P50, P200, and N300 components were larger in sleep than in wake, which accounts for the larger intercept values of the linear fits in sleep.

The slopes of the linear fits (Fig. 2b) measure the extent to which the three closely spaced tone intensities were distinguishable in the evoked response in wake (white) and in sleep (black). A slope of 0 means sound intensity does not modulate AEP amplitude. A positive slope value means that AEP amplitude increases with intensity. The amplitude versus intensity slopes of the P200 component were significantly different from 0 in both wake and sleep (wake, $P=0.001$; sleep, $P=0.001$), and were significantly steeper in wake than in sleep ($P < 0.005$). As Fig. 2b shows, in wake and in sleep, the amplitude of the P200 component increased the most among all AEP components (wake, 0.43 μV/dB; sleep, 0.31 μV/dB) as a function of tone intensity. Among the other AEP components, only the P50 (P1) slope in wake was significantly positive (0.06 μV/dB; $P < 0.05$). In sum, only the P200 (P2) was significantly sensitive to variation in intensity in both wake and sleep.

Pattern classification of tone intensity

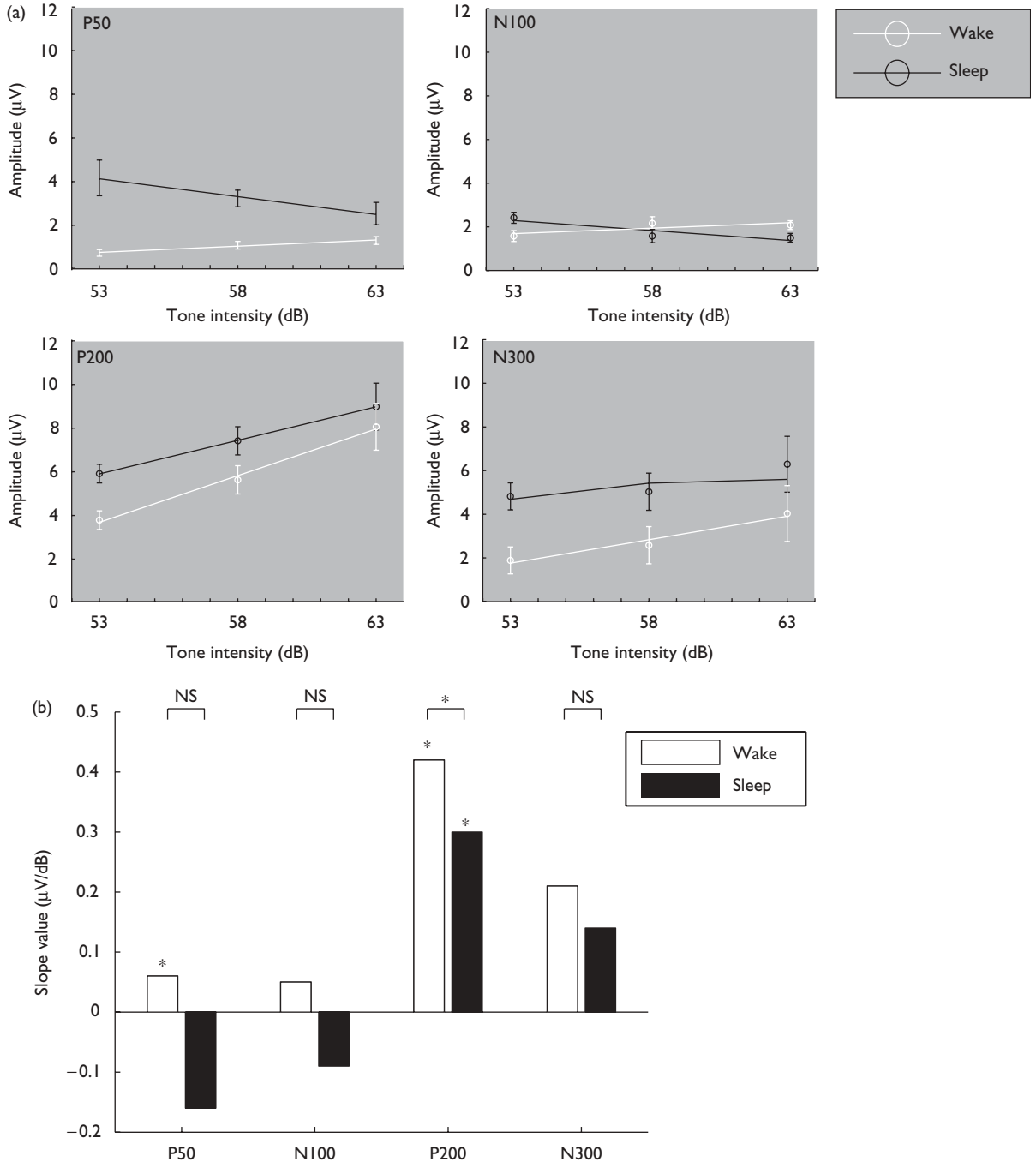
To confirm the above result, we trained and tested pattern classifiers to classify tone intensity on raw trial data on time periods corresponding to each of the four AEP components—P50, N100, P200, and N300 (Fig. 3, top panel). In order that the classification error reflects information about intensity in the response, and not classifier characteristic, we chose five different classifiers (see Methods) and averaged the errors of all. The

Fig. 1



Auditory-evoked potentials (AEPs) in wake and sleep. (a) AEPs in wake in response to a pure tone of three different test intensities are shown. (b) AEPs in light sleep in response to a pure tone of three different test intensities are shown. AEPs are the grand averages across all participants.

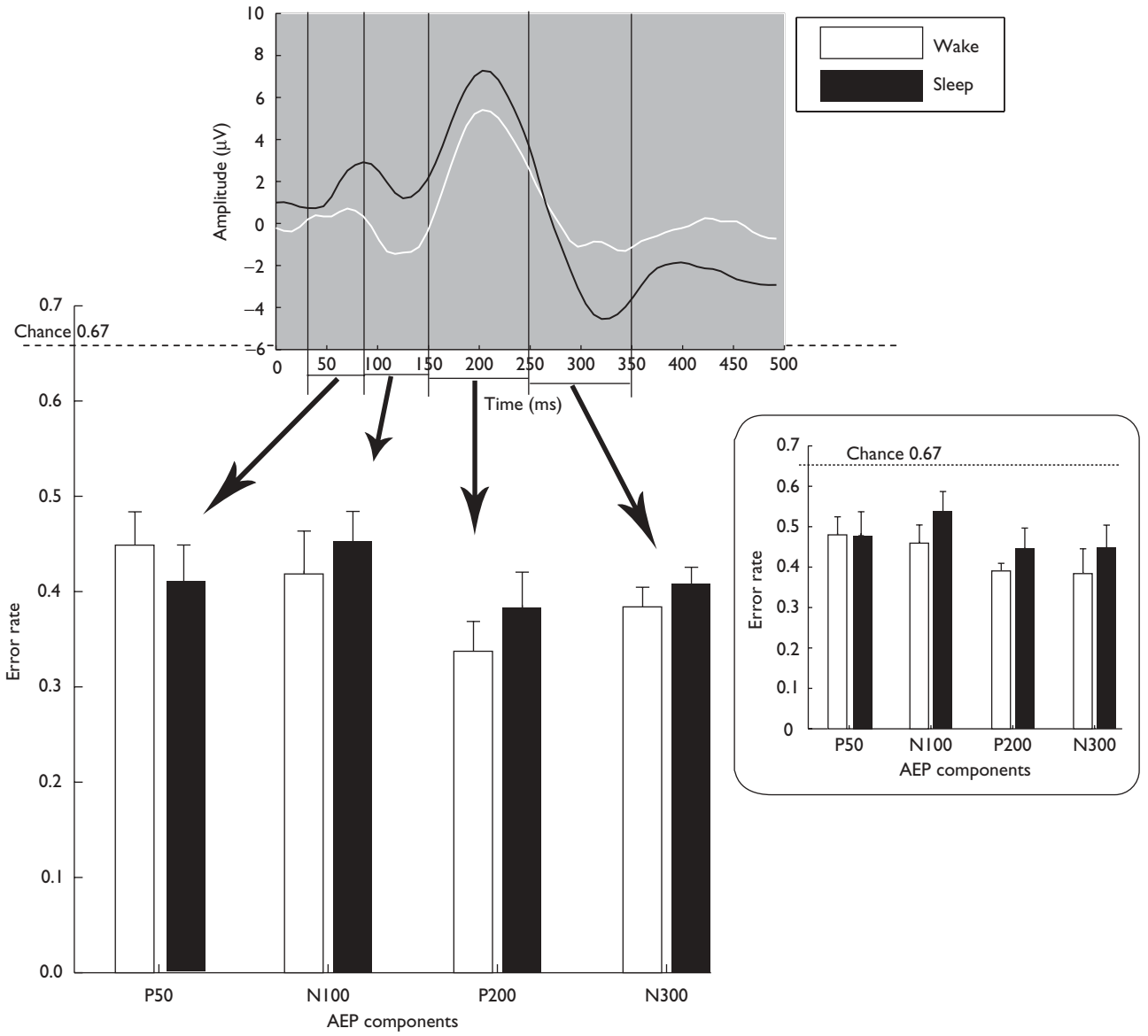
Fig. 2



(a) Slopes of amplitude versus intensity slopes of different auditory-evoked potential (AEP) components. (a) The graphs show the mean ± one SEM amplitudes and least-squares linear fits of the P50 (top left), N100 (top right), P200 (bottom left), N300 (bottom right) components in wake (white) and sleep (black). (b) The values of the slopes (microvolt per decibel) of the different AEP components in wake (white bars) versus sleep (black bars) and results of statistical comparisons are illustrated. NS, not significant; *, ($P < 0.05$).

classification study revealed two findings. Classification error in wake and, of importance, in sleep was substantially smaller than chance ($P = 2/3$; Fig. 3). Thus, even in sleep, there was information about sound intensity present in the evoked response. Consistent with the finding from the regression analysis, the classifiers trained on the time period corresponding to the P200 had the smallest average test error. In sum, there is information embedded in the evoked response of the sleeping brain concerning the intensity of the tone played.

Fig. 3



Pattern classification of tone intensity. Test error rate of the Fisher's linear classifier trained on four different time periods (top graph), each corresponding to a different auditory-evoked potential component (arrows) and arousal state (wake, white; sleep, black) is shown. Test error rates correspond to the performance of the Fisher's classifier. The inset shows the average error rate of all five classifiers on test data.

Discussion

Our findings suggest that the neural discrimination of sound intensity exists in light sleep, *albeit* to a somewhat reduced extent than in wake: the P200 component of the evoked response to sound is modulated monotonically as a function of its intensity and the ERP contains information about tone intensity. Past studies have shown that in sleep the brain does not stop responding to stimuli that are behaviorally or biologically relevant. In this study, participants passively listened to the tones and had no perceptual judgment relating to them to make. Furthermore, unlike past studies of auditory information processing in sleep [5,6], the sounds in this study were soft enough that arousals were extremely rare ($<<1\%$ of all trials) and a P300 component, which is absent during passive presentation of tones [14] and is known to signify behavioral or biological relevance of sound [3,15], was not seen. Thus, in contrast to previous studies, our sound stimulus was not behaviorally or biologically relevant or intrinsically salient, and yet its intensity affected systematically the response of the brain in sleep.

In cases such as ours when the P300 is absent from the evoked response, the amplitude of the P200 response

recorded at Cz has been shown to increase in a nearly linear manner with tone intensity in awake individuals [16]. Thus, the finding that the P200 is significantly sensitive to stimulus intensity and varies most among all AEP components as a function of tone intensity in wake and in sleep is in line with past reports of the singular importance of the P200 in auditory stimulus processing.

Two concerns commonly plague studies of sound processing in sleep. First, sleep and wake occur at different times of the day. Second, as the sound is repeated in the experiment, adaptation related decrease in AEP amplitude might occur that could be misattributed to change in brain state. However, because our recordings were in the afternoon, participants naturally transitioned from wake→sleep→wake, that is wake-straddled sleep, and was not all before it. This mitigates the above concerns. On the flip side, we could only investigate light sleep as our recordings were done in the afternoon. It is possible that discrimination of sound continues or ceases in deep sleep (stages III, IV) – either outcome is valuable information. Nonetheless, stage II sleep is important in its own right: adults spend 50% of their time in stage II sleep and older adults mainly experience stages I and II of sleep; also, stage II sleep is most tightly correlated of all stages with overnight enhancement of certain kinds of memories [17].

Conclusion

Selection pressures explain why the brain of a sleeping person continues to respond to behaviorally or biologically relevant stimuli. What the present study demonstrates, in addition, is that the sleeping brain, while unaware of the irrelevant sounds, nonetheless retains the capacity to detect and categorize them; this implies a more sophisticated and ongoing processing of the environment in sleep than was previously thought. The findings thus set limits on the utility of sensory awareness.

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