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Environmental novelty elicits a later theta phase of firing in CA1 but not subiculum

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Abstract

The mechanism supporting the role of the hippocampal formation in novelty detection remains controversial. A comparator function has been variously ascribed to CA1 or subiculum, while the theta rhythm has been suggested to separate neural firing into encoding and retrieval phases. We investigated theta phase of firing in principal cells in subiculum and CA1 as rats foraged in familiar and novel environments. We found that the preferred theta phase of firing in CA1, but not subiculum, was shifted to a later phase of the theta cycle during environmental novelty. Furthermore, the amount of phase shift elicited by environmental change correlated with the extent of place cell remapping in CA1. Our results support a relationship between theta phase and novelty-induced plasticity in CA1.

Keywords

Hippocampus; novelty; theta; rhythmic slow activity; encoding; retrieval

Theta-related activity in the hippocampal formation supports spatial memory and novelty detection. However, while the hippocampus proper (O'Keefe and Nadel, 1978; Meeter et al, 2004) and specifically region CA1 (O'Keefe and Nadel, 1978; Hasselmo et al., 1996; Lisman and Otmakhova, 2001), is often implicated in comparator/novelty processing, this function has also been ascribed to the subiculum (Naber et al., 2000; Gray, 1982). We explored how novelty affected theta-related principal cell firing in CA1 and subiculum. The phase of cell firing relative to theta may provide a metric for the temporal organization of neuronal firing. Supported by demonstrations that long-term plasticity is modulated by theta phase (e.g. Pavlides et al, 1988; Huerta and Lisman, 1993; Holscher et al., 1997), computational models have proposed that theta phase difference helps to separate encoding and retrieval modes in the hippocampus (e.g. Hasselmo et al., 2002; Manns et al., 2007). Thus, focusing on CA1, environmental novelty would favor the encoding mode by enhancing synaptic plasticity and the extrinsic inputs to CA1 from entorhinal cortex, while a familiar environment favors the retrieval mode in which the intrinsic inputs from CA3 to CA1 are enhanced and synaptic plasticity is reduced. These models therefore predict that

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environmental novelty should correspond to a change in the theta phase of neuronal activity in CA1 (or in subiculum under the alternative models of Naber et al., 2000 and Gray, 1982).

To investigate these predictions, we recorded unit and EEG activity from five rats in a study examining responses to environmental familiarity and novelty by employing a test trial series fully described in (Jeewajee et al., 2008), see Fig. 1A, B. Briefly, rats were run in an initially novel Environment A for five consecutive days, with four trials a day (T1 to T4). On the next day (Day 1*) rats were exposed for the first time to novel Environment B (T3, T4) and novel Environment C (T6) interleaved with further trials in Environment A (T1, T2, T5). The present report is based on 187 CA1 cells and 95 subicular cells taken from Day 1, Day 2, and Day 1*. The spikes recorded from a given region in a given trial (hereafter referred to as an ‘ensemble’ of spikes) were analysed with respect to the phase of theta at which they were fired. See Detailed Methods.

The theta-phase distribution of firing was significantly non-uniform in all ensembles, indicating theta-modulated firing, and justifying our use of the circular mean phase of firing (hereafter the ‘preferred firing phase’ of the ensemble). Due to electrode movement across different days, and uncertainties in electrode positioning relative to the cell layer, we report *relative* changes in preferred theta phase, and defer comment on absolute theta phase reference till the end of this report. We calculated the preferred theta phase on a baseline trial for each day and rat and region, and assigned ‘zero phase’ to that baseline trial preferred phase, expressing other trial ensembles’ preferred phase relative to that baseline. See Detailed Methods. The baseline trial used was always the day’s last trial in Environment A (i.e., the most familiar environmental setting for that day’s trials). Four rats were available for CA1 ensemble analysis, and four rats for subiculum ensemble analysis (CA1 and subiculum recorded simultaneously in three rats). See Tables 1 and 2 in Detailed Methods for cells/spikes data breakdown.

We asked if environmental novelty altered the preferred firing phase in either region. We investigated two types of novelty: that associated with within-day trial order, and that associated with the introduction of new environments within the common test setting. As described below, both types of novelty elicited a later preferred phase of firing in CA1 ensembles (Fig. 1C shows a schematic overview of our finding).

We first looked at the influence of trial order on days 1 and 2. Four rats were available for repeated measures analysis of CA1 ensembles recorded in the four trials per day. ANOVA showed a clear effect of trial-within-day ($F_{3, 21} = 7.93$, $p = 0.001$), caused by a significantly later preferred phase in trial 1 than in each of the other three trials (mean difference 18° ; Fisher’s LSD tests: $p = 0.03$). This same analysis showed no such effect in subiculum ($F_{3, 21} = 0.25$, $p = 0.86$). Subicular preferred phase values did not differ across trials within-day. See Fig. 1D and Fig. 2A.

A similar pattern of phase shift was seen on Day 1*. CA1 preferred phase in the first trial in Environment A was shifted later in the theta cycle relative to the two subsequent trials in that environment, (4 rats, Trial 1, $+21 \pm 4^\circ$; Trial 2, $+3 \pm 8^\circ$; Trial 5, 0° ; effect of trial, $F_{2, 6} = 6.60$, $p = 0.03$; Fisher’s LSD tests: trial 1 vs 5, $p = 0.01$; trial 1 vs 2, $p = 0.06$). Again, no such effect was seen in subiculum ($F_{2, 6} = 1.77$, $p = 0.25$).

In a complementary analysis, the expected positive correlation was seen between preferred firing phase in CA1 and trial primacy (1 for the first trial of the day, 0 for the other trials; $n = 32$, $r = 0.649$, $p = 0.00006$ for days 1-2; $n = 12$, $r = 0.717$, $p = 0.009$ for Day 1*).

We then compared preferred firing phase in the familiar and novel environments on Day 1*. The novel Environment-B and Environment-C trials were paired with the most temporally-

adjacent Environment-A trials. Paired-t tests (4 rats, 3 trial pairs per rat: T3-T2, T4-T5, T6-T5) showed that the preferred phase of CA1 ensembles increased by 33° in novel environment trials ($t_{11} = 3.63$, $p = 0.004$), while there was no such effect in subiculum ($t_{11} = 0.98$, $p = 0.35$), see Fig. 1E and Fig. 2B. Restricting this analysis to simultaneously-recorded ensembles in three rats gave similar results for the phase difference in novelty (CA1 = $+28^\circ$, $t_8 = 3.63$, $p = 0.035$; Subiculum = $+4^\circ$, $t_8 = 0.85$, $p = 0.42$), as did including even the first trial in Environment A as a baseline familiarity trial (3 trial pairs: T1-T3, T2-T4, T5-T6, CA1 = $+27^\circ$, $t_{11} = 2.72$, $p = 0.02$; subiculum = $+3^\circ$, $t_{11} = 0.70$, $p = 0.50$).

In a complementary analysis, the expected positive correlation was seen between preferred theta phase and novelty status on day 1* (1 for trials in environments B and C, 0 for trials in environment A; $n = 24$, $r = 0.472$, $p = 0.02$).

Thus environmental novelty appears to elicit a shift in CA1 firing phase. Placing a rat into a new environment changes the location and rate of firing of CA1 place cells ('remapping': Muller, 1996; Wills et al, 2005; Leutgeb et al, 2005), with the likelihood and extent of remapping increasing with the extent of the environmental change (Muller, 1996; Shapiro et al, 1997; Lever et al, 2002; Anderson and Jeffery, 2003; Leutgeb et al, 2005). If a novelty-induced change in CA1 firing phase corresponds to increased plasticity in the CA1 place cell representation, consistent with previous experimental (e.g. Huerta and Lisman, 1993) and theoretical work (e.g. Hasselmo et al, 1996; Lisman and Otmakhova, 2001; Hasselmo et al., 2002; Meeter et al, 2004), then the extent of phase shift should correlate with the extent of remapping.

In support of this idea, we noticed that on Day 1*, for each of the four rats with CA1 data, levels of remapping (relative to Environment A) were higher in novel Environment C than in novel Environment B, and correspondingly, that the theta phase-increase effect was larger in Environment C than in Environment B (Fig. 1F). Using spatial correlation to measure the similarity of the firing pattern across two trials, we found that the extent of remapping correlated significantly with theta phase increase (ensemble spatial similarity score vs preferred phase, four trial pairs per rat, $n = 16$, $r = -0.625$, $p = 0.010$), as predicted. See Detailed Methods.

Could any behavioural changes elicited by the new environments explain the increase in preferred theta firing phase seen in CA1? We found no significant correlations between firing phase and behavioural variables such as running speed (preferred phase vs mean running speed: $n = 24$, $r = 0.16$, $p = 0.45$) or rearing frequency ($n = 24$, $r = 0.15$, $p = 0.49$) on Day 1*.

We also asked if the preferred phase changes in CA1 simply reflected variations in excitability. Globally, theta phase likely controls CA1 excitability; the discharge probability of CA1 pyramidal cells is maximal around/just after the trough of pyramidal-layer theta (Kamondi et al., 1998; Csicsvari et al., 1999). We tested for relationships between preferred theta phase and global firing rate, calculated by dividing all spikes in a trial by the total number of cells active on that day, and dividing this by trial duration. Introducing novel environments did not change the mean global firing rate (0.54 Hz in Environment A; 0.53 Hz in Environments B and C). We first examined phase-increase associated with novel environments in the Day 1* dataset, but found no correlation between preferred phase and global firing rate ($n = 24$, 6 trials per rat, 4 rats, $r = -0.06$, $p = 0.78$). Further, we examined the phase-increase associated with the first trial of the day, but found no correlation between preferred phase and global firing rate over the trials in Environment A on days 1-2 ($n = 32$, 8 trials per rat, 4 rats, $r = -0.18$, $p = 0.33$), or on Day 1* ($n = 12$, 3 trials per rat, 4 rats, $r = -0.47$, $p = 0.12$). Finally, we note that this entire pattern of results was unchanged by

normalizing firing rates to account for inter-rat firing rate variance. The absence of a relationship between theta phase and firing rate argues against a simple explanation in terms of novelty-related variation in excitability, and is consistent with the (Manns et al, 2007) study of object-novelty effects in which there was no consistent relationship between changes in firing rate and changes in theta phase.

Up to this point, we have deferred mention of the absolute mean theta phase of CA1 spiking. This issue is important, because previous experimental work shows (e.g. Huerta and Lisman, 1993; Holscher et al., 1997) and theoretical work assumes (e.g. Hasselmo et al., 2002) that LTP-like synaptic plasticity in CA1 cells preferentially occurs at the peak of CA1 pyramidal-layer theta. A careful study in behaving rats indicated maximal firing of CA1 pyramidal cells at around 40° after the pyramidal-layer theta trough (a familiar environment was used: Csicsvari et al, 1999). Our novelty-elicited phase increase effect (c.15°-50° *later* than the baseline phase) would therefore result in a mean phase closer to the pyramidal-layer theta peak, consistent with the above-mentioned previous literature. The baseline mean phase of theta in this study averaged around 110°-150° after the local theta trough, but our theta-recording electrodes were mainly below the CA1 layer (e.g electrodes which had initially exhibited ripples and complex spikes, but then went through the layer). Interestingly, in one rat where the same theta-recording electrode was used throughout, and where tetrodes were not moved throughout days 1-6, the absolute preferred phase of CA1 spike ensembles decreased over days (rat A, 25 ± 2.4 cells/day, absolute preferred phase of ensembles in last & 2nd-last trials in Env A vs Day, $n = 12$, $r = -0.69$, $p = 0.01$). In other words, preferred theta phase of CA1 spiking became earlier and earlier in the theta cycle as environment A became progressively more familiar.

In summary, our data support the hypothesis that encoding and retrieval in CA1 preferentially take place at different stages of the theta cycle. Two kinds of novelty (the day's first trial, and novel environments) elicited an increase in CA1 preferred theta phase, such that firing was later in the theta cycle, and likely closer to the pyramidal-layer theta peak. Further, the degree of preferred phase increase (relative to the familiar environment) was correlated with the degree of spatial remapping (relative to the familiar environment). Preferred theta phase was also increased at the beginning of the day even in the highly-experienced Environment A (20 trials by the start of Day 1*). This could reflect encoding that occurs when an existing representation is 'updated' after a long delay (22 hours vs 20 minutes). This would be consistent with demonstrations that rats, when faced with two equally familiar spatial locations (or objects or odors), preferentially explore the location encountered longer ago, and that CA1 lesions impair this preference (Hunsaker et al, 2008).

The magnitude and direction of the effect warrant further examination. We emphasise that the preferred phase measure is an average over an entire trial (10 minutes in Envs A & B, 15 minutes in Env C). Larger effects might be seen with designs tailored to an encoding/plasticity versus retrieval/stability contrast. It seems likely, for instance, that some recall from previous sessions or earlier in a trial will occur in our novelty conditions. Theta phase *increase* in environmental novelty is consistent with Study 1 of (Manns et al, 2007). Interestingly, this increase is also consistent with preliminary indications that entorhinal-CA1 communication (utilizing fast gamma) is enhanced at a *later phase of theta* than CA3-CA1 communication (utilizing slow gamma) (Colgin et al, 2007, Soc. Neur. Abstr. 93.4), under the assumption that our novelty conditions enhance the entorhinal-CA1 inputs relative to the CA3-CA1 inputs, as the model by Hasselmo and colleagues postulates.

In summary, we have shown that the preferred theta phase of firing of CA1 principal cells shifts to a later phase of theta in novelty that is likely closer to the pyramidal-layer theta peak, and that this shift correlates with plasticity in the CA1 place cell representation. These

findings have implications for the neuronal mechanisms required for detecting and encoding novel information.

Detailed Methods

See (Jeewajee et al, 2008) for descriptions of EEG recording, testing environments, and test trial series, and (Wills et al, 2005) for descriptions of place cell recording and spatial firing analysis. For a cell to be included in the analysis, it had to have a pyramidal-like waveform, and fire with a locational peak rate of at least 1Hz (after smoothing, Wills et al, 2005) on at least one trial of the day. All CA1 and subiculum cells showed spatial firing.

Theta phase

The locally-recorded EEG signal was filtered using a 6-12Hz, 251-tap, Blackman windowed, band-pass *sinc* (sine cardinal) filter. An analytic signal was then constructed as: $s_a(t_k) = s(t_k) + iH[s(t_k)]$, where H specifies the Hilbert transform, $s(t_k)$ is the filtered EEG signal and $t_k = k\Delta$ where $k = 1, \dots, K$ and Δ is the inverse of the sampling rate. The phase of the analytic signal, $\phi(t_k)$, gives the phase of the EEG at time t_k and each spike was assigned the phase $\phi(t_k)$, if it occurred in the interval $[t_k - \Delta/2, t_k + \Delta/2]$. The analytic signal was filtered to remove periods of low quality EEG by discarding those regions with low signal power, which was identified as follows. Average power was calculated for each cycle in the analytic signal. Cycles with power below the 15th percentile in each trial and the spikes falling within them were discarded.

For each regional ensemble, we first calculated the circular mean phase of all the ensemble's spikes relative to the analytic theta signal (defining the trough as 180°, and peak as 0/360°). Rayleigh's-Z and Watson's-U² tests performed on each of the 56 CA1 and 56 subiculum ensembles (Days 1 & 2, n = 32 per region; Day 1*, n = 24 per region) showed that all were significantly non-uniform (Rayleigh's Z test, all p values < 0.0001 and generally << 0.0001; Watson's-U², all p values < 0.005). The circular mean phase of each regional ensemble ('preferred phase'; n = 112 ensembles in total) was thereafter treated as a single observation.

We then re-defined each preferred phase value such that, for each rat and region and day, the preferred phase on the baseline trial for that rat/region/day was 0°. This was done simply by subtracting from each mean phase value, the mean phase value of the baseline trial (last trial in familiar Environment A). On Days 1 and 2, the baseline trial was the 4th trial in Environment A (T4), and on Day 1* it was the 3rd trial in Environment A (T5). Preferred phase values were then linearly transformed where necessary (e.g. 350° became -10°). The range of preferred phases across any given set of comparisons was generally less than a quarter-cycle of theta, and never exceeded 110 degrees. Accordingly, linear statistics were used for the preferred phase comparisons.

We give a worked example of the assignment of preferred phase: On Day 2, the preferred phase of CA1 ensembles in rat A relative to the local theta (CA1 stratum radiatum, where trough = 180°, peak = 0/360°) were as follows: T1 = 353.0°, T2 = 335.3°, T3 = 322.3°, T4 = 328.0°. On day 2, T4 was the baseline trial, so after subtraction of 328.0°, these values became T1 = 25.0°, T2 = 7.3°, T3 = 354.3°, T4 = 0.0°. After linear transformation, these values became T1 = +25.0°, T2 = +7.3°, T3 = -5.7°, T4 = 0.0°. These last values were used for statistical comparisons.

There was no requirement for a minimum number of spikes to be fired by a cell to be included in the analysis: i.e. if a cell fired a single spike, that spike contributed to the calculation of the 'preferred phase' of the ensemble for that region. Accordingly, the number

of cells contributing to the spike ensemble on a given trial was generally equal to, or approached, the total number of cells recorded on that day.

Comparing remapping with changes in preferred theta phase of firing

The spatial firing similarity score across pairs of trials on Day 1* was derived using standard topological transformation and spatial bin-by-bin correlation methods (Wills et al., 2005), with the ensemble value for each rat being the mean spatial correlation across cells. A high score indicates spatial firing across a pair of trials is similar, a low or negative score that spatial firing is dissimilar (remapped). Since the theta phase-increase effect was seen for the first trial of the day in Environment A on Day 1*, this trial was excluded from the remapping vs preferred phase analysis. The second trial in Environment A served as the Baseline trial. For each trial X on Day 1*, preferred phase on trial X minus preferred phase on the Baseline trial gave the difference in preferred phase. This difference was compared to the spatial firing similarity score across trial X and the Baseline trial.

Behaviour

Running speed was calculated from 50-Hz position tracking data, with 400ms boxcar smoothing. Rearing frequency was counted using manual cell counters during each trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Preferred theta phase of firing in CA1, but not subiculum, increases in novelty (A) Laboratory recording setup. (B) Test trial series showing order of trials across and within days. Environment A was a square-walled box, Environment B a cylinder of different material to A (room cues visible), Environment C was a raised platform, the ‘floor’ of Environments A and B. (C) Schematic diagram of main result. Preferred theta phase of firing in CA1 ensembles occurs later in the theta cycle in novelty than familiarity. Indication of absolute preferred phase for illustrative purposes only. (D) Preferred theta phase in CA1, but not subiculum, occurred later in the theta cycle in the first trial of the day in Environment A relative to subsequent trials in that same environment. * $p = 0.03$ (see main text). (E) Preferred theta phase in CA1, but not subiculum, increased in trials in the novel environments B & C, relative to trials in the familiar environment A (first trial of day excluded). *** $p = 0.004$ (see main text). Note that 0° is defined for each ensemble as the preferred theta phase of the last trial of the day in the familiar environment, i.e. T4 in (D); T5 in (E). (F) Increase in theta phase preference is associated with remapping (ie. low spatial similarity score).

Figure 2. Novelty-elicited increase of preferred phase of firing in CA1 ensembles

Rose histograms show distribution of CA1 spikes over the theta cycle for each trial in 10°-bins. Representative spike ensembles show preferred phase is later in the theta cycle: **(A)** on the day's first trial relative to later trials (Day 1, rat C and Day 2, rat A); **(B)** in novel environments compared to familiar environment (Day 1*, rat A). Phase of spiking normalised such that mean phase in Trial 4 (A) and Trial 5 (B) is 0°. μ is mean phase, κ is Von Mises' κ (concentration), Z is Rayleigh's Z test statistic. κ is inversely related to the dispersion of the data, and is an index of the depth of theta modulation (Cacucci et al, 2004).

TABLE 1

Total number of cells recorded from each region. The number in parentheses indicates cells recorded simultaneously with cells from the other region.

	CA1	Subiculum
Day 1	66 (30)	35 (22)
Day 2	54 (31)	33 (18)
Day 1*	67 (46)	27 (20)
Total	187 (107)	95 (60)

TABLE 2

Cells/day and spikes/trial breakdown for individual rats. All Values expressed as means $\pm$ SEM. There were 3 testing days and 14 testing trials per rat. Number of spikes per trial are shown before and after filtering out spike-data associated with lowest-power theta (see 'Theta Phase' below).

Rat	A	B	C	C	D	D	E	E
Region	CA1	SUBIC	CA1	SUBIC	CA1	SUBIC	CA1	SUBIC
Cells per day	26.7 ± 4.7	10.7 ± 2.0	5.0 ± 1.7	9.0 ± 1.0	11.0 ± 0.6	8.0 ± 1.0	19.7 ± 3.5	4.3 ± 1.2
Spikes per ensemble before filtering	7823 ± 761	47169 ± 1478	2635 ± 251	17774 ± 810	4300 ± 385	24870 ± 1405	8987 ± 614	11809 ± 1793
Spikes per ensemble after filtering	6712 ± 656	40418 ± 1309	2260 ± 213	15093 ± 664	3700 ± 338	21127 ± 1198	7696 ± 535	10178 ± 1543