Hippocampal gamma oscillations increase with working memory load

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Abstract

Functional imaging has implicated a diverse network of brain regions supporting working memory—the capacity to maintain multiple item representations over brief intervals. However, considerable controversy surrounds the role of the hippocampus in working memory. To simultaneously examine both hippocampal and neocortical contributions to working memory, we recorded electroencephalographic activity from 1,454 widespread cortical and subcortical sites as 20 neurosurgical patients performed working memory tasks involving either letters or faces. These recordings revealed significant increases in 28–128 Hz gamma oscillatory power and significant decreases in 4–9 Hz theta oscillatory power with increasing memory load for both letters and faces. Sites exhibiting gamma increases with memory load appeared primarily in the hippocampus and medial temporal lobe, whereas those exhibiting decreases in theta activity appeared primarily in visual and temporal cortex. These findings implicate theta and gamma oscillatory activity in the maintenance of both letters and faces in working memory, and they provide the first direct evidence for modulation of hippocampal gamma oscillations in a working memory task.

Key words: oscillations, working memory, ECoG, memory, EEG, hippocampus

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1 Introduction

Working memory refers to keeping information in mind to guide subsequent behavior (Postle, 2006). This form of memory has been posited to be functionally and neurally distinct from episodic memory, which refers to memories that are accompanied by distinct details about the context in which the memory was encoded (Atkinson and Shiffrin, 1968; Baddeley and Hitch, 1974). Whereas episodic memory depends on the hippocampus and medial temporal lobe (MTL) structures, working memory is thought to be largely mediated by the prefrontal cortex (PFC, Curtis and D’Esposito, 2003; Goldman-Rakic, 1987) with little or no hippocampal involvement (Baddeley and Warrington, 1970; Squire et al., 1993). However, several recent studies have shown hippocampal involvement in working memory for complex stimuli (e.g., Holdstock et al., 1995; Nichols et al., 2006; Ranganath, 2006; Stern et al., 2001).

Spectral analyses of intracranial electroencephalography (iEEG) recordings during working memory have revealed distinct oscillatory correlates of memory load in the gamma (28–128 Hz) frequency band (Axmacher et al., 2007; Howard et al., 2003; Meltzer et al., 2008). In a study on two patients, Howard et al. (2003) found increases in gamma power with working memory load for
letters in frontal and temporal cortex. A larger study of working memory for digits by Meltzer et al. (2008) showed increases in gamma power with memory load during the delay period in widespread cortical regions. In a study of working memory for faces, Axmacher et al. (2007) found that gamma activity in MTL increases with memory load. Their gamma effect was significant in the rhinal cortex, but not statistically reliable in the hippocampus (although they did observe significant load-dependent decreases in the D/C potential in the hippocampus). In addition to load-related changes in gamma oscillations, theta oscillations have also been found to correlate with memory load, but the direction of those correlations is not consistent across brain regions, stimulus materials, or even participants (Meltzer et al., 2008; Michels et al., 2008; Jensen and Tesche, 2002; Howard et al., 2003).

None of the studies cited above have explicitly shown load-dependent oscillatory activity in the hippocampus, an issue that is of crucial importance for the debate over the involvement of the hippocampus in working memory. A related question is whether hippocampal involvement differs depending on the nature and verbalizability of the stimuli. To address these questions, we analyzed a large iEEG dataset on working memory for two classes of stimuli: faces and letters. We developed region-of-interest (ROI) analyses to assess the regional specificity of oscillatory effects in key regions that have been previously implicated in episodic memory and working memory functions.

[Fig. 1 about here.]
2 Methods

Twenty neurosurgical patients (12 male, Mean age=32, SD=13) participated in a Sternberg (1966) short-term recognition memory paradigm involving lists of synthetic faces (Figure 1) or the consonants of the Roman alphabet (henceforth letters). We conducted the study while patients were being monitored with subdural and/or depth electrodes for seizure activity and functional mapping. Patients were enrolled at the epilepsy monitoring units of the Hospital of the University of Pennsylvania, Philadelphia, Brigham & Women’s Hospital in Boston, and the Universitats Klinikum Freiburg in Germany. Our research protocol was approved by the institutional review boards at these institutions, and informed consent was obtained from all participants. Electrode placements were determined solely on the basis of clinical considerations. The 20 participants contributed a total of 1,454 electrodes distributed across varied brain regions.

Figure 1 illustrates the sequence of events during each trial of the experiment. Following the appearance of an fixation stimulus (an asterisk that appeared for 1000–1075 ms, jittered) participants viewed a short series of letters or faces for study (each appeared for 700-775 ms followed by a 275-350 ms inter-stimulus interval). Following a 3 s – 3.3 s retention interval, a probe item appeared and participants indicated with a keypress whether the probe was a member of the just presented series (a target) or an item not shown on the current list (a lure).
After each trial, participants were given accuracy feedback. The participant advanced to the next trial with a key press. Temporal jitter was employed to avoid spurious correlations between ongoing oscillations and the structure of the task. The experiment was programmed in the freely available Python Experimental Programming Library PyEPL (http://pyepl.sourceforge.net; description in Geller et al., 2007).

Lists comprised 1, 2, 3, or 4 letters, or 1, 2 or 3 faces (fewer faces were used because accuracy drops off quickly when going from three to four faces), and the face and letter trials were presented in separate blocks of 15 lists long. Study items were constrained such that none could be repeated on successive lists. Lists were constructed so that the frequencies of targets and lures and each of the list lengths were matched. In addition, targets were equally likely to match a study item from each serial position. Every session was preceded by two 16-trial training blocks, plus 40 additional one-item lists to familiarize the participant with the face stimuli. Participants were given feedback on their average accuracy and RT at the end of each block. Incorrect trials and trials with RTs shorter than 200 ms or longer than 3500 ms were removed from the analysis.

Rather than using photographs of faces, we adopted a set of synthetic faces derived from real photographs but filtered to remove removes components like hair and skin texture while preserving the shape, size and position of key facial features (Wilson et al., 2002). These faces are nonetheless realistic
enough to generate strong responses in the fusiform face area (Loffler et al., 2005). Detailed information concerning the specific set of faces used in the present study are provided in Pantelis et al. (2008).

The iEEG signal was amplified and digitally recorded at sampling rates between 250 and 1024 Hz (depending on the clinical site). Data were subsequently notch-filtered with a Butterworth filter with zero phase distortion between 48 and 52 Hz or 58 and 62 Hz to eliminate the relevant line noise. Intervals of interest in the EEG signal were scanned for artifacts by means of a kurtosis threshold; events were discarded if their kurtosis exceeded a threshold of 5 (Delorme and Makeig, 2004).

To synchronize the electrophysiological recordings with behavioral events, the experimental computer sent pulses through the parallel or USB port via an optical isolator into an unused recording channel or digital input on the amplifier. The time stamps associated with these pulses aligned the experimental computer’s clock with the iEEG clock to a precision well under the sampling interval of the iEEG recording (< 4 ms). For all participants, the locations of the electrodes were determined by means of co-registered post-operative CTs and pre-operative MRIs, or from post-operative MRIs, by an indirect stereotactic technique, and converted into MNI coordinates. Localization in the hippocampus was done manually through clinicians’ inspection of the post-operative MRIs.
Data analysis. Oscillatory power was computed using six-cycle Morlet wavelets (Schiff et al., 1994) at logarithmically spaced frequencies ($2^{x/4}$ Hz for $x \in 4, \ldots, 48$). We then converted absolute power into $z$-scores normalized by the mean and standard deviation of the power during the fixation interval preceding each trial. We combined oscillatory power in 7 frequency bands (delta 2–4 Hz; theta 4–9 Hz; alpha 9–14 Hz; beta 14–28 Hz; low gamma 28–48 Hz; mid-gamma 48–90 Hz; high gamma 90–128 Hz). During the maintenance interval, we computed mean power between 750 and 3000 ms after onset of the last study item (to avoid stimulus-related processing in the first 750 ms after stimulus onset).

We then regressed each trials’ list length on the $z$-transformed oscillatory power for each channel and frequency, and determined the $t$-statistic and $p$-value for these regression coefficients. For every regression, a corresponding permutation distribution with 1000 iterations was computed by randomly re-assigning each oscillatory power value (dependent variable) to each list length (independent variable) and recomputing the regression coefficients. This created a distribution of regression coefficients corresponding to the null hypothesis that there is no relation between oscillatory power and memory load. Within each frequency band, we then took the regression coefficient with the minimum $p$-value for the subsequent analysis, in which we compared it to the permutation distributions of $p$-values at the corresponding frequency.

Wavelets are currently the standard method for quantifying oscillatory brain activity. See van Vugt et al. (2007) for a detailed comparison of oscillation detection methods, including wavelets, multitapers and $P_{epi,ode}$. 

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All our analyses were done in regions of interest that have been previously associated with memory and attention (Table 1). For the individual-electrode analyses, we counted the number of electrodes in each region with a significant $p$-value in positive or negative direction. Significance was compared to a threshold of $p = 0.0002$, which corresponds to a false discovery threshold of 0.001. A false discovery rate (FDR) of 0.001 indicates that 0.1% of the significant Brodmann areas across all frequency bands and time intervals are false positives. Note that this differs from conventional $p$-value testing, where a $p$-value of 0.05 indicates that on average 5% of cases that in fact do not reject the null hypothesis will be labeled as significant.

To determine whether any region of interest showed an aggregate effect, we adapted methods developed by Sederberg et al. (2007a). For each participant, we aggregated across all electrodes in each region of interest by taking the mean of the inverse normal transformed $p$-values (thereby turning them into $z$-values), and the same was done for the permutation distribution. We then combined across participants by summing the thus-obtained $z$-values, for both the empirical data and the permutation distribution. The significance of each Brodmann area and frequency combination was determined by finding where each empirical summed-$z$-value fell within the permutation distribution. In
other words, a summed-$z$-value larger than all permuted summed-$z$-values would have a very small $p$-value. The resulting $p$-values were corrected for multiple comparisons using a FDR threshold of 0.001.

3 Results

Participants’ mean accuracy was 92.1% for letters and 67.2% for faces. Their mean reaction time (RT) for correct trials was 1283 ms for letters and 1742 ms for faces. The finding of superior accuracy and shorter response times for letters than for faces replicates several previous studies (Jacobs et al., 2006; van Vugt et al., submitted; Hwang et al., 2005).

[Fig. 2 about here.]

We first asked whether oscillatory activity during the maintenance interval varied with memory load across individual electrodes in five brain regions of interest (ROIs): dorsolateral PFC (DLPFC), perceptual regions, temporal cortex (TC), MTL, and hippocampus (see Table 1 for Brodmann areas associated with each of these regions). Electrodes in widespread cortical and subcortical regions (see Table 1 for Brodmann areas associated with each of these regions and Table 4 for the number of electrodes and participants in each of these regions) showed increases in high-frequency oscillatory power and decreases in low-frequency oscillatory power with memory load. Figure 2 shows the proportion of electrodes exhibiting a significant correlation ($p < 0.0002$,
corresponding to a false discovery rate of 0.001) between $z$-transformed oscillatory power and memory load across frequency band (delta, theta, alpha, beta, and gamma; see Methods), ROI, and stimulus class (faces vs. letters). Overall, theta power decreased ($\chi^2(1)=4.6$, $p < 0.05$), while gamma power showed a trend to increase with memory load.

The increase in gamma with memory load was statistically significant in all ROIs except DLPFC and perceptual regions (all other $\chi^2(1) > 4.57$). The decrease in theta with memory load was statistically significant in the DLPFC, perceptual regions, and temporal cortex ($\chi^2(1) > 3.9$, $p < 0.05$), and it was marginally significant in the hippocampus ($\chi^2(1)=3.6$, $p < 0.1$). The theta load effect was not significant in the MTL. Relative to other areas, the theta load effect was largest in left perceptual regions ($\chi^2(1)=14.1$, $p < 0.001$).

As described above, we observed significant increases in gamma and decreases in theta with memory load in most of the ROIs considered (Figure 2). We next asked whether these effects differed for faces and letters. Overall, we did not observe a significant difference in the numbers of electrodes exhibiting gamma increases for letters vs. faces ($\chi^2(1)=0.25$, n.s.), nor a difference in the number of electrodes exhibiting theta decreases with memory load for letters vs. faces ($\chi^2(1)=2.4$, n.s.). When looking specifically at the hippocampus, we also did not observe any difference between faces and letters for either the gamma or the theta memory load effects. We did, however, observe a somewhat stronger gamma effect for faces in the MTL ($\chi^2(1) = 7.37$, $p < 0.01$) and a stronger
Taken together, our individual electrode analyses reveal that gamma activity increases with memory load and theta activity decreases with memory load in a number of brain regions, and in particular in the hippocampus. We next sought to determine whether consistent memory load effects within ROIs generalize across participants, whose electrode distributions are highly variable due to clinical considerations. To do this we first determined the aggregate load effect for each participant within an ROI and then statistically evaluated the consistency of these effects across participants (see Methods).

Consistent with the individual electrode analyses shown in Figure 2, the aggregate ROI analysis also revealed significant increases in gamma power with memory load in a number of ROIs, including the hippocampus (see Table 3). This analysis also confirmed that theta power decreases with memory load across most ROIs, including the left hippocampus.

4 Discussion

For letters and abstract faces, we demonstrated that in the human hippocampus, 28–128 Hz gamma oscillatory power increased with memory load during the maintenance interval of a working memory task. At the same time, hip-
Pocampal 4–9 Hz theta power decreased with working memory load. This theta-gamma pattern was also observed in other attention- and memory-related regions. In the hippocampus, the theta decrease and gamma increase did not significantly differ between faces and letters.

A previous iEEG study of working memory for faces (Axmacher et al., 2007) did not observe statistically significant load-dependent hippocampal oscillatory activation. The stimuli in that study comprised trial-unique photographs of faces, whereas in our study we used a relatively small stimulus set. Using the same set of stimuli repeatedly, as we have, requires participants not only to remember whether they have seen probe stimuli before but also on which list. As such, in our experiment participants had to rely more strongly on retrieval of the relevant list context, a component of episodic memory (e.g., Dennis and Humphreys, 2001; Howard and Kahana, 2002; Polyn and Kahana, 2008), which might promote hippocampal involvement.

Lesion studies in animals and humans (Sidman et al., 1968; Wickelgren, 1968; Cave and Squire, 1992; Mayes et al., 2002) have shown that short-term maintenance of stimuli does not require the hippocampus. Neuroimaging studies have shown similar results (e.g., Zarahn et al., 2005). Hence a dominant viewpoint is that working memory is hippocampus-independent, whereas episodic memory requires the hippocampus (Baddeley and Warrington, 1970; Squire et al., 1993). However, recent studies (e.g., Axmacher et al., 2007, 2009; Holdstock et al., 1995; Ranganath and Blumenfeld, 2005; Ranganath, 2006; Schon
et al., 2004; Stern et al., 2001) have started to question this assumption. They found that the hippocampus and MTL may be required for working memory as well, especially in the maintenance of complex or trial-unique visual stimuli that do not lend themselves to easy verbal rehearsal. This is supported by studies that find impairment in short-term memory performance (delayed non-match to sample) for visual, but not verbal stimuli in patients with hippocampal lesions (Owen et al., 1997). Based these studies, we expected to see load-dependent hippocampal activation, and increased hippocampal load dependent activity for non-verbalizable stimuli than for letters (Ezzyat and Olson, 2008).

In addition, we expected differences in lateralization of the effects, given that Coleshill et al. (2004) found that stimulation of the left hippocampus in epileptic patients impaired verbal recognition memory, whereas stimulation of the right hippocampus impaired face recognition memory. These hypotheses were not supported by our data. We did observe load-dependent hippocampal activation, but lateralization was only found in other brain areas. For faces, there were more regions that showed a load-related gamma increase in the left hemisphere than in the right (Table 3). For letters, the opposite applied. Faces and letters are likely to use very different mechanisms of maintenance, in which letters can easily be verbally repeated, and faces cannot (Cabeza and Nyberg, 2000; Baddeley, 2003). It has been proposed that gamma increases with memory load reflect maintenance of the to-be-remembered stimuli (Jensen et al.,
2007; Jokisch and Jensen, 2007; Klimesch et al., 2007), or alternatively for-
formation of episodic memories, when those gamma increases are found in the
MTL (Fell et al., 2001; Sederberg et al., 2007b). However, the similarity of
hippocampus load-related activity for faces and letters in our study suggests
that both invoke the use of a common mechanism such as tagging the to-be-
remembered stimulus with an episodic context (Howard and Kahana, 2002).
Adding a context tag to a to-be-remembered item will allow it to be retrieved
by cueing with context later on.

In addition to the gamma effect, we observed decreases in theta with memory
load in many regions. Generally, increases of oscillatory power with memory
load have the intuitive interpretation of reflecting an increased used of cogni-
tive and neural resources when the task is more difficult. Conversely, in our
study we observed decreases in oscillatory theta power for the more difficult
condition, mirroring findings in scalp EEG by Jacobs et al. (2006). Decreases
in oscillatory power could reflect more localized activity, since less synchro-
nization of oscillatory activity corresponds to a reduction in oscillatory power.
More localized oscillatory activity could serve to reduce interference (Doesburg
et al., 2008). This is a particularly attractive hypothesis given that theta oscil-
lations are known to be synchronized over relatively large distances compared
to faster oscillations (Bullock et al., 1990), and are therefore thought to bring
together information from distant brain areas. More localized theta could thus
reduce interference from on-going external and internal stimulation.
In brief, we showed oscillatory correlates of memory load in a wide variety of brain regions, including most notably the hippocampus. Theta oscillatory power decreased and gamma oscillatory power increased with memory load, for both letters and faces. These findings provide the first direct evidence for modulation of hippocampal gamma oscillations by memory load in a working memory task.

References


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2  **Single electrode analyses by brain region during the maintenance interval.** Fraction of electrodes showing a significant increase (solid) or decrease (dotted) of oscillatory power with memory load. The frequency bands are $\delta$=delta (2–4 Hz), $\theta$=theta (4–9 Hz), $\alpha$=alpha (9-14 Hz), $\beta$=beta (14–28 Hz), $\gamma_1$=gamma$_1$ (28–48 Hz), $\gamma_2$=gamma$_2$ (48–90 Hz), $\gamma_3$=gamma$_3$ (90–128 Hz).
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<thead>
<tr>
<th>Region</th>
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<td>perceptual (perc)</td>
<td>BA 19, 37</td>
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<tr>
<td>temporal cortex (TC)</td>
<td>BA 20, 21, 22</td>
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<tr>
<td>MTL</td>
<td>BA 28, 35, 36</td>
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<tr>
<td>hippocampus (hipp)</td>
<td>hippocampus</td>
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Table 1
Definitions of the regions of interest.
Table 2
Number of electrodes and unique participants in each region of interest.

<table>
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**Load effects in regions of interest (ROIs).** For every combination of frequency (columns), hemisphere (columns) and brain region (rows), significant effects are indicated with F(ace) or L(etter). Significant increases of oscillatory power with memory load for faces are indicated with F+, and decreases with F-. For letters, the coding is L+, and L-. The superscripts in the gamma columns indicate the sub-bands of gamma: low (28–48 Hz), medium (48–90 Hz), or high (90–128 Hz). The ROIs are the dorsolateral prefrontal cortex (DLPFC), perceptual regions (perc), temporal cortex (TC), medial temporal lobe (MTL) and the hippocampus (hipp).