**Distribution Agreement**

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature: _____ Date: _____

John Trimper

Object Recognition Memory and Gamma Synchrony in the Rat Hippocampus

By

John B. Trimper

Master of Arts

Psychology

_____

Joseph Manns

Advisor

_____

Patricia Bauer

Committee Member

_____

Robert Hampton

Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.

Dean of the James T. Laney School of Graduate Studies

_____

Date

# Object Recognition Memory and Gamma Synchrony in the Rat Hippocampus

By

John B. Trimper

B.A., SUNY Buffalo, 2009

Advisor: Joseph R. Manns, Ph.D.

An abstract of

A thesis submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Master of Arts

In Psychology

2012

Abstract

Object Recognition Memory and Gamma Synchrony in the Rat Hippocampus

By John B. Trimper

Neuronal oscillations are believed to play a critical role in memory processing. These rhythmic fluctuations in voltage facilitate the dynamic routing of information to and from various brain regions by transiently linking distinct groups of cells. A recent proposal is that intra-hippocampal oscillatory coherence in the slow (30 – 55 Hz) and fast (65 – 90 Hz) gamma bands reflect the processes of retrieval and encoding, respectively (Colgin & Moser, 2009). We sought to test this idea by recording local field potentials simultaneously from hippocampal subregions CA1 and CA3 in rats (*n*= 5) as they performed variants of a novel object recognition memory task. Analyses failed to confirm the hypothesized relationship between slow gamma coherence and memory retrieval, indicating instead that slow gamma coherence relates to the processing of spatial and relational memories. As predicted, oscillatory synchrony in the fast gamma range was related to memory encoding. However, our results suggest that this relationship was restricted to the encoding of nonspatial information. The current study advances our understanding of the functional relationship between hippocampal gamma coherence and memory, demonstrating a connection between oscillatory synchrony and memory content.

*Keywords:* hippocampus; memory; gamma; coherence; object recognition

# Object Recognition Memory and Gamma Synchrony in the Rat Hippocampus

By

John B. Trimper

B.A., SUNY Buffalo, 2009

Advisor: Joseph R. Manns, Ph.D.

A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Arts
in Psychology
2012

Table of Contents

# Object Recognition Memory and Gamma Synchrony in the Rat Hippocampus

The hippocampal memory system is important for declarative memory in both humans and experimental animals (Cohen & Squire, 1980; Eichenbaum, Otto, & Cohen, 1992; Squire, 1992). Declarative memory depends on interactions between many regions of the hippocampal memory system, including CA1, CA3, and the entorhinal cortex. An important question is how the hippocampal memory system mediates these interactions in the service of successful memory. One idea gaining momentum recently is that cellular activity during memory processing is organized by neuronal oscillations (Buzsaki, 2006; Fries, 2005; Hasselmo, Bodelon, & Wybie, 2002 ).

Several oscillations are prominent in field potentials of the hippocampus (Buzsaki, 2006; O'Keefe, 2006). Among them are the theta (6 – 12 Hz), beta (13 – 25 Hz), gamma (30 – 90 Hz), and "sharp wave ripple" (~200 Hz) frequency bands, each of which has its own behavioral correlates. The gamma band, which has been found to be important for perception and attention (Gray & Singer, 1989; Singer & Gray, 1995; for review, see Martinovic & Busch, 2011), has received increased attention lately in the memory literature, as several studies have indicated that it is related to successful memory performance (Fell et al., 2001; Jutras, Fries, & Buffalo, 2010; Montgomery & Buzsaki, 2007).

The gamma frequency band can be subdivided into two components; slow gamma (30 – 55 Hz) and fast gamma (55 – 90 Hz). Both are prominent, transient oscillations in the hippocampal CA1 subfield and are believed to originate from distinct inputs (see Colgin & Moser, 2009 for a review). The slow gamma oscillation arises from the CA3 recurrent-collateral system (Colgin et al., 2009), which is internal to the hippocampus

proper, while fast gamma is generated in the entorhinal cortex (Charpak, Pare, & Llinas, 1995), which is the hippocampus's primary source of cortical input (Amaral & Witter, 1989).

Oscillations, including slow and fast gamma, provide an effective mechanism for the routing of information, as they allow for the activation of select groups of neurons while filtering out the activity of others (Hasselmo et al., 2002; Fries, 2005). It has been suggested that, through oscillations, the hippocampus is able to alternate between states of retrieving previously stored information from CA3 and encoding new information received from the entorhinal cortex (Hasselmo et al., 2002). As CA3 and the entorhinal cortex generate slow and fast gamma, respectively, Colgin and Moser (2009) proposed that this alternation between retrieval and encoding should be paralleled by an increase in slow and fast gamma power, respectively, in the CA1 local field potential. Support for this claim is provided by a study in which the experimenters recorded local field potentials simultaneously from both CA1 and either CA3 or the medial entorhinal cortex in awake behaving rats during 10-30 minutes of open-field exploration (Colgin et al., 2009). Subsequent analyses revealed that CA1 disproportionately synchronized with CA3 in the slow gamma range and disproportionately synchronized with the medial entorhinal cortex in the fast gamma range.

The current study sought to further investigate the mechanisms underlying hippocampal processing by recording local field potentials simultaneously from CA1 and CA3 throughout performance of an object recognition memory paradigm. Rats ran several pairs of laps around a circular track and encountered the same, or different, objects on lap 2 as compared to the objects encountered on lap 1. We hypothesized that

encountering a novel object should bias the rat's hippocampus more towards an encoding state. Thus, we expected to observe greater fast gamma coherence for encounters with novel objects as compared to encounters with previously explored objects. Likewise, we hypothesized that on lap 2, we would observe an increase in slow gamma coherence for encounters with repeated objects, compared to novel objects, as the system would be more biased towards the process of retrieval. Our results failed to confirm the hypothesis that slow gamma coherence expressly relates to memory retrieval, and instead indicated that slow gamma coherence primarily reflects the processing of relational and spatial memories. Our analyses for fast gamma coherence were in line with the prediction that fast gamma coherence relates to memory encoding. However, the data suggest that this relationship was restricted to the encoding of nonspatial information.

## Method

### Subjects

Subjects were five adult, male Long Evans rats, weighing between 375 g and 425 g, individually caged and maintained on a 12-hour light/dark cycle. At the outset of training, rats were food-deprived to no less than 90% of their ad libitum weights. Water was available at all times in the home cage. All surgical and experimental procedures were approved by Emory University's Institutional Animal Care and Use Committee.

### Apparatus

**Circular track.** Rats were trained and tested on an elevated (83.0 cm) circular track. The outside diameter of the track was 91.4 cm and the width of the track was 7.6 cm. The circular track was divided into 12 equal sections and a wooden flap was affixed with a hinge to 11 of the possible 12 sections. No flap was attached at the end of the start

arm (the 6 o'clock position). Hinges were used to connect each flap to the track so that, when desirable, flaps could be raised to track level for object presentation. Objects were presented on the flaps outside the track so rats wouldn't have to deviate from their normal path to inspect the objects and so that the objects wouldn't obstruct the rat's normal path.

**Objects.** Rats were presented with examples of the objects during training but were not exposed to any of the actual test objects before testing began. Objects came from a collection of approximately 1500 glass, plastic, wood, and metal junk objects, each of which was typically larger than 7 cm x 7 cm x 7 cm but smaller than 12 cm x 12 cm x 12 cm. After each test session, each object employed, along with all duplicate copies of that object, were washed with a 1:1 mixture of ethanol and water.

**Data acquisition.** Behavior during the experimental session was recorded using a high-resolution video camera mounted above the circular track. Videos were acquired, and subsequently analyzed, at 30 frames/second.

Neural data was recorded from tetrodes chronically implanted in regions CA1 and CA3 of the dorsal hippocampus. Each tetrode was a bundle of four thinly insulated nichrome wires (12.5 μm diameter; each wire) whose tips were gold plated to lower impedances to 200 kΩ at 1 kHz. All neural activity was filtered, monitored, and saved with nspike (nspike.sourceforge.net).

Guided by known hippocampal electrophysiological hallmarks (complex spikes, 200 Hz "ripples", etc.; Buzsaki, 1986), tetrodes were gradually adjusted to maximize the number of pyramidal neurons being recorded as a means for verifying that tetrodes were positioned in the pyramidal layer of either CA1 or CA3. Testing began the day after this number was sufficiently high. Local field potentials were recorded with a bandpass filter

from 1 to 400 Hz and were acquired with a continuous sampling rate of 1500 Hz.

**Procedure**

Rats were trained to run clockwise laps around the circular track for a small chocolate reward upon returning to the start arm at the completion of each lap. After reaching a criterion of 80 laps in 40 minutes, rats were implanted with a recording headstage above the right dorsal hippocampus, centered at 3.3 mm posterior to bregma and 2.3 mm lateral.

The details of the surgical procedure were as follows. All surgeries were performed under aseptic conditions. Rats were anaesthetized with isoflurorane (1-3%) and given buprenorphine (.05 mg/kg) for analgesia. Rats were then placed in ear-bars to level the head. When rats were unresponsive to a firm toe-pinch, the scalp was opened to reveal the skull. Eight anchor screws were placed in the rat's skull to hold the drive in place. A craniotomy was performed using a dental drill, revealing the dorsal surface of the brain at a distance posterior (3.3 mm) and lateral (2.3 mm) to bregma. The dura mater was carefully removed under a microscope and the drive was slowly lowered until it made contact with the brain. A reference screw was placed in the rat's skull above the cerebellum. Dental acrylic was used to cover the reference screw and to connect the drive to the eight anchor screws on the skull. Antibiotic was applied on all sides of the implant and one to two stitches were put in both anterior and posterior to the recording drive. Each tetrode was then lowered to ~ 1 mm above its target recording site. After waking up from anesthesia, buprenorphine (.05 mg/kg) and meloxicam (2 mg/kg) were given for analgesia. Rats received additional doses of each analgesic the following morning.

Rats were given one full week to recover. Following this week, rats were re-

trained to pre-surgery performance levels and these performance levels were subsequently maintained by running rats daily around our circular track daily for approximately 75 laps.

Each rat was tested in three different experimental sessions over three days, and data were combined for each object condition across days. Each experimental session consisted of 24 blocks with three laps in each block. On the first lap of each block (lap 0) no objects were placed around the track. On the second lap (lap 1), three objects were placed in pre-determined, random locations around the track. Objects were never placed on either flap immediately adjacent to the start arm (the 5 and 7'oclock positions).

Figure 1 shows a schematic of the three conditions of interest in the object recognition memory task. In the same object/same location condition, a novel object on lap 1 was replaced with an identical object in the same location on lap 2. In the same object/different location condition, a novel object on lap 1 was replaced with an identical object in a new location on lap 2. In the different object/same location condition, a novel object on lap 1 was replaced with a novel object in the same location on lap 2.

The same object/same location condition was used on all three days of testing. The same object/different location and different object/same location conditions were each used on two of the three days. Additional conditions were also introduced (similar object/same location and same object/similar location), but were not included in this analysis.

**Data Analysis**

**Behavior.** Frame-by-frame behavioral coding of each video was performed to find the exploration time for each object encounter. Exploration times were found by

calculating the time passed between the initiation and offset of exploration for each object encounter. Initiation of exploration was flagged when the animal's nose was within 2 cm of the object and he was demonstrating active investigation (e.g., sniffing or directed attention). Offset was marked when the rat began to turn away from the object or it became clear that the animal was no longer engaging in active exploration (e.g., all movement ceased). An exploration time of zero seconds was recorded each time the rat passed an object without stopping to explore it. All subsequent behavioral analysis was performed using custom programming in MATLAB (Mathworks).

**Local field potential.** All local field potential analysis was performed using custom programming in MATLAB and through Chronux (http://chronux.org; Bokil, Andres, Kulkarni, Mehta, & Mitra, 2010), an open source, spectral analysis MATLAB toolbox. Local field potential analysis focused on a 0.5 second window around the onset of each object exploration. A 0.5 second window was selected based on the idea that for a rat to use memory information to guide his decision of whether or not to inspect an object, it was highly likely that he had access to that information before the exploration onset.

For calculation of coherence, multi-taper Fast Fourier Transforms were used. Coherence, the absolute value of coherency, is a number between zero and one that reflects both phase and amplitude covariation for a given frequency range. A coherence value of one indicates that two oscillations have a constant phase relationship and that their amplitudes covary. A value of zero indicates that the two signals have no phase relationship. The multi-taper method was chosen over standard Fourier analysis because it calculates multiple, independent estimates from each sample, providing a value that is

less biased by the variability in any one trial (for an extensive review, see Mitra & Bokil, 2008). Up to $K=2TW-1$ tapers are suitable for use, where $T$ is the time length (.5s) and $W$ is the frequency bandwidth (6Hz) (Chronux). Thus, five tapers were employed.

**Histology.** After the completion of testing, rats were anaesthetized with isofluorane (3%) and a 40 μA current was passed through each recording tetrode for 20 sec to create small lesions around the tetrode tips. Following tetrode marking, rats were given a lethal dose of pentobarbital (100 mg/kg) and transcardially perfused with 0.9% saline, followed by 4% formalin. The animals' brains were then extracted and placed in 4% formalin for at least 48 hours before being transferred to 30% sucrose. After 24 – 48 hrs, the brains were removed from sucrose, frozen, sectioned into 50 μm thick coronal slices with a sliding microtome, and transferred to a phosphate buffer solution (1%). Slices were later transferred onto glass slides and Nissl stained to facilitate the localization of recording sites.

## Results

### Histology

The position of each recording tetrode relevant to this report was verified under microscope to be within the targeted dorsal hippocampal pyramidal cell layer. All CA1 and CA3 tetrodes (one of each per rat) were localized to the intermediate portion of their respective CA region's proximal-distal extent.

### Behavior

To test our hypothesis that rats would display behavioral evidence of memory, we performed a 2 x 3 repeated measures ANOVA with lap number and object condition as independent variables and exploration time as the dependent variable. Figure 1 shows the

change in exploration time for each object condition from lap 1 to lap 2. The analysis revealed a significant main effect for lap ($F$[1,4]=7.32, $p$=.054, $\eta_p^2$=.647) and a significant interaction between lap number and object condition ($F$[2,8]=4.67, $p$=.045, $\eta_p^2$=538), but no main effect for object condition ($F$[2,8]=2.21, $p$=.171, $\eta_p^2$=.357). The presence of a lap x object condition interaction indicates that exploration times differed across laps, and that this effect was mediated by object condition.

To further investigate which exploration time differences were driving the observed interaction, we compared lap 1 and lap 2 exploration times separately for each object condition. Paired sample t-tests indicated that, for the same object/same location condition, lap 1 exploration times ($M$=3.13, $SEM$=1.18) were significantly greater than lap 2 exploration times ($M$=1.57, $SEM$=0.62), ($t$[4]=2.68, $p$=.025, one-tailed, $d$=1.24). Lap 1 exploration times ($M$=3.98, $SEM$=1.41) were also significantly greater than lap 2 exploration times ($M$=2.18, $SEM$=0.73) for the same object/different location condition ($t$[4]=2.43, $p$=.036, one-tailed, $d$=1.07). No significant reduction between lap 1 exploration times ($M$=2.10, $SEM$=0.55) and lap 2 exploration times ($M$=2.11, $SEM$=0.67) was observed for the different object/same location condition ($t$[4]= -.04, $p$=*.484*, one-tailed). The findings suggest that rats habituated to the repeated presentation of object identities, but not to the repeated presentation of object locations.

We performed several additional comparisons aimed at investigating any possible differences in average lap 2 exploration times between the three object conditions. Previous research suggests that rats explore objects possessing some novel trait (e.g., identity or location) more than objects repeated in an entirely identical fashion. Paired sample t-tests revealed that lap 2 exploration times for the different object/same location

condition (*M*=2.11, *SEM*=0.67) were significantly greater than lap 2 exploration times for the same object/same location condition (*M*=1.57, *SEM*=0.62), (*t*[4]=-2.70, *p*=.054, *d*=1.21). Lap 2 exploration times for the same object/same location condition (*M*=1.57, *SEM*=0.62) were statistically equivalent to lap 2 exploration times for the same object/different location condition (*M*=2.179, *SEM*=0.733), (*t*[4]=1.40, *p*=.233, *d*= 0.63). This behavioral evidence indicates that rats explored novel objects on lap 2 more than repeated objects on lap 2, suggesting they detected changes to object identity. While it is possible that rats also detected changes in location, the behavioral analyses did not indicate that they altered their exploration rates to reflect this knowledge.

**Local Field Potentials**

CA1-CA3 coherence values were calculated for the 0.5 seconds of neuronal data centered around the onset of object exploration. The decision to include the 0.25 seconds *before* exploration began was based on the logic that, if rats used memory to decide whether or not to inspect an object, that memory information would likely have been accessed prior to the onset of object exploration.

Figure 3 shows CA1-CA3 coherence, in the 25 – 100 Hz range, for the first and second laps in each object condition. Several differences are notable in Figure 3 within and across the object conditions. In the same object/same location condition, slow gamma coherence appears to increase from lap 1 to lap 2, while high gamma coherence decreases from lap 1 to lap 2. The line graphs also reveal differences between lap 1 and lap 2 for both object manipulation conditions, with high gamma decreasing across laps in the same object/different location condition and increasing across laps in the different object/same location condition. Our initial local field potential analyses sought to confirm the

statistical significance of these observations.

We hypothesized that slow and fast gamma coherence reflect retrieval and encoding, respectively. To test these hypotheses, we averaged the coherence values within each frequency range and looked for changes in these averages from lap 1 to lap 2 for each object condition. These lap 1- lap 2 comparisons were performed based on the idea that, if slow gamma reflects retrieval, it should be stronger on lap 2, when objects and/or locations are repeated, and if fast gamma reflects encoding, it should be stronger on lap 1, when all objects are novel. We expected novelty on lap 1 to bias the memory system more towards a state of encoding, and repetition on lap 2 to bias the system more towards a state of retrieval. We also asked whether there might be differences in coherence across the three object conditions, as the nature of the information remembered may differ dependent on which manipulations were introduced.

We conducted a 2 x 2 x 3 repeated measures ANOVA, with lap number, gamma range, and object condition as independent variables and coherence as the dependent variable. The analysis yielded a significant main effect for object condition ($F$[2,8]=9.45, $p$=.008, $\eta_p^2$=.703), and a significant two-way interaction between lap number and object condition ($F$[2,8]=5.70, $p$=.029, $\eta_p^2$=.588). The finding of a significant lap x object condition interaction indicates that coherence values differed across laps as a function of object condition. The main effects for lap number and gamma range were both nonsignificant ($F$[1,4]=0.34, $p$=.592; $F$[1,4]=.001, $p$=.976, respectively), as was the gamma range x object condition interaction ($F$[2,8]=.864, $p$=.457), the gamma range x lap number interaction ($F$[1,4]=1.46, $p$=.293), and the three-way interaction ($F$[2,8]=1.827, $p$=.222).

To further investigate which differences might be driving the lap x object condition interaction, we performed all possible pairwise comparisons across object conditions for both lap 1 and lap 2, in addition to comparing lap 1 coherence values against lap 2 coherence values for each object condition. A total of nine post-hoc tests were performed and the results of each were evaluated against a Bonferroni corrected alpha level ($\alpha$=.006). All pair-wise comparisons were statistically nonsignificant (*all p values* > .034).

We next considered that, given the transient nature of gamma oscillations and the rapid dynamics of memory processing, our failure to detect significant differences with the analysis of variance might have been a result of averaging coherence across too large of a time window (i.e., 0.5 seconds). To assess this possibility, we performed a sliding window analysis on the 2 seconds of local field potential centered around each exploration onset. We used a sliding window of 0.5 seconds, stepping in intervals of 0.05 seconds, effectively dividing the 2 second time-span into 31 partially overlapping bins, and assessing coherence separately for each. Using this technique, we calculated coherence values for each lap and found the coherence change across laps by subtracting coherence values on lap 1 from coherence values on lap 2. A 95% confidence interval was calculated around the mean of the coherence change across all frequencies for each of the 31 half-second time windows, and all effects outside of these uncorrected confidence intervals, containing at least six contiguous data points, were plotted in the moving window spectrogram shown in Figure 4.

Several significant changes are noteworthy in Figure 4 and will be described in the following paragraphs (for complete descriptions of each cluster on each graph, see

Tables 1-3). Clusters that are higher in frequency than 90 Hz, or within the utility frequency band (55 – 65 Hz) are not included in the current discussion. All mentions of increases and decreases in coherence in the following paragraphs are referring to coherence on lap 2, as compared to coherence on lap 1.

Slow gamma coherence increased in both conditions in which object location was repeated. These increases are present from about 0.35 seconds before the onset of object exploration to 0.25 seconds after exploration onset. The coherence increase in the same object/same location condition, both in terms of intensity (max = .094) and frequency bandwidth within the slow gamma range (33 Hz – 54 Hz), appears to be much greater than the parallel increase in the different object/same location condition (max = .086; bandwidth within slow gamma= 30 Hz – 32 Hz).

In contrast to the two conditions in which object location was repeated, a notable decrease in slow gamma coherence is evident throughout almost the entire time window in the same object/different location condition. The coherence change reaches a maximum difference of approximately .14 units at about 0.60 seconds after exploration onset.

Fast gamma coherence seems to be more variable than slow gamma coherence. In the same object/same location condition, both significant increases and significant decreases are apparent, with the primary significant decrease centered around onset of exploration, spanning from about 82 – 87 Hz, and the primary increase beginning approximately 0.35 seconds after exploration onset and spanning from 76 – 85 Hz.

A significant decrease in fast gamma coherence is also evident in the condition in which object location was manipulated (same object/different location). This effect

appears to begin at the onset of exploration and last approximately 0.30 seconds, spanning from about 79 – 88 Hz.

In the different object/same location condition, a relatively strong increase in fast gamma coherence can be seen around 80 Hz, centered in time around the onset of object exploration. A few smaller and less intense decreases are also apparent in the low end of the fast gamma range.

In sum, several coherence differences were revealed through our sliding window analyses, both between lap 1 and lap 2, and across object conditions. Slow gamma coherence increased from lap 1 to lap 2 for both conditions in which object location was repeated, but this increase was greater for the condition in which both object identity and object location were repeated. Slow gamma coherence decreased across laps for the condition in which only object identity was repeated. Fast gamma coherence, on the other hand, primarily decreased across laps for both conditions in which object identity was repeated, but significantly increased for the condition in which only location was repeated. A notable fast gamma coherence increase was also present after the initiation of object exploration in the same object/same location condition.

## Discussion

A recent proposal is that intra-hippocampal oscillatory coherence in the slow (30 – 55 Hz) and fast (65 – 90 Hz) gamma bands, which originate from CA3 and the entorhinal cortex, respectively, reflect distinct memory processes. Specifically, the idea was put forth that increased slow gamma coherence reflects retrieval from memory, while increased fast gamma coherence reflects the encoding of new memories (Colgin & Moser, 2009). We sought to test these ideas about the functional roles of slow and fast

gamma by recording local field potentials from hippocampal subregions CA1 and CA3 in rats during performance of an object recognition memory task in which separate conditions were employed to emphasize memory for object identity, memory for object location, and memory for object-location pairings.

Our local field potential analyses did not agree with the interpretation, offered by Colgin & Moser (2009), that slow gamma coherence expressly relates to the process of memory retrieval. Instead, our results suggest that slow gamma coherence primarily reflects memory content, with the most synchrony observed during the processing of relational memories (i.e., certain objects in certain locations), and, to a lesser extent, during the processing of spatial information.

Our analyses of fast gamma coherence were largely in line with the hypothesis that synchrony in the fast gamma range reflects memory encoding. However, our data suggest this relationship was restricted to the encoding of memories for object identity.

**Slow gamma coherence relates to spatial and relational information content**

If, as predicted, slow gamma coherence reflected the process of memory retrieval, we should have observed greater slow gamma coherence on lap 2, as compared to lap 1, for both conditions in which object identity was repeated, as the same reduction in exploration time, indicative of memory, was revealed for each. The finding that the pattern of coherence change differed between the two conditions suggests that slow gamma coherence is related to memory content, more so than stage of processing.

The amount and direction of slow gamma coherence change varied across the three object conditions, each of which emphasized memory for a different type of information. A relatively strong increase in slow gamma coherence was observed when

the condition emphasized relational memory (i.e., same object/same location). A smaller increase was observed when spatial memory was emphasized (i.e., different object/same location), and a decrease in slow gamma coherence was observed when nonspatial memory was emphasized (i.e., same object/different location).

The finding that slow gamma coherence increased across laps only for the spatial and relational memory conditions is consistent with the idea that the hippocampus is critically involved in memory for spatial (O'Keefe & Nadel, 1978) and relational (i.e., associative) information (Eichenbaum, Otto, & Cohen, 1992). Indeed, several studies report impairments on spatial (Morris, Garrud, Rawlins, & O'Keefe, 1982; Morris, Schenk, Tweedie, & Jarrard, 1990) and relational (Barker & Warburton, 2011; Bussey, Duck, Muir, & Aggleton, 2000; Gaffan, 1994; Mumby, Gaskin, Glenn, Scharamek, & Lehmann, 2002; Parkinson, Murray, & Mishkin, 1988) memory tasks after lesions to the hippocampus.

Electrophysiological evidence offers further support for a hippocampal role in spatial and relational memory processing. Subregions CA1 and CA3 are known to contain pyramidal neurons that respond to discrete regions of space (i.e., 'place cells') (O'Keefe, 1976; O'Keefe & Dostrovsky, 1971). Recent evidence suggests that hippocampal pyramidal neurons also play an associative role, representing nonspatial information in conjunction with spatial information (Manns & Eichenbaum, 2009; Wood, Dudchenko, & Eichenbaum, 1999). Taken together, the interpretation that slow gamma coherence primarily relates to the processing of spatial and relational information is consistent with the widely accepted functional roles of the hippocampus in processing spatial and associative memories.

When only object identity was repeated, we observed a *decrease* in slow gamma coherence across laps. Indeed, several studies suggest that object recognition, void of a spatial or relational component, is reliant upon extra-hippocampal regions, such as the entorhinal and perirhinal cortices (for review, see Mumby, 2001; Steckler, Drinkenburg, Sahgal, & Aggleton, 1998). One explanation, then, for the observed decrease in slow gamma coherence across laps for the condition in which only object identity was repeated is that, on lap 1, a memory for the object-location pairing was encoded, requiring communication between CA1 and CA3. However, on lap 2, processing by CA1 and CA3 was not required to recognize the object (i.e., retrieve the memory only for object identity), as this task was achieved by extra-hippocampal regions instead.

In sum, our results suggest that slow gamma coherence primarily reflects the type of information processed, rather than the stage of processing. It appears that an increase in slow gamma coherence relates primarily to the processing of relational memories and secondarily to the processing of spatial memories. One idea for future research is to investigate how increasing the relational and spatial task demands will impact coherence in the slow gamma range.

**Fast gamma coherence relates to the encoding of nonspatial information**

Based on the proposal of Colgin & Moser (2009), we predicted that CA1-CA3 oscillatory coherence in the fast gamma range would relate to the process of memory encoding. Indeed, our results suggest a relationship between memory encoding and fast gamma coherence, but further indicate that this relationship was restricted to the processing of nonspatial information. Fast gamma coherence during exploration onset

was greater on lap 1, relative to lap 2, for both conditions in which object identity was repeated.

A relevant consideration for the current interpretation is the finding that fast gamma coherence was *greater* on lap 2, relative to lap 1, for the condition in which object identity was altered between laps. If novel objects are presented on both laps, and fast gamma coherence expressly relates to the encoding of object identity information, then one may rightly expect that no change in fast gamma coherence should occur across laps. However, it is also possible that, because the system detected the presence of a novel object in that same location on lap 1 just moments prior, an even greater level of fast gamma coherence during the object encounter on lap 2 may be needed to "over-ride" the previously formed object memory. Further experimentation including a condition in which rats encounter novel objects several times (i.e., more than two) in the same location will be required to assess this possibility.

A further question regards the fact that the medial entorhinal cortex, which is believed to generate the fast gamma oscillation (Charpak et al., 1995), is the hippocampus's primary source of spatial information (Hargreaves et al., 2005), not nonspatial information. This claim is supported by the existence of medial entorhinal 'grid cells' (Fynh et al., 2004), which topographically map out all areas explored by the rat in a tessellated grid pattern and are resistant to external landmark manipulations (Hafting, Fyhn, Molden, Moser & Moser, 2005). Considering the role of the medial entorhinal cortex in spatial processing, one could have expected that, if a preference existed at all, fast gamma coherence would specifically relate to the processing of spatial information. Our data, however, appear to support the opposite conclusion.

One explanation is that the fast gamma oscillations observed in the CA1 and CA3 local field potentials originated in the lateral entorhinal cortex, not the medial entorhinal cortex. The lateral entorhinal cortex has received considerable attention as the hippocampus's primary source of nonspatial information. It is the recipient of dense projections from the perirhinal cortex (Burwell & Amaral, 1998), which, when lesioned, leads to drastic impairments in visual object recognition (Meunier, Bachevalier, Mishkin, & Murray, 1993; Mumby & Pinel, 1994). It may be the case that the lateral entorhinal cortex, whose intrinsic organization is similar to that of the medial entorhinal cortex (Hamam, Amaral, & Alonso, 2002; Hamam, Kennedy, Alonso, & Amaral, 2000; Tahvildari & Alonso, 2005), is also a source of fast gamma oscillations, although this hypothesis has yet to be confirmed.

An interesting question is whether or not the same relationship between fast gamma and object identity information would be observed if our task more strictly required that rats keep track of object location information. Perhaps, in this scenario, a greater relationship between fast gamma coherence and spatial memory would be revealed, reflecting increased communication between the hippocampus and the medial entorhinal cortex. Future experiments, requiring strict attention to spatial cues, will be necessary to investigate this possibility.

**Conclusions**

The data failed to confirm the hypothesized relationship between slow gamma coherence and memory retrieval, suggesting instead that slow gamma coherence primarily reflects memory content, with slow gamma coherence highest for the processing of relational memories, but also increased during the processing of spatial

memories.

Our results for fast gamma coherence are in line with the hypothesized relationship between fast gamma and memory encoding, but further indicate that this relationship between fast gamma and encoding is restricted to memory for object identities.

The current study contributes to a rapidly growing body of literature positing a role for gamma oscillations in memory (for review, see Nyhus & Curran, 2010). Several studies have reported that gamma oscillations contribute to the encoding (Fell et al., 2001; Gruber, Tsivilis, Montaldi, & Muller, 2004; Jutras, Fries, & Buffalo, 2010), and retrieval (Gruber et al., 2004; Montgomery & Buzsaki, 2007; Mormann et al., 2005) of memories. This study builds upon the previous literature by focusing on slow and fast gamma coherence as separate entities, and revealing a possible relationship between coherence in each range and the content of information processed.

Future studies will include additional object conditions, such as one in which lap 2 object presentation is novel in both location and identity, to ask what coherence changes, if any, might be observed. An additional avenue of research we plan to pursue is to investigate oscillatory coherence along the longitudinal axis of the hippocampus, as research suggests that the functional role of the hippocampus varies from the septal to temporal pole (Moser & Moser, 1998).

# References

Amaral, D.G., & Witter, M.P. (1989). The three-dimensional organization of the hippocampal formation: A review of anatomical data. *Neuroscience, 31*(3), 571-591.

Bokil, H., Andres, P., Kulkarni, J.E., Mehta, S., & Mitra, P.P. (2010). Chronux: A platform for analyzing neural signals. *Journal of Neuroscience Methods, 192*, 146-151.

Burwell, R.D., & Amaral, D.G. (1998). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *The Journal of Comparative Neurology, 398, 179-205.*

Buzsaki, G. (1986). Hippocampal sharp waves: Their origin and significance. *Brain Research, 398,* 242-252.

Buzsaki, G. (2006). Rhythms of the brain. New York, NY, Oxford University Press.

Charpak, S., Pare, D., & Llinas, R. (1995). The entorhinal cortex entrains fast CA1 hippocampal oscillations in the anaesthetized guinea-pig: Role of the monosynaptic component of the perforant path. *European Journal of Neuroscience, 7,* 1548-1557.

Cohen & Squire, 1980. Preserved learning and retention of pattern-analyzing skill in amnesia: Dissociation of knowing how and knowing that. *Science, 210*(4466), 207-210.

Colgin, L.L., Denninger, T., Fyhn, M., Hafting, T., Bonnevie, T., Jensen, O.,… Moser, E.I. (2009). Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature, 462,* 353-357.

Colgin, L.L, & Moser, E.I. (2009). Gamma oscillations in the hippocampus. *Physiology, 25,* 319-329.

Eichenbaum, H., Otto, T., & Cohen, N.J. (1992). Two functional components of the hippocampal memory system. *Behavioral and Brain Sciences, 17*(3), 449-472.

Fell, J., Klaver, P., Lehnertz, K., Grunwald, T., Schaller, C., Elger, C.E., & Fernandez, G. (2001). Human memory formation is accompanied by rhinal-hippocampal coupling and decoupling. *Nature, 4*(12), 1259-1264.

Fries, P. (2005). A mechanism for cognitive dynamics: Neuronal communication through neuronal coherence. *Trends in Cognitive Science, 9*(10), 474-480.

Fynh, M., Molden, S., Witter, M.P., Moser, E.I., & Moser, M-B. (2004). Spatial representation in the entorhinal cortex, *Science, 305,* 1258-1264.

Gray, C.M., & Singer, W. (1989). Stimulus-specific neuronal oscillations in orientation columns of cat visual cortex. *Proceedings of the National Academy of Science (USA), 86,* 1698-1702.

Gruber, T., Tsivilis, D., Montaldi, D., & Muller, M.M. (2004). Induced gamma band responses: An early marker of memory encoding and retrieval. *Neuroreport, 15*(11), 7-11.

Hafting, T., Fyhn, M., Molden, S., Moser, M-B., & Moser, E.I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature, 436,* 801-806.

Hamam, B.N., Amaral, D.G., & Alonso, A.A. (2002). Morphological and electrophysiological characteristics of layer V neurons of the rat lateral entorhinal cortex, *The Journal of Comparative Neurology, 451,* 45-61.

Hamam, B.N., Kennedy, T.E., Alonso, A.A., & Amaral, D.G. (2000). Morphological and electrophysiological characeteristics of layer V neurons of the rat medial entorhinal cortex. *The Journal of Comparative Neurology, 418,* 457-472.

Hargreaves, E.L., Rao, G., Lee, I., & Knierim, J.J. (2005). Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. *Science, 308*, 1792-1794.

Hasselmo, M.E., Bodelon, C., & Wyble, B.P. (2005). A proposed function for hippocampal theta rhythm: Separate phases of encoding and retrieval enhance reversal of prior learning. *Neural Computation, 14*(4), 793-817.

Jutras, M.J., Fries, P., & Buffalo, E.A. (2010). Gamma band synchronization in the macaque hippocampus and memory formation. *The Journal of Neuroscience, 29*(40), 12521-12531.

Manns, J.R. & Eichenbaum, H. (2009). A cognitive map for object memory in the hippocampus. *Learning and Memory, 16,* 616-624.

Martinovic, J., & Busch, N.A. (2011). High frequency oscillations as a correlate of visual perception. *International Journal of Psychophysiology, 79*(1), 32-38.

Meunier, M., Bachevalier, J., Mishkin, M., & Murray, E.A. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys, *The Journal of Neuroscience, 13*(12), 5418-5432.

Mitra, P.P, & Bokil, H. (2008). Observed brain dynamics. New York, NY, Oxford University Press.

Montgomery, S.M. & Buzsaki, G. (2007). Gamma oscillations dynamically couple hippocampal CA3 and CA3 regions during memory task performance, *Proceedings of the National Academy of Science (USA), 104*(36), 14495-14500.

Mormann, F., Fell, J., Axmacher, N., Weber, B., Lehnertz, K., Elger, C., & Fernandez, G. (2005). Phase/amplitude rest and theta-gamma interaction in the human medial temporal lobe during a continuous word recognition memory task. *Hippocampus, 15,* 890-900.

Morris, Garrud, Rawlins, & O'Keefe, (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297,* 681-683.

Morris, R.G.M, Schenk, F., Tweedie, F., & Jarrard, L.E. (1990). Ibotenate lesions of hippocampus and/or subiculum:Dissociating components of allocentric spatial learning. *European Journal of Neuroscience, 12*(12), 1016-1028.

Moser, M-B., & Moser, E.I. (1998). Functional differentiation in the hippocampus. *Hippocampus, 8,* 608-619.

Mumby, D.G., & Pinel, P.J. (1994). Rhinal cortex lesions and object recognition in rats. *Behavioral Neuroscience, 108*(1), 11-18.

O'Keefe, J. (1976). Place units in the hippocampus of the freely moving rat. *Experimental Neurology, 51,* 78-109.

O'Keefe, J. (2006). Hippocampal neurophysiology in the behaving animal. In: Andersen, P., Morris, R.G.M., Amaral, D.G., Bliss, T.V.P., O'Keefe, J., editors. The hippocampus book. Oxford: Oxford Neuroscience, pp 475-548.

O'Keefe, J., & Dostrovsky, J. (1971). The hippocampus as a spatial map: Preliminary evidence from unit activity in freely moving rats. *Brain Research, 34,* 171-175.

O'Keefe, J., & Nadel, L. (1978). The hippocampus as a cognitive map. Oxford: Clarendon Press

Nyhus, E., & Curran, T. (2010). Functional role of gamma and theta oscillations in episodic memory. *Neuroscience and Biobehavioral Reviews, 34*¸1023-1035.

Rolls, E.T (1996). A theory of hippocampal function in memory. *Hippocampus, 6*, 601-620.

Singer, W., & Gray, C.M. (1995). Visual feature integration and the temporal correlation hypothesis. *Annual Reviews in Neuroscience, 18,* 555-586.

Squire, L.R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychological Review, 2,* 195-231.

Tahvildari, B., & Alonso, A. (2005). Morphological and electrophysiological properties of lateral entorhinal cortex layers II and III principal neurons. *The Journal of Comparative Neurology, 491,* 123-140.

Wood, E.R., Dudchenko, P.A., & Eichenbaum, H. (1999). The global record of memory in hippocampal neuronal activity. *Nature, 397,* 613-616.

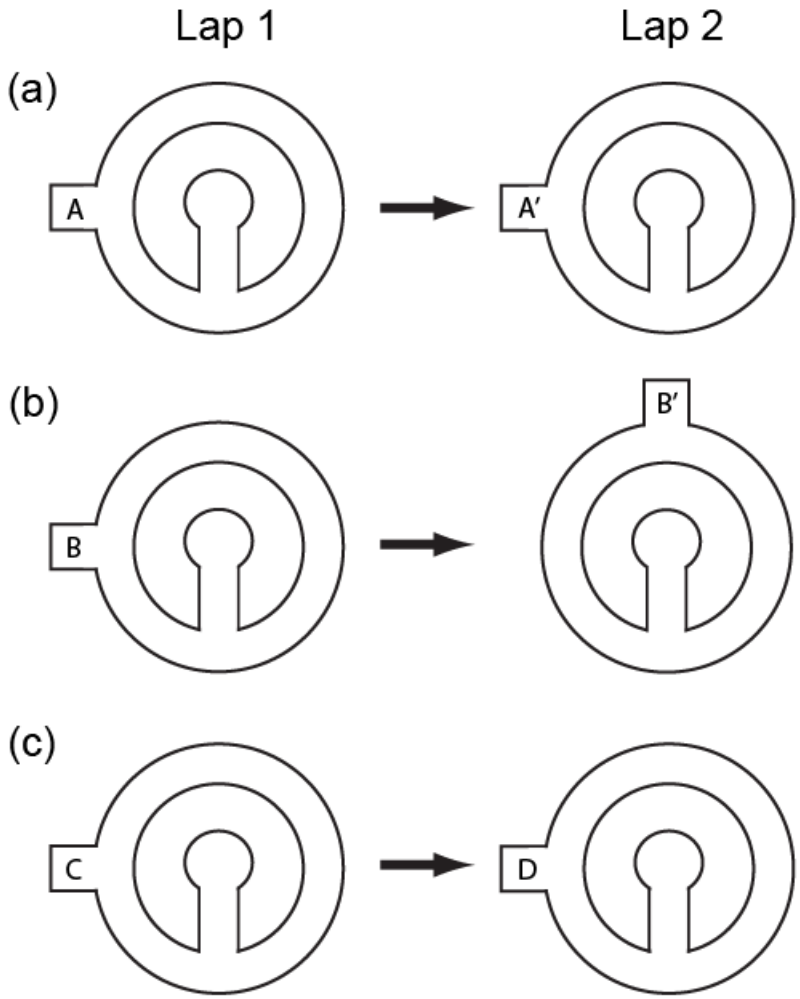


*Figure 1*. Task schematic for each of the three object manipulations between lap 1 and lap 2. Objects presented on lap 1 are replaced, on lap 2, by either (a) the same object in the same location condition, (b) the same object in a different location, or (c) a different object in the same location.

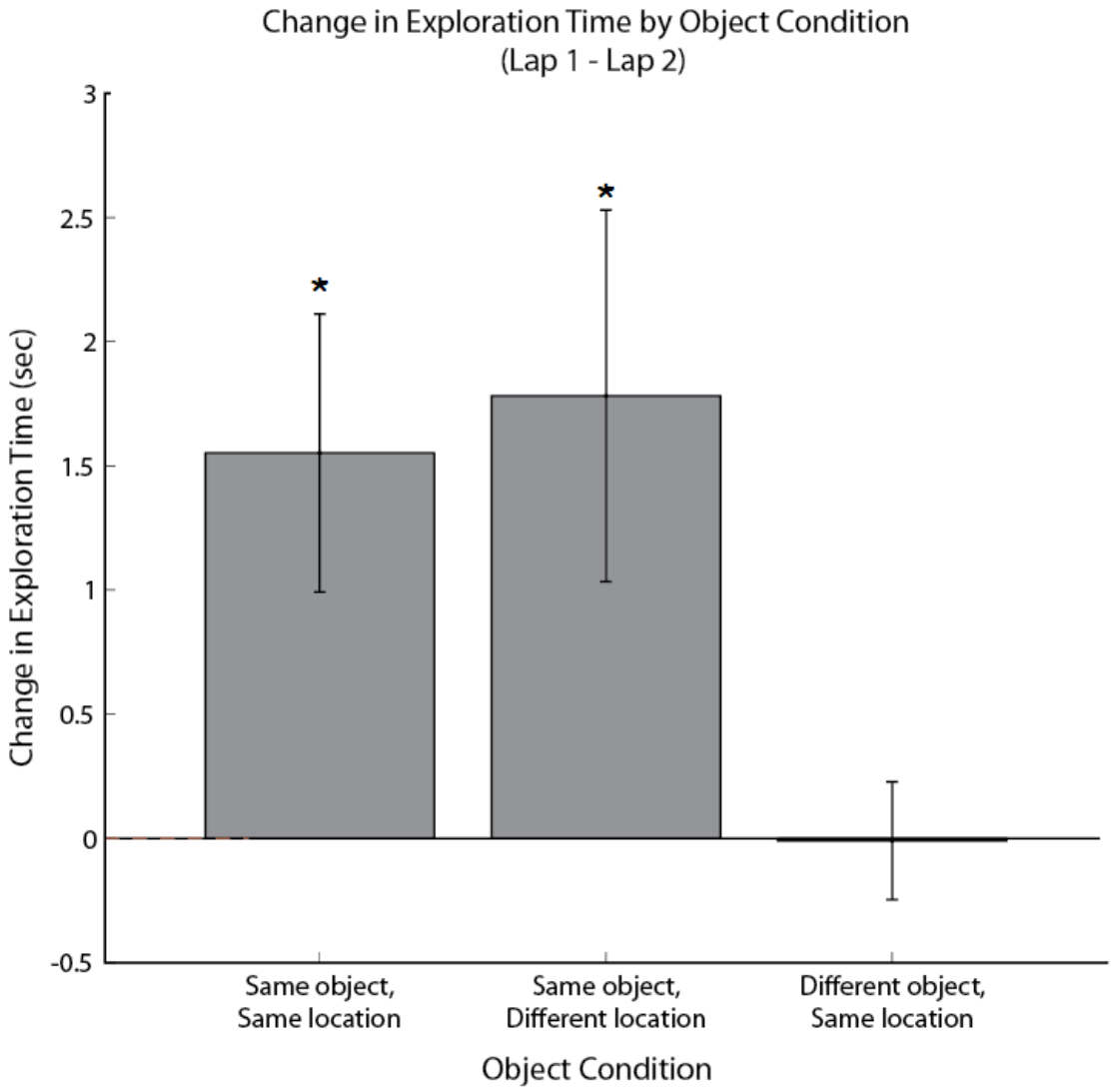


*Figure 2.* Average changes in exploration time between lap 1 and lap 2 as a function of object condition. Rats significantly decreased their exploration times for both conditions in which object identity remained constant, but failed to demonstrate a reduction when objects were repeatedly presented in the same location. Asterisks denote that the change in exploration time is significantly different from zero ($p<.05$). Brackets show SEM.

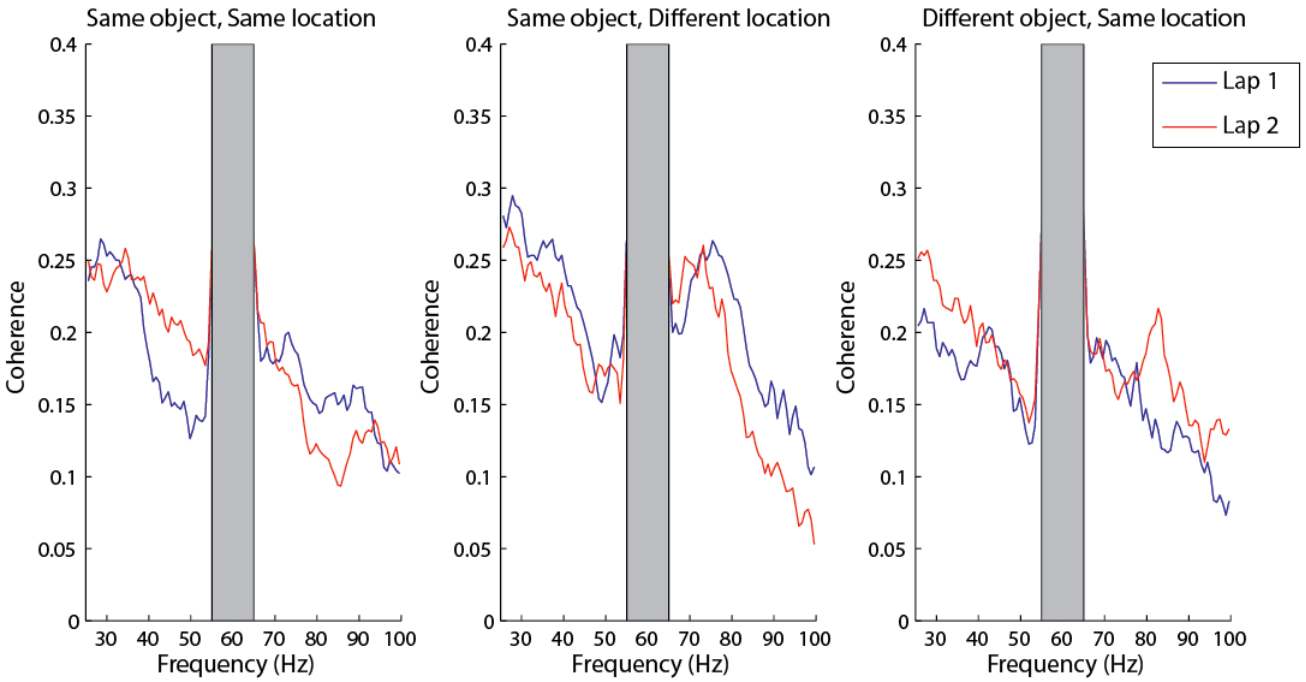


*Figure 3.* Coherence in the 25 – 100 Hz range on laps 1 and 2 for each object condition averaged across rats. For the same object/same location condition, slow gamma appears to increases on lap 2 while high gamma appears to decrease. In the same object/different location condition, high gamma appears to decrease across laps, while in the different object/location condition, high gamma appears to increase. Grey bars cover the utility frequency range (55-65 Hz).

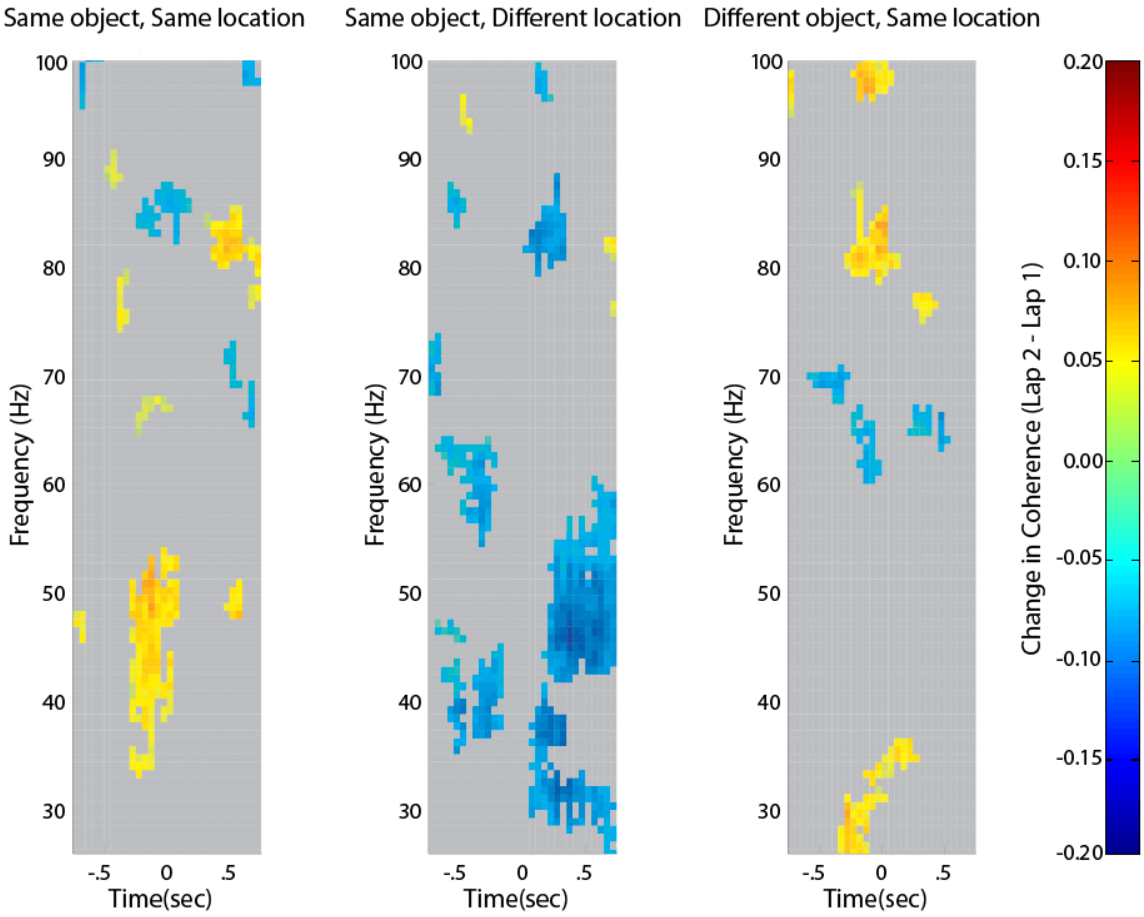


*Figure 4.* A moving window plot of the average coherence change between lap 1 and lap 2 as a function of time and object condition. All effects surpassing an uncorrected 95% confidence interval and containing at least six contiguous data points were plotted. Time zero indicates onset of object exploration. Note the increases in slow gamma coherence for conditions in which object location was repeated and the decrease in fast gamma coherence for both conditions in which object identity was repeated.

Table 1

*Detailed descriptions of the significant coherence changes from lap 1 to lap 2 for the same object/same location condition*

| | | Frequency | | Time | | Most Extreme Value | | |
|---|---|---|---|---|---|---|---|---|
| Gamma | Change | Low | High | Start | End | Value | Time | Frequency |
| Slow | Increase | 32.9 | 53.5 | -0.30 | 0.05 | .095 | -0.15 | 48.34 |
| | Increase | 45.41 | 47.60 | ≤ -0.75 | 0.70 | .056 | -0.95 | 46.88 |
| | Increase | 47.61 | 50.54 | 0.45 | 0.55 | .075 | 0.55 | 47.61 |
| Fast | Increase | 64.45 | 67.38 | -0.25 | 0.00 | .043 | -0.10 | 67.38 |
| | Decrease | 65.19 | 68.85 | 0.60 | 0.65 | .052 | -0.40 | 65.92 |
| | Decrease | 68.85 | 72.51 | 0.45 | 0.55 | -0.04 | 0.50 | 71.78 |
| | Increase | 73.97 | 79.1 | 0.40 | 0.35 | .052 | -0.40 | 75.44 |
| | Increase | 76.17 | 84.96 | 0.30 | ≥ 0.75 | .080 | 0.45 | 81.3 |
| | Decrease | 82.03 | 87.16 | -0.10 | 0.15 | -.058 | 0.00 | 85.69 |
| | Decrease | 82.76 | 85.69 | -0.25 | -0.10 | -.048 | -0.10 | 84.76 |
| | Increase | 87.16 | 90.09 | -0.50 | -0.40 | .038 | -0.45 | 87.89 |

Table 2

*Detailed descriptions of the significant coherence changes from lap 1 to lap 2 for the same object/different location condition*

| | | Frequency | | Time | | Most Extreme Value | | |
|---|---|---|---|---|---|---|---|---|
| Gamma | Change | Low | High | Start | End | Value | Time | Frequency |
| Slow | Decrease | 25.63 | 41.02 | 0.05 | ≥ 0.75 | -.116 | 0.60 | 40.28 |
| | Decrease | 35.16 | 43.21 | -0.65 | -0.50 | -.095 | 0.50 | 38.82 |
| | Decrease | 36.62 | 44.68 | -0.40 | -0.20 | -.083 | -0.30 | 36.62 |
| | Decrease | 41.75 | 60.06 | 0.15 | 0.75 | -.137 | 0.35 | 45.41 |
| | Decrease | 45.40 | 46.90 | -0.70 | -0.05 | -.043 | -0.55 | 46.9 |
| Fast | Decrease | 68.12 | 73.24 | ≤ -0.75 | -0.70 | -.073 | -0.70 | 68.12 |
| | Increase | 75.44 | 82.76 | 0.65 | 0.75 | .059 | 0.70 | 82.03 |
| | Decrease | 79.10 | 87.89 | 0.00 | 0.30 | -.096 | 0.05 | 82.76 |
| | Decrease | 83.50 | 87.16 | -0.60 | -0.50 | -.062 | -0.55 | 85.7 |

Table 3

*Detailed descriptions of the significant coherence changes from lap 1 to lap 2 for the different object/same location condition*

| | | Frequency | | Time | | Most Extreme Value | | |
|---|---|---|---|---|---|---|---|---|
| Gamma | Change | Low | High | Start | End | Value | Time | Frequency |
| Slow | Increase | 25.63 | 31.49 | -0.35 | 0.00 | .086 | -0.30 | 29.30 |
| | Increase | 32.23 | 35.89 | -0.15 | 0.25 | .063 | 0.10 | 35.16 |
| Fast | Decrease | 67.38 | 70.31 | -0.60 | -0.30 | -.069 | -0.50 | 68.85 |
| | Increase | 74.71 | 76.90 | 0.25 | 0.40 | .056 | 0.30 | 76.90 |
| | Increase | 78.37 | 87.16 | -0.30 | 0.10 | .090 | -0.05 | 83.50 |
| | Increase | 94.06 | 97.77 | ≤ 0.75 | 0.75 | .063 | 0.05 | 97.03 |
| | Increase | 95.54 | ≥ 100 | -0.25 | 0.05 | .090 | -0.15 | 95.54 |