The effects of sleep stages and time of night on NREM sleep ERPs

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Abstract

Event-related potential (ERP) is one of the best techniques for studying information processing during sleep because it does not require behavioral responses or consciousness awareness. Several ERP components have been identified during non-rapid eye movement (NREM) sleep, but the associated underlying processes of these waveforms remain unclear. The present study examines the effect of sleep stage and time of night on the NREM ERPs to further understand these processes. An oddball paradigm was conducted in 11 healthy subjects to elicit ERPs throughout the night. Polysomnographic recordings were also applied to identify sleep stages. The results showed that P220, N350, and P900 decreased during the second half of the night, when the NREM sleep drive is partially satiated. This finding is consistent with the notion that the NREM ERPs reflect an inhibitory process associated with sleep drive. P220 and P900 were also found to increase as subjects entering deep sleep. However, the N350 was not affected by the deepening of sleep and peaked earlier during stage 1 sleep. Although these components are all related to the process for sleep preservation, the N350 may be more associated with sleep–wake transition and the P220 and P900 with the process of deepening of sleep.

Keywords: NREM sleep; ERPs; Sleep stage; Time of the night

1. Introduction

Sleep is an altered state of consciousness in which the processes of external stimuli are attenuated from the waking state. In spite of the absence of behavioral responses to environmental stimuli during sleep, arousal thresholds have been shown to be lower for meaningful stimulus than neutral stimulus, indicating that the sleeping brain does process external information beyond the sensory level (e.g., McDonald et al., 1975; Shanon, 1979). However, the studies on information processing during sleep have been limited by the lack of measurable indices during sleep. One technique, event-related potential (ERP), has long been used to investigate information processing with or without overt behavioral responses, and with or without attention to the stimuli. Through recording and averaging the brainwaves immediately following stimulus presentation, it is possible to obtain a series of deflections in brainwaves that represent the brain activities associated with the processing of the stimulus presented. Since the analysis of ERP does not require behavioral responses and/or conscious awareness, it is an ideal technique to study the processing of sensory stimuli by the brain during sleep.

Several sleep-specific ERP components have been reported in previous studies. With the use of oddball paradigm, the standard P300 component is known to disappear around the time of sleep onset and is replaced by a series of sleep-specific ERP components during non-rapid-eye-movement (NREM) sleep. Components reported to form part of the NREM-specific ERP include P220, N350, P450, N550, and P900 (e.g., Atienza et al., 2001; Harsh et al., 1994; Hull and Harsh, 2001; Ujszaszi and Halasz, 1988; Winter et al., 1995; for a review, see Bastuji and Garcia-Larrea, 1999).

Although these NREM ERP components have been consistently observed across different studies, the underlying processes associated with theses waveforms have not been fully

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explored. Nonetheless, some preliminary hypotheses have been generated based on the features of the different components. For example, the NREM ERPs have been shown to increase their amplitudes in response to deviant stimuli compared to standard ones with oddball paradigm, indicating that these waveforms do not simply reflect a general reaction to sensory stimuli, but are at least associated with a primitive discriminating process of information (Bastien and Campbell, 1994; Harsh et al., 1994; Hull and Harsh, 2001; Nielsen-Bohlman et al., 1991). Moreover, when the subjects were asked to attend or respond to a target stimulus while they were still awake, after falling asleep the NREM ERPs (especially N350, N550 and P900) were consistently found to elevate to the target stimulus compared to non-target stimulus (Nielsen-Bohlman et al., 1991; Bastien and Campbell, 1994; Harsh et al., 1994). This elevation might reflect further process of the meaning of the stimulus. Based on the findings, some researchers have suggested that these waveforms are primarily associated with an arousal process that orientates the individual to process the psychological or biological relevance of sensory stimuli during sleep (Halasz, 1998; Bastuji and Garcia-Larrea, 1999; Atienza et al., 2001). However, habituation and/or refractory period might offer alternative explanations of this phenomenon. The shorter intervals between two standard stimuli might decrease the level of firing in the neuronal population because some of the neurons might be refractory after reacting to the previous stimulus (Bastien and Campbell, 1992; Harsh et al., 1994). When the probabilities of target and non-target were held constant through experimental manipulation, the amplitudes of P450, N550 and P900 elicited were found to be similar between the two types of stimuli (Colrain et al., 2000a; Hull and Harsh, 2001). The stimulus type effect is more associated with the probability of occurrence than the psychological or cognitive relevance of the stimulus. Furthermore, recent studies on the effects of sleep deprivation and sleep disruption on NREM ERPs also did not support the point of view that the waveforms reflect an arousal process. In one study, the amplitudes of the N350 and the P900 during sleep and the N550 during wake-to-sleep transition were found to increase during evening naps following one night of sleep deprivation (Peszka and Harsh, 2002). In another study, the amplitudes of N350 increased during the undisturbed sleep subsequent to disrupted ones (Nicholas et al., 2002). Given that sleep deprivation and sleep disruption enhances sleep drive (and therefore increases the threshold for arousal) during subsequent sleep, it is unlikely that the elevation of the amplitudes of these waveforms reflects an arousal reaction. On the contrary, they are more likely to reflect an inhibitory process that prevents or minimizes cognitive processing and/or cortical activation following the detection of a sensory event. These NREM specific ERP components have been suggested to represent the manifestation of underlying sleep pressure, as a mechanism that facilitates the initiation and maintenance of sleep (Nicholas et al., 2002; Peszka and Harsh, 2002). One study in particular reported that the percentage of slow wave sleep (SWS) increased in association with the N350 amplitude and suggested that this component may be associated with pressure of SWS (Nicholas et al., 2002).

The present study aimed to further explore the functional significance of the NREM ERPs by examining the effects of sleep stage and time of night on the different waveforms. Several issues were addressed in this study. Firstly, NREM ERPs were compared between the first and second halves of the night. It was reported that the auditory arousal thresholds during sleep were decreasing across the night, with increasing of frequency of awakening by auditory stimuli (Busby et al., 1994; Rechtschaffen et al., 1966; Watson and Rechtschaffen, 1969; Williams et al., 1964; Zimmerman, 1970). It indicates an elevation of arousal process or a reduction of inhibitory process to auditory inputs during the second part of the night when sleep drive has been partially satiated. Therefore, if the ERP waveforms reflect an inhibitory process associated with sleep pressure, they should be attenuated during the later part of the night. In contrast, if the waveforms are associated with an arousal process, they should be enhanced during the second half of the night. There were limited number of studies looked into the effects of time of night on NREM ERPs. The results were not very consistent. For example, both P220 (P2) and N350 during stage 2 sleep was reduced in amplitude during the second half of sleep as compared to the first half, as shown in the figure of an earlier study (Campbell et al., 1992, Fig. 7.5). In another study, the amplitude of N550 during stage 2 sleep was also reported to be higher during the first than the second part of the night, but the amplitude of P220 (P240) was not different between the two parts of the night (Plihal et al., 1996). Since the previous researches on the time-of-night effects on NREM ERPs were conducted only during stage 2 sleep and were limited to some ERP components, the present study further examined the time-of-night effects for different waveforms and during all NREM sleep stages.

Secondly, NREM ERP components in different sleep stages were compared. Several of the NREM ERPs were suggested to reflect the process associated with sleep deepening (Nicholas et al., 2002) or to be the phasic manifestations of SWS pressure (Peszka and Harsh, 2002). If this is the case, the waveforms should be enhanced as an individual gets into deeper sleep stages. Most of previous studies on NREM ERPs focused on the wake to sleep transition or stage 2 sleep under the context of K-complex; only a few studies have compared data derived from all NREM stages. An earlier study reported that the N350 (N300) and the P900 had a tendency to increase with the deepening of sleep (Ujszaszi and Halasz, 1988). The other study showed higher P900 (P700) amplitude during SWS than during stage 2 sleep (Wesensten and Badia, 1988). However, a later study showed that the N350 was not affected by sleep stage (Bastien and Campbell, 1992). The figure of another study showed a tendency of increasing amplitudes of P220 (P2) and N350 (N2) from stage 2 to SWS (Bastuji et al., 1995, Fig. 4). Overall, P220 and P900 were consistently found to be elevated in deeper sleep, N350 showed inconsistent findings. Although these studies have reported the stage effects, they did not consider the effect of time of night at the same time. Since the distributions of different sleep stages are not equal across the night, the stage differences could be confounded by the effects of time of night. Therefore, the present study examined the effects of depth of sleep and time of night at the same time to clarify this issue. It was expected that if a certain ERP component reflects processes associated with sleep deepening, it

should be increased in deeper sleep regardless of the proportions of the night.

2. Methods

2.1. Subjects

Eleven subjects (4 males, 7 females; mean age = 32.64 ± 11.47) participated in the study. Subjects were recruited from local community and a university campus. Inclusion criteria were (1) aged 20–55 years, (2) no present or past history of major medical, psychiatric, and/or sleep disorders, (3) no current use of prescribed or leisure drugs that may affect sleep, (4) non-shift worker with regular sleep–wake schedule. Potential subjects were screened for sleep disorders, psychiatric disorders, and major medical disorders with a clinical interview and a nocturnal polysomnography (NPSG). Nineteen potential subjects passed the clinical interview. Two of them were excluded due to sleep disorders detected by NPSG and six were excluded due to inadequate amount stage 1 sleep and/or SWS for ERP analysis. A written informed consent was obtained from all participants.

2.2. Procedures

Potential subjects who passed the screening interview were asked to record their daily sleep patterns on a sleep log for at least 1 week and then to attend the sleep laboratory on two consecutive nights for NPSG and ERP recordings. In the 3 days prior to attending the sleep laboratory, they were instructed to refrain from alcohol use and to limit caffeinated drink (to one cup/can a day before noon and none thereafter). In addition, they were requested to follow a regular sleep–wake schedule, as derived from their sleep logs. The subjects were instructed to come to the laboratory 1 h before their regular bedtime. They were allowed to go to bed after the preparation for recording and practice runs. The bedtime for the ERP night were delayed for some subjects due to the experimental procedures, with an averaged bedtime of 12:01 a.m. The subjects were woken up in the morning at the time they requested. Since most subjects needed to go home first and get ready to go to school or go to work in the morning, they tended to get up slightly earlier than their regular wake-up time. The averaged wake-up time was 6:16 a.m.

During the first night in the laboratory a standard clinical NPSG was conducted to serve as a further screening and to allow the subject to become accustomed to sleep in the environment. ERP was not collected at this time. The NPSG montage included (i) electroencephalogram (EEG) with C3, C4, O1 and O2 referenced to linked mastoids (A1 + A2), (ii) electrooculogram (EOG) recorded from outer canthus of both eyes, (iii) chin electromyogram (EMG), (iv) oral/nasal airflow measured by thermosensors, (v) thoracic and abdominal respiratory efforts, (vi) finger-probe oxymeter, (vii) anterior tibialis EMG, and (viii) electrocardiogram (EKG). Recordings were made with a Compumedics Sleep System and were scored manually in 30-s epochs by trained graduate students following the standard scoring system by Rechtschaffen and Kales (1968). Subjects who demonstrated symptoms of sleep disorders (i.e., apnea/hypopnea index >5 and/or periodic limb movement index >15) were excluded from the study.

Throughout the second night, NPSG and ERP recordings were conducted. The NPSG montage was reduced to EEG, EOG, and chin EMG only. Additional EEG sites (Fz, Cz, and Pz) were added for the recording of ERPs. Both vertical and horizontal EOG were recorded for the detection of artifacts. The signals were collected at a sampling rate of 500 Hz with NeuroScan NuAmps system. The low-cut filter for EEG recording was 0.05 Hz. The oddball paradigm modified from a previous study on the determinants of NREM ERPs (Hull and Harsh, 2001) was applied. Brief 1000 Hz and 1500 Hz tones (45 ms) at 80 dB SPL, with an inter-stimulus interval (ISI) of 1.5 s were presented through inserted earphones to both ears. One of the two types of tones served as the deviant tone and the other standard tone, counterbalanced across subjects. The tones were presented continuously throughout the night. The sequencing of the tones was randomized within every subsequent 40 tones, with the probability of the deviant to standard stimuli being 20 (8 tones) to 80 (32 tones). The subjects were instructed to count the number of deviant tones presented while they were aware of them, but also to not resist falling asleep. To make sure that the subjects did understand the instruction of the task, a 5-min practice run was conducted while the subjects were awake and sitting on a chair prior to bedtime. The number of deviant tones counted was checked after the practice run. The practice run was redone in two subjects due to a misunderstanding of the instructions. The experimental procedures were conducted following the ethical principles of psychologists of the Taiwanese Psychological Association.

The recordings were staged manually in 30-s epochs following the standard scoring system by Rechtschaffen and Kales (1968). The EEG data were then filtered with a high-cut filter of 30 Hz and segmented according to stimulus timing (150 ms prior to stimulus onsets to 1200 ms after). Baseline corrections were then conducted based on the average of the 150 ms EEG signal prior to stimulus onset. Segments containing EOG deflections over 75 μV above or below the baseline were then excluded from analysis. Potential subjects who did not have enough stage 1 sleep or SWS to provide adequate number of EEG sweeps (sweep number <30) for ERP analysis were excluded from data analysis. The numbers of accepted EEG sweeps for different sleep stages were as follows: (i) stage 1: 144–1192 sweeps with standard tones and 31–303 sweeps with deviant tones, (ii) stage 2: 3606–8469 sweeps

Table 1
Sleep parameters during the ERP nights

	Duration (min)		Percentage (%)[a]	
	Mean	S.D.	Mean	S.D.
Total bed time	375.4	70.9	–	–
Total sleep time	337.1	72.7	–	–
Sleep efficiency	89.5	5.8	–	–
Sleep onset latency	9.1	9.5	–	–
Wake after sleep onset	29.3	19.4	8.4	6.2
Stage 1 sleep	20.2	10.0	5.6	2.7
Stage 2 sleep	189.4	49.1	51.5	8.6
SWS	78.5	27.3	21.2	6.1
REM sleep	49.1	16.3	13.2	3.2

[a] The percentage is calculated as a ratio of elapsed time from sleep onset to the last epoch of sleep.

with standard tones and 1086–2044 sweeps with deviant tones, and (iii) SWS: 1031–2462 sweeps with standard tones and 253–635 sweeps with deviant tones. Because the number of accepted EEG sweeps were greater in stage 2 sleep than the other stages, one out of every four consecutive accepted sweeps in stage 2 sleep were randomly selected for averaging to balance the numbers of sweeps averaged.

Previous studies have shown that NREM ERP waveforms were different in trails with and without K-complexes. Trials with K-complexes were identified according to a definition modified from the criteria of the Rechtschaffen and Kales' (1968) standard and the standard used in a previous study (Cote et al., 1999). K-complex was defined as a large-amplitude negative–positive wave peaked greater than 75 μV, lasted longer than 0.5 s, and maximum over Fz or Cz. The negative wave peaked between 450 and 750 ms, and the positive wave between 800 and 1300 ms. Because K-complexes were elicited in only few sweeps for all the different conditions (0–0.92%; 0–12 sweeps), the sweeps were excluded from data analysis.

The windows of peak latency designated to different ERP components were as follows: 150–300 ms for P220, 250–475 ms for N350, 375–600 ms for P450, 450–700 ms for N550, and 600–1000 ms for P900. These windows were adjusted slightly for some subjects according to visual inspection of their averaged waveforms. When the peak of a waveform fell within the overlapping range of two windows, the peak was classified as belonging to the earlier window. Peak latencies and amplitudes for the ERP components were measured separately for stage 1 sleep, stage 2 sleep, and SWS (stage 3 and stage 4). The night was divided into two parts by the mid-point of the night. The trials were sorted and averaged according to types of tones, part of the night, and sleep stages. ERP measurements were compared between the first and second halves of the night.

Analysis of variance (ANOVA) was applied to compare the peak amplitudes and latencies of the NREM ERP waveforms in different conditions. In order to avoid the complexity in the interpretation of the results, the predominant electrode sites of each waveform were determined firstly by comparing the peak amplitudes of ERPs derived from different electrode sites. Subsequent comparisons were conducted only on the data from the electrode site with the highest amplitude. Three-way ANOVAs (2×3×2) with Greenhouse–Geisser adjustment were conducted to compare the measures among three different sleep stages (stage1 vs. 2 vs. SWS), two halves of the night (first vs. second halves), and two types of stimulus (standard vs. deviant tones) for P220, N350, and P900. Post hoc comparisons were conducted with LSD method. Due to the fact that P450 and N550 were not measurable in some conditions, statistic analyses were only applied to partial data (see Results for details).

3. Results

Table 1 shows the sleep parameters during the night of ERP recording. The subjects did acquire sufficient (although not optimal) sleep in light of the tone presentation throughout the night. Tables 2 and 3 show the means and standard deviations of peak amplitudes and latencies for P220, N350,

Table 2
Means and S.D.s (in parentheses) for peak amplitudes (microvolt) of (a) P220, (b) N350, and (c) P900

	Fz				Cz				Pz			
	First half		Second half		First half		Second half		First half		Second half	
	Standard	Deviant	Standard	Deviant	Standard	Deviant	Standard	Deviant	Standard	Deviant	Standard	Deviant
(a) P220												
Stage 1	5.01 (3.87)	6.23 (4.45)	3.01 (1.87)	3.57 (1.96)	6.14 (4.39)	6.94 (6.67)	2.89 (2.09)	3.75 (2.25)	4.03 (3.46)	4.14 (5.46)	1.97 (1.49)	2.60 (1.58)
Stage 2	5.22 (2.88)	7.30 (3.34)	3.70 (2.62)	4.64 (3.06)	5.77 (3.52)	8.00 (4.34)	3.97 (3.21)	5.80 (3.44)	4.47 (2.96)	5.81 (3.40)	3.03 (2.48)	4.02 (2.64)
SWS	7.71 (4.55)	9.94 (4.87)	6.45 (4.56)	8.34 (4.43)	8.21 (5.67)	11.41 (6.65)	6.69 (5.16)	10.26 (5.53)	6.99 (5.37)	9.74 (6.77)	5.34 (4.00)	9.21 (5.83)
(b) N350												
Stage 1	−5.62 (2.79)	−9.60 (4.61)	−3.87 (2.60)	−6.35 (4.84)	−5.90 (3.54)	−10.55 (5.28)	−3.65 (2.35)	−7.38 (5.39)	−3.41 (2.65)	−6.66 (3.89)	−2.00 (1.25)	−4.64 (3.26)
Stage 2	−3.96 (3.31)	−6.25 (4.34)	−2.80 (2.37)	−7.23 (7.07)	−5.18 (4.08)	−8.62 (4.71)	−3.51 (2.73)	−7.60 (7.00)	−3.92 (2.80)	−8.62 (4.71)	−2.30 (1.51)	−5.61 (4.89)
SWS	−4.83 (3.70)	−9.39 (4.59)	−2.24 (2.93)	−5.96 (6.32)	−5.78 (4.29)	−11.08 (5.44)	−3.39 (4.92)	−5.94 (7.27)	−3.49 (3.19)	−7.23 (4.14)	−2.71 (4.60)	−3.33 (6.91)
(c) P900												
Stage 1	2.47 (2.35)	5.88 (7.34)	2.49 (1.94)	4.71 (3.43)	2.69 (1.77)	5.15 (6.70)	2.36 (1.77)	3.85 (2.73)	2.19 (1.67)	3.79 (4.61)	2.15 (1.42)	3.00 (1.63)
Stage 2	6.43 (3.54)	11.54 (4.11)	4.75 (2.60)	7.75 (3.35)	5.78 (3.00)	9.49 (3.51)	4.57 (2.60)	7.32 (2.84)	4.86 (3.02)	7.47 (3.70)	3.91 (2.39)	6.09 (3.07)
SWS	9.27 (4.52)	16.13 (6.32)	8.00 (5.75)	12.73 (8.32)	9.61 (5.45)	15.95 (6.63)	8.78 (6.51)	13.25 (7.87)	8.77 (5.88)	13.28 (6.19)	7.82 (5.61)	11.09 (6.37)

The data were presented separately for different electrode sites (Fz, Cz, and Pz), different portions (first half and second half) of the night, different stimulus types (standard and deviant tones), and different sleep stages (stage 1, stage 2, and slow wave sleep).

Table 3
Means and S.D.s (in parentheses) for peak latencies (milliseconds) of (a) P220, (b) N350, and (c) P900

	Fz				Cz				Pz			
	First half		Second half		First half		Second half		First half		Second half	
	Standard	Deviant	Standard	Deviant	Standard	Deviant	Standard	Deviant	Standard	Deviant	Standard	Deviant
(a) P220												
Stage 1	234 (16)	236 (28)	230 (34)	243 (23)	232 (19)	236 (27)	226 (32)	244 (22)	225 (20)	229 (31)	226 (32)	243 (28)
Stage 2	246 (22)	237 (32)	234 (29)	250 (45)	246 (20)	241 (31)	233 (29)	249 (41)	230 (21)	231 (25)	242 (30)	240 (42)
SWS	234 (39)	241 (29)	225 (40)	239 (46)	227 (39)	242 (29)	229 (38)	241 (46)	230 (37)	242 (30)	227 (36)	240 (47)
(b) N350												
Stage 1	360 (34)	361 (30)	380 (26)	368 (47)	357 (29)	364 (30)	376 (29)	364 (46)	363 (27)	361 (29)	379 (29)	363 (45)
Stage 2	410 (33)	398 (32)	388 (29)	411 (28)	388 (17)	381 (24)	385 (24)	397 (30)	391 (18)	387 (26)	383 (32)	400 (30)
SWS	399 (36)	400 (30)	394 (41)	391 (40)	387 (32)	391 (25)	372 (53)	390 (34)	379 (24)	387 (25)	362 (63)	382 (50)
(c) P900												
Stage 1	841 (74)	864 (72)	803 (53)	855 (91)	810 (71)	855 (77)	821 (83)	862 (90)	834 (74)	870 (84)	814 (72)	873 (83)
Stage 2	769 (58)	786 (59)	783 (66)	818 (47)	764 (57)	778 (49)	770 (50)	799 (57)	776 (72)	769 (57)	765 (63)	797 (91)
SWS	745 (55)	761 (50)	769 (51)	787 (76)	752 (51)	753 (41)	766 (49)	800 (86)	752 (52)	765 (43)	791 (37)	811 (84)

The data were presented separately for different electrode sites (Fz, Cz, and Pz), different portions (first half and second half) of the night, different stimulus types (standard and deviant tones) and different sleep stages (stage 1, stage 2, and slow wave sleep).

and P900. Statistical results for each ERP components are as follows.

3.1. P220

P220 reflects a central distribution with the amplitude at Cz higher than at Pz, but no different at Fz from the other sites (F[1.8,17.8]=9.75, P<.005). Subsequent comparisons were conducted only with data at Cz. ANOVA results show that all three factors have significant main effects on the amplitude of P220; none of the interactions were statistically significant. Overall, P220 was larger during SWS than during stage 2 and stage 1 (F[1.4,14.1]=15.17, P=.001), and was larger during the first than the second half of the night (F[1,10]=8.96, P<.05). Also, P220 derived from a response to deviant tones were larger than those derived from standard tones (F[1,10]=20.82, P=.001). In terms of the peak latencies, ANOVA demonstrated a significant main effect of stimulus type (F[1,10]=5.28, p<.05). P220 responding to deviant stimuli peaked slightly slower than that responding to standard stimuli.

3.2. N350

The N350 was distributed fronto-centrally, with amplitudes at both Cz and Fz significantly larger than those at Pz (F[1.9,18.8]=9.45, P<.005). Subsequent comparisons were conducted with data recorded at Cz since the N350 amplitude at Cz was slightly larger than at Fz. ANOVA on N350 amplitudes revealed that the N350 is significantly affected by stimulus type (F[1,10]=17.20, P<.005) and time of the night (F[1,10]=12.62, P=.005), but not sleep stage (F[1.8,17.8]=0.33, n.s.). The amplitude of N350 increased more with deviant tones than standard tones and was higher during the first rather than the second half of the night. No significant interactions were indicated.

Three-way ANOVAs on the peak latencies of N350 show significant main effects with sleep stage (F[1.2,12.6]=6.14, P<.05). The N350 reached a peak earlier during stage 1 than stage 2 and SWS.

3.3. P900

With respect to P900 amplitudes, a fronto-central distribution was also demonstrated with the amplitude larger at Fz and Cz than at Pz (F[1.1,11.4]=11.08, P=.005). Subsequent comparisons were conducted with the ERP derived from Fz since the mean P900 amplitude at Fz was slightly higher than at Cz. ANOVA results showed significant main effects of all the three variables. The mean P900 amplitude was significantly higher during SWS than the other stages and was higher during stage 2 than stage 1 sleep (F[1.8,18.3]=14.64, P<.001). Also, it was larger following deviant stimuli than following standard stimuli (F[1,10]=40.68, P<.001), and was higher during the first half than the second half of the night (F[1,10]=6.11, P<.05). No interaction was significant statistically.

Comparisons of peak latencies showed significant sleep stage (F[2.0,19.6]=14.1, P<.001) and stimulus type (F[1,10]=6.28, P<.05) main effects, as well as a stage by time-of-night interaction (F[2.0, 19.6]=4.38, p<.05). The P900 latencies were longer during stage 1 sleep than during stage 2 sleep and SWS during the first half of the night and were longer during stage 1 sleep than SWS only during the second half of the night. Also, the P900 peaked later for deviant than standard tones.

3.4. P450 and N550

P450 and N550 were identifiable in only limited numbers of subjects under certain conditions: nine subjects in stage 1 sleep, four subjects in stage 2 sleep, and one subject in SWS. In this way, 2 (time of the night)×2 (stimulus type)×3 (electrode site) three-way ANOVAs were calculated separately for the amplitudes and latencies in stage 1 and stage 2 sleep. The statistical results showed that none of the effects were significant for both P450 and N550.

In terms of SWS, the P450 and N550 were measurable in seven subjects during the second half of the night but in only one subject during the first half. Therefore, 2 (stimulus type) × 3 (electrode site) two-way ANOVA was conducted for the second half of the night only. Again, no significant main effects or interactions were demonstrated for either amplitude or latency.

4. Discussion

The present study aims to examine the effects of sleep stages and time of night on NREM sleep ERP components, in order to further understand the underlying processes of the waveforms. Similar to the findings in previous studies, auditory stimuli during NREM sleep induced clear P220, N350, and P900 in our subjects, although P450 and N550 could only be identified in a limited number of subjects under certain conditions. The scalp distributions of these waveforms along the midline of the head were also consistent with previous findings, with frontal distribution for P900 and central distributions for P220 and N350 (e.g., Colrain et al., 2000b; Cote et al., 1999; Hull and Harsh, 2001). The P450 and N550, however, were found to be indiscernible in several of our conditions. The N550 was considered to be the negative component of the K-complex and was shown to be absent or greatly reduced when the trials with K-complex were excluded from averaging (Bastien and Campbell, 1992; Niiyama et al., 1995; Colrain et al., 1999; Gora et al., 2001). Previous studies have reported that K-complexes were not to be elicited with shorter ISIs (Bastien and Campbell, 1994; Colrain, 2005; Halasz, 2005). The N550 amplitudes were also attenuated with an ISI of 5 s in comparison to ISIs of 10, 20, and 30 s (Campbell et al., 1985). K-complexes were elicited in very few sweeps in the present study (0–0.92%; 0–12 sweeps) with an ISI of 1.5 s. It is not to be a surprise that the N550 was hardly identified in our data. The features of the P450 reported in previous studies were less consistent. Its amplitude has been shown to be affected by stimulus probability and attention in some studies (Nielsen-Bohlman et al., 1991; Salisbury et al., 1992), but not in others (Harsh et al., 1994; Hull and Harsh,

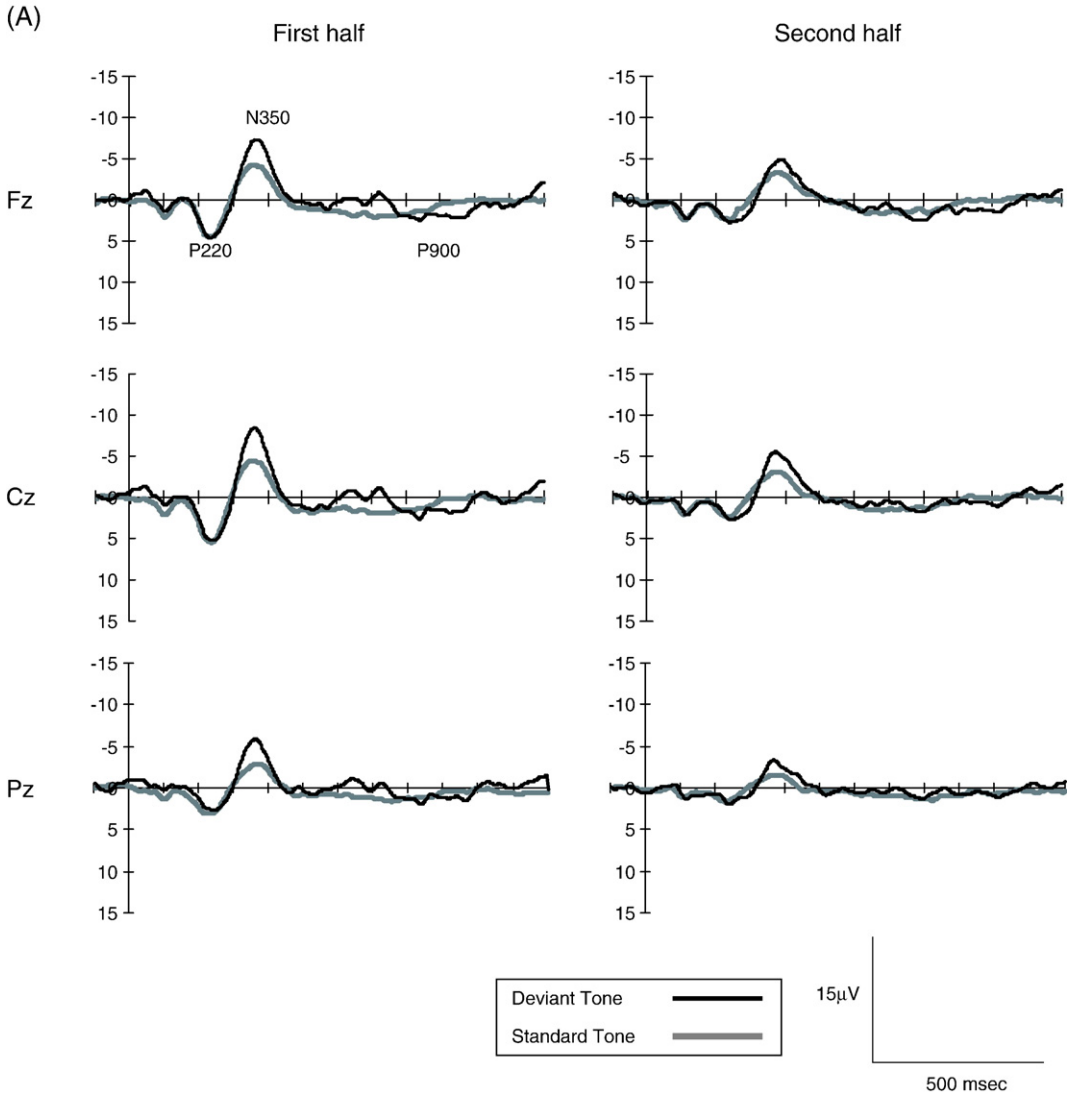

Fig. 1. Grand averages of NREM ERPs during (A) stage 1 sleep, (B) stage 2 sleep, and (C) slow wave sleep during the first half and second half of the night, recorded at Fz, Cz, and Pz (negative up). The darker line represents the waveforms elicited by deviant tone, and the gray line represents the waveforms elicited by standard tone.

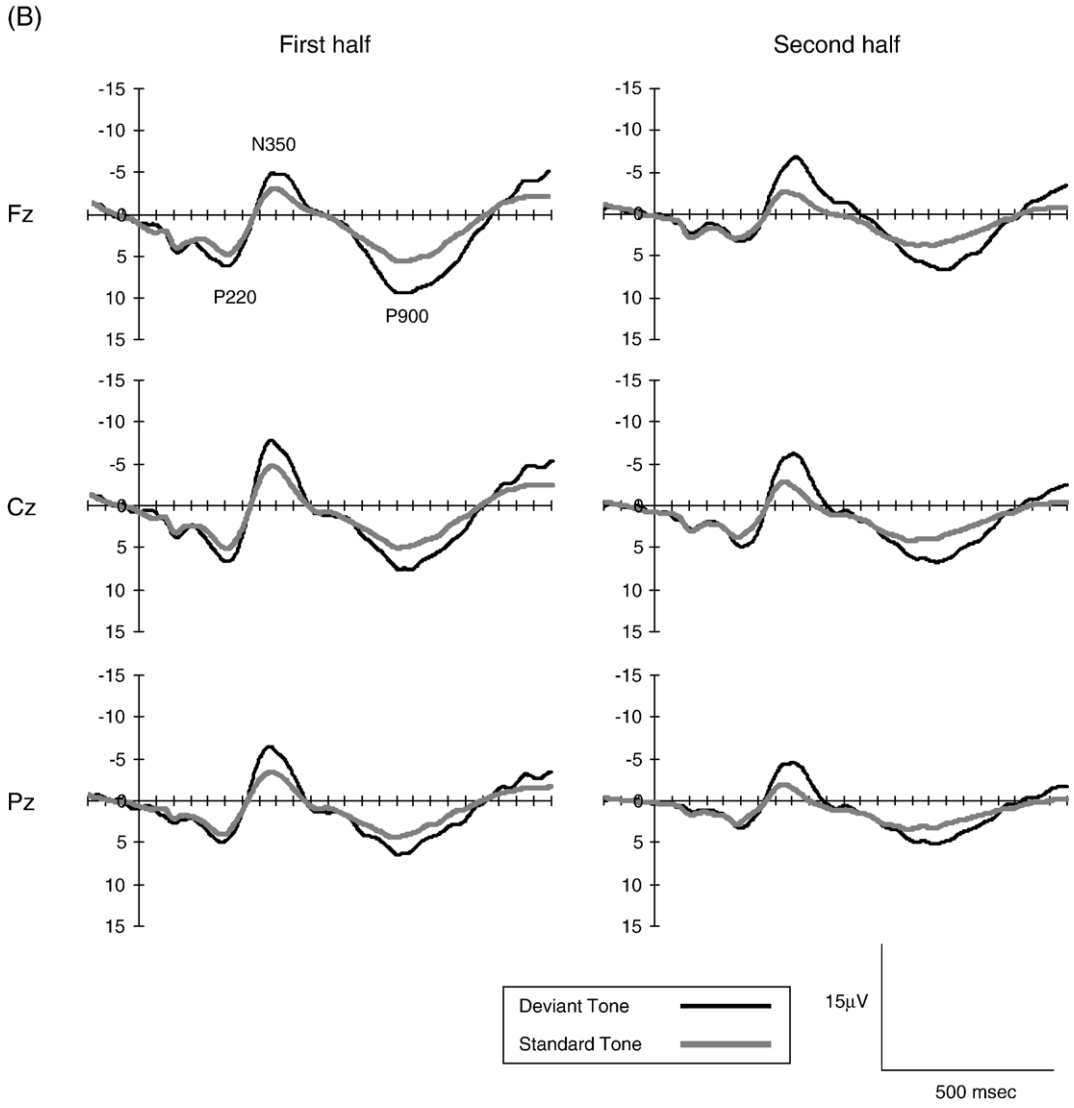

Fig. 1B. (*continued*).

2001). It seems that the P450 is not a very stable component and may simply be the trough between N350 and N550. It was difficult to be identified when the N550 was not clearly formed.

Consistent with findings reported in previous studies (Nielsen-Bohlman et al., 1991; Bastien and Campbell, 1994; Harsh et al., 1994; Hull and Harsh, 2001), most of the NREM ERP components were larger following target (deviant) than non-target (standard) stimuli, indicating an involvement of a difference detection process of the information. However, the results do not imply a differentiation of psychological meaning of the stimuli. As mentioned earlier, stimulus-specific habituation or refractory period may offer an explanation of the finding since the target and non-target tones occurred with different probability.

In terms of time-of-night effects, P220, N350, and P900 were shown to be larger during the first half than the second half of the night. These findings are in line with the decreased amplitudes of P220 and N350 during the second half of the night in previous studies (Campbell et al., 1992), but are not consistent with the report of stable P220 amplitude across the night in another study (Plihal et al., 1996). In addition, the current study demonstrated that the P900 amplitude was also attenuated during the second part of the night. The first part of the night is associated with higher NREM sleep propensity and lower arousal susceptibility. The current results support the hypothesis that the NREM ERP components may be more related to the inhibitory process for sleep-state prevention associated with sleep pressure.

The effects of sleep stages varied among different waveforms, suggesting that they may reflect different underlying processes. Previous studies have shown that the amplitude of N350 was larger at or near the time of sleep onset (Ornitz et al., 1967) and was larger during light sleep relative to SWS (Kallai et al., 2003). Furthermore, there was a close correlation between the emergence of N350 and reductions in behavioral responsiveness around sleep onset (Harsh et al., 1994) as well as the emergence of theta activity in stage 1 sleep (Gora et al., 1999; Colrain et al., 2000a). N350, therefore, has been proposed to be

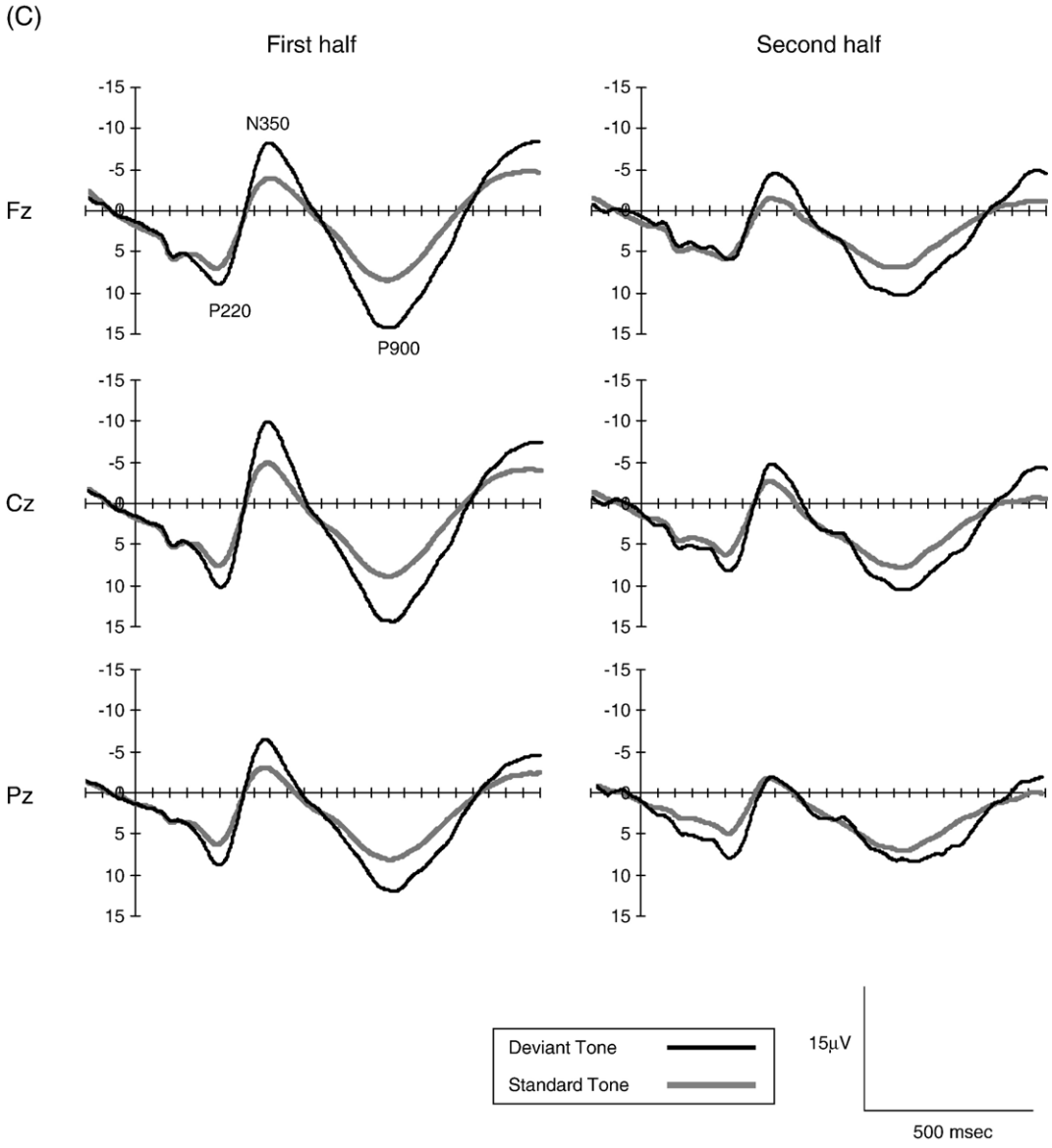

Fig. 1C. (*continued*).

associated with the process of sleep onset. In addition, based on the finding that the amplitude of the N350 increased following sleep deprivation, it was suggested that the N350 reflects the pressure of SWS (Peszka and Harsh, 2002). The current study found that N350 was larger during the first half of the night than the second half of the night, supporting that N350 is associated with the homeostatic drive of sleep. However, N350 showed no amplitude enhancement in deeper sleep. Also, it peaked earlier during stage 1 than the other stages. Our findings suggest that N350 does not reflect mechanisms associated with SWS or deepening of sleep. The results are more consistent with the view that the N350 is associated with the process of sleep onset or the initiation of sleep. Thus, the N350 may represent the mechanism to inhibit sensory arousal to prevent conscious awareness of sensory inputs. This process is most obvious during wake to sleep transition and becomes stable once the individual gets into continuous sleep. This process is enhanced when the pressure to sleep is heightened, but is not necessarily related to the depth of sleep. Although the N350 amplitude has been shown to be larger during SWS than stage 2 sleep in a couple of studies (Bastuji et al., 1995; Ujszaszi and Halasz, 1988), the studies did not compare data collected from different parts of the night and therefore may be confounded by the time-of-night effects.

On the contrary, P900 is greatly influenced by the depth of sleep. The P900 was barely identifiable during stage 1 sleep, was clearly recognized during stage 2 sleep, and became very large after getting into SWS. The peak latencies of the P900 also became faster as the subjects went into deeper sleep. Previous studies have also reported increased amplitude (Wesensten and Badia, 1988; Ujszaszi and Halasz, 1988) and shortened latency (Cote et al., 1999) of the P900 with the deepening of sleep. Thus, the P900 may be more associated with the inhibition of sensory inputs for the maintenance and deepening of sleep.

It is worth pointing out that both N350 and P900 were affected by the homeostatic drive of sleep, but only the P900 was associated with the deepening of sleep stages. Since the

N350 was considered to be an analogue of the vertex sharp wave (VSW) occurring during wake to sleep transition (Colrain et al., 2000a; Harsh et al., 1994; Sekine et al., 1998) and the N550 and the P900 were considered to respectively represent the early negative component and the late positive component of the K-complex (Atienza et al., 2001; Bastien and Campbell, 1992; Campbell et al., 1992; Ujszaszi and Halasz, 1986; for review, see Bastien et al., 2002; Colrain, 2005; Halasz, 2005), it has been suggested that there are at least two separate but associated neural generators during NREM sleep. The first one is responsible for vertex sharp waves and the N350 process predominantly in stage 1 sleep; the second one is for K-complexes and is enabled later following the activation of the N350 generator (Colrain et al., 1999). Our findings suggest that the first generator, reflected in N350, could be enhanced or reduced by the manipulation of the homeostatic drive for sleep (sleep deprivation or partial satiation of sleep drive) but not affected by the deepening of sleep. It is more responsible for the process in wake–sleep transition. The second generator (was thought to be reflected in K-complex) may be further separated into two processes or may contain two generators: one responsible for the N550 and the other for the P900. The N550 generator may have a longer refractory period and therefore is attenuated with shorter ISI; the P900 generator, on the other hand, is not as easily habituated and is modulated by both the homeostatic process and the depth of sleep stage. Studies on the topographic distributions of these two components have also shown that N550 and P900 have different patterns of scalp topography during different sleep stages and suggested that they have different sources (Cote et al., 1999), although earlier studies reported similar topographic distribution of the two components (Bastien and Campbell, 1992, 1994). Our data show that the P900 was very prominent during SWS, whereas the N550 was not identifiable. Given that P900 was clearly presented in some conditions when N550 was not, it seems unlikely that they are generated by the same process.

It was noted that the N350 recorded in the present study is relatively low in amplitude compared to that reported in previous studies. One possible reason for the difference is the short ISI used and the lack of K-complex in the present study. Previous study has reported a reduction of N350 amplitude by approximately 50% in trials without a K-complex (Bastien and Campbell, 1992). Another study used same ISI also showed lower amplitude of N350 in the figure (Fig. 1 in Hull and Harsh, 2001), although the N350 was not measured and analyzed in the study. Thus, the amplitude of N350 reported in the present study is within a reasonable range. The P900 amplitude in the present study, on the other hand, is relatively large compared to that was reported in previous studies (Bastien and Campbell, 1992; Bastien et al., 2002). It may also be due to the short ISI used in the current study. The P900 amplitude in stage 2 sleep reported by Hull and Harsh (2001) with an ISI of 1.5 s is very similar to what was found during stage 2 sleep in the present study. Because their study did not collect data during SWS, we were not able to compare the P900 during SWS in our study with their results. If the enhanced P900 is truly caused by short ISI, the protocol may offer an opportunity to study the underlying processes of the P900 that is independent of the K-complex and N550. It is also possible that the P900 observed in the present study may not be the same as the P900 observed in the context of a K-complex. The latency of the P900 observed in the present study seems to be slightly shorter than the ones reported in a K-complex. Also, the P900 within K-complex was reported not to be different between stage 2 and SWS (Cote et al., 1999). The P900 in the present study significantly increased from stage 2 to SWS. This issue requires to be clarified in future studies. Another finding worth being noted is that nine of the subjects showed low amplitude but measurable N550 during stage 1 sleep. All together, the results suggest that the NREM ERPs may be associated with the phasic events of EEG, but may not necessarily occur as parts of the events.

Consistent with previous finding (Bastuji et al., 1995), P220 was shown to be larger during SWS than the other stages. It suggests that the underlying mechanism of P220 is enhanced by the deepening of sleep. However, the association between P220 and the homeostatic drive for sleep is rather inconsistent. The present data showed that P220 decreased during the second half of the night when sleep pressure became lower. This result is in line with a previous study (Campbell et al., 1992) but not another one (Plihal et al., 1996). Also, previous studies have reported that sleep deprivation had no effect on the P220 during a nap the following night (Peszka and Harsh, 2002). Therefore, P220 may not be associated with the homeostatic drive of sleep directly, but may be modulated by the process of sleep deepening. In addition, P220 was found to be higher when responding to deviant tones than standard tones in the present study. Although the same pattern has been reported in previous studies, the opposite effect has been reported as well (Voss and Harsh, 1998). One study reversed the ratio between target and non-target tones and found that P220 was larger for non-target tones when it was lower in probability than target tones (Hull and Harsh, 2001). Therefore, P220 is more associated with the probability than the meaningfulness of the stimulus. Together with the findings of previous studies, the results suggest that P220 may reflect the process to detect distinct sensory stimuli. This process seems to be associated more with the deepening of sleep than the homeostatic aspect of sleep drive.

In summary, the present study further clarifies the underlying mechanisms of some of the NREM ERP components and illustrates the similarities and differences among these components. Both the N350 and P900 are likely to reflect the inhibitory processes associated with sleep pressure. However, the N350 is more associated with sleep initiation and the P900 more with the deepening of sleep. Also, the results suggest that N550 and P900 had different generators and both could be dissociated from the K-complex. The P220 was also found to be associated with sleep deepening, but its features were less consistent and require further studies. Some limitations should also be mentioned. Firstly, the present study only applied a small number of electrodes and, thus, was not able to explore the spatial distribution of the waveforms. Future studies can examine the topographic distributions of various components and the changes of the distributions across the night. Secondly, the sleep qualities of the subjects during the ERP nights were

adequate but not optimal (mean sleep efficiency = 89.5%), and were possibly interfered by the tones. Although we do not expect a large difference, but the results might be somewhat different if the subjects could maintain a better quality of sleep. Thirdly, the age range of our subjects were larger than most of the previous researches. Most of the previous studies on NREM ERPs were conducted on young subjects. Our subjects consist of 8 young and 3 middle-aged adults. Previous studies have reported decreased N350 and N550 amplitudes, increased P220 amplitudes, and a long-lasting positive EEG shift in elderly subjects (Crowley et al., 2002, 2004). However, inspection of the ERP data of our three middle-aged subjects did not reveal differences in their ERP measures comparing to the rest of the subjects. Also, the age range of our subjects (mean age = 32.64 years) was much younger than the ones in the studies reported age effects (mean ages were 66.3 and 75.6 years). Therefore, age is not likely to be a confounding in our data. Finally, the effect of circadian rhythm was not considered in the present study. Motor responsiveness to auditory stimuli during stage 2 sleep has been shown to correlate positively with body temperature (Lammers et al., 1991). Circadian rhythm may have an effect on NREM ERPs as well. Future studies are required to further explore the circadian effects on the various waveforms. With increased understandings of NREM specific ERPs, the techniques could be applied in future studies to study clinical populations in order to investigate the underlying pathological processes in patients with sleep disorders.

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