Attention to external stimuli during wakefulness and sleep: Evoked 40-Hz response and N350

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Abstract
Changes of two components of the auditory event-related potential, the evoked 40-Hz response and the N350, were studied during different stages of wakefulness and sleep. The evoked 40-Hz response has been proposed to represent an attention-modulating mechanism; the N350 seems to reflect an inhibitory process associated with reduced information processing. Because recent literature suggests that both components reflect opposite mechanisms, an inverse relationship was expected. Ten participants were presented with tone pips while reading, lying awake in bed, and during light sleep, slow wave sleep, and REM sleep. A significant evoked 40-Hz response was observed during reading and lying awake in bed. N350 was evident in all conditions and, as expected, peaked during light sleep. The hypothesized inverse relationship was confirmed. These findings support former proposals that relate both components to attention.

Possibly, the N350 is most pronounced during the sleep transition period because its inhibitory processes have to prevail over the attentional mechanisms (40-Hz response) to permit sleep onset.

Descriptors: Sleep, Event-related potentials, 40 Hz, Gamma activity, N350, Information processing

Measures of event-related potentials (ERPs) recorded from the human scalp provide a nonbehavioral technique for studying sleep-related changes in information processing. It is well known that sensory stimuli are processed not only during wakefulness but also during sleep and that information processing during sleep varies across sleep stages (e.g., Atienza, Cantero, & Escera, 2001; Campbell, Bell, & Bastien, 1992; Cote, Lometa, & Campbell, 2001; Harsh, Voss, Hull, Schrepfer, & Badia, 1994). Only little is known, however, about the central processes underlying these sleep-related changes. Investigating the degree to which a sleeping individual is aware of events in his or her external environment might help us to deepen our understanding of the mechanisms of sleep. Additionally, this might give us insights into certain disturbances of sleep that might be related to abnormal, that is, either extended or reduced, information processing, especially concerning falling asleep, maintaining sleep, terminating sleep, or sleep quality. Besides broadening our knowledge of sleep itself, eventually the investigation of sleep-related information processing might offer a better understanding of processes occurring during wakefulness. Sleep may be the only state in which subjects can truly ignore stimuli, as opposed to processing and rejecting them during wakefulness (Campbell & Colrain, 2002; Muller-Gass & Campbell, 2002). Thus, data collected in sleep research could supplement those from selective attention tasks performed by waking subjects.

Relating the changes in amplitude and latency of ERP components during different stages of sleep to information processing in waking conditions might provide a useful approach to attention and consciousness of the human brain in general.

The aim of the present study is to obtain further insights into the differences in information processing during wakefulness and sleep focusing mainly on the effects of three different components of the auditory event-related potential, the transient evoked 40-Hz response, the N100, and the N350. So far, all three components have been related to attentive processes (e.g., Atienza et al., 2001; Harsh et al., 1994; Muller-Gass & Campbell, 2002; Tiitinen, May, & Näätänen, 1997). To link these components during different situations in which human beings are to a variable degree aware of their environment might help us to understand the processes of attending, ignoring, or rejecting of information more precisely. The specific focus of this article lies primarily on the evoked 40-Hz response and the N350, because these two components appear to be antidromic in the course of their elicitation, but have not yet been examined together. Whereas studies dealing with the evoked 40-Hz response have mainly focused on waking individuals (e.g., May, Tiitinen, Sinkkonen, & Näätänen, 1994; Tiitinen et al., 1993), studies examining the N350 were performed on sleeping subjects (e.g., Harsh et al., 1994; Voss & Harsh, 1998). Investigating these two attention-related components together during stages of both wakefulness and sleep could yield a new contribution toward our understanding of attentional mechanisms in the human brain.

The auditory transient evoked 40-Hz response, so far primarily examined during wakefulness, is an oscillatory
response in the gamma frequency band (at about 40 Hz) that occurs due to sensory stimulation (cf. Galambos, Makeig, & Talmachoff, 1981; May et al., 1994; Tiitinen et al., 1993). It is hypothesized to originate from the synchronization of spontaneous 40-Hz activity, that is, the evoked 40-Hz response is not generated by the activation of 40-Hz oscillators but by already existing 40-Hz oscillations becoming phase-locked as a result of sensory stimulation (Herrmann & Mecklinger, 2000; Llinás & Ribary, 1992; Tiitinen, Sinkkonen, May, & Näätänen, 1994; Yordanova, Kolev, & Demiralp, 1997). The auditory evoked 40-Hz response is a response in the middle latency range. It commences shortly after stimulus onset and lasts for about 100 ms, reaching maximal amplitudes at fronto-central sites (e.g., Galambos, 1992; May et al., 1994; Tiitinen et al., 1993, 1994). It has been shown to be related to both selective (Tiitinen et al., 1993) and sustained attention (May et al., 1994). One of the propositions concerning its function is that the evoked 40-Hz response could be characterized as an attention-modulating response reflecting enhanced attentional resourcing. It might signal the occurrence of an incoming stimulus to higher-order attentional processes, regardless of the physical stimulus properties (Tiitinen et al., 1997). These higher-order processes might then proceed to the analysis of the physical properties and significance of the incoming stimuli, which might be reflected in long-latency low-frequency ERPs (such as N100 or N350). This implies that the mechanism underlying the evoked 40-Hz response reflects the detection of stimuli but not the processing of stimulus features (Tiitinen et al., 1994, 1997). Furthermore, Llinás and Ribary (1992) even presume that the evoked 40-Hz response offers the vehicle to achieve perceptual integration (binding) or conscious perception, that is, the evoked 40-Hz response is regarded as a preliminary stage to higher-order processing. In magneto-encephalographic studies, spontaneous 40-Hz oscillations have been observed to span over wide parts of the brain and to synchronize due to sensory stimulus events, presumably generating global functional states. Possibly, this serves the detection of multiple sensory inputs at different locations of the cortex and gives rise to their integration into a unified perception (Gobbelé, Waberski, Schmitz, Sturm, & Buchner, 2002; Herrmann & Mecklinger, 2001; Llinás & Ribary, 1992; Llinás, Ribary, Joliot, & Wang, 1994). Whereas these suppositions are mainly based on studies examining waking individuals (e.g., Galambos, 1992; May et al., 1994; Tiitinen et al., 1993), only little is known concerning the transient evoked 40-Hz response during sleep. Llinás and Ribary (1993) report an absence of the evoked 40-Hz response during slow wave sleep (SWS) and rapid eye movement (REM) sleep. However, it is unclear whether the evoked 40-Hz response is a continuous phenomenon, which gradually diminishes with increasing depth of sleep, or whether it represents an on–off phenomenon, only occurring during wakefulness. Therefore, one of the objectives of the present study was to obtain further information on the evoked 40-Hz response during different stages of wakefulness and sleep. Based on the literature on altered information processing during sleep (e.g., Campbell et al., 1992; Campbell & Colrain, 2002; Harsh et al., 1994), we expected a decline of its amplitude across the following wake/sleep stages: awake in chair (subjects are sitting up reading), awake in bed (subjects are lying awake in bed), light sleep, SWS. No differences were anticipated between SWS and REM sleep.

As the transient evoked 40-Hz response has been related to the auditory N100 of the ERP in earlier studies (May et al., 1994), and as both components have been proposed to reflect similar mechanisms in the modulation or triggering of attention (May et al., 1994; Näätänen, 1992), this study will also examine the N100 component. The auditory N100 is a negative deflection of the ERP occurring between 75 and 125 ms following stimulus onset, reaching maximal amplitudes at fronto-central sites (Atienza et al., 2001; Cote, de Lught, & Campbell, 2002; Muller-Gass & Campbell, 2002). In the waking ERP, it can be observed clearly changing its morphology depending on the physical characteristics of a stimulus, for example, intensity or stimulus presentation rate. Additionally, the N100 has been observed during both attend and ignore conditions (cf. Muller-Gass & Campbell, 2002). According to these observations, Näätänen (1990, 1992) interpreted the N100 as an exogenous or stimulus-driven ERP component that may act as a transient detector. In this view, it is assumed to trigger an automatic attentional system, switching attention to external events, which may lead to the conscious perception of stimuli (Atienza et al., 2001; Näätänen, 1990). Auditory stimuli might not be consciously experienced unless the pre-attentive processes underlying the N100 are activated (Atienza et al., 2001). In contrast, Campbell and colleagues (Campbell & Colrain, 2002; Campbell et al., 1992; Muller-Gass & Campbell, 2002) argue for an opposing hypothesis and propose the N100 to be entirely endogenous. For example, they explain the occurrence of the N100 during ignore conditions with the fact that alert subjects cannot completely ignore auditory input, even if asked to do so. Therefore, N100 amplitude may be enhanced directly by uncontrolled attentional influences. Accordingly, when subjects are in an unconscious state, such as sleep, N100 vanishes. It is consistently reported that its amplitude is at or near baseline level during non-REM sleep (Campbell & Colrain, 2002; Cote et al., 2002; Ogilvie, Simons, Kuderian, MacDonald, & Rustenberg, 1991). Sleep onset studies observed a gradual reduction of the N100 during sleep stage 1 with N100 amplitude being positively related to the subjects’ reactivity, that is, N100 decreases in amplitude when a subject stops to show behavioral reactions to external stimulus events in a reaction-time paradigm (Cote et al., 2002; Ogilvie et al., 1991). During REM sleep, N100 recovers slightly (Atienza et al., 2001; Bastuji, García-Larrea, Franc, & Mauguière, 1995; Campbell, 2000). However, both the exogenous and the endogenous hypothesis of the N100 suggest that this ERP component represents an important interposing part in the course of attentional processing in general, that might mediate between earlier and later processes as represented by the evoked 40-Hz response and the N350, respectively. Therefore, we intended to relate the N100 to the evoked 40-Hz response and the N350 with the objective to provide a fuller picture of the changes occurring in information processing during wakefulness and sleep.

Apart from investigating the components related with enhanced attentional resourcing as reflected by the evoked 40-Hz response and the N100, a further aim of the present study was to examine the N350 component of the ERP. The N350, a negative deflection of the ERP occurring about 350 ms after stimulus onset, has been observed during sleepiness and sleep (Harsh et al., 1994; Ogilvie et al., 1991; Peszka & Harsh, 1999; Voss & Harsh, 1998). This endogenous long latency component reaches maximal amplitude at the vertex and is a reliable indicator of information processing during sleep (Voss & Harsh, 1998). It is related to behavioral sleep, emerging when a subject stops reacting to sensory stimuli (Campbell et al., 1992; Harsh et al., 1994; Ogilvie et al., 1991) and varies with psychological...
stimulus properties (Voss & Harsh, 1998). Oddball studies have shown lower amplitudes to target stimuli compared to nontarget stimuli (Harsh et al., 1994) as well as an inverse relation between stimulus probability and N350 amplitude (Nielsen-Bohlmann, Knight, Woods, & Woodward, 1991). These observations led to the assumption that the N350 reflects a mechanism contrary to attention, preventing conscious processing of stimuli and facilitating falling asleep (Harsh et al., 1994; Voss & Harsh, 1998). The protection of sleep might be accomplished by the inhibition of nonspecific cortical activation typical for conscious processing of sensory stimuli (Voss, 2001). This mechanism could resemble the process that is filtering unimportant stimuli during wakefulness to allow specific attention. Selective attention tasks require the subject to actively ignore or blend out unimportant information. Successfully passing the wake–sleep transition may represent a similar task. For sleep to prevail, external stimuli have to be prevented from reaching conscious awareness.

Generally, the N350 component can be observed during both non-REM and REM sleep (Campbell et al., 1992; Ogilvie et al., 1991; Ornitz, Ritvo, Carr, La Franchi, & Walter, 1967; Weitzman & Kremen, 1965). However, there are contradictory results concerning the N350 during non-REM sleep. Whereas some studies observed maximum amplitudes during the sleep transition period (Broughton, 1988, cited in Ogilvie et al., 1991; Ornitz et al., 1967), other studies found increasing amplitudes with increasing sleep stage (Campbell et al., 1992; Fruhstorfer & Bergström, 1969; Weitzman & Kremen, 1965). The present investigation intends to provide further information on the N350 amplitude during wakefulness and sleep. Comparisons of N350 s are based on averages of a large number of sweeps (>300) to allow analyses on a single subject basis. Compared to earlier studies (e.g., Harsh et al., 1994; Ornitz et al., 1967; Weitzman & Kremen, 1965), which averaged only a small number of sweeps (e.g., 10 or 100 sweeps), this design should yield new information on the N350.

Finally, the main objective of the present study was to examine the relationship between the transient evoked 40-Hz response and the N350. As both ERP components have been related to attention, albeit in different ways (Harsh et al., 1994; Tititinen et al., 1997; Voss, 2001), the evoked 40-Hz response and the N350 might indicate opposite mechanisms. Therefore, the present study hypothesizes an inverse relationship between these two components of the ERP observed during different stages of wakefulness and sleep.

Method

Participants

Ten volunteer participants (six male and four female) between the ages of 19 and 35 were recruited from the student population at the University of Southern Mississippi. The participants were screened for health problems, medication use, and abnormal sleep/wake schedules. Each participant was paid $25 for his or her participation.

Apparatus

All participants were tested in a 9 ft. × 13 ft. (2.74 m × 3.96 m) room furnished with a bed and a straight-backed chair. Ag-AgCl electrodes were used for recording of all electroencephalographic (EEG) activity. The electrodes were referred to linked mastoids with a forehead ground. Electrooculogram (EOG) electrode placements were at the lower and upper outer canthus of either eye. Submental electrode placements were used for bipolar recording of electromyographic (EMG) activity. All electrode impedances were kept below 5 kΩ. For scoring stages of wakefulness and sleep, EEG activity at the sites C3 and O1 (International 10-20 Electrode Placement System; Jasper, 1958) as well as EOG and EMG activity was amplified and filtered with Grass Model 7P511 amplifiers using standard settings for sleep recordings. Midline recordings of evoked 40-Hz response, N100, and N350 were collected at frontal pole (Fpz), frontal (Fz), and vertex (Cz) scalp sites and amplified using Coulbourn High-gain Bioamplifiers. Output was taken from the back of the Coulbourn amplifiers to obtain an effective fall time constant of 1.1 s. High-cutoff filtering was accomplished following data acquisition using software filters as described in the data analysis. Recordings of the evoked 40-Hz response and N350 data were digitized (200 samples/s) and stored using an A/D board (Keithley MetraByte DAS1601) housed in a 486/50 computer. The recording epoch was 800 ms in duration including a 200-ms prestimulus baseline. Off-line averaging was controlled by a laboratory software program that included a routine for the correction of ocular artifact (Gratton, Coles, & Donchin, 1983). Recording of the data and tone presentation were controlled by a Visual Basic program using a counter/timer-board (Keithley MetraByte CTM-10). The creation of the tones was controlled by Coulbourn solid-state equipment, and the tones were presented binaurally through miniature earphones that could be taped in the participants’ ears. The tones were 45 ms, 1000 Hz (linear rise and fall times of 10 ms each) and were presented 3 s apart at fixed SOAs. Tone intensity was 60 dB (SPL), and the tones were presented against a continuous background of white noise at 65 dB. White noise was used to enable the participants to fall asleep easily and to maintain sleep in spite of the disturbing tones (Harsh et al., 1994; Hull & Harsh, 2001; Peszka & Harsh, 1999).

Procedure

Participants arrived at the laboratory on the test night 2 hr before their usual bedtime hour (range: 20.30–22.30 hr). They were instructed not to nap on the day of the test. Participants were asked to abstain from drugs and alcoholic beverages and to drink no more than the usual amount of any caffeinated beverage on the day before testing.

For each participant, the general procedures were explained and electrodes and earphones were attached. Participants were told that they would hear a tone every 3 s, which they should ignore. The recording of the physiological data was divided into three periods: During period one (awake in chair), participants sat in a straight-backed chair and read a self-selected book for 30 min. During period two (awake in bed), participants were asked to lie down on the laboratory bed. They were instructed to stay awake for 30 min, while keeping their eyes closed. If a participant fell asleep during this period, he or she was awakened. During period three, participants were allowed to go to sleep. During this last period, data for the conditions light sleep, SWS (sleep stages 3 and 4), and REM sleep were collected. Testing was terminated when a participant had had at least half an hour of light sleep, SWS, and REM sleep.

Data Analysis

Polygraphic records were scored in 30-s epochs for stages of wakefulness and sleep by two independent scorers using standard
criteria (Rechtschaffen & Kales, 1968). Interscorer agreement was greater than 90% for all stages. Disagreements were resolved by discussion. Data for the awake in chair condition were taken from the condition when the participants sat up and read in the self-selected book. Data for the awake in bed condition were taken from the corresponding condition. Only data that were recorded while the participants were awake according to the standard criteria were used. Light sleep was defined as stage 2 sleep, SWS was defined as sleep stages 3 and 4, and REM sleep was defined according to standard criteria.

Averages of evoked 40-Hz response, N100, and N350 were obtained for each of the five stages of wakefulness and sleep. They were based on at least 300 stimulus presentations. Waveforms for each participant and for each stage of wakefulness or sleep were first visually examined on a single trial basis: All trials exceeding ± 120 μV at any electrode site (Fpz, Fz, Cz) were excluded from analysis, so that trials with movement artifact or with K complexes were removed. The remaining trials were further analyzed.

To determine the evoked 40-Hz response, for each participant the sweeps for every single wake/sleep stage were corrected for eye movement artifact and averaged. This unfiltered ERP was the sweeps for every single wake/sleep stage were corrected for eye movement artifact and averaged. This unfiltered ERP was then digitally convoluted by a Gabor wavelet function, yielding a continuous measure of frequency-specific power over time to obtain synchronous 40-Hz activity, which is 40-Hz activity phase-locked to the stimulus (Sinkkonen, Tiitinen, & Näätänen, 1995). The Gaussian frequency gain function of the Gabor filter set was to 40 Hz with a SD of 8 Hz. The evoked 40-Hz response was defined as an increase in this synchronous 40-Hz activity during the first 100 ms after stimulus onset. It was measured as the mean power of synchronous 40-Hz activity during the time 0–100 ms after stimulus onset relative to baseline. To avoid any overlap between signal and baseline, the baseline period was defined as mean power of synchronous 40-Hz activity during the time −150 to −50 ms before stimulus onset. Baseline activity was subtracted from the signal before further analyses.

For determining the N100 and the N350, the same sweeps were analyzed in a different way. This time, the sweeps were corrected for eye movement artifact, filtered, and then averaged using laboratory software. High-cutoff filtering (20.67 Hz) was accomplished by a zero-phase filter (Ruchkin & Glaser, 1978). The ERPs were computer scored using a peak-picking program. The N100 was quantified as the maximum negative amplitude during the time window 50–150 ms, and the N350 as the maximum negative amplitude during the time window 250–500 ms after stimulus onset. Both components were computed for each participant and each wake/sleep stage relative to baseline activity (mean activity during the 200 ms preceding stimulus onset).

Averaged ERPs with less than 300 sweeps per average were not considered in the analysis and were treated as missing values (2 of 50). One participant had an insufficient number of sweeps for the condition awake in bed, another subject did not spend enough time in SWS. Missing values were replaced by means of the corresponding wake/sleep stage.

**Statistical Analyses**

Data for evoked 40-Hz response, N100, and N350 were analyzed separately. Each dependent variable was submitted into a two-way repeated-measures analysis of variance (ANOVA) with the factors Stage (awake in chair, awake in bed, light sleep, SWS, REM sleep) and Lead (Fpz, Fz, Cz). To compensate for violations of repeated-measures assumptions, for all ANOVAs the degrees of freedom were corrected by using Greenhouse–Geisser adjustments. In the Results section, original degrees of freedom and corrected probability values are presented. Post hoc tests are based on Bonferroni adjustments.

**Results**

**Evoked 40-Hz Response**

Synchronous 40-Hz activity over time averaged for all subjects during each of the five stages of wakefulness and sleep is shown in Figure 1A. As expected, distinct peaks of synchronous 40-Hz activity can be seen during the time window 0–100 ms after stimulus onset, especially at lead Cz during the two waking conditions, awake in chair and awake in bed. During the three sleeping conditions, light sleep, SWS, and REM sleep, peaks of synchronous 40-Hz activity during the first 100 ms after stimulus onset do not stand out clearly against earlier or later peaks at any of the three electrode positions.

However, although in the averaged analysis distinct evoked 40-Hz responses can be observed during the two waking conditions, it should be noted that this response could not be seen with every subject. Although some subjects showed clearly visible increases of synchronous 40-Hz activity due to stimulus onset during wakefulness, other subjects did not. Figure 2 shows the synchronous 40-Hz activity of 2 such subjects during the five different stages of wakefulness and sleep.

As can be seen in Figure 2, subject SP shows the expected result, that is, an increase in synchronous 40-Hz activity due to stimulus onset during wakefulness, whereas subject PE does not. Altogether there is a high variability across subjects, showing that an evoked 40-Hz response could not be observed in every single subject. Specifically, looking in detail at the synchronous 40-Hz activities during the two waking conditions at the three different electrode sites, the expected increases during the time window 0–100 ms can be seen with the following number of subjects: During the condition awake in chair (n = 10), it can be observed with 5 subjects at Fpz, with 5 subjects at Fz, and with 6 subjects at Cz. During the condition awake in bed (n = 9), an evoked 40-Hz response can be seen with 4 subjects at Fpz, with 5 subjects at Fz, and with 6 subjects at Cz.

Due to the fact that not every subject showed an evoked 40-Hz response during the two waking conditions, further analyses concentrated on 7 subjects who showed the expected response during either awake in chair or awake in bed. Three subjects (2 male, 1 female, aged 19, 25, and 26) with no evidence of an evoked 40-Hz response during any of these two waking conditions were omitted. For further analyses, the evoked 40-Hz response was defined as mean activity during the time window 0–100 ms after stimulus onset relative to baseline activity. The averaged evoked 40-Hz responses for the five different wake/sleep stages at the three different electrode sites are presented in Figure 3.

A Stage (awake in chair, awake in bed, light sleep, SWS, REM sleep) × Lead (Fpz, Fz, Cz) repeated-measures analysis of variance revealed significant main effects for both Stage, F(4,24) = 6.95, p = .00, ε = .72, and Lead, F(2,12) = 9.41, p = .00, ε = .95. As can be seen from Figure 3, at every electrode site, the most prominent evoked 40-Hz responses could be observed during awake in bed (mean power values at Fpz: 0.39, SD = 0.63; at Fz: 0.68, SD = 0.59; and at Cz: 1.01; SD = 0.69). At all three leads, the evoked 40-Hz response was slightly
Reduced during awake in chair and even more reduced during the three sleeping conditions, reaching its minimum power during SWS. Whereas the evoked 40-Hz responses during the conditions light sleep and REM sleep were about the same and reached values close to zero, during SWS negative values were observed, resulting from the averaged amplitude during the first 100 ms after stimulus onset being lower than baseline activity. The increase of the evoked 40-Hz response from awake in chair to awake in bed, the following decrease during light sleep, and SWS as well as the renewed increase during REM sleep is characterized by a significant cubic trend, $F(1,6) = 26.59, p = .00$. As expected, the evoked 40-Hz response changes significantly during the five stages of wakefulness and sleep. Results of post hoc tests concerning the main effect for factor Stage are shown in Table 1. The only significant differences between single stages can be found between awake in bed and SWS as well as between SWS
and REM sleep. Although the first difference is as expected, the latter contradicts the hypothesis.

Examining the observed main effect for factor Lead by comparing the evoked 40-Hz responses at the three different electrode sites revealed the most prominent responses at lead Cz, followed by responses at Fz. At Fpz the evoked 40-Hz responses were clearly reduced.

According to the hypotheses, an evoked 40-Hz response was expected during awake in chair, awake in bed, and light sleep, but not during SWS or REM sleep. Therefore, it was tested whether the observed evoked 40-Hz responses during the five different stages of wakefulness and sleep were significantly different from zero using one-sample \( t \) tests. The only significant evoked 40-Hz responses could be observed during awake in chair at Cz, \( t(6) = 2.68, p = .02 \), as well as during awake in bed at both Fz, \( t(6) = 3.02, p = .01 \), and Cz, \( t(6) = 3.91, p = .00 \). Because it was possible that sleep onset resulted in a latency shift of the evoked 40-Hz response, longer latencies of the evoked 40-Hz response

Figure 2. Synchronous 40-Hz activity of 2 subjects during five wake/sleep stages at sites Fpz, Fz, and Cz. (The ordinate shows the power obtained using the Gabor filter.)
were also considered, using averaged activity during the time periods 25–125 ms, 50–150 ms, 75–175 ms, and 100–200 ms relative to baseline activity. An evoked 40-Hz response could not be identified in these time windows, either.

**Evoked 10-Hz and 20-Hz responses**

Additional analyses were performed to determine whether the evoked 40-Hz response represents an independent phenomenon or whether it is just a harmonic multiple of other frequencies, namely phase-locked 10-Hz or 20-Hz activity. Therefore, these activities have been investigated with the same tools applied to the evoked 40-Hz response. Results indicate that the course of these evoked activities over the five stages of wakefulness and sleep are not the same as for the evoked 40-Hz response. Evoked 10-Hz activity demonstrates its highest values during awake in chair. It is clearly diminished during awake in bed and sleep, reaching its lowest values during SWS. Evoked 20-Hz activity shows its highest values during both awake in chair and awake in bed, and diminishes continuously through the three sleep stages light sleep, SWS, and REM sleep. Therefore, it can be assumed that the evoked 40-Hz response is not due to an evoked phase-locking in these frequency ranges but rather is a separate phenomenon.

**N100**

Averaged ERPs recorded during each of the five stages of wakefulness and sleep are shown in Figure 1B, indicating the N100 component only being clearly present during the two waking conditions awake in chair and awake in bed. A Stage (awake in chair, awake in bed, light sleep, SWS, REM sleep) × Lead (Fpz, Fz, Cz) repeated-measures ANOVA yielded significant main effects for both Stage, \( F(4,36) = 40.10, p = .00, \varepsilon = .65 \), and Lead, \( F(2,18) = 5.10, p = .05, \varepsilon = .56 \).

As one can see in Figure 4, N100 reached highest values during awake in chair and was slightly reduced during awake in bed. As expected, it strongly decreased during non-REM sleep, being completely absent during SWS, and recovered again somewhat during REM sleep (see also Figure 1B). Post hoc tests for this stage main effect are shown in Table 2.

Comparing the N100 at the different electrode sites during the two waking conditions, we found it proved to be most prominent at lead Fz. At both Fpz and Cz, N100 amplitude was marginally lower.

**N350**

A Stage (awake in chair, awake in bed, light sleep, SWS, REM sleep) × Lead (Fpz, Fz, Cz) repeated-measures ANOVA

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**Table 1. Power Differences of the Evoked 40-Hz Responses between the Five Stages of Wakefulness and Sleep (Adjustments for Multiple Comparisons According to Bonferroni)**

<table>
<thead>
<tr>
<th>Stages</th>
<th>Power Differences</th>
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<tbody>
<tr>
<td>Awake in chair</td>
<td>-0.26 (SE = 0.22)</td>
</tr>
<tr>
<td>Awake in bed</td>
<td>n.s.</td>
</tr>
<tr>
<td>Light sleep</td>
<td>0.36 (SE = 0.21)</td>
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<tr>
<td>Slow wave sleep</td>
<td>0.68 (SE = 0.24)</td>
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<tr>
<td>REM sleep</td>
<td>0.28 (SE = 0.21)</td>
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<tr>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>0.94 (SE = 0.18)</td>
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<tr>
<td></td>
<td>0.32 (SE = 0.17)</td>
</tr>
<tr>
<td></td>
<td>0.49 (SE = 0.18)</td>
</tr>
<tr>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>-0.13 (SE = 0.20)</td>
</tr>
<tr>
<td></td>
<td>-0.45 (SE = 0.08)</td>
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<tr>
<td></td>
<td>p = .02</td>
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<tr>
<td></td>
<td>n.s.</td>
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<td></td>
<td>n.s.</td>
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<td>p = .08</td>
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revealed again significant main effects for both Stage, $F(4,36) = 56.70, p = .00, \eta = .63$, and Lead, $F(2,18) = 15.40, p = .00, \eta = .61$. Additionally, a significant Stage x Lead interaction, $F(8,72) = 11.38, p = .00, \eta = .36$, could be observed.

As can be seen in Figure 5, averaged N350 amplitude relative to baseline activity was highest during light sleep. It was present but attenuated during SWS and even more attenuated during REM sleep. During awake in chair and awake in bed, N350 amplitudes were lowest. Nevertheless, the data depicted in both Figure 1B and Figure 5 suggest that an N350 component, albeit extremely small, was present during the two waking conditions. Results of post hoc tests for the stage main effect are shown in Table 3.

Whereas during the awake in chair, awake in bed, and REM sleep conditions no distinct differences in N350 amplitude at the three different electrode sites could be observed, during light sleep and SWS N350 s at Fz and Cz were clearly of higher amplitude than at Fpz. The highest values were reached at Cz (light sleep: $-8.84 \mu V, SD = 3.04$; SWS: $-7.28 \mu V, SD = 2.61$).

Looking at the N350 s of the single subjects, every subject had low N350 amplitudes during wakefulness but large ones during sleep. However, not all subjects had their highest N350 amplitude during light sleep. With 3 of 10 subjects, N350 amplitude was largest during SWS.

**Correlation between the Evoked 40-Hz Response and the N350**

As the evoked 40-Hz response and the N350 seem to reflect opposite mechanisms of information processing (cf. Harsh et al., 1994; Tiitinen et al., 1994), we expected an inverse relation between these two components of the ERP. Because the extents of the two components are measured in opposite directions (evoked 40-Hz response: positive values, N350: negative values), this inverse relationship would be represented by a positive correlation coefficient. As both the evoked 40-Hz response and the N350 proved to be maximum centrally, the correlational analysis was restricted to the vertex electrode. To yield a correlation coefficient representing all subjects, Pearson correlation coefficients were computed for each subject over the five stages of wakefulness and sleep and then summarized into a single coefficient. The averaged correlation coefficient for all 7 subjects was $r = .57$. The corresponding correlation coefficients of the single subjects ranged between $r = .20$ and $r = .83$. Because both components are pronounced at these respective sites, this finding supports the hypothesis of a relation between the evoked 40-Hz response and the N350.

Additional analyses for the 3 omitted subjects, who did not show any evoked 40-Hz response during the two waking conditions, yielded negative correlation coefficients between the two responses for all 3 of them.

**Table 2. N100 Amplitude Differences in Microvolts between the Five Stages of Wakefulness and Sleep (Adjustments for Multiple Comparisons According to Bonferroni)**

<table>
<thead>
<tr>
<th></th>
<th>Awake in chair</th>
<th>Awake in bed</th>
<th>Light sleep</th>
<th>Slow wave sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake in bed</td>
<td>$-0.99 (SE = 0.26)$</td>
<td>$-1.82 (SE = 0.32)$</td>
<td>$-1.06 (SE = 0.43)$</td>
<td></td>
</tr>
<tr>
<td>Light sleep</td>
<td>$-2.81 (SE = 0.35)$</td>
<td>$-2.88 (SE = 0.29)$</td>
<td>$-1.06 (SE = 0.43)$</td>
<td></td>
</tr>
<tr>
<td>Slow wave sleep</td>
<td>$-3.87 (SE = 0.36)$</td>
<td>$-2.88 (SE = 0.29)$</td>
<td>$-1.06 (SE = 0.43)$</td>
<td></td>
</tr>
<tr>
<td>REM sleep</td>
<td>$-2.46 (SE = 0.22)$</td>
<td>$-1.46 (SE = 0.25)$</td>
<td>$0.35 (SE = 0.35)$</td>
<td>$1.41 (SE = 0.47)$</td>
</tr>
</tbody>
</table>

Error bars indicate standard errors.
Discussion

Correlation between Evoked 40-Hz Response and N350

It was hypothesized that the evoked 40-Hz response and the N350 component represent two opposite mechanisms of information processing, the enhancement and the prevention of attention, respectively (cf. Harsh et al., 1994; Tiitinen et al., 1997). The expected inverse relation between these two components was confirmed by a positive correlation ($r = .57$) when both the evoked 40-Hz response and the N350 were measured at Cz. However, in spite of this expected finding, on a descriptive level, both components proved to be not completely opposing: whereas the evoked 40-Hz response reached its highest power during awake in bed and continuously decreased during non-REM sleep, reaching minimal values during SWS, averaged N350 was showing its highest amplitude during light sleep.

It might be speculated that the N350, which has been suggested to reflect a process preventing the conscious perception of a stimulus (Harsh et al., 1994; Voss, 2001), is very pronounced during sleep onset, because the attention-modulating mechanism represented by the evoked 40-Hz response might still be active to some degree. Although diminished, it may still announce the occurrence of stimuli. If the evoked 40-Hz response is a continuous phenomenon that is declining gradually during non-REM sleep, as was observed on a descriptive level in our data, its underlying mechanisms would be more active during light sleep than during SWS. Therefore, a process that tries to allow and protect sleep by preventing attention to sensory stimuli should be more marked during light sleep. That would explain why an N350 amplitude is higher during this sleep stage than during SWS. However, it has to be kept in mind that not all subjects showed their highest N350 amplitudes during light sleep. An attenuated N350 during REM sleep might be connected to the fact that the channeling of sensory signals is reduced during this stage because of changes in central sensory pathways.

Albeit the correlational analysis confirmed the hypothesis of an inverse relation between the evoked 40-Hz response and the N350, this has to be regarded only as a first step in analyzing the exact relationship between these two components. Suggesting that the N350 is most pronounced during light sleep because the mechanisms underlying the 40-Hz response would still be active at this time actually leads to the new hypothesis that the relation between these two components should change depending on the ongoing sleep stage. Specifically, the observed inverse relationship should not be present during sleep onset. During sleep onset, N350 could be more pronounced when stimuli are signaled to

Table 3. N350 Amplitude Differences in Microvolts between the Five Stages of Wakefulness and Sleep (Adjustments for Multiple Comparisons According to Bonferroni)

<table>
<thead>
<tr>
<th></th>
<th>Awake in Chair</th>
<th>Awake in Bed</th>
<th>Light Sleep</th>
<th>Slow Wave Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake in bed</td>
<td>0.14 (SE = 0.36)</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light sleep</td>
<td>5.76 (SE = 0.55)</td>
<td>5.62 (SE = 0.39)</td>
<td>$p = .00$</td>
<td></td>
</tr>
<tr>
<td>Slow wave sleep</td>
<td>5.15 (SE = 0.45)</td>
<td>5.01 (SE = 0.55)</td>
<td>$p = .00$</td>
<td>$-0.61 (SE = 0.66)$</td>
</tr>
<tr>
<td>REM sleep</td>
<td>1.22 (SE = 0.27)</td>
<td>1.08 (SE = 0.45)</td>
<td>$p = .02$</td>
<td>$-4.55 (SE = 0.57)$</td>
</tr>
</tbody>
</table>

Figure 5. Averaged N350 amplitudes ($n = 10$) at lead Fpz, Fz, and Cz during five wake/sleep stages. (N350 amplitude is measured as the maximal amplitude during the time window 250–500 ms after stimulus onset relative to baseline activity.) Error bars indicate standard errors.
higher-order processes via the evoked 40-Hz response than when they are not, as these stimuli are probably more disturbing for allowing or maintaining sleep. The same might be true for tasks of selective attention during wakefulness. Therefore, further analyses are needed on the exact relationship between these two components comprising only separate stages of wakefulness or sleep. In any case, such analyses have to be based on within-subject analyses, as both the evoked 40-Hz response and the N350 proved to be varying across subjects.

**Evoked 40-Hz Response**

As expected, significant 40-Hz responses could be observed during awake in chair and awake in bed, replicating previous findings (e.g., May et al., 1994; Tiitinen et al., 1993). Mean power of synchronous 40-Hz activity during the first 100 ms after stimulus onset relative to baseline activity proved to be significantly different from zero at Cz during awake in chair as well as at Fz and Cz during awake in bed. On a descriptive level, the evoked 40-Hz response during awake in bed demonstrated to be of higher amplitude than during awake in chair. The finding that the evoked 40-Hz response was more prominent while subjects were lying awake in bed than while they were sitting up while reading can be interpreted as confirmation of earlier proposals connecting the evoked 40-Hz response to attentional mechanisms, especially selective attention (cf. Tiitinen et al., 1993, 1994).

Although, during both conditions, subjects were asked to ignore the presented tones, obeying this instruction might have been easier while reading. The reading task might have facilitated the subjects’ distraction from the tones more than just lying awake in bed with eyes closed and, therefore, a stronger evoked 40-Hz response might have resulted during the latter condition. Therefore, it may be that the evoked 40-Hz response indicates not just the occurrence of a stimulus, but that it is also itself modulated by ongoing activity.

Turning toward sleep, it is still unclear whether the evoked 40-Hz response is an on–off phenomenon just occurring during wakefulness but not during sleep or whether it is a continuous phenomenon reducing gradually with increasing depth of sleep. When we examine the data of the synchronous 40-Hz activity per sleep stage, there is no evidence of an evoked 40-Hz response during either light sleep, SWS, or REM sleep, that is, there is no increase in synchronous 40-Hz activity due to stimulus onset. However, on a descriptive level, differences in the power values of the evoked 40-Hz responses during the three examined sleep stages can be observed indicating a continuous decrease over the stages awake in bed, light sleep, and SWS. This decrease represents an evoked 40-Hz response during awake in bed (positive values) and no definite evoked 40-Hz response during light sleep (values close to zero) as well as some kind of desynchronization during SWS (negative values). However, it remains questionable whether this linear decrease during non-REM sleep is of functional significance or whether it is related to an increase in slow wave activity. During sleep, EEG background activity is distinctly of higher amplitude than during wakefulness (Schandra, 1996), possibly influencing the resolution of low amplitude responses. Future research with a larger number of sweeps in the single sleep stages is needed to resolve this issue. Nevertheless, our data lead to the supposition that the evoked 40-Hz response is not simply reflecting an on–off mechanism, but that there might be some additional processes involved as indicated by the SWS data suggesting a possible desynchronization of 40-Hz activity. The absence of the evoked 40-Hz response during both SWS and REM sleep replicates the findings reported by Llinás and Ribary (1993). However, contrary to expectations, post hoc tests indicated a significant difference between SWS and REM sleep. This difference is due to the fact that during SWS, the power of the 40-Hz response after stimulus onset proved to be lower than baseline activity, indicating that 40-Hz activity became desynchronized due to the stimuli. Although this result is unexpected, it does not contradict the Llinás and Ribary findings of no reset of 40-Hz activity during these two sleep stages. However, the resulting negative values for the evoked 40-Hz response during SWS are surprising and should be addressed in future studies.

Generally, our results concerning the evoked 40-Hz response during wakefulness and sleep have to be interpreted cautiously: An unusual finding of the present study is the fact that not all subjects showed reliable 40-Hz responses. During the two waking conditions, 3 subjects did not show the expected increase in synchronous 40-Hz activity at all, and, therefore, had been excluded from further analyses. Böttger, Herrmann, and von Cramon (2002) found that 40-Hz responses were significantly reduced for older age groups. However, our omitted subjects were inconspicuous in age. Furthermore, the remaining 7 subjects also exhibited a large variability in their amplitudes of the evoked 40-Hz responses and did not all show the expected response at every lead during both the awake in chair and awake in bed conditions. Such a variability of the evoked 40-Hz responses across subjects has not yet been mentioned in other studies. Several reasons may account for this unexpected finding in our investigation.

First, it might be that the number of sweeps used (at least 300) was too small to achieve sufficient resolution of the small 40-Hz signal. A methodic constraint of the present study was that subjects cannot be asked to read and then lie awake in bed with eyes closed for hours in order to collect more data during these periods. To obtain evoked 40-Hz responses as prominent as possible, the ISI was set to 3 s, referring to findings by Makeig (1990) indicating that the evoked 40-Hz response was larger with a wider ISI. However, an evoked 40-Hz response of a higher amplitude due to a large ISI does not compensate for the advantages of a large number of sweeps. The low number of sweeps might have resulted in unstable response patterns leading to less reliable 40-Hz response values.

It might also be taken into account that the sampling rate of 200 Hz was rather low for the study of the evoked 40-Hz response. Although other studies have used similar sampling rates successfully (e.g., May et al., 1994; Tiitinen et al., 1993), the low sampling rate in combination with the low number of sweeps may have contributed to the variability in our data. Using higher sampling rates could possibly identify 40-Hz oscillations more reliably.

Another explanation could be that the signal (60 dB in 65 dB noise) was too weak to evoke the transient 40-Hz response. The background noise was used to help the subjects fall asleep and maintain sleep. It has proved to be helpful in other sleep studies, showing no remarkable influences on traditional ERP components such as N350. However, no data have yet been collected on its effect on the evoked 40-Hz response. This response might be critically dependent upon signal intensity. With decreasing stimulus intensity, the probability that a stimulus is signaled to higher-order attentional processes via the mechanism reflected by the evoked 40-Hz response might be reduced, possibly resulting in a higher variability. This should be considered in future studies.
Evoked 40-Hz response and N350

The results concerning the N100 were as expected and replicated previous findings (e.g., Bastuji et al., 1995; Campbell, 2000). N100 was most prominent during awake in chair, slightly reduced during awake in bed, and vanished during non-REM sleep. During REM sleep, it increased again.

Although both the evoked 40-Hz response and the N100 have been related to an attention-modulating function in sending interrupt signals to attentional mechanisms (May et al., 1994; Näätänen, 1992), the fact that N100 was most prominent during a different stage as well as at a different lead (awake in chair at Fz) than the evoked 40-Hz response (awake in bed at Cz), suggests that the corresponding underlying mechanisms are different. However, during sleep, both components developed concurrently. These observations extend previous findings by May et al. (1994), who showed that both responses attenuated simultaneously during long-term stimulation in awake subjects, relating both components to sustained attention.

The observation that the N100 is higher during awake in chair than during awake in bed suggests that this component is more dependent on the actual stage of alertness. Although subjects were awake during both conditions, they were most likely more alert when they were reading than when they were just lying awake in bed with eyes closed. By contrast, the evoked 40-Hz response was more prominent during awake in bed. A 40-Hz response during the reading condition could not be evoked so easily. These findings suggest that the evoked 40-Hz response might be more involved in selective attention, as it was shown to attenuate while reading, that is, performing an activity distracting from the stimuli.

As expected, prominent N350 components could be observed during light sleep, SWS and REM sleep, but not during awake in chair or awake in bed. Albeit the N350 amplitude was clearly reduced during these two waking conditions, it was already visible. This finding suggests that the N350 may not be a component that is restricted to sleep. It strengthens the proposal linking the cognitive processes during sleep onset to the processes necessary for selective attention.

Earlier studies (e.g., Harsh et al., 1994; Ogilvie et al., 1991) showed that the N350 usually rises during sleep onset. However, by looking at this component more specifically through the means of single-subject analyses, it could be demonstrated that its emergence varied across subjects depending on the subjects’ reactivity to stimuli (Harsh et al., 1994). Thus, the emergence of the N350 does not depend on the ongoing sleep stage only, but rather on the subject’s capability to react to sensory stimulus events. This finding also could lead to the assumption that N350 need not necessarily be restricted to sleep, but also could emerge during waking conditions, if a stimulus is truly ignored. However, as discussed by Muller-Gass and Campbell (2002), it may be extremely difficult to reach truly “ignore situations” in waking conditions, as the instructions to ignore a stimulus usually involve some kind of processing, like classifying a stimulus as irrelevant. In any case, it is not really surprising that an N350 may be elicited when subjects are reading or just passively listening to tones without any further instructions as they might be in situations where they do not consciously perceive the tones after a while anymore. Therefore, future studies should examine whether the mechanism underlying the N350 corresponds with the filtering of irrelevant stimuli during wakefulness in order to allow selective attention. Specifically, the proposal should be addressed that the N350 reflects a process preventing a cortico-thalamic activation reaction after a stimulus has been evaluated concerning its relevance.

Although the highest N350 amplitude across all subjects could be observed during light sleep, single-subject data showed higher N350 amplitudes during SWS for 3 out of 10 subjects. An analysis of the single subjects in the present study is quite adequate concerning the large number of averaged sweeps. Earlier studies (e.g., Harsh et al., 1994; Ornitz et al., 1967; Weitzman & Kremen, 1965) often used a small number of sweeps depending on their designs (e.g., 10 or 100 sweeps). The finding that some subjects reached their highest N350 amplitude during light sleep and others during SWS might indicate that N350 amplitude is not dependent on sleep stage only. For example, the amplitude of an N350 response may depend on the predominating background EEG activity, for example, showing its highest amplitudes only during a theta phase but not during delta activity. Few studies already consider these different EEG activities in their scoring of the sleep stages (e.g., Peszka & Harsh, 1999). Furthermore, there is a relation between N350 amplitude and K complexes (Campbell et al., 1992; Ujzsiási & Halász, 1986). Campbell et al. (1992) found, for example, that N350 amplitude is lower when it is not followed by a K complex. Therefore, varying N350 amplitudes through different sleep stages might be related to varying amounts of K complexes. This should also be addressed in future studies. Understanding the reasons why the N350 is with some subjects higher during light sleep and with others during SWS may critically enhance our comprehension of attentional mechanisms during wakefulness and sleep.

Conclusions

Our study indicates that all three examined components of the auditory ERP changed across the different stages of wakefulness and sleep. Whereas the evoked 40-Hz response and the N100 were present during wakefulness and clearly reduced during sleep, the opposite was the case for the N350. N350 was low during wakefulness and present during all sleep stages. Although both the evoked 40-Hz response and the N100 have been proposed to serve an attention-modulating function in sending interrupt signals to attentional processes, the fact that they were differentially peaking at awake in bed and awake in chair, respectively, suggests that their underlying specific functions might be slightly different. The evoked 40-Hz response seems to be higher when subjects are more “attending” to stimuli (passively listening) than when they are performing a distracting task (reading). In contrast, N100 is of higher amplitude when subjects are reading. Therefore this component might be more related to the actual alertness of a subject. However, both components attenuated during sleep. In contrast, the averaged N350 was most prominent during light sleep, supporting its proposed attention-preventing function. During sleep onset, attention-enhancing mechanisms (e.g., as indicated by the evoked 40-Hz response or N100) might still be active to a certain degree. The large amplitude N350 response might reflect an increased effort to counteract these attentional mechanisms in order for sleep to prevail. However, N350 also was already visible during wakefulness, indicating that this component might not be restricted to sleep only and proposing that the cognitive processes during sleep onset might be linked to the processes necessary for selective attention.