Septotemporal variation in dynamics of theta: speed and habituation

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Abstract

Theta (6-12 Hz) field potentials and the synchronization (coherence) of these potentials present neural network indices of hippocampal physiology. Theta signals within the hippocampal formation may reflect alterations in sensorimotor integration, the flow of sensory input and/or distinct cognitive operations. While the power and coherence of theta signals vary across lamina within the septal hippocampus, limited information is available about variation in these indices across the septotemporal (long) or areal axis. The present study examined the relationship of locomotor speed to theta indices at CA1 and DG sites across the septotemporal axis as well as in the entorhinal cortex. Our findings demonstrate the dominant relationship of speed to theta indices at septal sites. This relationship diminished systematically with distance from the septal pole of the hippocampus at both CA1 and DG sites. While theta power at entorhinal sites varied in relation to speed, there were no differences across the areal axis of the entorhinal cortex. Locomotor speed was also related to changes in theta coherence along the septotemporal axis as well as between the hippocampus and entorhinal cortex. In addition to the speed related variation, we observed a decrease in theta power at more temporal hippocampal sites over repeated behavioral testing within a single day that was not observed at septal sites. The results outline a dynamic and distributed pattern of network activity across the septotemporal axis of the hippocampus in relation to locomotor speed and recent past experience.

Keywords: Theta rhythm, hippocampus, entorhinal, synchrony, EEG, rat
Introduction

The laminar organization of the hippocampus (HPC) provides an ideal anatomy for the generation of large amplitude local field potentials (LFPs). Theta (6-12 Hz) LFP oscillations are generated by the summation of synchronous excitatory and inhibitory potentials as well as intrinsic membrane potentials (e.g., Green and Arduini, 1954; Petsche et al., 1962; Leung, 1985; Brankack et al., 1993; Bragin et al., 1995; Buzsaki, 2002). In general, the theta signal reflects moment-by-moment variation in the synchronization of afferent input impinging upon the somatodendritic field of hippocampal neurons. Studies have linked changes in theta LFPs to cognitive variables in several mammals (e.g., Ulanovsky & Moss, 2007; Rizzuto et al., 2006; Montgomery et al. 2009; see Jutras and Buffalo, 2010 or Nyhus and Curran 2010 for reviews), while variation in the theta signal in the rodent has often been linked to locomotor speed and sensorimotor integration (see Bland and Oddie, 2001; Wyble et al., 2004; Sinnamon, 2006 for reviews).

Early reports on the behavioral correlates of theta noted its prominence during locomotion and relation to running speed (Vanderwolf, 1969; Teitelbaum and McFarland, 1971; Whishaw and Vanderwolf, 1973; Feder and Ranck, 1973; McFarland et al., 1975). The increase in theta power in relation to speed has been confirmed in studies examining the septal pole of the HPC (Rivas et al., 1996; Bouwman et al., 2005), although Maurer and colleagues (2005) suggested variability in the role of speed at more temporal aspects of the HPC.

There is considerable topographic organization in the afferent input to the HPC. Inputs from the entorhinal cortex (EC) define three domains along the septotemporal (long) axis based upon the band of origin in the EC (Dolorfo and Amaral, 1998a; 1998b; Witter, 2007).
Prominent amygdala inputs preferentially target more temporal levels of the HPC (see Pitkanen et al., 2000 for review) while several subcortical neuromodulatory inputs exhibit variation across the septotemporal axis (Amaral and Kurz, 1985; Gage and Thompson, 1980; see also Thompson et al., 2008 for review).

The present study examined the relationship between speed and various measures of theta across the septotemporal axis of both CA1 and DG, as well as in medial (MEA) and lateral (LEA) entorhinal areas. We demonstrate that locomotor speed is a prominent predictor of theta power and coherence in the septal-most aspect of the HPC and that there is a decrease in the relationship between speed and theta with distance from the septal pole. Further, we observed decreases in theta power over repeated behavioral testing within a single day at more temporally located electrode sites without any decrement at septal sites. This habituation related decrease was unrelated to locomotor speed. The latter suggests that theta power can be sensitive to the recent past experience of the animal. The results are discussed with regards to anatomy, septotemporal differentiation within the HPC as well as the utility of theta measures as indices of neural network function.

**Materials and Methods**

**Animals and Surgical Procedures**

Six adult male Fisher-344 rats were used in this study. The animals were individually housed in a temperature-controlled room and maintained on a 12-h/12-h light-dark cycle. All procedures were performed in accordance with the guidelines set forth by University of Connecticut’s Institutional Animal Care and Use Committee and NIH.
Rats were anesthetized with a ketamine cocktail (4 ml/kg consisting of 25 mg/ml ketamine, 1.3 xylazine mg/ml, and 0.25 acepromazine mg/ml). After a midline scalp incision, burr holes were drilled in the skull over the HPC and three electrode arrays were positioned along the septotemporal extent of the HPC, while a fourth array was positioned in the entorhinal cortex. The following coordinates were used for each of the four arrays: Septal HPC (AP -3.0, ML 2.5, DV 3.0); Intermediate HPC (AP -5.0, ML 5.0, DV 5.0); Temporal HPC (AP -6.5, ML 5.5, DV 7.0); Entorhinal Cortex (AP – 6.0 - 8.0, ML 6.5, DV 6.5 - 7.5). Each electrode array consisted of four linearly spaced 50 µm tungsten wires (16 total electrodes; California Fine Wire Co., Grover Beach, CA), arranged and spaced using fused silica tubing (Polymicro Tubing, Phoenix, AZ). All electrodes were attached to female pins (Omnetics, Minneapolis, MN) secured in a rectangular five by four pin array. Two stainless steel watch screws driven into the skull above the cerebellum served as indifferent and ground electrodes. Two or more additional support screws were positioned over the anterior aspect of the skull and the entire ensemble was secured with dental acrylic. Rats were allowed to recover for one week following surgery.

**Behavioral Performance and Electrophysiological Data Acquisition**

Rats were food deprived to 85% of their ad libitum weight and trained to run on a linear track (10 X 140 cm) for chocolate sprinkles. Recording sessions consisted of five individual recordings sessions separated in time within a single day (Fig. 1A). The end of the first recording session marked Time 0 (T0 or baseline). The subsequent four sessions were initiated at T5, T20, T60 and T120 minutes. Each of the five sessions required the rat to complete a minimum of 50 trials, where a single behavioral trial consisted of the rat running...
from one end of the track to the other end. After the rat completed 50 trials, it was returned to its home cage on a table adjacent to the linear track until it was time to start the next recording session. No changes were made to the track or the room in between recordings.

Wide-band electrical activity was recorded (1-1894 Hz, 3787 samples/sec) during each recording session using a Neuralynx data acquisition system (Bozeman, MT). Light emitting diodes attached to the headstage were tracked via a camera (33 samples/sec) positioned over the linear track, thus allowing for an offline record of the animals position over time. In order to calculate locomotor speed, the positional difference between successive tracking samples was calculated and then lowpass filtered (cutoff = 0.25 Hz) in order to minimize the contribution of head movements and movement artifacts to the overall speed of the rat. A representative filtered position versus time trace is shown in Figure 1B. Such traces were used to calculate instantaneous and mean speed during designated intervals.

All data analysis was conducted using custom written programs in MatLab (The MathWorks, Natick, MA) or in SPSS (SPSS Inc., Chicago, Illinois). Movement related data was visualized as a state-space plot (position versus velocity; see Fig. 1C). In order to exclude data recorded during consumption of sprinkles and turning behavior, a physical threshold 14 cm from each end of the maze was set (Fig. 1B, C) and any trial during which the rat’s speed decreased below 5 cm/sec was discarded. The resulting dataset contained an average of 46.4 +/- 0.88 (SEM) trials per recording.

**Spectral Indices**

Power spectral density estimates were obtained using Welch’s averaged modified periodogram method (Welch, 1967). For each trial run, the average power in the 6-
12 Hz band and the corresponding mean speed for each non-overlapping 1.5 second interval was calculated. Theta phase was obtained from the Hilbert transform of the theta bandpass filtered signal (6 – 12 Hz) and then the instantaneous frequency was determined by calculating the change in phase divided by the change in time between each sample. Average theta frequency was calculated over each 1.5 second trial segment (see also Jeewajee et al., 2008).

In order to calculate coherence, trials (as defined above) were sorted based on mean speed (slowest to fastest) and the EEG signals were concatenated into a single continuous string of data (Roark and Escabi, 1999, see also Sabolek et al., 2009), such that each recording session (at T0, T5, etc) generated a series of twenty-second long data strings with different mean speeds. Thus, the slowest trials totaling twenty seconds were concatenated, the next slowest totaling twenty seconds were concatenated and so forth for all trials (number of concatenated data strings per recording = 5.47 +/- 0.11 (mean +/- SEM)). Coherence values (Bullock et al., 1990) for each channel pair were computed using the Welch periodogram estimation procedure with a spectral resolution of ~ 2 Hz (see below).

Each spectral index (power and coherence) from each electrode (or electrode pair) was separately subjected to a simple linear regression analysis that included the mean speeds and the spectral index for the corresponding period of time in order to assess the relationship between locomotor speed and each of the spectral indices. Thus each electrode or electrode pair yielded a single correlation coefficient (r-value) for each spectral index. R-values near 1 indicate that locomotor speed and the corresponding spectral index are linearly associated, such that a net increase in speed leads to a linear increase in the spectral index. Values near zero indicate no relationship between locotomor speed and spectral index.
Electrode recordings within each septotemporal quartile of the DG and CA1 (e.g., septal 25%; Fig. 1F) as well as those in the MEA and LEA were grouped separately to determine whether that region had a mean r-value different than zero using a t-test. A non-zero mean for a region’s speed r-value distribution indicates that that spectral index is significantly speed modulated either positively or negatively (Lorch and Myers, 1990). Additionally, a simple linear regression analysis was conducted on the speed r-values for DG and CA1 along with the distance from the septal pole (in millimeters, mm) as an explanatory variable, thus demonstrating whether the speed modulation of each spectral index varied along the septotemporal axis.

**Statistics: Coherence Analysis**

A significance estimation procedure was devised in which the coherence estimate was compared to that of signals with identical magnitude spectrum but with zero phase coherence. For each channel pair, the cumulative distribution of the frequency-dependent coherence values was created by randomizing the phase spectrum of the signals while preserving the magnitude spectrum, calculating the coherence for the phase randomized signals, and bootstrapping the procedure 250 times (Efron and Tibshirani, 1993). This procedure guarantees that the signal magnitude spectrums are identical but have no linear association, because the phase or time information has been removed. The coherence distribution obtained via bootstrapping the procedure was used to determine a significance threshold for each frequency band (2 Hz resolution), below which 95% of the shifted null hypothesis coherence values fell (i.e., the Null hypothesis; see also Sabolek et al., 2009).
Only regions of the non-shuffled signal coherences falling above the 95% threshold were considered significant. For each channel pair, the statistically significant area in the theta (6-12 Hz) range was calculated and normalized by the frequency ranges (expressed as average coherence value per Hz) to facilitate comparison of different frequency ranges. Finally, the coherence value was normalized for bandwidth and a new zero point, with the resulting normalized coherence values fall between 0-1.

Statistics: Habituation Analysis

In order to assess any changes in theta power over the repeated recording sessions, a linear regression analysis was conducted that included the mean speeds and four orthogonal dummy coded categorical variables for the five recording timepoints (eg, T0, T5, etc) as explanatory variables. Each electrode site yielded a single standardized regression coefficient ($\beta$-value, where $\beta = b \frac{SD_y}{SD_x}$) for each of the explanatory variables. The resulting $\beta$-values indicate how theta power changes in relation to the baseline recording while controlling for speed. Thus a significant non-zero mean $\beta$-value distribution indicates that theta power increased/decreased significantly from baseline. The resulting distributions of $\beta$-values were assessed by septotemporal position (distance from the septal pole; Fig. 5B) and grouped by septotemporal quartiles (see Fig. 1F; see also Sabolek et al., 2009) for both CA1 and DG sites, or for MEA or LEA sites for EC electrodes. Repeated measures ANOVA and individual t-tests were used to compare the distribution of $\beta$-values at T5, T20, T60 and T120 for each anatomical region.
Histology

Following the completion of recordings, rats were anesthetized with Euthasol (sodium pentobarbital solution) and transcardially perfused with ice-cold saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2). Brains were sliced (50 µm sections) using a vibratome (Vibratome Series 1500), mounted, and Nissl stained using thionin. All electrode positions were verified and categorized according to laminar and septotemporal position. Septotemporal distances between electrode positions were determined by noting the location of each electrode position on a flatmap representation of the HPC (Swanson et al., 1978). Each section of a flatmap represents approximately 200 µm of tissue, and so fairly accurate approximations of the relative distance between electrodes could be determined by counting the number of sections between two electrodes. Photomicrographs of relevant electrode tracks were captured using a Nikon microscope connected to a Spot RT camera system, digitized and prepared for presentation using Adobe Photoshop 7.0.

Results

Electrodes were positioned at sites primarily within stratum moleculare or stratum granulosum of the DG (N = 15), while CA1 (N = 25) sites spanned from the ventral aspect of stratum pyramidale to stratum lacunosum moleculare with the majority of sites within stratum radiatum (Fig. 1E). In the areal direction, DG placements ranged from 1.6 – 4.8 mm from the septal pole (within the septal and mid-septotemporal regions), while placements in CA1 ranged more broadly from 1.3 – 7.6 mm from the septal pole. Electrode placements within the superficial layers of the EC included sites in the medial (n = 7) and lateral (n = 6) subdivisions
that were dispersed across lateral, intermediate and medial bands (Fig. 1E for MEA sites: see also Supplemental Figure 1 for LEA EC sites).

At all electrode sites, visual inspection and power spectral density confirmed the presence of theta while rats shuttled between ends of a linear track for food reward (Fig. 1B, C, D). The power of theta varied according to laminar position, as has been well described in the septal HPC (see Bragin et al., 1995), with sites nearest the hippocampal fissure within stratum moleculare of DG and stratum lacunosum moleculare of CA1 yielding the largest amplitude signals.

We observed a slight decrease in theta power of roughly 3-5 dB when comparing sites in the septal most quartile (both DG and CA1) to sites in homotopic positions in the second and third quartiles (Fig. 1G, 2A, Table 1). To some extent the difference in theta power may reflect anatomic differences in the density of afferent inputs involved in the generation of LFPs and/or the density of the dendritic fields in which those afferents terminate. Despite the differences in absolute power, sites across the septotemporal axis of both DG and CA1 exhibited similar amounts of variability in theta power (see Table 1).

**Theta power and locomotor speed across the septotemporal axis of the HPC**

All electrode sites located in the septal-most HPC exhibited a prominent increase in theta power as a function of running speed. Initial visual inspection of the relationship between speed and theta power for all electrodes within a given animal exhibited clear variation in this relationship throughout the hippocampal formation (Fig. 2B, 3A,D). Examples from two rats during the baseline recording show the relationship between speed and power from simultaneously recorded electrodes at multiple septotemporal levels of CA1 and DG, as
well as in EC (Fig. 2B, 3A). As can be seen, the strength of the correlation between speed and theta power varies with distance from the septal pole in CA1 and DG for these examples. Results are also shown from three simultaneously recorded CA1 sites (Fig. 3A) where the strength of the relationship varies systematically along the long axis. For additional information on the distribution of regression coefficients (b-values, slopes) at CA1 and DG sites across the long axis as well as EC see Supplemental Figure 2.

Thus, speed accounted for a significant portion of the variability in theta power at the septal most electrode sites, while sites located more temporally in each individual animal exhibited a clear decrease in the relationship between speed and theta power. In order to quantify this change along the septotemporal axis of DG and CA1, we analyzed the data two ways. First, we conducted a regression analysis using the septotemporal position of each electrode (millimeters from septal pole) and the corresponding r-value (for speed vs power) for that electrode. The relationship of speed to theta power significantly decreased along the septotemporal axis of CA1 (N = 25, r = -0.69, p < 0.0005; Fig. 3B). While there was a clear trend for a decrease in the relationship of speed to theta power along the septotemporal axis of the DG, this relationship was not significant (N = 15, r = -0.38, p = 0.16; Fig. 3B). Note that DG sites did not extend beyond roughly 5 mm from the septal pole and no DG sites were located in the temporal 50% of the HPC (Fig. 1F, 3B).

Although the speed modulation of theta power decreased along the septotemporal axis, this does not demonstrate whether sites at more temporal levels exhibited a significant relationship between speed and theta power. In order to determine whether theta power at different septotemporal levels of DG and CA1 was significantly related to speed, we grouped electrodes for each HPC quartile and conducted t-tests on the distribution of correlation coefficients (r-values). A significant relationship between theta power and speed was
observed for electrode sites within the first two quartiles of CA1 ($^{1\text{st}}$: $t(4) = 9.25$, $p < 0.001$; $^{2\text{nd}}$: $t(11) = 3.79$, $p < 0.005$), but not for electrode sites in the third quartile ($^{3\text{rd}}$: $t(7) = -0.08$, $p = 0.94$; Fig. 3C). In DG, a significant relationship between speed and theta power was observed only for electrode sites in the septal most quartile ($^{1\text{st}}$: $t(7) = 5.71$, $p < 0.001$; $^{2\text{nd}}$: $t(6) = 1.04$, $p = 0.34$; Fig. 3C).

In summary, a positive relationship between speed and theta power was quite evident in all septal ($^{1\text{st}}$ quartile) electrode sites with speed accounting for considerable variation in theta power at both CA1 (mean $r$-value for CA1 sites in $^{1\text{st}}$ quartile = 0.66 +/- 0.07) and DG (mean $r$-value for DG sites in $^{1\text{st}}$ quartile = 0.31 +/- 0.05) sites. Notably, locomotor speed accounted for more of the variability at CA1 sites than at DG sites within the first and second quartiles of the HPC ($p$'s < 0.001; Fig. 3C). Note that the more prominent relationship between speed and theta power in CA1, as compared to DG sites, has been previously demonstrated by Montgomery and colleagues (2009: see Fig 3). Their study noted clear differences across distinct laminar positions with greater variability across laminar subfields of CA1 (eg, pyramidal versus lacunosum moleculare). We also observed subtle differences across distinct laminar positions (see Supplemental Figure 3). Regardless of laminar location within CA1, a prominent decrease in the speed to power relationship was observed across the septotemporal axis.

**Theta power and locomotor speed within the EC**

Theta LFPs were recorded at 13 electrode sites within the superficial layers of the EC in both the medial ($n = 7$) and lateral ($n = 6$) subdivisions of the EC (Fig. 1F). The power of
theta signals from both MEA and LEA were moderately lower than those observed in the HPC, but no differences were observed between MEA and LEA sites (see Table 1). Sites in both MEA and LEA showed significant speed modulation of theta power (MEA: 
\[ t(6) = 3.81, p < 0.01; \] LEA: \[ t(5) = 15.10, p < 0.0001 \] ) with no difference between MEA and LEA sites \( (t(11) = 1.09, p = 0.30; \) Fig. 3D,E). Locomotor speed predicted roughly 10-20\% (mean \( r^2=0.18 \) for all EC sites) of variability at electrode sites in the MEA and LEA. Thus the relationship of speed to theta power was less than that observed at septal HPC sites, suggesting that the relationship of speed to theta power within septal HPC sites is not necessarily a consequence of this phenomenon at EC sites. Certainly, our analysis was limited to 14 sites across the areal axis of the EC (see Fig 1 as well as Supplemental Figure 3). Given the topography of EC to HPC projections, one might expect locations in the most lateral band of the EC (subjacent the rhinal sulcus; Dolorfo and Amaral, 1998a) to exhibit the largest speed to power relationship. Clearly, we did not observe any obvious differences among the more laterally located electrode sites, but most were located in the medial band of MEC and LEC with only two sites approaching the region subjacent the rhinal sulcus. Further assessment of variation in the speed to power relationship across the areal surface of the EC is thus warranted.

Theta frequency and locomotor speed throughout the HF

The relationship between theta frequency and locomotor speed was investigated in the same manner as the relationship between theta power and locomotor speed described above. Theta frequency was found to increase as a function of locomotor speed in all regions explored (Figure 4). This positive relationship between locomotor speed and theta
frequency can be seen for three simultaneously recorded sites across the septotemporal axis of CA1 in Figure 4A. In opposition to the septotemporal gradient of the influence of locomotor speed on theta power, sites across the septotemporal axis of both CA1 and DG showed significant speed modulation of theta frequency. The relationship between locomotor speed and theta frequency did not vary across the septotemporal axis of either CA1 (r = -0.35, p = 0.09) or DG (r = -0.18, p = 0.53; Fig. 4B). Assessing the speed modulation of theta frequency within individual quartiles of both CA1 and DG indicated that all regions of the hippocampus explored displayed significant speed modulation of theta frequency (CA1 1st: t(4) = 18.73, p < 0.001; 2nd: t(11) = 13.52, p < 0.001; 3rd: t(7) = 12.32, p < 0.001; DG 1st: t(7) = 11.43, p < 0.001; 2nd: t(6) = 11.61, p < 0.001; Fig. 4C). In EC, theta frequency was speed modulated in both MEA and LEA (MEA: t(6) = 3.25, p < 0.05; LEA: t(5) = 2.95, p < 0.05; Fig. 4D). Thus theta frequency was positively modulated by the locomotor speed of the animal in all regions of the hippocampal formation explored. Notably the present results support relatively independent mechanisms for regulating hippocampal theta amplitude as compared to theta frequency. A variety of evidence indicates a prominent role for the medial septal inputs in controlling theta amplitude, while a network including the nucleus reticularis pontis oralis (RPO) and hypothalamic nuclei (e.g., supramammillary nucleus) controlling theta frequency (see Lee et al., 1995; Vertes and Kocsis, 1997; Vertes et al., 2004; Pan and McNaughton, 2004 for reviews).

Rhinal-hippocampal and intrahippocampal theta coherence and locomotor speed
The relation between locomotor speed and theta coherence was examined between EC and DG sites (EC-DG pairs = 32) as well as CA1 (EC-CA1 pairs = 48). MEA and LEA sites were grouped together since there was no statistical difference between the two areas (p’s > 0.50). Similar to changes in theta power, theta coherence between paired EC and HPC sites significantly increased as a function of locomotor speed when the pair included a septal HPC electrode site (Fig. 5A). Regression analysis of the septotemporal position (distance from septal pole) and the speed-related r-value showed that theta coherence between EC-CA1 pairs and EC-DG pairs exhibited less relation to speed when HPC electrode sites were located at more temporal levels (r = -0.44, p < 0.005 for EC-CA1 pairs; r = -0.55, p < 0.005 for all EC-DG pairs regardless of areal position of the EC electrode (Fig. 5B). Examining the data grouped by hippocampal quartiles, theta coherence increased in relation to locomotor speed only between EC-CA1 and EC-DG pairs within the septal most quartile of the HPC (EC-CA1 (t(3) = 5.04, p = 0.02; EC-DG (t(12) = 6.30, p < 0.0001; Fig. 5C). Thus the speed-related increase in theta coherence between any EC electrode and any HPC electrode varied along the septotemporal axis. Importantly, the intrahippocampal coherence in relation to speed also exhibited variation along the septotemporal axis. Plotting the speed-related correlation coefficient among HPC electrode pairs by quartile, Figure 5D illustrates that coherence among the septal most electrodes increased, while coherence among more temporally located pairs decreased as a function of locomotor speed.

Theta power across repeated behavioral sessions

We collected data during an initial run session (baseline) of at least 50 trials and then returned rats to the linear track to run additional sessions of at least 50 trials at +5, +20, +60
and +120 minutes after the initial run session (Fig. 1A). In examining data across repeated recording sessions within a single day, it became obvious that there was a systematic decrease in theta power over sessions at more temporally located electrode sites. A clear downward shift in the linear best-fit line for the relationship between speed and theta power was observed at the more temporal aspects of both DG and CA1 without any significant change in the slope of the lines (Fig. 6A). This shift was not evident at septal HPC sites (Fig. 6A). In order to examine if this decrease had any relation to running speed or time (habituation), we conducted a linear regression analysis factoring out speed across time points. The resulting standardized regression coefficients (β-values) reflect the change in theta power means from baseline to the post baseline recordings controlling for the speed of the animal. The β-values were assessed using a within-electrode repeated measures ANOVA. A significant time-dependent reduction in theta power was observed in the second and third quartiles of CA1, as well as within the second quartile of the DG (CA1 Q1 F(3,12)=3.49, p=0.05; CA1 Q2 F(3,33)=12.08, p<0.001; CA1 Q3 F(3,21)=6.99, p<0.005; DG Q1 F(3,21)=1.76, p=0.19; DG Q2 F(3,18)=7.58, p<0.005; Fig. 6B). A significant decrease in theta power was observed among electrodes in the first quartile of the DG (p < 0.01; Fig. 6B), but this decrease did not vary across time points. No significant changes were observed among electrodes in the first quartile of CA1 (Fig. 6B). Thus the reduction in theta power observed at more temporally located recordings sites appears to represent habituation to sensory, motor or motivational aspects of the behavior without any obvious relationship to differences in the overt motor act (eg, speed of locomotion).

Discussion
Dynamic variation in LFP signals (eg, power and coherence) may serve as useful measures of change in the highly laminar and topographically organized afferent zones of hippocampal afferents. Such changes can be related to ongoing sensorimotor experience (eg, speed), recent past behavioral experience (eg, habituation over repeated testing) or cognitive processes (eg, Kay, 2005; Ulanovsky and Moss, 2007; Rizzuto et al., 2006; Montgomery et al., 2009; Tort et al., 2009; Shirvalkar et al., 2010).

The present findings demonstrate substantial septotemporal variation in indices of theta LFPs as a function of sensorimotor experience and recent past behavioral experience. Foremost, we observed that theta power increased linearly as a function of speed at electrode sites in the septal HPC, accounting for 40-80% of the variability in theta power. The strength of this relationship diminished with distance from the septal pole. Second, the relationship between speed and theta power, and its decline across the long axis was more prominent at CA1 sites as compared to sites within the DG. Third, theta power increased with speed at all EC sites, including both LEA and MEA sites. The relationship between speed and theta power did not vary across the areal axis of the EC (LEA as compared to MEA). Fourth, theta coherence between EC and HPC also exhibited a septotemporal gradient in its relationship to speed, such that coherence between EC-septal HPC sites was positively speed modulated, while coherence between EC – temporal HPC sites was not speed modulated. Fifth, theta coherence increased as a function of speed between pairs of septally located HPC electrodes, while theta coherence decreased between temporally located HPC pairs. Last, we observed significant changes in theta power as a function of behavioral habituation (repeated runs on a linear maze). This habituation-related decrease in theta power was minimal at CA1 sites in the septal HPC and was most prominent at sites located distant from the septal pole.
Our most basic finding is that speed is a prominent predictor of theta indices (both power and coherence) in the septal HPC accounting for 40-80% of the variability in theta power at septal CA1 sites. Speed, however, had a limited relation to variation in theta power at more temporal locations. The latter is consistent with previously observed differences in the speed to power relationship across the longitudinal axis observed by Maurer and colleagues (2005) with regards to both amplitude of theta field potential and the change in place field firing rate as a function of locomotor speed (see Fig 4; Table 6 in Maurer et al., 2005). Differences in the speed to power relationship in the present study were not directly attributable to a decrease in the variability of the theta signal, but likely reflects a fundamental difference in the mechanisms/circuitry that govern theta across the long axis. We observed significant changes in theta power associated with repeated behavioral testing within a daily session that also exhibited regional (septotemporal) variation. These data suggest that the theta LFP signal is highly dynamic, reflecting clear variation in the septotemporal synchronization of synaptic input across the dendritic field of hippocampal neurons. Such variation can be compared in many ways to alterations in the BOLD signal of human fMRI studies, where changes across the anterior-posterior axis of the HPC have been observed in relation to novelty, familiarity and habituation (Stern et al., 1996; Strange et al., 1999; see Kumaran and Maguire, 2009 for review).

Anatomic differences across the septotemporal axis

Areal variation in theta signals may reflect variation in the topography of several afferent inputs that synapse on the dendritic field of CA1 and DG neurons. There are three major populations of excitatory glutamatergic afferents that are critical to theta current
Speed and hippocampal theta generation. First, projections from layer 2 EC neurons target the distal dendrites of dentate granule neurons (as well as local GABAergic neurons). Second, projections from layer 3 EC neurons target the distal dendrites of CA1 neurons. These EC projections originate from segregate areal bands that project in a topographic manner along the septotemporal axis (Dolorfo and Amaral, 1998a, 1998b). Thus, the EC projections provide a potential source of variation in the theta signal across the long axis. Third, intrinsic hippocampal associational projections (CA3 to CA1 and mossy cells to DG) project extensively across the long axis (Ishizuka et al., 1990; Amaral and Witter, 1995) providing a potential mechanism for synchronizing neural activity.

While the topography of EC afferents could contribute to the observed differences in theta coherence, we found no variation in the relationship between speed and any theta index across the areal surface of the EC. While our mapping of the EC was limited, we have no evidence to support areal variation in the speed to power relationship at EC sites. Deshmukh and colleagues (2010) have illustrated clear differences in theta power and the theta modulation of EC neurons between the MEA and LEA. Our study was limited to fourteen sites across the areal surface of the EC and few of the sites (see Fig 1F) extended to the lateral band of the EC. Neurons in the most lateral band of the EC in both the MEA and LEA, subjacent the rhinal sulcus and at the dorsocaudal extreme of the EC innervate the septal 50% of the HPC (see Dolorfo and Amaral, 1998a; 1998b). Thus, additional mapping of variations in the theta signal and its relation to speed will be required to determine the influence of EC inputs on variation in the speed to theta power across the septotemporal axis of the HPC.

In addition to the excitatory EC inputs, any number of studies point to the prominent role of subcortical afferents including glutamatergic afferents (eg, supramammilary nucleus),
GABAergic afferents (most prominently from the medial septum) and several neuromodulatory inputs in generating and influencing the theta signal via direct afferent input to the HPC and/or indirectly via medial septal afferents (see Lee et al., 1994; Bland, 1986; Bland and Oddie, 2001; Vertes and Kocsis, 1997; Vertes et al., 2004).

While many studies have demonstrated variation in the discharge rate of subcortical neurons across theta states (e.g., theta versus non-theta), few studies have systematically examined changes in neuronal firing rate as a function of locomotor speed. King and colleagues (1998) described that many (65%), but not all, medial septal neurons exhibit a linear increase in burst discharge rate as a function of speed. Thus, medial septal inputs may provide a key source of variation in relaying speed information across the septotemporal axis. Such speed information may be transmitted to septal neurons via supramammillary, mid-line thalamic, or other brain stem afferents. Importantly, there is considerable topographic variation in the organization of ascending subcortical afferents both in their direct projections to the HPC as well as in their indirect inputs to medial septal neurons, which also project topographically throughout the septotemporal axis (Amaral and Kurz, 1985; Amaral and Witter, 1995).

Further our analyses indicated that while there were differences in the relationship between speed and theta power across the septotemporal axis, we did not observe any differences in the relationship of speed to theta frequency. While theta frequency increases in relation to locomotor speed, there was no variability in this relationship across the septotemporal axis of the HPC or across distinct sites within the EC. The fundamental difference between the variations in speed to power as compared to speed to frequency across the septotemporal axis supports the idea that distinct subcortical circuits regulate theta power and frequency. A variety of evidence indicates that a network including the
nucleus reticularis pontis oralis (RPO) and hypothalamic nuclei (eg, supramamillary nucleus) contribute to theta frequency independent of theta power (see Vertes and Kocsis, 1997; Vertes et al., 2004; Pan and McNaughton, 2004 for reviews).

**Functional differentiation across the septotemporal axis**

Historically significant emphasis has been placed on examining the functionality of distinct hippocampal subregions within the trisynaptic circuit (DG>CA3>CA1) rather than functional differences across the areal or longitudinal expanse of the HPC, which in the rodent is referred to as the septotemporal axis. The rodent septal pole is most similar to the posterior HPC in humans, while the temporal pole is similar to the anterior aspect of the HPC. The septotemporal axis of the HPC is analogous to an area region of the neocortex and receives topographically organized input from distinct areal regions of the EC. Thus, the septal 50% of the HPC receives input from lateral band EC neurons subjacent to the rhinal sulcus (Dolorfo and Amaral, 1998a, 1998b). The topographic organization of EC inputs may convey relatively segregate domains of associative sensory input to different septotemporal levels of the HPC (Burwell and Amaral, 1998; Lavenex and Amaral, 2000). A variety of behavioral studies based largely on lesion data in rodents and neuroimaging data in humans support functional differentiation of hippocampal circuits along the long axis (Moser et al., 1995; Strange et al., 1999; see Bannerman et al., 2004 for review). Additionally there is considerable septotemporal variation in neuronal markers (eg, Gusev et al., 2005), neuromodulation of plasticity (Maggio and Segal, 2007) and differences in the experiences that influence neurogenesis along the long axis (Snyder et al., 2009).
Our laboratory is particularly interested in synchronicity in theta along the septotemporal axis of the HPC and its relation to the EC input, with the general hypothesis that under distinct sensory or behavioral conditions theta coherence will increase among functional interactive domains, or decrease between less interactive domains. While theta is clearly coherent across the long axis, we have reported that there is a general decrease in theta coherence across the long axis during REM sleep (Sabolek et al., 2009; see also Royer et al., 2010). The present findings demonstrate a general increase in power and coherence in relation to locomotor speed across the most septal aspect of the HPC that is sustained over repeated behavioral experience (see Fig. 6). In contrast, there is a minimal alteration in theta power in relation to locomotor speed in the more temporal aspects of the HPC and a systematic decrease over repeated behavioral experience (see Fig. 6). Typically, rats are trained and tested as in this case in the same environmental context, so the observed changes occur in an environment that is highly familiar to the animal. Recent findings in our laboratory demonstrate that novel spatial environments (while the rat is performing the exact same behavior, running along a linear path) increase theta power and theta coherence across much of the septotemporal axis (Penley and Chrobak, unpublished observations). Thus, it appears that changes in theta power and coherence are sensitive to both novelty and habituation. As observed in the human neuroimaging studies, the LFPs signal variation in rodents also demonstrates considerable variability across the septotemporal axis.

**Summary**

Theta LFP signals within the HF reflect the dynamic interaction of competing and cooperating inputs, which vary across the dendritic field of HPC and EC neurons. In this
regard, systematic assessment of this signal at various laminar and septotemporal positions can provide a spatial and temporal window into the dynamic flow of afferent input. Systematic changes in theta potentials have been linked to changes in sensorimotor integration, the flow of sensory input, as well as cognitive/memory functions. The present findings demonstrate dynamic and distributed pattern of theta LFPs across the septotemporal axis of the hippocampus in relation to locomotor speed and recent past experience.
Acknowledgements: This work supported by NSF (0090451) to JJC and MAE.
References


Dolorfo CL, Amaral DG (1998b) Entorhinal cortex of the rat: organization of intrinsic


Figure 1. Experimental timeline and electrode locations A) A series of five recordings within a single day were obtained from each rat. The end of the baseline recording marked Time zero (T0) and then recordings were initiated at T5, T20, T60 and T120. Each recording required the rat to complete 50 traversals of the linear track. In between recordings rats sat in their home cage on a table adjacent to the linear track. No changes were made to the experimental environment in between recordings. B) The position of a rat along the 140 cm linear track over time during six consecutive trials is shown. Black lines overlaid on the gray trace indicate the portion of each track traversal that was considered as an individual trial. C) The rat’s velocity as a function of position on the track is shown for the six trials shown in A) plus the following 6 consecutive trials. Velocity is depicted instead of speed in order to illustrate the similarity in running behavior in both directions. Again the black lines indicate the portion of each traversal that was included as a single trial in further analyses. D) Distribution of mean trial speeds for an entire single recording session. E) Photomicrographs of representative recording sites in septal DG (top left), septal CA1 (top right), midseptotemporal DG (bottom left) and EC (bottom right) are shown. F) Flatmap representation of the hippocampal formation with all recording locations indicated by black stars. The septal pole of the hippocampus is located at the top and the temporal pole at the bottom. The thick lines mark the boundaries between the hippocampal quartiles, as well as the boundary between MEA and LEA. G) Baseline theta power values as a function of distance from the septal pole. Theta power tended to be slightly lower in both CA1 (blue) and DG (red) with greater distance from the septal pole. H) Despite the lower theta power values with greater distance from the septal pole, the standard deviation of theta power values at a given site did not vary as a function of distance from the septal pole.

Figure 2. Variable speed modulation of theta power. A) Local field potential traces simultaneously recorded from two septotemporal levels of CA1 and DG, as well as one site in EC from a single rat. Note the prominent theta oscillations present at all recording locations. The distance from the septal pole is indicated for the CA1 and DG recording sites. B) Each scatter plot shows the relationship between mean trial speed and theta power for the five simultaneously recorded sites shown in A) during the baseline recording only (~50 trials). The distance of the CA1 and DG recording sites from the septal pole are displayed at the top of each plot and the correlation coefficient (r) is displayed at the bottom of each plot. Note the strong relationship between speed and theta power at the septal CA1 and DG sites, as well as the EC site, but that relationship decreases at the more temporal sites. On the right is a flatmap representation of the hippocampal formation with the black stars indicating the five recording locations.

Figure 3. Variation in speed modulation of theta power throughout the hippocampal formation. A) The relationship between mean trial speed and theta power for three simultaneously recorded sites along the septotemporal axis of CA1 is shown during the baseline recording only (~50 trials). The distance of each recording site from the septal pole is displayed in each plot along with the correlation coefficient. Note the decrease in the correlation coefficients with increasing distance from the septal pole. B) The relationship between distance from the septal pole and the speed vs theta power correlation coefficients for each CA1 (red) and DG (blue) electrode is shown in the scatter plot. A significant decrease in the CA1 correlation coefficients as a function of distance from the septal pole can be seen, while there was a trend for a decrease in DG. C) All CA1 and DG electrodes were grouped according to septotemporal quartile in order to assess whether theta power was significantly speed modulated in each region. Theta power was significantly speed modulated in the first and second quartiles of CA1, but only in the first quartile of DG. Additionally, there was greater speed modulation of theta power in CA1 than in DG. D) Scatter plots, as in A), of the relationship between mean trial speed and theta power for two simultaneously recorded electrode sites in EC, with one site
in MEA and the other in LEA during the baseline recording only (~50 trials). E) Mean correlation
coefficients for MEA and LEA where theta power was significantly modulated by speed in both areas.

Figure 4. Speed modulation of theta frequency throughout the hippocampal formation. A) The
relationship between mean trial speed and theta frequency for three simultaneously recorded sites
along the septotemporal axis of CA1 is shown during the baseline recording only. The distance of
each recording site from the septal pole is displayed in each plot along with the correlation coefficient.
Note the there is no change in the correlation coefficients with increasing distance from the septal pole.
B) The positive relationship between locomotor speed and theta frequency is observed across the
septotemporal axis of both CA1 and DG as indicated by the nonsignificant correlation between each
electrodes distance from the septal pole and the speed vs theta frequency correlation coefficients for
each CA1 (red) and DG (blue) electrode. B) All CA1 and DG electrodes were grouped according to
septotemporal quartile in order to assess whether theta frequency was significantly speed modulated in
each region. Theta frequency was significantly speed modulated in all quartile investigated. C) Theta
frequency was also found to be speed modulated in both MEA and LEA.

Figure 5. Rhinal-hippocampal and intrahippocampal theta coherence and locomotor speed A) Middle,
flatmap showing a recording site in EC and four hippocampal sites. The scatter plots show the
relationship between mean trial speed and theta coherence between the EC site and each of the four
different hippocampal sites. Distances displayed in each plot are the distance of the hippocampal
electrode sites from the septal pole. Also displayed in each plot is the correlation coefficient for the
relationship between speed and theta coherence for that electrode pair. Note that theta coherence
between EC and the more temporal hippocampal electrodes is less speed modulated than theta
coherence between EC and the septal hippocampal electrodes. B) The speed modulation of EC-CA1
(blue) and EC-DG (red) theta coherence significantly decreases as a function of the distance from the
septal pole of the HPC electrode. C) Theta coherence between EC and the first quartile of CA1 and
DG is speed modulated, while theta coherence between EC and all other quartiles is not speed
modulated. D) Theta coherence within the hippocampus is speed modulated when the electrodes are
within the septal half, while theta coherence within more temporal aspects of CA1 actually decreases
with increasing locomotor speed.

Figure 6. Theta power habituates at temporal levels A) Flatmap displays five simultaneous recording
sites (same as in Figure 2). Scatter plots show theta power as a function of mean trial speed during the
baseline recording (black) and the fifth recording session of the day that was initiated 120 minutes after
the cessation of the baseline recording (red). The distance from the septal pole is indicated for the
hippocampal sites, as well as the standardized regression coefficients (β) for each site. A clear
downward shift in the linear best fit line can be seen in the more temporal DG and CA1 sites
demonstrating a within day habituation of theta power. B) Mean β-values for all regions investigated.
There was a significant effect of time in the second and third quartiles of CA1 and the second quartile
of DG, while the first quartile of DG showed a decrease in theta power without an effect time. *
indicate a significant time-dependent reduction in theta power for an individual quartile as assessed by
repeated measures ANOVA (p’s < 0.01). † indicates a significant decrease in theta power from
baseline for an individual quartile (p’s < 0.01).

Figure S1. Electrode placements in the lateral entorhinal area (LEA) spanning from approximately 5.8
(A), 6.8 (B) and 7.8 (C) mm AP. Images (left) illustrate location of electrode track within the LEA,
while close-up images (right) illustrate tip of electrodes in superficial layers of EC.
Figure S2. A) The slope (b), indicating the potential amount of change in theta power as a function of locomotor speed, of the theta power versus speed relationship decreases as a function of distance from the septal pole (compare with Figure 3B). B) Theta power vs speed slopes were significantly positive in the first quartile of both CA1 and DG and the second quartile of CA1, but not in the second quartile of DG or the third quartile of CA1 (compare to Figure 3C). C) In both MEA and LEA the theta power vs speed regression slopes were significantly positive (compare to Figure 3E).

Figure S3. Relationship between locomotor speed and theta power for each layer of CA1 and DG. A-E) Each plot shows the relationship between distance from the septal pole and the speed vs. theta power correlation coefficient for electrode in each of the layers of CA1 (A-C) and DG (D-E). Note that the strength of the relationship, as measured by the correlation coefficient, decreases with increasing distance from the septal pole in all layers. Although there are slight differences in the strength of the influence of locomotor speed in septal hippocampus, the differences seen across the septotemporal axis tend to exceed the differences observed across the layers in septal hippocampus.
**Table 1: Theta Power by HPC Quartile and within MEA/LEA**

<table>
<thead>
<tr>
<th></th>
<th>CA1-Q1</th>
<th>CA1-Q2</th>
<th>CA1-Q3</th>
<th>DG-Q1</th>
<th>DG-Q2</th>
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<tr>
<td>Baseline</td>
<td>46.3 ± 2.2</td>
<td>40.1 ± 1.4</td>
<td>40.7 ± 1.0</td>
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<tr>
<td>Baseline</td>
<td>1.3 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.9 ± 0.1</td>
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<tr>
<td>(Standard Deviation + SEM)</td>
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<tr>
<td>Baseline</td>
<td>6.2 ± 0.9</td>
<td>7.5 ± 0.4</td>
<td>7.2 ± 0.6</td>
<td>7.9 ± 0.7</td>
<td>9.0 ± 0.4</td>
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<td>(Range + SEM)</td>
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<tr>
<th></th>
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<th>LEA</th>
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<tr>
<td>Baseline</td>
<td>33.5 ± 1.9</td>
<td>32.4 ± 1.1</td>
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<tr>
<td>Baseline</td>
<td>2.0 ± 0.1</td>
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<tr>
<td>Baseline</td>
<td>7.5 ± 0.4</td>
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<td>(Range + SEM)</td>
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</table>

Baseline values expressed as dB relative to 1µV.
A

CA1 2.6 mm

\[ r = 0.93 \]

CA1 4.9 mm

\[ r = 0.32 \]

CA1 6.7 mm

\[ r = 0.12 \]

B

Correlation Coefficient (r)

C

Hippocampal Quartile

Q1 Q2 Q3

Correlation Coefficient (r)

D

Theta Power (dB)

MEA

\[ r = 0.41 \]

LEA

\[ r = 0.36 \]

E

Correlation Coefficient (r)
A

Normalized Coherence vs Mean Speed (cm/sec)

- EC-DG (1.9 mm) $r = 0.33$
- EC-DG (4.8 mm) $r = 0.73$
- EC-CA1 (1.3 mm) $r = 0.32$
- EC-CA1 (4.9 mm) $r = 0.53$

B

Correlation Coefficient (r) vs Distance from Septal Pole (mm)

- EC-CA1
- EC-DG

C

Bar Graph

- Correlation Coefficient (r)
- Q1 - Q3
- * indicates significance

D

Correlation Coefficient (r) vs Distance from Septal Pole (mm)

- CA1-CA1
- DG-DG
A

![Graphs of Theta Power (dB) vs Mean Trial Speed (cm/sec) for DG, CA1, and EC with regression coefficients β.](image)

B

![Bar charts of standardized regression coefficients (β) for CA1, Dentate Gyrus, and Entorhinal Cortex with time points (+5 minutes, +20 minutes, +60 minutes, +120 minutes).](image)