EVENT-RELATED POTENTIALS REPRESENTING AUDITORY PERCEPTUAL LEARNING OF FOREIGN LANGUAGE FEATURES IN NOCTURNAL SLEEP

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Whereas infants' language learning is effortless and automatic even so that enhancement in auditory discrimination has been found to happen during sleep, in adulthood learning a foreign language has been thought to require active practice. So far only few studies have been conducted on sleep learning in adults. Event-related potentials (ERPs) provide a way to reveal processing of foreign language features during sleep. To master a language, learning to discriminate speech sounds is necessary, which can be measured with the ERP component mismatch negativity (MMN). In the present study the EEG of 20 Finnish speaking participants naive for Chinese was recorded during four consecutive nights. Oddball condition was used to play the auditory tonal stimuli in sleep stage II. The sound series consisted of frequently occurring tone and two rare stimuli of different tonal change. For the first time it was shown that language features can be learned during sleep, as measured by the late negative wave and MMN that appeared on the fourth night. Furthermore the brain was able to process the auditory stimulus change during stage II as observed in the amplitude modulation of the ERP components MMN, P2, P400 and the late negative wave. P2, P3 and the late negative wave indicated that stimulus discrimination between the frequent stimulus and the larger tonal change occurred, whereas the late negative wave was the only component that differentiated between the frequent stimulus and the smaller tonal change as well as between the two tonal changes on the fourth night indicating sleep learning. Therefore not only does the sleeping brain consolidate memories, it seems to be able to process new information and is capable of stimulus discrimination to the extent that perceptual learning is possible.

KEY WORDS: mismatch negativity (MMN), perceptual learning, lexical tones, event-related potentials (ERP), sleep learning, oddball paradigm

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Vastasyntyneillä, joilla kielen oppiminen tapahtuu vaivatta ja automaattisesti myös kuuloärsykkeiden erottelukyvyn on todettu parantuvan unen aikana. Sen sijaan aikuisuudessa vieraan kielen oppimisen on ajateltu vaativan aktiivista harjoittelua. Tähän päivään mennessä aikuisilla unessa tapahtuvaa oppimista onkin tutkittu vain vähän. Aivojen sähköiset jännitevasteet mahdollistavat vieraan kielen piirteiden prosessoinnin tutkimisen jopa unen aikana. Olennainen osa kielen oppimista on puheäänteiden erottelun oppiminen, mikä on mitattavissa poikkeavuusnegatiivisuus vasteen avulla. Tutkimuksessamme mitattiin 20 suomalaisen koehenkilön aivosähkökäyrää (EEG) neljänä peräkkäisenä yönä. Yön aikana tutkittaville soitettiin kiinan kielen toonivaihteluita sisältäviä puheäänteitä (/a/) S2 univaiheessa. Tutkittavilla ei ollut aiempaa altistumista kiinan kielelle. Tämä tutkimus osoittaa ensimmäistä kertaa, että kielen piirteitä on mahdollista oppia unen aikana, mikä näkyi muutoksina myöhäisessä negatiivisessa aallossa sekä poikkeavuusnegatiivisuus vasteen ilmaantumisena neljäntenä yönä. Lisäksi aivot näyttivät prosessoivan ääniärsykkeitä S2 univaiheessa, minkä osoittivat komponentit MMN, P2, P400 sekä myöhäinen negatiivinen aalto. Ärsykkeiden erottelu toistuvan toonin ja suuremman poikkeavan toonimuutoksen välillä näkyi komponenteissa P2, P3 ja myöhemmässä negatiivisessa aallossa. Ainoa komponentti, joka erotteli toistuvan ja pienemmän poikkeavan toonimuutoksen toisistaan sekä erotteli toonimuutokset neljäntenä yönä, oli myöhempi negatiivinen aalto. Myös tämä viittaa unenaikaiseen oppimiseen. Tulokset osoittavat, että unen aikana aivot eivät ainoastaan konsolidoi muistoja vaan myös kykenevät prosessoimaan uutta tietoa ja erottelemaan ärsykkeitä, mahdollistaen unen aikaisen havainto-oppimisen.

AVAIN SANAT: poikkeavuusnegatiivisuus (MMN), havainto-oppiminen, leksikaaliset toonit, herätevasteet (ERP), unen aikainen oppiminen, oddball paradigma

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1. INTRODUCTION

Learning of speech sounds

Starting from even before birth infants are sensitive to a variety of language characteristics and are capable of accurate auditory discrimination (Liu & Kager, 2014). This perceptional process is guided by infants' surrounding environment and during the first year their focus shifts to contrasts within their native language and less on non-native language characteristics (Liu et al., 2014), referred to as the sensitive period.

Whereas for infants, language learning is fairly automatic and effortless, learning a foreign language in adulthood may require active practice. It is widely accepted that receptive language learning requires attention paid to acoustic patterns that differ from one's native language which can be accomplished by perceptual training and linguistic experience (e.g. Francis & Nusbaum, 2002; Guion & Pederson, 2007). Perceptual learning refers to improved performance on different sensory tasks following training (Tsodysk & Gilbert, 2004), and is due to persistent changes occurring in the perceptual system (Goldstone, 1998). It is an implicit form of learning that develops automatically under frequent exposure to the stimuli (Tsodysk et al., 2004). Differentiation is a mechanism of perceptual learning by which perceptions are learnt to discriminate (Goldstone, 1998).

Lexical tones are variations in pitch that change the meaning of the word, a linguistic function special for tone languages such as Mandarin Chinese (Liu et al., 2014). Studies using infants learning a tonal language and a non-tone language have suggested the perception of tonal contrasts to appear between 4 and 9 month olds (Liu et al., 2014). Following infants shift of tuning in to one's native language characteristics, the ability to discriminate non-native vowels and consonants deteriorates (e.g. Bosch, & Sebastián-Gallés, 2004). In learning tonal languages differentiation of tones is crucial for the semantic meaning of the words. Since Finnish is a non-tone language it is optimal to study learning of tones with Finnish participants free of previous exposure or ability to distinguish different tones.

During infancy, enhancement in auditory discrimination has been found to happen also during sleep, independent from attention (Cheour, Kushnerenko, Ceponiene, Fellman & Näätänen, 2002; Cheour et al., 1995). However whether it is possible for adults to process and learn to discriminate auditory stimuli automatically during nocturnal sleep is still controversial. For learning to be possible in sleep, the brain would have to be able to process new information during the sleeping period.

Auditory processing during sleep

Sleeping involves different levels of auditory processing since the sleeping period consists of sleep stages that alternate throughout the night in cycles. These stages can be divided into rapid-eye-movement sleep (REM) and non-REM (nREM) (Ibáñez, Martín, Hurtado & López, 2008). Non-REM can be further on divided into four consecutive stages of different depth of sleep, which are characterized by changes in EEG. Comparing the electroencephalography (EEG) of being awake and the first stage of sleep (stage I) a decrease in frequency (to 4-8 Hz) and increase in amplitude (to 50-100μV) can be seen. In stage II sleep spindles (10-12 Hz, 50-150 μV) that last few seconds and K-complexes occur frequently. The last two stages, stage III (2-4 Hz, 100-150 μV) and stage IV (0.5-2Hz, 100-2000 μV), are known as the slow-wave sleep (SWS) and the EEG is characterized with slower waves. SWS and REM dominate different ends of sleep (Rasch & Born, 2013). In the beginning of sleep, SWS takes place in more intense and longer stages and the further on the sleeping period continues, the longer and the more intense the REM sleep stages become. Nearly half of the sleep of an adult consists of stage II sleep. Due to previous studies being able to record event-related potentials (ERP) such as mismatch negativity (MMN) (Sallinen, Kaartinen & Lyytinen, 1994) in this stage of sleep, stage II was used in this experiment. Furthermore, during stage II the background EEG isn't too large in amplitude to hide the ERPs to the auditory stimuli.

It is clear that the way the brain processes information isn't the same in the wake brain and during sleep. Changes happening in sensory processing during different states of vigilance have long been studied. The sensory neurons continue to send messages about the external

environment across waking states, however, this synaptic transmission of external information was long suggested to, to some extent, end at the thalamus as the subjects fall asleep (Hennevin, Huetz & Edeline, 2007). In recent years, with the use of neuroimaging techniques and studies with event-related potentials it has been now been established that auditory processing happens also during sleep. Wetter (2001) further concluded in their functional Magnetic Resonance Imaging (fMRI) study that following auditory stimulation the pattern of brain activation in wakefulness and nREM sleep is similar, although during sleep most of the brain regions are less active.

Since some relevant stimuli do get processed, it seems that there is still sensory integration and discrimination continuing to happen while asleep (Hennevin et al., 2007). For example a recent study on word categorization during sleep, based on semantic or lexical properties of the word, revealed it to be possible to process task-relevant external information and furthermore prepare for a motor response according to it, all while asleep (Kouider, Andrillon, Barbosa, Goupil, & Bekinschtein, 2014). Studies on auditory processing happening in other states of consciousness, e.g. coma, have also been conducted, with some level of auditory processing being found to take place, although it is debated how similar this processing is to what goes on in the sleeping brain (Kotchoubey, 2005).

It is known that even a stimulus with very low intensity if meaningful to a person can interrupt sleeping. This has been approached with studies interested in the detection of subject's own name while asleep. A study done using auditory evoked potentials revealed that subjects responded differently to one's own name than to other first names (Perrin, García-Larrea, Mauguière & Bastuji, 1999), further on supporting the brains ability to categorize to some extent external stimuli during sleep. Hearing one's own name during sleep was comparable to the same processes happening while awake in the brain. Similarly in an EEG and fMRI study by Portas et al., (2000) the sleeping brain was discovered to react differently to subjects' own name than to a meaningless auditory stimulus.

ERP's during sleep

One way of studying brain's cognitive and auditory processing during sleep is by using event-related potentials (Ibanez et al., 2008). With the use of ERPs, cognitive processing can be studied without the subject's conscious or behavioral response. Due to ERPs great temporal resolution, the way brain processes different properties of the stimuli at a certain moment, can be measured. Across different states of vigilance, the latency and amplitude of the ERPs have been found to alter indicating variations in the information processing (Coenen, 2012). For example during sleep the latencies of the responses has often been found prolonged (Hennevin et al., 2007). The use of a paradigm with rare "deviant" stimuli, interspersed with repeated standard stimuli in a so called oddball condition, makes it possible to evaluate brain's discrimination ability during sleep stages (Bastuji & García-Larrea, 1999). In this condition N1, MMN and P3 responses are elicited. Most ERP studies during sleep have focused on components N1, MMN, P2, P3, N350 and the late negative wave that are found to link to auditory processing. Also there have been indications showing that stimuli discrimination is possible to some extent in stage II of sleep seen in components MMN, P2, P400 and the late negative wave.

A negative ERP appearing between 75 and 150ms after the presented auditory stimulus is referred to as auditory N1 (Ibanez et al., 2008). N1 is influenced by physical characteristics of the stimulus and the general state of vigilance, but it is also thought to be involved in the detection and registration of the stimuli (Campbell, 2010; Campbell & Colrain, 2002). During sleep a decrease in the amplitude of N1 has been seen which is thought to be due to the changes in the pre-cortical information processes across sleep stages (Ibanez et al., 2008). Reduction in the amplitude can be seen already in stage I (de Lugt, Loewy & Campbell, 1996). Especially in nREM sleep, loss of amplitude in N1 has been apparent as well as an increase in latency, the deeper the sleep phase (Paavilainen et al., 1987), even so that N1 has been often found close to or at baseline in stage II (Nielsen-Bohlman, Knight, Woods & Woodward, 1991; Cote, de Lugt & Campbell, 2002; Colrain, Parsia & Gora, 2000). Similar problems have been found in detecting N1 during coma, due to its diminished amplitude (Fischer, Morlet & Giard, 2000).

Originally found in auditory modality, mismatch negativity (MMN) is a negative ERP that occurs 100-200ms after the stimulus presentation (Näätänen, Gaillard & Mäntysalo, 1978).

It is evoked by a deviant stimulus occurring among more numerous standard stimuli. MMN is believed to represent sensory memory processing that occurs very briefly (Campbell & Colrain, 2002). Repeatedly occurring stimuli is held in the sensory memory and automatically compared to a new deviant stimuli that is processed, generating MMN if the stimuli differ from one another. While awake sensory memory is thought to last for few seconds, whereas during sleep sensory memory duration diminishes.

Although studies on MMN during sleep have yielded varying results, mismatch negativity has been found in some stages of sleep. MMN has been observed in stage 1 (Nashida et al., 2000; Nittono, Momose & Hori, 2001) and REM sleep (Nashida et al., 2000; Loewy, Campbell & Bastien, 1996; Atienza, Cantero & Escera, 2001) in multiple studies. The first ones to report MMN in stage II were Campbell, Bell, and Bastien (1992), who used large deviation in the frequency of the auditory stimuli to successfully elicit a small MMN. Sallinen et al. (1994) were also able to elicit a MMN-like deflection in sleep stage II when followed by a K-complex. Nevertheless these findings could not be replicated by Sallinen and Lyytinen (1997). In an attempt to elicit MMN, Sabri, de Lugt and Campbell (2000) used larger and smaller deviants with a rapid rate of presentation, to pass the fading of sensory memory known to happen during sleep. A negativity related to the larger deviant was seen in wakefulness but also during stage II of sleep, only with diminished amplitude. However it has been debated whether this corresponds to MMN, although it has several similar characteristics such as having largest scalp distribution in the fronto-central area, occurring in the same time window and having a polarity inversion at the mastoid site. MMN was reported to be found also in slow-wave sleep in a study by Sabri and Campbell (2002). Interestingly on human infants mismatch negativity has been detected during all sleep states (e.g. Cheour- Luhtanen et al., 1995; Alho, Sainio, Sajaniemi, Reinikainen, & Näätänen, 1990).

Nevertheless in some cases, studies have had difficulties in finding MMN in sleep stage II (e.g. Nashida et al., 2000). Using stimuli of only small deviation from the standard stimuli might help explain why some studies have been unsuccessful in eliciting MMN in sleeping subjects (Paavilainen et al., 1987). It is thus suggested that when using smaller deviants MMN declines to close to baseline levels at sleep onset and stage I, but with larger deviants MMN might be elicited later on as well (Campbell & Colrain, 2002). It has also been noted that MMN recorded in unconscious states comes with a variety of challenges since other event-related

potentials and background EEG activity might get overlapped and summated with the MMN (Sabri & Cambell, 2002). This could help explain the inconsistency in studies on MMN during stage II. Also methodological aspects such as filtering and presentation rate have been proposed to influence the varying results on MMN during nREM (Ibanez et al., 2008).

P2 component that appears 200ms following stimulus presentation during sleep, has been found in several studies (Ibanez et al., 2008), and is found to be distributed in the fronto-central area (Crowley, Trinder & Colrain, 2002; Nittono et al., 2001). It is thought to be sensitive to features of the stimuli such as its novelty and saliency (Ibanez et al., 2008). In most studies an increase in the P2 amplitude has been found at sleep onset continuing into stage II and in some studies further into NREM sleep (Ogilvie, Simons, Kuderian, MacDonald & Rustenburg, 1991; Nielsen-Bohlman et al., 1991; Winter, Kok, Kenemans & Elton, 1995; de Lugt et al., 1996). In response to deviant stimuli, P2 has been found in NREM sleep in several studies (Sallinen et al., 1994; Sallinen et al., 1997), however, others have reported eliciting P2 wave following both standard and deviant stimuli (Winter et al., 1995). Also Nielsen-Bohlman et al. (1991) found that both standard and deviant auditory stimuli generated P2 in stage II of sleep with the amplitudes of the components being greater following the deviant stimuli. Thus P2 seems to be a known sleep component even though its function and importance have not been fully established (Crowley & Colrain, 2004).

While awake the P3 component has been associated with the processing of deviant stimuli (Kotchoubey, 2005). As attention is paid to the deviants the amplitude of the component has been found to grow. Interestingly P3 has also been found without a task requirement in several studies with this passive P3 being to some extent smaller (Lang & Kotchoubey, 2002). This could suggest it might be possible to obtain P3 during sleep as well, which have been done using passive response paradigms (Ibanez et al., 2008). Results on recording P3 during sleep have varied depending on the sleep stage that has been studied. For example in stage I and REM sleep a parietal P3 response has been found (Cote et al., 2002; Cote & Campbell, 1999). Nevertheless multiple studies have consistently not been able to find the component P3 in non-REM sleep (Koutchoubey, 2005; Cote et al., 2002). However a positive ERP response peaking around 400ms following deviant stimuli found in stage II, has been suggested to resemble P3 (Niiyama, Fushimi, Sekine, & Hishikawa, 1995; Pratt, Berlad & Lavie, 1999). These parietal P3 like waves have been found on few occasions with oddball paradigms. Often these waves found

are longer in latency and smaller in amplitude than the P3 seen in wakefulness (Atienza et al., 2001). For example in stage II P420 response was found to the deviant stimuli (Nielsen-Bohlman et al., 1991). Also Salibury, Maloney and Squires (1992) were able to elicit a posterior P3-like wave in stage II using deviant stimuli that were louder and more invasive. Similarly using semantic stimuli such as one's own name in stage II most likely an equivalent to the parietal P3 component was observed by Perrin et al. (1999). Thus it was suggested that P3 during sleep might be involved in automatic evaluation of rare stimuli during sleep not only an indicator of active cognitive processing.

Similarities between P400 and P3 of wakefulness have attracted research (Ibanez et al., 2008). P400 component with the latency of 400-450ms has been reported in several studies. For example following the deviant stimuli Cote, Etienne and Campbell (2001) found it peaking at 420ms on parieto-occipital sites and in an earlier study peaking at 450ms over occipital regions (Cote, De Lugt, Langley & Campbell, 1999). However these P3 like positive responses often do not have the latency, responsiveness to targets or parietal topography typical to wake P3 (Cote, 2002). It has been reasoned that to be considered equivalent to P3 of the wake state the sleep component has to have a similar parietal scalp distribution and peaking point, detect deviants and have amplitudes varying inversely according to the target presentation (Cote, 2002). Besides since during wakefulness the P3 component requires attention being paid to the target deviants to appear (Polich, 2009), it seems unlikely that sleep P400 is straight equivalent to wake P3. Niyama et al. (1995) argued that it seems like these positive responses are likely to link to K-complexes which are induced by similar stimuli as P3. Thus P400 seems to be more part of the K-complex than equivalent to P3 of the sleeping brain (Ibanez et al., 2008).

Sleep is characterized with very long ERPs peaking from 300 to 900ms after the stimulus onset (Cambell, 2010). A negative ERP between 300 and 400ms, N350, is followed by the 'late negative wave' N550 that occurs 500-750ms following the stimulus (Coenen, 2012). These components are found in stages I, II and during slow-wave sleep (Cote et al., 1999). Both of these components have been hypothesized to link to processes protecting sleep (Colrain & Campbell, 2007). Several studies have demonstrated N350 and N550 components to be independent from one another, be involved in different processes and have a dissimilar scalp distribution (Atienza et al., 2001). The amplitude of N350 has been indicated to be affected by the meaningfulness of the stimuli, such as one's own first name (Perrin et al., 1999) but also

stimulus intensity and novelty, whereas the component N550 has been shown to be responsive to contextual characteristics of the stimuli and especially changes in its novelty and saliency (Atienza et al., 2001). For example Niiyama et al. (1995) and Colrain, Webster and Hirst (1999) found N550 to be larger in amplitude following rare stimuli than the frequent stimuli. It was thus concluded that this long negative wave might reflect some form of automatic information processing following external stimuli during sleep. The scalp distribution of N550 wave has been reported to be maximal over fronto-central areas (Colrain et al., 1999; Niiyama et al., 1995).

A connection with the late negative wave and the appearance of a K-complex has also been established in several studies (Bastien & Campbell, 1992; Colrain et al., 1999). Niyama et al. (1995) noted in their study that if a K-complex appeared along with the late negative wave, the difference wave of the stimuli was found to be larger than when K- complexes were not present. Since introducing a deviant stimulus among repeating stimuli, the likelihood of this eliciting a K-complex increases (Sallinen et al., 1994; Sallinen et al., 1997). Also rare stimuli have been found to evoke larger K-complexes. This has been thought to suggest the brain to be able to detect to some extent the stimuli characteristics and distinguish the different stimuli also in NREM sleep. Also the long latency of the late negative wave has been proposed to indicate a delay in the processing of the external information (Coenen, 2012).

Learning during sleep

Since it seems to be possible to process auditory material during sleep, an obvious further question is whether exposure to foreign language features during sleep also promotes perceptual learning. There has been a growing interest on studying learning during sleep in recent years. Although it is well known that a relationship between memory consolidation and sleep exists (Stickgold, & Walker, 2005), whether it is possible to learn new information while sleeping is unclear. Studies have also been done on learning during different states of consciousness such as with subjects with disorders of consciousness (DOCs) using trace conditioning (Bekinschtein et al., 2009).

The dilemma of studying sleep learning has been approached especially with studies on olfactory stimuli due to it not disturbing subjects' sleep. In a study by Arzi, Shedlesky, Ben-Shaul, Nasser, and Oksenberg (2012) subjects were conditioned to associations of odors and tones in their sleep with ERP's showing that the brain processed the stimuli. When the sniffing response to these tones was tested later, the sniff volume was larger following a tone that previously was associated with a pleasant odor than an unpleasant odor. Arzi et al. (2014) also conducted another study of associative sleep learning using olfactory stimuli to find out whether smoking of cigarettes would be altered following aversive conditioning. The odor of cigarettes was conditioned with an unpleasant odor to induce implicit learning during sleep which indeed resulted in reduction of cigarette smoking following just one night of exposure. These results indicate that it seems to be possible to learn new associations during sleep. Since the brain seems to be capable of associative learning during sleep, perhaps also perceptual learning of speech sounds could be a possible form of sleep learning that is yet to be discovered.

Language learning and mismatch negativity

Differentiation of speech sounds is a necessary part of language learning both with infants learning their first language and with adults learning a foreign language. For example in tone-languages learning to differentiate between the different lexical tones will change the semantic meaning of the word entirely. The ERP component MMN has been suggested to be part of an automatic change detection function, thus it has been used as a tool to measure brains ability to differentiate language characteristics. For example using Finnish and Estonian participants Näätänen et al. (1997) found larger MMNs following native language phonemes than non-native phonemes. In several studies on language learning, MMN has been used as a tool to examine plasticity on a neurophysiological level that occurs following training since practice and exposure to language environments have been found to affect the amplitude, latency and duration of MMN (Cheour, Leppänen & Kraus, 2000). For example Kraus et al. (1995) showed speech discrimination training to be linked to an increase in the MMN amplitude and duration. Larger and earlier ERPs following training could be due to grown number of neurons, cell assemblies

that are firing at the same time, following certain stimuli (Kraus et al., 1995). Results supporting changes in MMN after foreign language training that reveal changes to have happened on the neural level have been reported also by Winkler et al. (1999). In their study, phonemes native to Finnish language elicited MMN with Finnish participants and Finnish-speaking Hungarians but not with Hungarians foreign to Finnish. Since MMN works as a tool to reveal learning of speech sounds and it works independent from conscious awareness, it might provide the means to measure learning occurring during the sleeping period. There are MMN indications of infants discriminating auditory stimuli during sleep, but it is not yet know whether it's also possible for adults. What remains unknown is whether changes in MMN can be found after exposure to unfamiliar speech sounds on multiple consecutive nights that could suggest learning to happen in adult subjects as they sleep.

As seen above, several studies have approached the subject of auditory processing in different states of vigilance and provided evidence of the brain continuing to process external information. Multiple ERP components reveal the brains ability to discriminate, to some extent, the deviant stimuli from the standards in the sleeping brain. These studies range from using sleeping participants and coma patients, and have already found indications of associative learning during sleep, to have happened. Even so, to this day only few studies have been done on sleep learning. This study could help bring more understanding to what extent learning of speech sounds during sleep is in fact possible, and whether it can be revealed and measured with ERP components. If perceptual learning was possible in the sleeping brain, it could change the training of stimulus discrimination which is necessary for the learning of new languages.

Research questions and hypothesis

The aim of this study is to explore to what extent changes in Chinese lexical tones are processed in sleep and whether perceptual learning of these tones occurs during sleep. To this end, we will expose Finnish participants naive to Chinese to changes in lexical tones on four consecutive nights while their brain responses to these sounds are recorded with EEG. We assume that exposure to speech sounds during stage II will elicit ERP components MMN, P2, P400, N350

and the late negative wave from the first night. This hypothesis is based on previous findings on ERP responses measured during sleep (Ibanez et al., 2008). However we do not presume to find N1 component since previous studies have not found it in stage II of sleep (e.g. Nielsen-Bohlman et al., 1991). Further, we assume that ERP components will be modulated by the exposure indicating sleep learning in the form of perceptual learning. This hypothesis is supported by findings of associative learning in the sleeping brain (Arzi et al., 2012; Arzi et al., 2014).

2. METHODS

Participants

20 subjects (4 men, 16 women) participated in the experiment. Mean age of the participants was 23 years, SD 1.93. They were recruited via Jyväskylä University email lists. All subjects were Finnish speaking and right handed with no neurological or psychiatric disorders or history of brain operations. Excluding criteria for the participants also included previous exposure to Chinese language or other tonal languages for 3 months. The hearing of the participants was tested before the experiment to make sure it was normal. Also a written consent was gotten from all the participants before the experiment. The EEG-recordings were carried out during March-May 2014. One participant was excluded due to insufficient exposure to the auditory stimuli during the nights.

Procedures

The participants slept in the laboratory for 4 consecutive nights. However, the first night slept in the laboratory was excluded from the statistical analysis. It has been shown in several studies that sleeping in a new environment may cause subjects to experience more sleep disturbances. This first-night effect appears as an asymmetry in hemispheric sleeping depth so that one of the

hemispheres stays more vigilant while asleep and larger ERPs to deviant stimuli are seen (Tamaki, Won Bang, Watanabe & Sasaki, 2016). These enhanced responses to ERPs during the first night could influence this experiment and the changes in the ERPs following subsequent nights could get distorted.

The mean duration of the sleeping period per each night was 8h starting at around 11pm and finishing at around 7am. The subjects were allowed to read a book before choosing to start to sleep. Electrodes had a cord long enough for the subjects to be able to change their position throughout the night. Also there was a microphone connection to the monitoring room from the subjects' rooms the whole night.

The polysomnography of the subjects' was observed throughout the night by two trained researcher assistants to make sure the subjects were exposed to the auditory stimuli at stage II of non-REM sleep. When sleep spindles or K-complexes were present the sleep could be scored as stage II of sleep (Tatum, Husain, Bendis, & Kaplan, 2007). The speaker was 50 cm from the participants' heads and the auditory stimuli was played at the volume of 50 dB. The volume of the stimuli grew slowly within 3 minutes to the intended volume, not to disturb the participants' sleep. The minimum exposure time to the auditory stimuli was intended to be 2h per night. The average exposure time on night 2 was 137min (SD = 15.9), on night 3; 127min (SD = 23.3) and on night 4; 132min (SD= 23.7).

Stimuli

Three tonal stimuli were used in the experiment: as the standard stimulus a frequent tone and as deviant stimuli two tonal changes with a different degree of physical difference, a larger and a smaller (figure 1.). The stimuli were isolated vowels /a/ differing in lexical tones. The original stimuli were recording from a native female Chine speaker at a sampling rate of 44.1 kHz. SoundForge (SoundForge, Sony corporation, Japan) was used to digitally edit the vowels each having a duration of 200ms. Pitch tier transfer was performed with the Praat software so that the lexical tones could be isolated and the rest of the acoustic features kept identical. Following this method two stimuli /a2/ and /a4/ were generated, both being identical except for the pitch

contour difference. These two correspond to the real tones in Mandarin Chinese /a2/ being a rising tone and /a4/ being a falling tone. The stimuli /a2/ and /a4/ were used as the endpoint stimuli in order to make a 10-interval lexical tone continuum (figure 2.). A morphing technique was used in Matlab with STRAIGHT (Kawahara, Masuda-Katsude, & de Cheveigné, 1999) in 10 equal intervals. All 11 stimuli were normalized in RMS intensity. Oddball design was used in presenting the frequent stimuli and the two tonal changes of different degree with the probability of 80%, 10% and 10%.

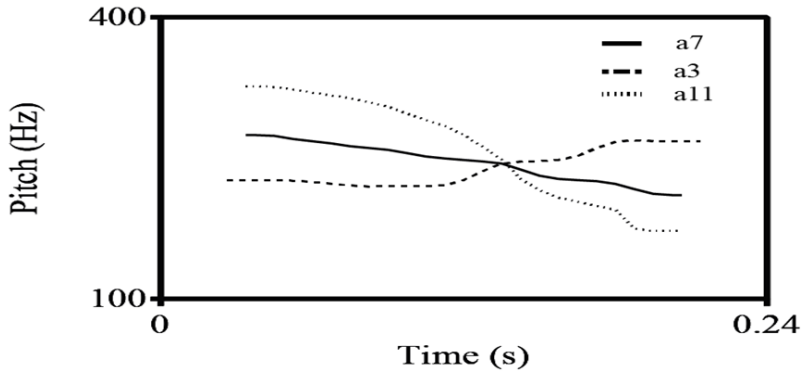

Figure 1. The tonal stimuli used in the experiment: /a3/ being the frequent tone, /a7/ the smaller tonal change and /a11/ the larger tonal change.

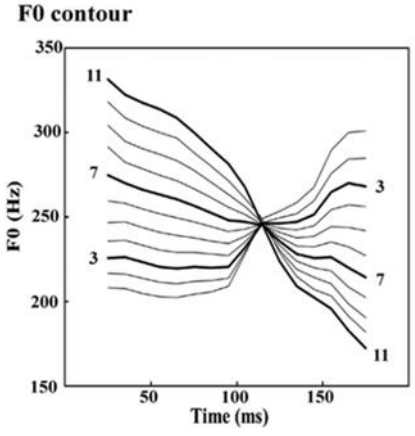

Figure 2. The lexical tone continuum of vowel /a/. Mandarin Chinese rising tone /a2/ corresponds to 1 on the continuum and falling tone /a4/ to 11.

EEG recording and analysis

Raw electroencephalography (EEG) was recorder with Neurone using electrodes C3, C4 and FPz that were arranged according to a typical polysomnography recording. C3 and C4 electrodes were attached to the scalp using glue. Also electrooculography (EOG) and electromyography (EMG) were measured with electrodes. Neurone sampling rate was 500Hz, filter settings were 0,1-200Hz and IMP was greater than 5kΩ. During the experiment vivago watches and first beat heart beat monitors were used for heart rate variability measurement (not reported here). The data analysis was carried out using Besa Research 6.1. The epochs were 500ms pre-stimulus and 1000 post-stimulus. The baseline correction was -100-0 ms. Low cut-off filter used was 1 Hz and 12 db/oct with the high cutoff being 30 Hz and 24 db/oct. The artifact rejection was done on amplitudes greater than 120 µV, gradients greater than 75 and low signal lesser than 0,01. The sleep stage II was re-evaluated from the sleep EEG by two research assistants working in co-ordinance with the study. The mean averages were calculated for each participant each night individually after which the grand averages were calculated for each of the nights.

Statistical analysis

Statistical analysis was carried out using SPSS. Repeated measures ANOVA was performed and the findings further analyzed by using paired samples T tests. Four time windows were chosen based on visual inspection of the grand average responses: MMN (120-200ms), P2 (200-280ms), P400 (340-420ms) and late negative wave (620-700ms). The data was analyzed with repeated measures analysis of variance (ANOVA) with stimulus type (standard tone, smaller tonal change, larger tonal change), night (2,3,4) and electrodes (C3,C4 and FPz) being within-subjects factors. Huynh- Feldt test was chosen to report the results. Statistically non-significant results ($p>.05$) will not be reported.

3. RESULTS

MMN (120-200ms)

ANOVA showed main effect of stimulus type, F (2,36) = 6.94, p = 0.003, η_p^2 = 0.28. There was also an interaction effect of stimulus type, electrode and night (F(8,144) = 2.4, p = 0,029, η_p^2 = 0.12). Bootstrap for paired samples t-tests showed no significant difference between smaller and the larger tonal change on any of the electrodes or nights so these were combined to form a new variable for average tonal change.

During the 2nd night on electrode C4 t-tests showed a difference, t(18)= 2.30, p = 0.008, between the frequent tone (M= -0.0026, SD= 0.227) and the tonal change (M= -0.277, SD= 0.395). Bootstrap 95% confidence interval was 0.101-0.459. Between the frequent tone (M= -0.0226, SD= 0.231) and the tonal change (M= -0.262, SD= 0.420), there was also a difference of t(18)= 2.67, p = 0.021 on electrode C3. Bootstrap 95% confidence interval was 0.0645-0.414.

During the 3rd night on electrode C4 between the frequent tone (M= 0.0189, SD= 0.225) and the tonal change (M= -0.316, SD= 0.468), a difference, t(18)= 3.27, p= 0.005, was found also during night 3. Bootstrap 95% confidence interval being 0.144-0.556. Bootstrap for paired samples t-tests showed a difference, t(18)= 2.92, p = 0.007, between the frequent tone (M= 0.0342, SD= 0.201) and the tonal change (M= -0.261, SD= 0.421) on electrode C3 during night 3 as well. Bootstrap 95% confidence interval was 0.0948-0.481.

On the 4th night electrode C4 showed a difference of t(18)= 2.72, p= 0.018, also on night 4 between the frequent tone (M= -0.0432, SD= 0.145) and the tonal change (M= -0.272, SD= 0.415) with bootstrap 95% confidence interval of 0.0692-0.397. On electrode C3 between the frequent tone (M= -0.0405, SD= 0.172) and the tonal change (M= -0.247, SD= 0.339) the difference was t(18)= 2.78, p = 0.027. Bootstrap 95% confidence interval was 0.0700-0.340. T-test showed a difference in amplitudes between the frequent tone (M = -0.0779, SD = 0.194) and the tonal change (M = -0.316, SD = 0.325) on FPz electrode only during night 4, t(18) = 2.94, p = 0.02. Bootstrap 95% confidence interval was 0.0708 - 0.388.

P2 (200-280ms)

Main effect was found with ANOVA only for the stimulus type, $F(2, 36) = 4.83$, $p = 0.014$, $\eta_p^2 = 0.212$. No significant interaction effects were found. Since no effects were found for the electrode or nights, their information was averaged for post hoc test. Paired samples t-tests with bootstrap were performed to compare the mean amplitudes for the frequent tone, the smaller and the larger tonal change. The frequent stimuli ($M = 0.332$, $SD = 0.347$) elicited larger responses than the larger tonal change ($M = 0.067$, $SD = 0.423$) with the difference of $t(18) = 3.15$, $p = 0.003$. Bootstrap 95% confidence interval was 0.105- 0.417. A marginally significant difference, $t(18) = 2$, $p = 0.062$, was found between the frequent tone ($M = 0.332$, $SD = 0.347$) and smaller tonal change ($M = 0.178$, $SD = 0.576$) responses. Bootstrap 95% confidence interval was 0.0124- 0.295.

P400 (340-420ms)

Repeated measures ANOVA showed no significant main effects but showed an interaction effect of stimulus type and electrode, $F(4,72) = 3.94$, $p = 0.026$, $\eta_p^2 = 0{,}18$. Since no effect was found for the night the data was combined to compute a new variable. Bootstrap for paired samples t-tests showed no difference between the frequent tone and the smaller tonal change on any of the electrodes C4, C3 or FPz. T-tests for the frequent tone and the larger tonal change showed differences on electrode FPz and C3. On FPz between the frequent stimuli ($M = 0.0999$, $SD = 0.295$) and larger tonal change ($M = 0.388$, $SD = 0.643$) paired samples t-tests showed a difference ($t(18) = -2.4$, $p = 0.028$). Bootstrap 95% confidence was -0.505- (-0.0371). On C3 the difference was marginally significant, $t(18) = -2.1$, $p = 0.054$), between the frequent tone ($M = 0.168$, $SD = 0.339$) and the larger tonal change ($M = 0.378$, $SD = 0.694$). Bootstrap 95 % confidence interval was -0.409- (-0,0154).

The late negative wave (620-700ms)

Repeated measures ANOVA showed main effect of stimulus type, $F(2, 36) = 10.5$, $p = 0,00$, $\eta_p^2 = 0.368$. There was a marginally significant interaction effect of stimulus type, electrode and night, $F(8, 144) = 2.14$, $p = 0,06$. No other statistically significant interaction effects were found.

During the 2nd night paired samples t-tests with bootstrap showed differences on electrode C4. The frequent tone had larger responses ($M = 0.0195$, $SD = 0.329$) than the smaller tonal change ($M = -0.292$, $SD = 0.597$) with a difference of $t(18) = 2.26$, $p = 0.036$. Bootstrap 95% confidence interval being 0.0532 - 0.565. Also between the frequent tone ($M = 0.0195$, $SD = 0.329$) and the larger tonal change ($M = -0.418$, $SD = 0.559$) a difference of, $t(18) = 3.35$, $p = 0.004$, was found. Bootstrap 95% confidence interval was 0.193- 0.666. On electrode C3 t-tests showed a difference of $t(18) = 2.15$, $p = 0.042$, between the frequent tone ($M = 0.0142$, $SD = 0.34$) and the smaller tonal change ($M = -0.24$, $SD = 0.485$) with bootstrap 95% confidence interval of 0.0095-0.465. Also between the frequent stimuli ($M = 0.0142$, $SD = 0.34$) and the larger tonal change ($M = -0.331$, $SD = 0.579$) a difference of $t(18) = 2.68$, $p = 0.015$, was found. Bootstrap 95% confidence interval was 0.098-0.591. Also electrode FPz had a difference of $t(18) = 2.88$, $p = 0.022$, between the frequent tone ($M = -0.04$, $SD = 0.377$) and the larger tonal change ($M = -0.383$, $SD = 0.504$). Bootstrap 95% confidence interval was 0.125-0.591.

During the 3rd night on C4 paired samples t-tests with bootstrap showed a difference of $t(18) = 2.96$, $p = 0.007$, between the frequent tone ($M = 0.0463$, $SD = 0.0895$) and the smaller tonal change ($M = -0.332$, $SD = 0.522$). Bootstrap 95% confidence interval being 0.129- 0.639. On electrode C3 there were differences on the frequent tone and the two tonal changes. The frequent stimuli ($M = 0.0705$, $SD = 0.0885$) and the smaller tonal change ($M = -0.396$, $SD = 0.434$) had difference of $t(18) = 4.29$, $p = 0.000$, with bootstrap 95% confidence interval of 0.282-0.659. Between the frequent tone ($M = 0.0705$, $SD = 0.0885$) and the larger tonal change ($M = -0.231$, $SD = 0.425$) the difference was $t(18) = 2.69$, $p = 0.015$. Bootstrap 95% confidence interval was 0.101-0.529.

On the 4th night on electrode C4 the frequent tone ($M = 0.0205$, $SD = 0.137$) and the larger tonal change ($M = -0.452$, $SD = 0.547$) had a difference of $t(18) = 3.98$, $p = 0.001$. Bootstrap 95% confidence interval was 0.268- 0.707. A difference $t(18) = 2.34$, $p = 0.034$,

between the smaller (M = -0.16, SD = 0.52) and the larger tonal change (M = -0.452, SD = 0.547) was also found on electrode C4. Bootstrap 95% confidence interval was 0.0653- 0.551. On electrode C3 between the frequent tone (M = 0.0158, SD = 0.162) and the larger tonal change (M = -0.466, SD = 0.467) a difference of t(18) = 4.48, p = 0.001 was found, with bootstrap 95% confidence interval of 0.288-0.687. There was a marginally significant difference of (t(18) = 2.01, p = 0.053) between the frequent tone (M = 0.0158, SD = 0.162) and the smaller tonal change (M = -0.148, SD = 0.336). Bootstrap 95% confidence interval was 0.00638-0.317. Also on C3 between the smaller (M = -0.148, SD = 0.336) and the larger tonal change (M = -0.466, SD = 0.467) there was a difference on night 4, t(18) = 2.63, p = 0.016. Bootstrap 95% confidence interval was 0.0937-0.547. On electrode FPz T-tests showed a difference, t(18) = 2.4, p = 0.026, between the frequent stimuli (M = -0.0105, SD = 0.162) and the smaller tonal change (M = -0.301, SD = 0.589) with bootstrap 95% confidence interval of 0.0385-0.532.

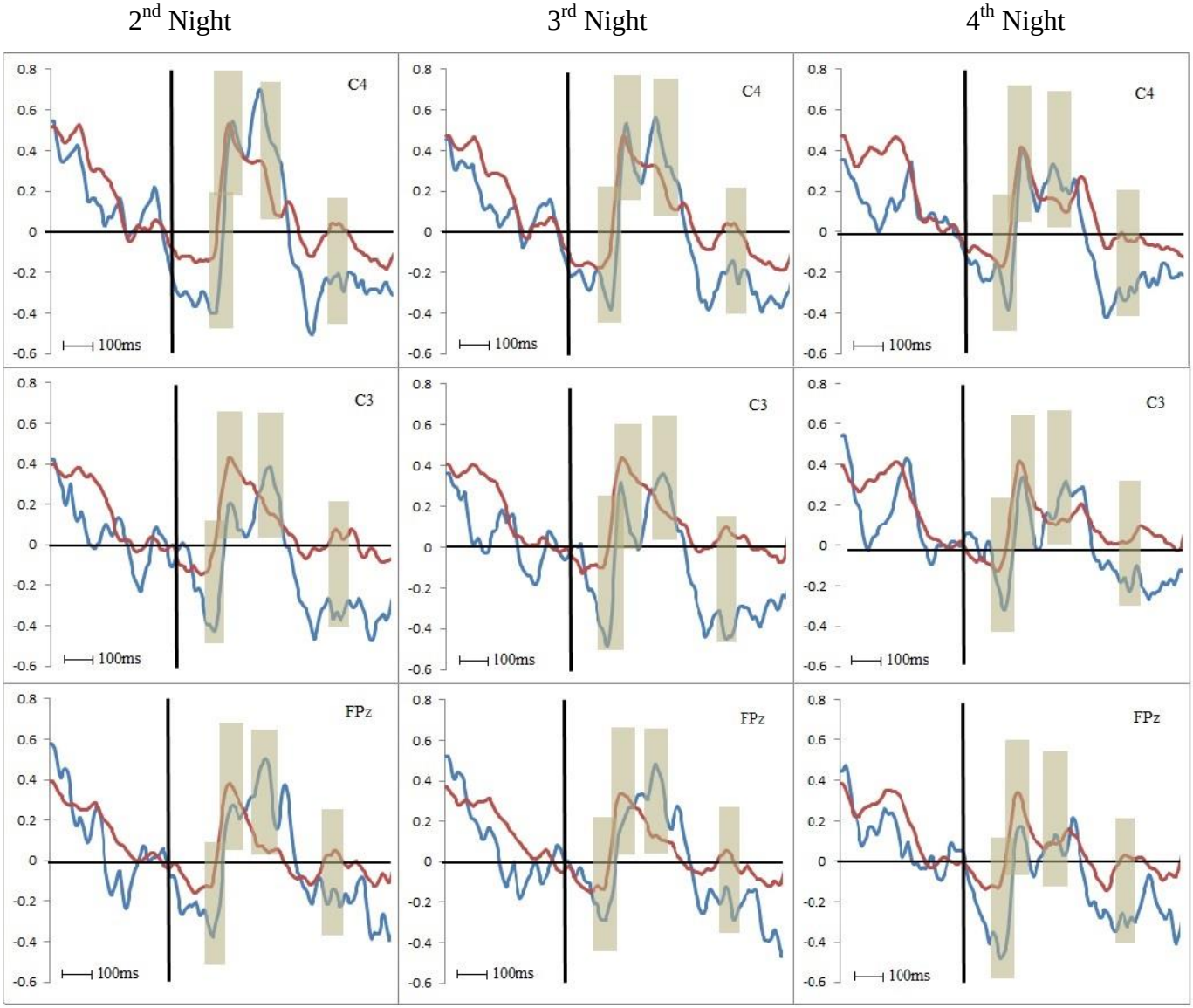

Figure 3. Grand average responses to the smaller tonal change (blue) and to the frequent tone (red) on electrodes C4, C3 and FPz on night 2, 3 and 4 shown in time windows: MMN (120-200ms), P2 (200-280ms), P400 (340-420ms) and late negative wave (620-700ms).

Sleep learning was seen in the change between the 2nd and the 4th night in MMN, in the difference between the frequent tone and the combined tonal change, but also in the late negative wave, in the difference between the large and small tonal change.

2nd Night 3rd Night 4th Night

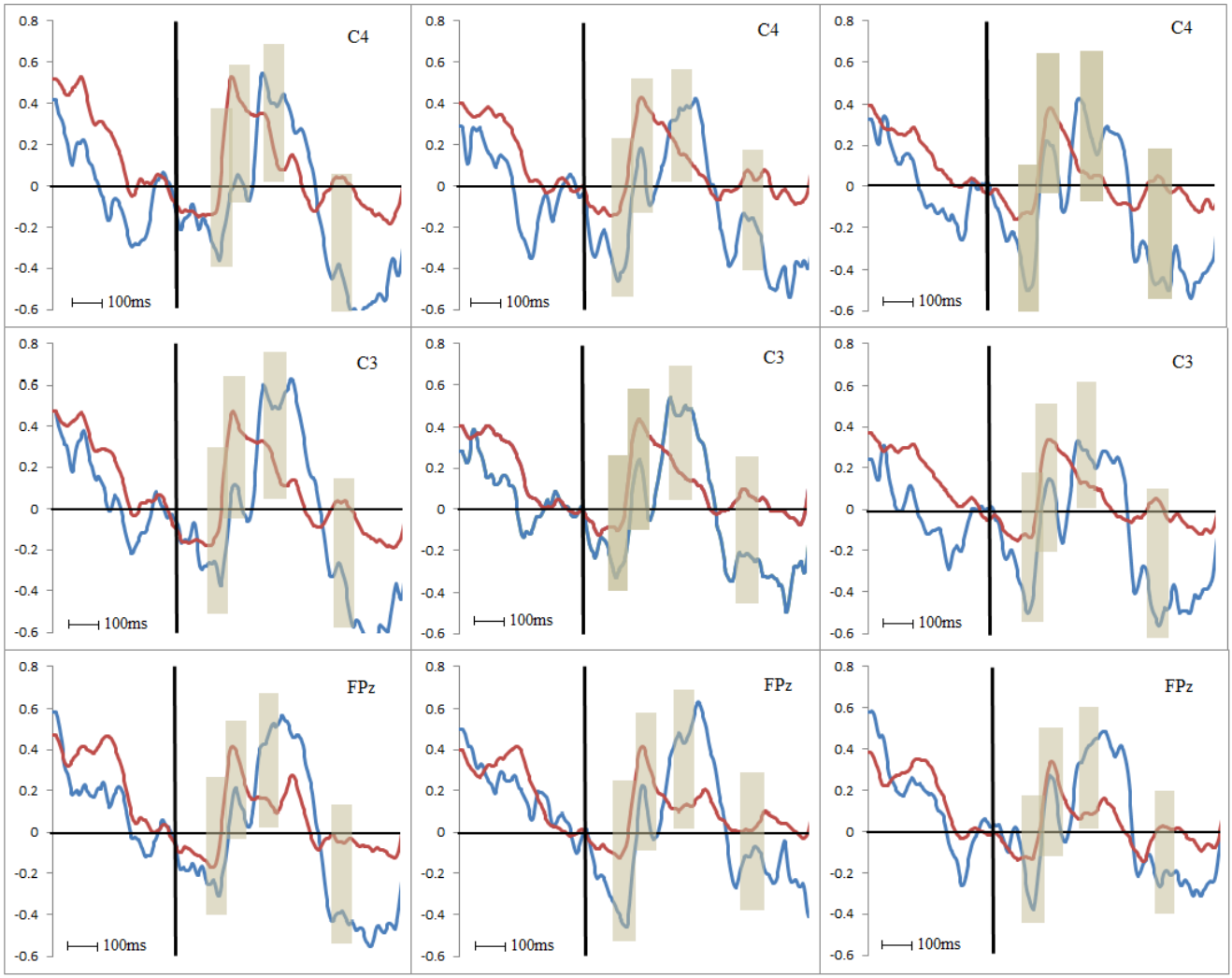

Figure 4. Grand average responses to the larger tonal change (blue) and to the frequent tone (red) on electrodes C4, C3 and FPz on night 2, 3 and 4 shown in time windows: MMN (120-200ms), P2 (200-280ms), P400 (340-420ms) and late negative wave (620-700ms).

4. DISCUSSION

In this study we exposed subjects to Chinese tonal stimuli during four consecutive nights to find out to what extent the brain was able to differentiate between different tones while asleep and whether there were indications of sleep learning that could be measured with ERP components. We found that the brain is capable of stimulus discrimination between the frequent tone and the infrequent larger tonal change in stage II of sleep seen in P2, P400 and the late negative wave. However, the brain responses between the smaller tonal change and the frequent tone were significantly different only for the late negative wave and marginally significant for P2. Interestingly the difference between the nights was only apparent in components MMN and the late negative wave. MMN component showed a difference between the frequent tone and the rare tone indicating perceptual learning, whereas the late negative wave was the only component that revealed the brain to differentiate between the infrequent tonal changes, also indicating enhancement having happened in the stimulus discrimination.

According to our hypothesis ERP components MMN, P2, P400 and the late negative wave were elicited following exposure to Chinese tones in stage II of sleep. Also as assumed N1 component did not appear. However unlike assumed N350 was not apparent in the data either. Our second hypothesis concerned whether these components would be altered indicating perceptual learning to have happened. The MMN component's amplitude differed from zero on the 4^{th} night of the experiment which could be interpret as an indication of perceptual learning to have happened and the brain ability to differentiate sounds to have enhanced. Also the late negative wave showed a significant difference between the tonal changes that appeared on the fourth night furthermore indicating sleep learning to have occurred.

In accordance with previous studies, component N1 was not apparent in stage II. The time window of 120-200ms most likely represents MMN rather than N1 in this study since in stage II N1 is often found at baseline or close to it (Nielsen-Bohlman et. al, 1991; Cote et al., 2002; Colrain et al., 2000). The amplitude of N1 has been found to decrease and the latency has been found to increase the deeper the sleep phase in nREM (Paavilainen et al., 1987).

In previous studies there have been inconsistent results on MMN found during stage II of sleep and it's highly debated whether it is at all possible (e.g. Nashida et al., 2000). Our results

not only give proof of MMN being successfully elicited in the sleeping brain but also indicate that perceptual learning is possible during sleep. Previously MMN has been found with sleeping infants (e.g. Cheour- Luhtanen et al., 1995) but these results provide evidence that the same takes place in the adult brain too. It has been suggested that studies that have not reported MMN in stage II, the deviant stimuli used may not have had a large enough deviance for the brain to detect during sleep (Paavilainen et al., 1987). So far there haven't been other MMN studies exposing participants for several consecutive nights.

The component P2 was apparent in the data, although the results weren't consistent with previous studies (e.g. Sallinen et al., 1997; Nielsen-Bohlman et al., 1991) due to the responses being larger to the standard stimuli than to the deviant of larger deviance. Although the small response to the deviant in this study might be, however, due to the previous MMN component that causes a large negative wave following the deviant just before P2. Although the function of P2 is not yet well-known, based on these results it is evident that this occurring sleep component reflects stimulus discrimination during sleep.

The latency of the response at 340- 420ms is similar to P400 response found by several other studies done in stage II (Ibanez et al., 2008). It was clear that here too P400 response showed the brain to differentiate rare tonal stimuli during sleep. However, this study cannot settle the debate whether P400 could be equivalent to P3 of the sleeping brain. Much like the wake P3 component the P400 response showed the brain to elicit a larger response to infrequent rather than frequent stimuli. The latency of the sleep response is later than in the wake P3, although it has been suggested, that the latencies of ERP components might appear later during sleep, probably due to changes in the processing in the sleeping brain (Hennevin et al., 2007). Nevertheless, it seems unlikely that these two responses are comparable since the wake P300 requires attention to be paid to the infrequent target stimuli to appear (Polich, 2007), a process that cannot happen in the sleeping brain. The P400 might reflect some other process that takes place in the sleeping brain that is not yet known. Furthermore it has been suggested that P400 might rather be connected to the K-complexes that are generated by similar stimuli as is the component P3 explaining their similarities (Niyama et al., 1995).

Unlike the N350 component that was not clear on the data, the late negative wave that appears 500-750ms following the stimulus was clearly visible (Coenen, 2012). Throughout the nights the difference between the different tonal changes and the frequent tone was apparent,

with the amplitudes following rarely occurring tonal changes being larger, supporting previous studies. The late negative wave was the only other component that showed a difference between the nights besides MMN. Interestingly during the last night for the first time the difference between the smaller and the larger tonal change became apparent. This might also indicate learning of speech sound features and their discrimination to have happened. Previously it has been suggested that the late negative wave reflects automatic processing of external information during sleep (Niiyama et al., 1995), thus the differentiation of the two tonal changes on the fourth night might indicate improvement to have happened in this function due to sleep learning.

Strengths and limitations

Previous sleep ERP studies have often been conducted comparing ERP components of pre and post EEG measuring. However here, online recording was used to capture the ERP responses as they occurred during sleep each night. This allowed the comparison of sleep ERPs instead of comparing wake ERPs following the sleeping period. Besides showing that MMN appears during sleep in adult participants not only with infants, this study also provides evidence of sleep learning in the form of ERP components. Here the late negative wave and MMN were shown to have changed following four nights of exposure. Especially changes in MMN give clear indications of perceptual learning to have occurred during sleep.

Conducting sleep EEG studies in general can be challenging in few ways. In our study, the simultaneous observing of sleep stages not only allowed the exposure to the stimuli to happen in the right sleep stage but also minimized disturbing the subjects' sleep. Furthermore only few electrodes were used here in the EEG measuring for more ideal sleeping conditions although that made it more difficult to draw conclusions on the topography of the ERP components. To our knowledge, there aren't previous studies done on measuring sleep EEG on several consecutive nights. This measuring of four nights allowed the exclusion of the first night, which could have influenced the results. It has been shown that sleeping in a new environment appears as hemispheric asymmetry that affects the ERPs to external stimuli especially deviant stimuli (Tamaki et al., 2016), that was a crucial part of this experiment. In generalizing the

results of the study it should be noted that since the subjects that took part in the experiment were mostly students, these results can be only generalized to young adults. However, the results indicate that most probably similar processes happen in the whole adult population. Although in the number of the participants was fairly low, the long exposure to the tonal stimuli each night is a definitely a strength in this study. This large amount of data collected enabled reliable statistical analyses for the responses.

Although these results clearly indicate the brain to process external information during the sleeping period, these results should be replicated before final conclusions can be made. ERPs during sleep haven't been as thoroughly studied as the ones occurring in the wake state, so not much is yet known about their function and characteristics. Therefore sleep EEG studies such as this one are important. ERP components occurring in the wake brain and during sleep might not be automatically equivalent to one another. There are ERP components that are unique for the sleeping brain e.g. N550, N350 and P2, as there are components that seem to demand awareness e.g. P3, which can only occur in the wake brain. Previously inconsistent results on ERPs recorded in sleep studies have been explained by reasons such as differences in the stimuli characteristics, definitions of awake and sleep stages, tasks used in the experiment and successfully being able to exclude K-complexes that overlap with ERP components (Pratt, 1999).

Conclusions

It is widely known that during sleep consolidation and reactivation of memories takes place, however, these results indicate that the brain is also capable of active processing of external information during sleep to the extent that sleep learning is possible. Nevertheless, one of the main functions of sleep is to provide the brain an ideal condition for the consolidation processes to take place, since the processing of external information is diminished (Rasch & Born, 2013). Although these results provide unlimited new possibilities in utilizing sleep learning, another challenge for the future might be, how to do this without disturbing other functions of sleep, as it has been suggested that encoding new information and consolidation might be mutually exclusive due to same neuronal sources. So far only few studies have been done on sleep

learning making these results remarkable. Not only is the brain able to learn associations during sleep, as seen in previous studies using olfactory stimuli, the brain is also capable of perceptual learning during sleep through auditory stimuli. This opens up new opportunities for language learning as well as teaching, as learning of speech sounds is necessary to master a language.

For future studies interesting would be to see which other forms of learning could be possible in the sleeping brain besides associative and perceptual learning with all age groups. Also more studies ought to be done on sleep ERP components to fully understand their function and characteristics and how they differ from similar wake ERP components. Another interesting idea for the future line of research could be to take the results from this study into developing new learning applications and experiments that take advantage of not only sleep learning but also passive learning.

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