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Spike Field Coherence (SFC) for Ripples in Rat Hippocampus

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Abstract

The aim of this project was to determine coherence between two types of neural recordings which can be obtained from the rat hippocampus: spikes and local field potentials. Extracellular recording makes it possible to determine spiking activity from individual neurons in the vicinity of the recording electrode. Local field potential recording gives a combined activity of many neurons (thousands) at once to determine an overall picture of the coordination of the cells in real time. Here we examine the relationship between these two signals, focusing on place cells which spike at their maximal rate only at certain positions in physical space. Examining these cells using spike-field coherence techniques we determined that during running and rest behaviors, the place cells have different patterns of firing. During run sessions on a linear track, they would fire maximally at their preferred position on the track and showed high spike-field coherence with the theta band (4 Hz – 10 Hz) of the LFP. During rest sessions on the same track, when ripples occur, the place cells would fire sequentially, essentially replaying back the run session firing at a much faster timescale and with high spike-field coherence in the gamma band (100 Hz – 250 Hz) of the LFP.
Introduction

Navigation

Navigation can be simply understood as going from point A to point B. Taking this simple concept and expanding it further leads to traversing the layout of environments both natural and manmade by many mediums. How does this seemingly innocuous task become registered in the mind? As a mental process it must have a cognitive basis upon which it is imprinted and then later read when needed. Rats are shown to have impairment on navigation with lesions in the hippocampus (Morris et al., 1982).

Spatial Memory

In the hippocampus, cells which are appropriately named “place” cells have been discovered which sheds light on some of the mystery. As an animal traverses the environment, the firing rate of hippocampal place cells changes. Usually, there is a localized spatial location, a preferred place, where the neuron has a maximal firing rate (O’Keefe and Dostrovsky, 1971; O’Keefe, 1976). Due to the number of place cells existing in the layers of the hippocampus and the loss of function when a lesion is present in the hippocampus, spatial mapping seems to occur in the hippocampus (O’Keefe and Nadel, 1978).

Episodic Memory

In addition to spatial memory, there is evidence that episodic memory may be represented in the hippocampus of rats (Wood et al., 1999). Episodes consist of different types of information which can be bridged into sequences to make a structured based network known as semantic memory (Eichenbaum et al., 1999). Both are categorized under a higher subdivision known as declarative memory (Suzuki and Eichenbaum, 2000). A famous example of impairment in this type of memory can be seen from the studies done on Patient H.M. who had parts of his brain surgically removed to attenuate his epileptic seizures. One of the structures included the hippocampus which made him...
unable to form new memories (Scoville and Milner, 1957) while keeping his visuomotor skills (Milner, 1962) intact. This provided evidence that different types of memory exist in the brain, giving credibility that declarative memory (conscious information of facts and events) is different from procedural memory (skill-based information) (Cohen and Squire, 1980; Squire, 2009).

**Link between Memories**

Recently, efforts have been done to link two types of memory: spatial and episodic. A phenomenon known as replay is said to occur in the rat hippocampus during periods of rest after spatial experience (Foster and Wilson, 2006). Replay contains spatial information, namely place cell firing in a reverse order as an event or “episode”. This firing occurs within the same timeframe of a “ripple” event in the local field potential (100Hz –250 Hz oscillations lasting for 100 msec.) which is the measure of local activity of multiple neurons including place cells near the recording site.

**Anatomy and Main Circuit of the Hippocampus**

Before delving deeper into the replay and ripple interplay, it is necessary to step back and take a look at the anatomy of the hippocampus. In the rat brain, the hippocampus is composed of mainly three parts: the Cornu Ammonis (CA 1, CA 2, and CA 3), Dentate Gyrus (DG) and the Enthorinal Cortex (EC). The EC receives processed sensory information from other cortical areas in the brain. It essentially functions as a repository of centralized sensory information which sends its excitatory pyramidal cell axons to the excitatory granule cells of the DG. These granule cells send their axons up to the excitatory pyramidal cells in CA 3 which in turn send their axons up to the excitatory pyramidal cells in the CA 1. The pyramidal cells in the CA 1 send their axons back into the EC via an intermediary structure known as the Subiculum, thus completing the circuit (Amarell and Lavenex, 2006) (Fig. 1). The information flow that is described in the circuit is mainly unidirectional and throughout the different parts of the
hippocampus, inhibitory neurons known as interneurons exist to maintain the integrity of the circuit (Freund and Buzsaki, 1996).

**Figure 1:** The anatomy and circuit of the hippocampus as drawn by Ramon y Cajal.

*Spikes and LFP*

The cells which are given the name place cells are mainly the pyramidal cells in the CA 1 and CA 3 region. As mentioned previously, they fire spikes at their highest when the rat is at a specific position in space. Multi-unit recordings make it possible to determine spikes coming from multiple individual neurons simultaneously to make position decoding possible. While this technique discriminates each neuron as an individual unit, local field potential averages a pool of neuronal activity near the recording electrode (Kajikawa & Schroeder, 2011). It is a very useful technique as it gives an overall picture of the electrical activity of the structure. There are two states or patterns of oscillation (Fig. 2) in the rat hippocampus that the LFP can be characterized by. The first is rhythmical slow wave activity (RSA) or theta rhythm (4 Hz – 10 Hz) which can be seen during running and REM sleep (Buzsáki, 2002). The second is a broad spectrum of large irregular activity (LIA) which persists in behaviors such as grooming and eating (Vanderwolf, 1971; O’Keefe and Nadel, 1978). There are short-lived events in this spectrum known as sharp wave ripples (SWR) that exist in the 100 Hz – 250 Hz frequency range (O’Keefe and Nadel, 1978; Buzsáki et al., 1983; Ylinen et al., 1995).
Physiology of Theta Activity

Theta activity (4 Hz – 10 Hz) is oscillatory with a sinusoidal like shape in nature (Fig. 2). A number of theories exist to explain the oscillation such as recurrent inhibition loops and feed forward inhibition (Buzsaki, 1984; Buzsaki and Eidelberg, 1982). Generally, there is a synchronized activation of pyramidal cells in the CA region which is then followed by inhibition by the interneurons. After this inhibition, there is a rebound excitatory phase (O’Keefe and Nadel, 1978).

Physiology of Ripple Activity

SWR occur due to the concerted activity of the pyramidal cells in the CA 3 region, which gives a sharp wave characteristic in the stratum radiatum of the CA 1 region (Buzsáki, 1986; Csicsvari et al., 2000). The activity in the CA 3 region leads to the activation of pyramidal cells and interneurons in the CA 1 and this gives rise to the 100 msec. SWR (100 Hz – 250 Hz) oscillation in the CA 1 pyramidal layer (Ylinen et al., 1995) (Fig. 2).

Consequence and Motive

Due to its suggested implications in memory consolidation and retrieval (Carr et al., 2011), replay is an area of active research. Replay has been further seen to occur sequentially either forward
or in reverse and to not only code for experiences that occurred right before replay but also remote events (Anoopum et al., 2010). It has been established that theta power is high when the rat undergoes a motor behavior such as running (Whishaw and Vanderwolf, 1973). Ripple events occur when the theta wave is absent (Buzsaki and Silva, 2012) such as when the rat is at rest. Consequently, replay is shown to occur during these ripple events (Foster and Wilson, 2006). Given these points, the thesis will take a new approach and calculate the coherency between LFP and place cells (ones assumed to be involved in replay) during ripple events.

Coherence

As stated previously, the LFP reflects the local activity of neurons, if an individual neuron is firing at a rhythm which matches the oscillation of the LFP; it safe to assume that it is involved in a coordinated activity. Spike-field coherence (SFC) measures phase synchronization between the local field potential (LFP) and spike times as a function of frequency (Fries et al., 2001) (Fig. 3).

Methods

Data that was analyzed was previously recorded from the Buzsaki Lab which is publicly available online (Mizuseki et al. 2014).

All analyses were performed using custom-written MATLAB (MathWorks) code.

Subjects and recording. Long Evans rat was anesthetized with isoflurane (1-1.5%). 4 or 8 shank silicon probe(s) were implanted in the right dorsal hippocampus and recorded from CA1, CA3 or dentate gyrus,
and another 4-shank silicon probe was implanted in the right dorsocaudal medial entorhinal cortex (Mizuseki et al. 2014). Detailed information about the angle of insertion of the electrodes can be found in the paper referenced.

Behavior. After a week of recovery from surgery, physiological signals were recorded while the rat ran a linear track (Fig. 4). The rat was water deprived 24 hrs. prior to the start of the recording. For tracking the position of the animal, two small light-emitting diodes, mounted above the headstage, were recorded by a digital video camera at 30 Hz resolution (Mizuseki et al. 2014).

![Figure 4: Linear track which is 250 cm x 7 cm with a water reward on both ends of the track.](image)

Data Collection. 4 or 8 shank silicon probe(s) contained 8 recording sites each giving 32 or 64 site silicon probes. Signals from the recording sites were amplified (1,000×), bandpass-filtered (1 Hz–5 kHz) and acquired continuously at 20 kHz (DataMax system; RC Electronics). After recording, the signals were down-sampled to 1,250 Hz (DataMax system) for the local field potential (LFP) analysis (Fig. 5). Spike (800 Hz–5 kHz) sorting was done using KlustaKwik yielding clusters which were used for further analysis (Fig. 6) (Mizuseki et al. 2014).

![Figure 5: LFP recording on one recording site of an electrode.](image)

![Figure 6: Classification of a single neuron based on similar waveform spikes recorded simultaneously on 8 recording sites of an electrode.](image)
**Position Information.** Acquire raw Data (Fig. 7). Interpolate missing intermittent position data; normalize track length to 0-1 scale (Fig. 8), and separate into trials with bounds set from .1 to .9. Set up basis function with Gaussian distribution for position data (function count: 10, width: .05) (Fig. 9).

![Raw Position Data](image1.png) ![Normalized Position Data](image2.png)

**Figure 7:** Raw data position taken from only the x coordinate. **Figure 8:** Normalized position data removing inconsistent position information.

![Basis Functions](image3.png)

**Figure 9:** Basis functions for position data. Shows 10 basis curves for the 10 basis functions, the second set of red, blue, and green curves are separate functions and not associated with the first set of rbg curves.

**Place Field Analysis.** Linear Poisson Regression Place Tuning Curve for each place field was estimated using the model below (Stevenson et al. 2012) (Fig. 10). Results of the analysis were sorted by peak max position (Fig. 11). Place fields were filtered by spike counts in leftward direction trials. Cells with spike count of more than 200 spikes were discarded to remove putative interneurons from analysis; cells with
spike count of less than 10 were discarded to ensure activity within trial. Cells that were common to all leftward trials or all but one were used in replay detection (Table 1).

Equation 1: The estimated firing rate for each neuron $\lambda_i$ is a function of the external variable $x$, which is the position basis functions. $\beta_0$ is the baseline firing rate, $\alpha$ is baseline basis fitting (Stevenson et. al. 2012).

Figure 10 (left): Fitting of tuning curve over the position with the greatest rate of spikes to generate place field.

Figure 11 (right): Cells sorted using the analysis described, presence of a diagonal pattern from 35 to 100 which shows successful sorting of cells with a higher peak position firing rate than the cells before it.

Table 1: Color coded table of cells which were present in the 6 leftward trials, only 22 cells passed the test and were used for further analysis.

**LFP filtering:** theta band, and Ripple detection. Run LFP in a Butterworth band-pass order 4 filter (4 Hz – 10 Hz). Use theta filtered LFP for later analysis. Run LFP in a Butterworth band-pass order 4 filter (100 Hz – 250 Hz). Standardize filtered LFP data and find ripple regions which exceed 2 st. dev., have ripple peak which exceeds 5 st. dev., has an inter ripple interval of at least 30 msec. and has a ripple duration of at most 100 msec. (FMA toolbox) (Fig. 12) (Fig. 13). Use ripple filtered LFP for later analysis.
Figure 12 (top left, top right, bottom left): Progression of LFP shape from raw form to standardized form. The dashed red lines represent the 2nd st. dev. and 5th st. dev. which ripple regions and ripple peaks need to exceed respectively.

Figure 13 (bottom right): Red diamonds show some examples of ripples that were selected using the ripple detection criteria.

**Filtered LFP over Trials.** Compute spectrogram for raw LFP and the two filtered LFPs from the prior sections.

**Coherency analysis.** The coherency spectrum between two signals, $x$ and $y$, is defined as

$$\text{Coherency}_{xy}(f) = \frac{S_{xy}(f)}{\sqrt{S_{xx}(f)S_{yy}(f)}}$$

Equation 2. $S_{xy}(f)$ denotes the cross-spectrum, and $S_{xx}(f)$ and $S_{yy}(f)$ denote the auto-spectra of each signal. These were computed using the multitaper method (Ray and Maunsell, 2011).
Signal x is the continuous LFP data and signal y is the point spike times, the coherence analysis was computed using the Chronux Toolbox.

*Relationship between running and Theta band.* Select 4 randomized 1 sec. segments when rat is running down the track for each leftward trial. Calculate coherency between cell spikes from the Place Fields Analysis section and raw LFP from the LFP filtering section for each running segment (Chronux Toolbox). Parameters used for coherency: winseg = .4 Fpass = [0,250] tapers = [3,5] pad=2.

*Relationship between rest and Ripple band.* Calculate coherency between cell spikes from the Place Fields Analysis section and raw LFP from the LFP filtering section for each ripple time segment from the Ripple Detection section (Chronux Toolbox). Parameters used for coherency: Fpass = [0,250] tapers = [3,5] pad=2.

*Statistical testing between theta and ripple.* Calculate peak coherence frequency for run and rest segments. Calculate mean coherence across theta and ripple band for rest and run segments. Compute the theta-ripple coherence ratio between the rest and run segments.
Results

Place Field Results. The Poisson Regression generated the sorted place fields of 80 cells from a pool of 100 cells. As seen in the previous section. Further filtering so that each cell fired in the leftward trials (follow <200 spikes and > 10 spikes criteria) brought the cell count to 22 cells (Fig. 14).

Figure 14: (Top) shows red boxes of the 6 trials considered, they were chosen because the rat only moved in one direction and there was a considerable amount of the rest after the run. (Bottom) raster plot for the 22 cells for the 6 trials, notice the pattern of spikes and run.
Sample Replay Event.

Replay was assumed to occur for every candidate ripple event (Fig. 15).

Figure 15: Boxed area shows a sample replay event during a ripple event.

Ripple Results. Running the ripple algorithm explained in the previous section generated these results for one channel:

After detection by thresholding: 728 events.

After ripple merge: 443 events.

After peak thresholding: 200 events.

After duration test: 179 events.

After selected time filtering: 90 events.
LFP Spectogram.

Figure 16. Spectrogram of filtered and unfiltered LFPs over the entire data set. Red dotted boxes represent the 6 trials considered.

**Theta-Running Results.** Computing the coherency between the 22 cells and the LFP generated for the 24 1 second run segments these results (Fig. 17).

Figure 17. Coherency of LFP and spikes, green line boundary represents theta band frequency (4 Hz – 10 Hz) and red line to the end represents the ripple band frequency (100 Hz – 250 Hz). p>0.05.
**Ripple-Rest Results.** Computing the coherency between the 22 cells and the LFP generated for the 90 ripple events is split into three graphs (Fig. 18).

![Graphs showing coherency](image)

Figure 18. Coherency of LFP and spikes, green line boundary represents theta band frequency (4 Hz – 10 Hz) and red line to the end represents the ripple band frequency (100 Hz – 250 Hz). *p* > .05. Ripple Duration between 10 and 25 msec: event # 1 – 15. Ripple Duration between 25 and 50 msec: event # 16 – 68. Ripple Duration between 50 and 100 msec: event # 69 – 90. *p* > .05 for all three graphs

**Note:** Since the ripple coherency was broken up into three segments, they were averaged together to represent ripples that last from 10 to 100 msec.

**Peak Coherence.** Peak coherence during running occurs at frequency 9.8 Hz. Peak coherence during rest occurs at frequency 189.6 Hz.
**Mean Coherence.** The mean coherence across the theta band is .3724, the mean coherence across the ripple band is .2813 for run segments. The mean coherence across the theta band is .3364, the mean coherence across the ripple band is .3766 for rest segments.

**Coherence Ratio.** The theta-ripple coherence ratio for run segments was 1.323 and the theta-ripple coherence ratio for rest segments was .8862.
Discussion

The ripple-replay correspondence has been shown to be significant in many instances. This project attempts to shed light on the phase coherence between the ripple and replay events. One thing to state is replay was not calculated in the paper, it is assumed that replay occurs during rest segments when ripples occur. It is important to make sure the cells which are undergoing replay are place cells and this why a considerable amount of effort was done to ensure successful detection of place cells. From the initial 100 cells presented in the data set, the rigorous amount of filtering brought the count down to 22. Figure 14 shows the firing pattern for these 22 cells and how it matches the movement of the rat during that time. Similarly, a considerable amount of focus was spent to detect ripples, from the initial 728 events; the count was brought down to 90 events by running the ripple detection algorithm. One caveat is that the detection was only done on one channel whereas the place cells were distributed in areas where the one channels electrode was out of range to detect the activity. Only 3 out of the 22 cells were near channel 10 electrode who’s LFP was taken for replay detection. More channels as well as adding a replay detection algorithm can be incorporated to bring more accuracy to the results.

The theta filtered spectrogram in figure 15 shows regions of high theta activity during the boxed trials, the activity is generally high when the rat is running along the track and lower otherwise. From this observation and the literature that exists on theta studies, place cells will be more coherent during the theta rhythm than any other frequency. From the results obtained, this seems to be the case since the peak SFC during running segments was when the frequency was 9.8 Hz which falls into the 4 Hz – 10 Hz theta range. The mean coherence also supports this claim.

The aim of the project was to determine if there was any coherence between the ripple events and spiking where it was assumed replay occurs, and if so at what frequencies. The coherency was split into three graphs because each comparison used different bin sizes for frequency, in the end the
coherency was averaged across the three graphs and the peak coherency for rest segments occurred at 189.6 Hz which is in the 100 Hz – 250 Hz ripple range. This suggests that place cells fire more in phase when the rat is at rest to the ripple band more than any other frequency. The mean coherence also supports this claim.

By computing a ratio of the mean coherence for the two bands, run and rest segments can be compared directly. The theta-ripple coherence ratio for run segments is 1.323 and for rest segments is .8862. Because the ratio is > 1 during run segments, it is more coherent during theta. Because the ratio is <1 during rest segments, it is more coherent during ripples.

In closing, this project supports the idea that place cell activity is different during running and rest. When the rat is running, the place cells fire in a pattern (spatial memory) which corresponds to the rat’s current position (Fig. 14) and are coherent to the theta band of the LFP (Fig. 17). On the contrary, when the rat is at rest, the place cells exhibit a replay activity (Fig. 15) (episodic memory) which occurs at a much faster timescale and is coupled with the ripple band of the LFP (Fig. 18).
References


**Software**


**Data Set**

HC-3: CRCNS.org.http://dx.doi.org/10.6080/K09G5JRZ

**Custom Toolboxes**

Chronux Toolbox: chronux.org

FMA Toolbox: fmatoolbox.sourceforge.net