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The Effect of low frequency oscillation phase on memory encoding

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THE EFFECT OF LOW FREQUENCY OSCILLATION PHASE ON MEMORY ENCODING

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Dedication

To Rezvan,

my grandmother,

who has done everything for me and means everything to me.
Abstract

Oscillatory activity is a ubiquitous property of brain signals. The importance of the phase of EEG for processing naturalistic stimuli, which have typically long duration, is still not clear. In this study, we presented word-nonword pairs, each of which was visible for five seconds and measured the effect of EEG phase during stimulus onset on later memory recall. The task consisted of an encoding phase in which 20 word-nonword pairs were presented, followed by a testing phase in which subjects were shown one of the seen words with four target nonwords to choose from. We found that memory recall performance was higher when the words during encoding were presented at a descending phase of the theta oscillation. This effect was the strongest over the frontal cortex. These results suggest that the phase of ongoing cortical activity can affect memorization of persistent stimuli which are an integral part of daily tasks.
Acknowledgements

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Finally, I would like to thank my family and friends who have been the best source of energy and inspiration.
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
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<tr>
<td>iEEG</td>
<td>Intracranial Electroencephalography</td>
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<td>MEG</td>
<td>Magnetoencephalography</td>
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<td>LTM</td>
<td>Long Term Memory</td>
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<td>LIA</td>
<td>Large Irregular Activity</td>
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<td>PET</td>
<td>Positron Emission Tomography</td>
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<td>Short Term Memory</td>
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1. Introduction

1.1 Memory formation, storage and retrieval

Memory consists of the act of registration (learning or encoding), followed by storage which will lead to future retrieval. These three stages have been largely adopted by behavioral and cognitive neuroscientists. The processes that mediate between the experience of an event and the memory formation constitute the encoding phase. It is difficult to differentiate the encoding and storage stages. Based on the modal model (Murdock, 1967), memory storage can be classified into three levels: sensory storage, short-term memory (STM) and long-term memory (LTM). The information can enter the sensory stores whether the subject is paying attention or not and there is an immediate registration of the stimulus within the appropriate sensory dimensions (Atkinson & Shiffrin, 1968). Through control processes in each stage, the information flows from sensory registers to STM and then LTM (Figure 1).

Figure 1. The information flow according to the memory model from Atkinson and Shiffrin (1971)
In the early 1970s, there was evidence that the Modal model had at least two problems. The evidence suggested that if an item is merely held in STM, learning does not necessarily happen, but that learning depends on the level of the processing that an item goes through (Craik & Lockhart, 1972). The second problem for this model was that it had been assumed that STM is crucial for long-term learning. A series of experiments conducted by Baddeley and Hitch (1974), suggested that LTM was not totally dependent on STM. Instead, Baddeley and Hitch replaced STM by a more complex system called “working memory”. In their proposed memory system, there are two short-term stores (phonological loop and visuospatial scratchpad) and a control system (central executive) (Figure 2).

![Diagram of Baddeley and Hitch model of working memory from Baddeley and Hitch (1974)](image)

Figure 2. Baddeley and Hitch model of working memory from Baddeley and Hitch (1974)

The information transfer from STM to LTM is called memory consolidation, which means the memory has been stabilized and becomes more resistant to interference (Stickgold, 2005). Consolidation is thought to originate from the reactivation of the encoded neuronal memory representations during slow-wave sleep (SWS). Retrieval of a memory is the process through which memory attributes are taken from the storage (Estes, 2014). During retrieval, neural states which were experienced during encoding are reactivated (Ritchey, Wing, LaBar, & Cabeza, 2012).
According to the information above, encoding and retrieval can be similar. Based on a computational model, this similarity is relevant to the role of the hippocampus in guiding a replay of the encoded information across neocortex (Sutherland & McNaughton, 2000). In this model, hippocampal neural activity is characterized by two states: theta and large irregular activity (LIA). In the theta state, the activity is driven by the external stimuli, and in the LIA state the patterns of neural activity during the theta state are replayed. In a human study (Ritchey et al., 2012), it has been shown that memory success was correlated with encoding-retrieval pattern similarity in frontal and posterior cortices. This means that if the encoding process is performed properly, the stimuli might be recalled better.

1.2 Electroencephalography (EEG) oscillations and memory

Cortical cell assemblies communicate through brain oscillations (Klimesch, 1996). In the last three decades, researchers have investigated the role of these oscillations in memory formation. Most studies have focused on simple properties of a specific oscillation in a frequency band, for example, power of alpha (Doppelmayr, Klimesch, Stadler, Pöllhuber, & Heine, 2002; Klimesch, 1999). However, the emergence of complex computational methods made it possible to investigate other frequency bands and various oscillatory features, for example, phase-amplitude coupling. Canolty et al. (2006) showed that high gamma power is modulated by theta phase across a range of behavioral tasks including a working memory task.
Neuronal information can flow from one structure to another in the brain. The synaptic path length and effective connectivity are the factors that decide the possible routing schemes for information (Buzsáki, 2006). The frequency of the oscillations is a determinant factor in how far the information can propagate in one step. Hence, low-frequency oscillations can travel further, and therefore, it has been suggested that the low frequency oscillations, for example theta oscillation, favor in synchronizing networks over longer spatial ranges, whereas high frequency oscillations are thought to synchronized the neural assemblies over a shorter distance (Buzsáki, 2006; Jensen & Colgin, 2007). An overview of studies has shown that different frequency oscillations might demonstrate opposing changes in their power in memory-related tasks (Hanslmayr & Staudigl, 2014).

### 1.2.1 Oscillator synchrony

As noted above, the relation between phases of various oscillations has been recently investigated using more complex computational approaches. In recent years, studies ranging from single-unit recordings in animals to EEG and magnetoencephalography (MEG) studies in humans have demonstrated the pivotal role of phase synchronization in memory processes (Fell et al., 2011). Etymologically, ‘synchronize’ stems from the Greek word ‘synchronizein’, meaning ‘be of the same time’. The word ‘synchronization’ may be used differently depending on the context in the neuroscience literature. There are different forms of synchronization among oscillators, and therefore, it is important to distinguish which type of synchronization is the aim of
investigation. As it is explained by Buzsáki (2006), these heterogeneous mechanisms of synchronization are as follows:

a) Mutual entrainment: if two or more oscillators have different frequencies, an intermediate resonating frequency, which is the global frequency of the network, may be produced when they are connected together. Therefore, the system is entrained to oscillate at a frequency that stabilizes the network.

b) Coherence: if there is a constant phase difference between two signals or there is a fixed phase relationship with a third signal that is considered as reference for each, the signals are called coherent.

c) Phase-locking: if two oscillators interact in a way that the phase difference is kept fixed regardless of the amplitude changes, they are phase-locked. For phase-locking or phase-coupling, the events can be non-oscillatory, such as a phase-locked discharge of irregularly spiking neuron and an oscillator. In these cases, the term ‘entrainment’ is used instead.

d) Cross-frequency phase synchrony: if there are two or more oscillators with various integer frequencies, they can be phased-locked at multiple cycles. This is referred to as cross-frequency phase synchrony. In case two oscillators differ in frequency and cannot maintain a fix phase difference, they can produce a transient and systematic interaction, called phase precession or phase retardation.
e) Phase reset: if one or many coupled or independent oscillators of one or many coupled or independent oscillators are enforced to restart at the same phase, phase reset occurs.

1.2.2 Phase synchronization

Phase synchronization is a related phenomenon to phase reset, but with a different mechanism. This phenomenon is a stimulus-induced oscillation in two or more structures with transient coherent phase. One of the phase synchronization mechanisms used for communication is cross-frequency phase-phase coupling. When this coupling happens, in addition to the entire cycle, individual phases of a single cycle of an oscillation with a higher frequency are locked to specific phases of an oscillation with a lower frequency (Fell & Axmacher, 2011). Jensen and Lisman (2005) argued that the cross-frequency phase-phase coupling has an important role in representations (without interference) of multiple items in working memory via consecutive gamma cycles, which are locked to specific theta phases.

Phase-amplitude coupling is another form of synchronization where the phase of a low-frequency oscillation may be synchronized both with the phase and the amplitude of another (usually higher frequency) oscillation. This phenomenon was initially described based on recordings from the rat hippocampus (Bragin et al., 1995). Phase-amplitude coupling was also observed in the human brain using scalp (Demiralp et al., 2007) and intracranial EEG recordings (Canolty et al., 2006).
1.2.3 EEG Acquisition

Neuronal oscillations have a broad spectral content ranging from 0.05 to 500 Hz in the mammalian forebrain (Buzsáki & Draguhn, 2004). These oscillations are characterized by their amplitude, frequency, and phase. In order to study these properties in the time domain, namely phase and amplitude, a temporal resolution of 2 ms to 20 s is required.

EEG, MEG, and intracranial EEG (iEEG) are common recording methods for studying the dynamics of synchronous neuronal oscillations and cognitive processes, such as memory and attention (Ward, 2003). iEEG recordings provide a high signal-to-noise ratio compared to EEG. However, the former is an invasive method that is applied in epilepsy surgeries for preoperative evaluation and functional mapping (Wang, Yan, Wen, Yu, & Li, 2016). Methods such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) record changes in blood flow and the metabolic activity, respectively. These measures have a time resolution varying from seconds to minutes. Hence, in spite of their high spatial resolution, they blur events in time.

Although EEG does not provide a high spatial resolution, it gives a temporal resolution on the order of milliseconds. This temporal resolution makes it easier to track the rapid shifts in brain functioning. Numerous studies have used EEG to study the effect of the amplitude of EEG oscillations on memory recall (Jensen & Tesche, 2002; Klimesch, 1999; Onton, Delorme, & Makeig, 2005). Compared to other methodologies in cognitive research, EEG allows temporally accurate measurements of intracranial current...
flows, is inexpensive, and noninvasive. For these reasons, in our study we made extensive use of 128-channel dense array EEG system at the University of Lethbridge to investigate EEG dynamics in a visual memory task regardless of the underlying functional anatomy of memory encoding and retrieval.

1.2.4 Phase Analysis

One of the properties of the oscillations is phase. The instantaneous phase of a complex function is defined as:

$$\varphi(t) = \arg[s(t)]$$  \hspace{1cm} (1)

Where $\arg$ is argument function (Fig 1). For a real-valued function, the phase is calculated as:

$$\varphi(t) = \arg[s_a(t)]$$  \hspace{1cm} (2)

where $s_a(t)$ is the analytic representation of the function. An analytical function can be expressed in the form of time-varying magnitude and phase and can be written as:

$$s_a(t) = a(t) \exp[j\varphi(t)]$$  \hspace{1cm} (3)

where $a(t)$ is the instantaneous amplitude and $\varphi(t)$ is the instantaneous phase of the real signal $s(t)$ (Picinbono, 1997).

In order to measure the instantaneous phase, two methods have been used for neural signals: wavelet transform and Hilbert transform (le Van Quyen et al., 2001;
Olkkonen, 2006). For wavelet analysis, the convolution of the signal with a complex wavelet is computed. When using Hilbert transform, the analytical function is obtained by convolving the signal with the function \( \frac{1}{\pi t} \). The output of this function in combination with the original signal gives the analytic signal (3) that is a complex valued signal with no negative frequency components. From this function, the instantaneous phase \( \varphi(t) \) is obtained.

Wavelets are localized in both the time and frequency domains, unlike sine and cosine, which are used as basis functions in fast Fourier transform (FFT) and are localized in frequency but infinitely extended in time. Shrinking the wavelet in time results in a more localized resolution in time and will cover a wider frequency range, whereas stretching the wavelet will result in less localization in time and more localization in frequency. Hence, wavelets follow the Heisenberg Uncertainty Principle. This property of wavelet makes them a good choice for analyzing the neuroelectric events that are closely spaced in time.

As mentioned earlier, the Hilbert transform gives the analytic signal of the real signal. In order to calculate a meaningful instantaneous phase some conditions must be met: the signal has to be monocomponent, zero mean locally and symmetric with respect to the zero mean (Huang et al., 2009). However, EEG is not a monocomponent signal. In order to reliably calculate the instantaneous phase, EEG signal should be bandpass filtered to a narrowband. As a result, applying the Hilbert transform necessitates designing bandpass filters for different frequency bands.
Despite the fact that there are minor differences between wavelet and Hilbert transforms, it has been shown that for studying phase synchrony and phase-locking in neuroelectrical signals both methods can be equivalently used with comparable computational complexity (Le Van Quyen et al., 2001).

1.2.5 Filter design

Filtering is one of the preprocessing steps for analyzing EEG data. Although filtering attenuates undesired components of the signal, such as noise or off-target frequencies, it might introduce unintended distortions to the signal.

The first step is to decide what type of filter is needed for analyzing the EEG signal. According to our desired frequency range, we can choose low-pass (attenuating high-frequency band), high-pass (attenuating low-frequency band), band-pass (passes the signal within a certain band), or band-stop (opposite of a band-pass). In our study, we were interested in band-pass filtering the signal. In order to ideally filter the signal to a desired frequency band, a sharp filter with narrow transition band is needed. The steeper the filter is in the frequency band, the longer the order of the filter will be in the time domain. It has been suggested to separately apply a steep high-pass and a shallow low-pass filter over a band-pass filter with steep high-pass and low-pass transition (Widmann, Schröger, & Maess, 2015). If the length of EEG data, as the input, is large enough compared to the order (or length) of the filter (usually three times larger), it is possible to design a steep band-pass filter.
In theory, an ideal filter has a finite bandwidth in frequency domain, and therefore requires an infinitely long filter in the time domain. This is impossible as all recorded data are finite. The solution is to multiply the impulse response of the filter by a window to make it finite, but this introduces sidelobes outside the desired frequency band. The choice of the window can minimize these adverse effects. From the common window choices, such as rectangular, Hamming, Hanning, Blackman, and Kaiser, we chose the Kaiser window, as it gives acceptable attenuation outside the desired frequency band and minimum passband ripple (deviation from desired frequency response within the passband), which minimizes signal distortion.

We next needed to decide to use either a finite impulse response (FIR) or an infinite-impulse response (IIR) filter. In order to make this decision, there are some factors to consider. Although IIR filters are computationally more efficient, they have a non-linear phase, which introduces different delays to different frequency bands. However, with FIR it is always possible to achieve a linear phase, which introduces an equal (group) delay at all frequency bands. This means that a certain amount of delay is applied to all the samples of the input signal in time domain. This property enables us to use a two-pass filtering (filtering the output of the filter in the reverse direction) approach and compensate for the filter delay, which can be achieved through using the `filtfilt` function in MATLAB.

Taking into account these considerations, we chose to design a linear phase FIR band-pass filter with the Kaiser window using `fdatool` from MATLAB.
2. Phase of the Low Frequency Brain Oscillations

2.1 Introduction

Oscillations have been documented in the brains of mammalian species, ranging from very slow (with periods of minutes) to very fast (with periods of milliseconds). In rats, all frequencies from 0.02 to 600 Hz are continuously present (Buzsáki, 2006). Frequency band categorization in humans, however, follows the classification of the International Federation of Societies for Electroencephalography and Clinical Neurophysiology from 1974, which were based on the limitations of the recording devices used at the time. Nonetheless, we adhered to this convention, and the frequency band borders were chosen as: delta: 0.5 - 4 Hz, theta: 4 – 8 Hz, alpha: 8 - 12 Hz, beta: 12 - 30 and gamma: >30 Hz.

Slow wave oscillations during sleep play a crucial role in memory consolidation (Buzsáki, 1998; Marshall, Helgadóttir, Mölle, & Born, 2006). SWS (< 1 Hz) is characterized by slow sequences of hyperpolarization (down state) and depolarization (up state) with high amplitude (Achermann & Borbely, 1997; Steriade, McCormick, & Sejnowski, 1993). During the down state, neurons are silent (Plenz & Kitai, 1998) whereas the up states are characterized by irregular spike discharge at low frequency as well as by burst firing (Wilson & Groves, 1981). The enhancement of memory consolidation during sleep depends on the timing of the stimuli. If the stimuli are delivered in phase with the ongoing rhythmic occurrence of the up states of slow wave oscillation, memory consolidation is facilitated (Ngo, Martinetz, Born, & Mölle, 2013). In another study, presenting milliseconds long stimuli at the trough of the alpha oscillation
suppressed the cortical activation after the stimulus onset and made participants less likely to detect the target in a metacontrast masking paradigm (Mathewson, Gratton, Fabiani, Beck, & Ro, 2009). Batterink, Creery, and Paller (2016) showed that during sleep, the phase of slow oscillations at the time of targeted memory reactivation affected later recall. Participants were asked to learn objects paired with a characteristic sound, for example, cat – meow. Then, half of the sounds were presented during SWS periods of sleep. Cues were more likely to be forgotten or remembered depending on the phase of slow oscillation prior to stimulus presentation. This demonstrated that there is an optimal phase for memory consolidation during sleep. However, it has not been shown whether this holds true when the participants are awake.

2.2 Phase of theta oscillations

Theta oscillation (4-8 Hz) can contribute to successful encoding. Klimesch, Doppelmayr, Russegger, and Pachinger (1996) showed that the amplitude of brain oscillations in the theta frequency positively affected later retrieval success. Pre-stimulus thalamic theta power predicted successful and unsuccessful encoding in humans (Sweeney-Reed et al., 2016). Between-area phase synchrony in theta frequencies has been reported to be predictive of short-term memory performance (Liebe, Hoerzer, Logothetis, & Rainer, 2012). Successful memory formation was linked with tight coordination of spike timing with the local theta oscillation (Rutishauser, Ross, Mamelak, & Schuman, 2010). Theta oscillations have been observed in many structures, including hippocampus (Buzsáki, 2002; Cantero et al., 2003; Rutishauser et al., 2010) and amygdala (Rutishauser et al., 2010). The phase of low frequency oscillations at the onset of stimuli seems to play
a significant role, not only during sleep but also when the participants are performing a memory-related task during daytime. However, whether the phase of theta oscillations at the onset of the stimuli modulates encoding of information has not been determined.

In this study we used EEG to test the hypothesis that low frequency oscillations, namely theta, might play a role in encoding sensory information. We recorded the EEG signal over the scalp while human subjects performed a word-nonword pair matching experiment. We tested if stimuli presentation at a particular phase of low frequency oscillations would affect later recall.

2.3 Methods

2.3.1 Participants

Undergraduate students (n=18) from the University of Lethbridge were recruited from introductory neuroscience courses and participated for course credit. Participants provided informed written consent. All procedures were in accordance with the declaration of Helsinki and were approved by the University of Lethbridge Human Subjects Review Committee. Subjects reported normal vision and no neurological conditions. Only EEG data from participants who correctly responded at a rate higher than chance (>25% correct) were analyzed. Thus, 11 participants (6 female; average age 19.8, SD = 2) contributed to the data analysis.

2.3.2 Stimuli and Procedures

Subjects were comfortably seated 57 cm away from a 17-inch LCD monitor, with a refresh rate of 60 Hz. Figure 3 shows the structure of the modified word-nonword pair
matching task adapted from (Mander et al., 2013) using Psychophysics Toolbox Version 3. The task consisted of four blocks, each of which was divided into two phases: learning and testing. The learning (or encoding) phase contained 20 trials. In each trial a word-nonword pair was presented to the participant for five seconds followed by a one second interstimulus interval. Word-nonword pairs appeared with a white fixation square at the centre of the monitor. EEG collection was time-locked to the onset of stimulus. Each word-nonword pair was comprised of a word and a nonword which were derived from a normative database (Buchanan, Holmes, Teasley, & Hutchison, 2013) and the ARC nonword database (Rastle, Harrington, & Coltheart, 2002), respectively. All stimuli were presented on a light grey background. In the self-paced testing phase, the participant was asked to match the word with the nonword in the format of a multiple choice question as it is shown in Figure 3. Twenty word-nonword pairs which were previously seen during the learning phase were presented along with 20 new words. Thus, there were 40 multiple choice questions in the testing phase.

Each participant completed four blocks. Short rest breaks (no more than one minute) were given between the blocks. Participants were given verbal instruction before the experiment began and the participants were asked to minimize their eye and body movements.
2.4 EEG Acquisition and Analysis

EEG was recorded with 128 Ag/Ag-Cl electrodes in an elastic net (Electrical Geodesics Inc., Eugene, OR, USA). Scalp voltages were recorded at a 500 Hz sampling rate and impedances were maintained under 100 kΩ. Data were high-pass filtered at 0.1 Hz to remove DC offset, re-referenced offline to average and analyzed using the BESA software package (Megis Software 5.3, Grafelfing, Germany). All the channels
were visually inspected for poor signal. The signal from a small number of electrodes (10 or less) showing poor quality was replaced with an interpolated signal from neighboring sites. Because of the length of the trials, eye movement artifacts occurred in a majority of trials. Therefore, eye movement artifacts were corrected using the adaptive artifact correction algorithm (Ille, Berg, & Scherg, 2002). The channels from the standard montage were used for analysis. Data were exported from BESA and further analyzed in MATLAB (MATLAB version 8.3.0.532; The Mathworks Inc., 2014, Natick, MA, USA) using custom scripts and EEGLAB functions (Delorme & Makeig, 2004).

For phase analysis, we first bandpass filtered the data to 0.5 and 30 Hz. For this reason, we designed a FIR ban-pass filter with Kaiser window using \texttt{fdatool} from MATLAB (Figure 4). EEG data were then epoched from -200 ms before to 1000 ms after each stimulus. The period from -200 ms to 0 ms was considered as the baseline, and the average EEG amplitude in the period was subtracted from each trial. In order to calculate the phase angle and power for each trial we used Morlet wavelet transform of single trials using \texttt{timefreq} function of EEGLab. Wavelet transformation was computed with one Hz steps and cycles starting from 0.5 for 0.5 Hz and 15 for 30 Hz. The output of this function is a matrix of complex numbers. Thus, for calculating the phase angle of each trial we used \texttt{angle} function from MATLAB. In order to ensure that our results were not dependent on this particular wavelet transform, we also used the Hilbert transform to calculate phases at the onset of the stimuli (Le Van Quyen et al., 2001). Both methods gave consistent results.
2.5 Results

2.5.1 Behavioural results

In the word-nonword pair matching task, if the participant correctly matched the previously studied word with the paired nonword, it was labeled as ‘hit’, and if the participant incorrectly matched the word with other choices it was labeled as ‘nonhit’. As an example, consider Figure 3.A; if the participant would choose the nonword ‘hirmbth’ in the testing phase, it is called a hit and all other choices, nonhits. In our word-nonword pair matching task, the participants correctly matched (64.7 ± 5.9 SEM) % of the words over all the four blocks (chance level = 25%, Figure 5).
2.5.2 P200 component

Event-related potentials (ERPs) provide a way to compare the differences in average EEG amplitude between experimental conditions. We investigated the difference between one of the components of the grand average ERP (Figure 6.A). In particular, we investigated the P200 component, which has been reported to be modulated by different cognitive processes such as short-term memory (Golob & Starr, 2000) and selective attention (Hillyard, Hink, Schwent, & Picton, 1973). Consistent with other studies (Kenemans, Kok, & Smulders, 1993; Key, Dove, & Maguire, 2005), we observed a pronounced P200 component at the frontal sites around 150-200 ms after the stimulus onset (Figure 6.A). Based on paired two-tailed t-test results, there was no significant difference in the magnitude of the P200 component for hits ($M = 2.41 \mu V, SEM = 0.41 \mu V$) and nonhits ($M = 2.62 \mu V, SEM = 0.35 \mu V$); $t_{(10)} = 0.43, p = 0.68$. This suggests that
the amplitude of P200 component, which is a typical EEG measure of cognitive processes, is not sensitive to performance in our memory encoding task.

**2.5.3 Phase of the EEG signal at the onset of the stimuli and memory performance**

Next we investigated if other features of the EEG signal correlate with performance in our memory task. In Figure 6.A, we show that the ERP amplitude of hits and nonhits is different at [-38, -26] ms before the onset of the stimulus (p<0.05 for all points in that interval; two-tailed paired t-test). Considering previous work (Batterink et al., 2016), we hypothesized that this pre-stimulus difference could be the result of different EEG phases at stimulus onset, which could affect memory encoding. To obtain EEG phase information, we used wavelet decomposition (see Methods). For each subject we calculated the average phase at the stimulus onset for hit and nonhit trials. We found that for the frequency band ~ 4-5Hz at a frontal channel (F3), hit trials had a tendency to start at a descending phase (Figure 6.B, C), while nonhit trials did not show specific phase preference.
Figure 6. (A) Grand average ERP for channel F3 plotted as a function of time. Time = 0 corresponds to the onset of the stimuli. Shaded regions demonstrate the SEM at each time point. Horizontal bar denotes the interval [-38, -26] ms, where the amplitude of the hit and nonhit are significantly different before the onset of the stimuli. (B) Circular plot for hits (blue) and nonhits (red) for channel F3, f ~ 4-5 Hz at the onset of the stimuli. Each blue circle on the unit circle demonstrates the average phase of the hit trials for a subject. For visualization, the red circles, which show the average phase of the nonhits for the subjects are depicted on a circle with a smaller radius. The blue and the red bar show the preferred phase of successful memory encoding.
direction and magnitude of the average phase over all the subjects for hits and nonhits, respectively. (C) One cycle of a sinusoidal wave illustrating the preferred phase of successful memory encoding.

In order to determine if this effect is spatially restricted, we used the Rayleigh test to check whether the phase angles (across trials) are uniformly distributed for each condition. The test was performed on the phase angle of all the hit versus nonhit trials, and consisted of 569 hit and 311 nonhit trials for each channel. Figure 7 shows the p-values for $f \sim 4-5$ Hz at the onset of the stimuli for 19 channels on the scalp. As seen from the figure, the angle phase of hit trials is significantly directional ($p < 0.01$) compared to the nonhit trials. This effect was most pronounced in the frontal regions on the scalp.

Figure 7. P-values from the Rayleigh test for 19 channels on the scalp depicted using topographic maps for (A) hit and (B) nonhit trials at the onset of the stimuli calculated for $f \sim 4-5$ Hz. The angles for each condition were tested to check whether they were uniformly distributed using Rayleigh test. The color bars indicate p-value, and a lower p-value means higher directionality in the phase distribution.
3. Discussion

The present study investigated the effect of the phase of slow oscillations on memory recall using a word-nonword pair matching experiment. We demonstrated that the phase of the theta oscillations at the onset of the stimuli affects memory recall. The phase of the hit and nonhit trials was calculated and compared using the Rayleigh test. We observed that if the stimuli were delivered at a specific phase of theta oscillations, the participants were more likely to match the nonword with the correct word. This is the first study to show that for a memory task similar to many daily tasks, the phase of theta oscillation affects the success of later recollection.

Our results support the idea that theta oscillations play a crucial role in memory-related processing, as was previously postulated by studies done in monkeys (Liebe et al., 2012) and humans (Rutishauser et al., 2010). Theta oscillations are associated with induction of synaptic plasticity (Huerta et al., 1995), which underlies memory formation. Increases in theta power before the onset of the stimuli in the hippocampus and neocortex predict heightened memory performance in human participants with implanted electrodes (Sweeney-Reed et al., 2016). Theta coupling has been shown between cortical area V4 and prefrontal cortex in a monkey study on working memory maintenance (Liebe et al., 2012). In our study, we have shown that the phase of the theta oscillation in the occipital and frontal regions is a good indication of the performance of the participants.

Our results provide evidence that phase synchronization of theta oscillation is not prominent over the entire scalp. This effect is seen in the frontal and occipital regions on
the scalp (Figure 7). Since both regions are phase-locked to the onset of the stimuli, this suggests that there is a phase-related functional connectivity between these regions. It has been shown that neuronal communication is facilitated by oscillatory synchronization within a group of neurons sending a message along with coherence (or phase-locking) between the oscillations in the sending and receiving group (Fries, 2005). There is also abundant evidence that the prefrontal cortex, in particular the medial prefrontal cortex (mPFC), is involved in memory consolidation (Lynch, 2004; Marshall et al., 2006; Peyrache, Battaglia, & Destexhe, 2011). This suggests that the frontal regions are involved in encoding or gating the information.

In our study, we found no significant difference between hits and nonhits regarding the P200 component. Dunn, Dunn, Languis, and Andrews (1998) demonstrated that lowrecallers generate greater frontal P200 amplitude and smaller parietal/occipital amplitudes than highrecallers while encoding the words across two different (serial-order and category) memory tasks. However, in our study we focused on comparing two conditions rather than comparison across subjects with high and low memory performance. It has been shown that the amplitude of the P200 component was correlated with memory load over the parietal electrodes (Missonnier et al., 2007). Although in our task memory load does not vary across trials, it can be further investigated whether there is a significant difference in P200 amplitude in parietal regions of the brain. If P200 merely indexes mechanisms of selective attention (Hillyard et al., 1973), it can be argued that a significant difference in the amplitude of P200 should be expected between hit and nonhit trials. However, our comparison is performed across all the subjects, and since
there are strong individual differences in the P200 component (Dunn et al., 1998), the difference within conditions might have been averaged out.

Taken together, our findings suggest that phase of the low frequency oscillations, namely theta, has a role in gating the information during the encoding phase. If the stimuli are presented at a specific phase of theta oscillations, the participants are more likely to recall the word-nonword pairs later. We have shown that the phase of theta oscillation at the onset of the visual stimuli enhances the encoding of information during wakefulness. At the population level, information in cortical areas is thought to be processed not continuously but in the form of discrete packets with a sequential structure (Luczak, McNaughton, & Harris, 2015). We speculate that if the stimuli are not delivered at the correct phase of the occurring packets, it might be less optimal to integrate the information into the ongoing process. Hence, our observations are consistent with the proposition that the phase of low frequency oscillations in frontal regions could be a fundamental component in the encoding of information.
4. Conclusion

Brain oscillations are fundamental to neuronal communication within cell assembly networks. Low frequency oscillations have been shown to play an important role in memory formation. Theta oscillations, although mostly investigated in rodents, have implications in memory formation in humans (Cantero et al., 2003; Kahana, Sekuler, Caplan, Kirschen, & Madsen, 1999; Rutishauser et al., 2010). Our word-nonword pair matching memory task provided further evidence that the phase of theta oscillations might affect encoding and later retrieval, accordingly.

In this study, we showed that if the stimuli were delivered at a specific phase of theta oscillations, the recall success increased. The fact that theta oscillations affect the memory performance is in line with previous research that showed theta oscillations have been implicated in memory formation (Klimesch, 1999; Klimesch et al., 1996; Rizzuto, Madsen, Bromfield, Schulze-Bonhage, & Kahana, 2006). More importantly, the phase of theta oscillations at the onset of the stimuli contributed to encoding the information so that it could be recalled later. This is in line with a study conducted by (Rutishauser et al., 2010) in which they showed that neurons in the hippocampus and amygdala were firing phase-locked to the theta oscillations (±45° around the peak or trough) when participants remembered the stimulus. This phase locking effect was most pronounced in the frontal regions of the scalp, which supports the results of Siapas et al., (2005) where they suggested that neurons in the medial prefrontal cortex of freely behaving rats fire phase-locked to the hippocampal theta oscillations, which may be important for the formation of long-term memory.
These findings suggest that the phase of ongoing cortical activity can affect the ability to remember persistent stimuli, which are an integral part of many daily tasks.
References


