Environmental Novelty is Signaled by Reduction of the Hippocampal Theta Frequency

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ABSTRACT: The hippocampal formation (HF) plays a key role in novelty detection, but the mechanisms remain unknown. Novelty detection aids the encoding of new information into memory—a process thought to depend on the HF and to be modulated by the theta rhythm of EEG. We examined EEG recorded in the HF of rats foraging for food within a novel environment, as it became familiar over the next five days, and in two more novel environments unexpectedly experienced in trials interspersed with familiar trials over three further days. We found that environmental novelty produces a sharp reduction in the theta frequency of foraging rats, that this reduction is greater for an unexpected environment than for a completely novel one, and that it slowly disappears with increasing familiarity. These results do not reflect changes in running speed and suggest that the septo-hippocampal system signals unexpected environmental change via a reduction in theta frequency. In addition, they provide evidence in support of a cholinergically mediated mechanism for novelty detection, have important implications for our understanding of oscillatory coding within memory and for the interpretation of event-related potentials, and provide indirect support for the oscillatory interference model of grid cell firing in medial entorhinal cortex. © 2007 Wiley-Liss, Inc.

KEY WORDS: hippocampus; EEG; acetylcholine; exploration; rat; associative mismatch

INTRODUCTION

Detection of novelty is crucial to the everyday life of all mammals, and is a prerequisite for the efficient encoding of events into memory. The hippocampal formation (HF) has long been linked with novelty detection, especially where experience differs from expectation given the experimental context or other stimuli (a.k.a., "contextual" or "relational" novelty, "associative mismatch" or "comparator" processing, see e.g., Sokolov, 1963; Hasselmo et al., 1996; Honey et al., 1998; Aggleton and Brown, 1999; Strange et al.,

1999; Gray and McNaughton, 2000; Lisman and Otmakhova, 2001; Vinogradova, 2001; Kohler et al., 2005; Kumaran and Maguire, 2006), consistent with its role in context-dependent episodic/declarative memory (O'Keefe and Nadel, 1978; Squire and Zola-Morgan, 1991; Cohen and Eichenbaum, 1993). The electrophysiological correlates of contextual novelty detection have been the focus of extensive experimental investigation (Halgren et al., 1980; Rugg and Coles, 1995; Knight, 1996; Fried et al., 1997; Grunwald et al., 1998; Axmacher et al., 2006; Grunwald and Kurthen, 2006; Rutishauser et al., 2006), paralleled by intense theoretical speculation regarding the underlying electrophysiological and neuro-pharmacological mechanisms (Myers et al., 1996; Gray and McNaughton, 2000; Lisman and Otmakhova, 2001; Ranganath and Rainer, 2003; Yu and Dayan, 2005; Hasselmo, 2006). However, the precise mechanism of novelty detection in the HF remains elusive.

Environmental novelty is a particularly salient form of contextual novelty for foraging mammals, and the HF both represents environmental layout, via the firing of "place cells" (O'Keefe and Nadel, 1978; Muller, 1996) and directs exploration of spatial alterations to environmental layout—i.e., of unexpected rearrangement or absence of objects, but not replacement of an object by a new one—(O'Keefe and Nadel, 1978; Save et al., 1992a,b; Lee et al., 2005). This exploration is thought to enable environmental representations to be updated (O'Keefe and Nadel, 1978), and seems to result from discrepancy between current experience and a stored (spatial) representation rather than the absolute novelty of individual stimuli. Indeed, some place cells ("misplace" cells) specifically fire at the location of an unexpectedly displaced or missing object (O'Keefe, 1976; Fyhn et al., 2002; Lenck-Santini et al., 2005), but not when an object is replaced by a new one (mirroring the effects of lesions, Lenck-Santini et al., 2005). In addition, a sufficiently changed environment induces rapid global "remapping" of the entire place cell representation (Bostock et al., 1991; Wills et al., 2005; Fyhn et al., 2007): producing a new attractor state in memory (Nakazawa et al., 2002; Leutgeb et al., 2005; Wills et al., 2005).

Here, we examine the electroencephalogram (EEG) of rats during their initial foraging in a novel environmental setting, as it subsequently became familiar, and finally in two new environments unexpectedly encountered within the now-familiar setting. The most pro-

minent feature of the hippocampal EEG of foraging rats is the 7–11 Hz "movement-related theta" rhythm (Vanderwolf, 1969; Kramis et al., 1975). This rhythm is generated in the HF under the influence of cholinergic and GABAergic innervation from the medial septum, see (Buzsaki, 2002; O'Keefe, 2006) for reviews, and the involvement of the cholinergic septo-hippocampal system in spatial memory has long been known (Givens and Olton, 1995). In addition, empirical studies have demonstrated the importance of theta oscillations for efficient memory operations (Sederberg et al., 2003; Fell et al., 2004; McCartney et al., 2004; Ekstrom et al., 2005; Mormann et al., 2005; Jacobs et al., 2006; Osipova et al., 2006; Rizzuto et al., 2006) and there has been theoretical interest in the possible role of the theta rhythm in controlling memory encoding and retrieval, see e.g., (Myers et al., 1996; Gray and McNaughton, 2000; Borisyuk et al., 2001; Lisman and Otmakhova, 2001; Hasselmo et al., 2002; Meeter et al., 2004; Hasselmo, 2006). Accordingly, we sought to examine the link between environmental novelty and theta, and we specifically set out to test the prediction of a recent model of neural firing patterns in the HF: that environmental novelty might be signaled by a drop in theta frequency (Burgess et al., 2007).

MATERIALS AND METHODS

Subjects

Four male Lister Hooded rats, weighing 315–390 g at time of surgery were used as subjects. They were maintained on a 12:12-h light:dark schedule (with lights off at 3 pm). Food deprivation was maintained such that subjects weighed 85–90% of free feeding weight.

Electrode Implantation

The surgical procedures used in this study were as previously described (Cacucci et al., 2004). Briefly, rats were chronically implanted with two microdrives under deep anaesthesia. These microdrives allowed four variously-spaced tetrodes to be vertically lowered through the brain after surgery. Tetrodes were constructed from 25 μm HM-L coated platinum-iridium wire (90%/10%, California fine wire), and were aimed at CA1 or subiculum. Coordinates for the CA1 target insertion zone were: 3.0–3.1 mm posterior to bregma, 1.8–2.0 mm lateral to the midline.

Electrophysiological Recording and Data Acquisition

Rats were allowed a week to recover postoperatively before screening sessions began. Microelectrodes were lowered towards hippocampal regions over days/weeks, and then left to stabilize before recording commenced. Electrophysiological recording techniques have been previously described (Cacucci et al., 2004). Briefly, the electrode wires were AC-coupled to unity-gain buffer amplifiers (headstage). Lightweight wires (3 m) connected the headstage to a preamplifier (gain 1000). The outputs of the preamplifier passed through a switching matrix, and then to the filters and amplifiers of the recording system (Axona, UK). Electrodes used for EEG were located as follows; Rats 1 and 2, CA1 throughout; Rat 3, CA1 (days 1–4) and subiculum (thereafter); Rat 4, CA1 (days 1–3) and dentate (thereafter). (We note that theta frequency does not vary with recording location within the HF, unlike amplitude and phase (Bullock et al., 1990)).

EEG signals were amplified 10,000–20,000 times, band-pass filtered at 0.34–125 Hz and sampled at 250 Hz. Two arrays of small, infrared light-emitting diodes (LEDs), one array brighter and more widely projecting than the other, were attached to the rat's head to track head position and orientation, using a video camera and tracking hardware/software (DACQ2, Axona, UK). The two arrays were positioned such that the halfway position between the two arrays was centered above the rat's skull. Offline analysis defined this halfway position as the position of the rat (TINT, Axona, UK). Positions were sampled at 50 Hz.

Screening and Training Procedures Before the Test Trials

Before the period of formal recording of the test trial series, rats were screened and acclimatized to recording apparatus and rice-foraging, in a room separate from both the home cage room and the testing lab. Electrode activity was monitored over a 2–5 week period. During screening, the rat rested on a square holding platform (39 cm sides, 5 cm high ridges) containing sawdust. Rats were pretrained in this screening room to forage for rice on a black square platform (94 cm sides, 2 cm high ridges). Each rat was given a few sessions (10–20 min long) of training until it had acquired the random foraging task such that it spent most of its time moving over all areas of the platform. These training procedures were done so that, as far as possible, the novelty afforded by the first exposures to Environment "a" did not include non-environmental novelty associated with rice-related cues/contingencies, continuously locomoting with the headstage, and so on. Once electrodes were appropriately positioned and acclimatization complete, the rat was brought to the testing lab, and placed on a holding platform (similar to the one in the screening room), see Figure 1a. All the rats had 2–3 h of exposure to the holding platform in the testing lab over 1–2 days before beginning the test trial series.

Test Trial Series

A diagram of the environments used and the order of testing in the test trial series are shown in Figures 1a,b. Experiments were conducted within a black-curtained circular testing arena 2.3 m in diameter (except that the curtains were opened for Environment "b," as described below). The centre of each testing environment had the same location relative to the arena. An external white cue card (102 cm high, 77 cm wide) which was prominent in the testing arena provided directional con-
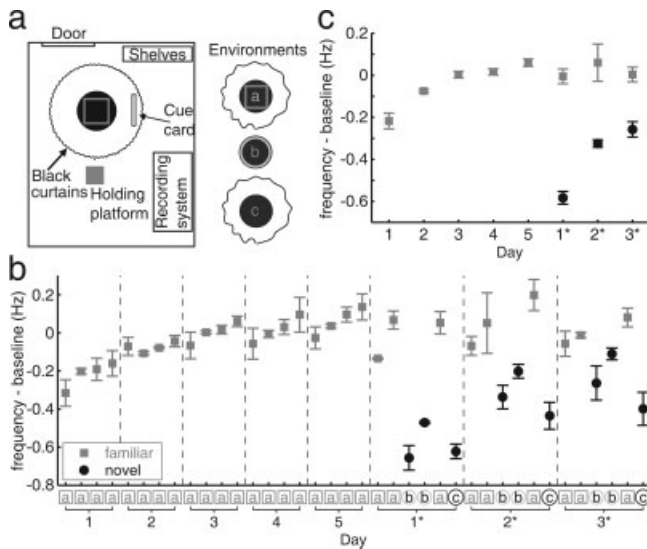
FIGURE 1. Effect of novelty on theta frequency. (a) Experimental set-up. (b) Theta frequency increases with days of experience in environment "a" (days 1–5), and change to an environment within a common setting produces an even greater initial decrease in frequency than first exposure to environment "a" (cf. frequency in environments "b" and "c" on days 1*–3*, black points, to frequency in environment "a" on days 1–3, grey points). The difference in theta frequency from "baseline" (i.e. the mean frequency over days 2–5 for a given rat) is shown for each trial. (c) The novelty effect is shown most clearly when each day's trials within familiar environment "a" (grey points) or novel environments "b" and "c" are averaged (black points). Points show the mean across rats, error bars show s.e.m.

stancy throughout the test trial series, as did standardized procedures for translating the rat and placing it into the box, and various background cues. For every trial, the rat was passively displaced, facing "lab north," about 2 m in the "lab north" direction, and placed at the centre of the given environment. During trials the rat searched for grains of sweetened rice randomly thrown into the box about every 30 s. At the end of each trial, the rat was removed from the given recording environment, and placed back on the holding platform. Rats were kept on a holding platform outside the arena before and after every trial. The inter-trial interval was 20 min.

Three environments were used in the test trial series. Environment "a" was a square "morph" box, as previously described (Lever et al., 2002), with 62 cm sides. The black curtains were fully drawn so as to surround Environment "a." Environment "b" was a circular-walled, wooden light-grey enclosure, 79 cm in diameter, with "lab-north" and "lab-south" seams. For Environment "b," the black curtains were opened, such that various extra-arena cues were visible. The "floor" of Environments "a" and "b" was a circular black platform, raised 27 cm above the actual floor of the testing arena. Environment "c" consisted simply of this circular open platform, which was 90 cm in diameter, with the arena curtains fully drawn as for Environment "a." The platform was thus the floor for all three environments, and was cleaned before every trial.

During the habituation phase (days 1–5) there were four trials per day, all in Environment "a." This was followed by the manipulation phase, on days 6, 8, and 10 (referred to as days 1*, 2*, and 3*, respectively, where the asterisk indicates day within the manipulation phase). No testing was performed on days 7 and 9. There were six trials on days 1*–3*, organized as follows. Trials 1, 2, and 5 were conducted in Environment "a," trials 3 and 4 in Environment "b," and trial 6 in Environment "c." The black curtains were opened 90 s before the beginning of trial 3, and closed 90 s after the end of trial 4. We took care that testing time was similar for all rats, and similar across the test trial series for a given rat. Across all days and all rats, the starting time of the day's first trial ranged from 2.25 pm to 4.25 pm.

Recording Sites and Histology

Details of EEG electrode recording sites were reconstructed using records of electrode movement, physiological markers and postmortem histology. The rats were given an overdose of sodium pentobarbital (Lethobarb; 10 mg) and perfused transcardially with saline followed by 4% paraformaldehyde. The brain was sliced coronally into 40-μm thick sections, which were mounted and Cresyl-Violet Nissl-stained for visualization of the electrode tracks/tips (see Fig. 2). Physiological markers included the presence of ripple/sharp wave activity, and complex spike cell firing.

Analysis

To restrict analysis to movement-related theta, avoiding potential behavioral confounds such as rearing, EEG data were filtered to admit only portions of EEG where the rat spent ≥ 0.5 seconds at speeds above 5 cm/s. We also refiltered the data with an additional constraint: that the median speed in each trial was constant and equal to the median speed of the rat across all trials in the experiment k. Thus, speed limits $s_1 > 5$ cm/s and $s_2 < \infty$ were chosen such that the length of ordered data was symmetric (and maximal) about k. After filtering approximately half to three-quarters of the data remained for analysis.

Power spectra were calculated by finding the fast Fourier transform of the concatenated filtered data, where the square-modulus of each Fourier frequency coefficient represents the signal power at that frequency. The power spectrum was smoothed using a Gaussian kernel with standard deviation 0.375 Hz. Results were robust to variations in kernel size and shape. Theta frequency was taken as the frequency with peak power in a broad band including movement-related theta (5–11 Hz). All analyses were conducted using MatLab R12.1, (The MathWorks).

To visualize systematic variation in theta frequency across rats we defined, for each rat, a baseline frequency as the mean of theta over trials in habituation days 2–5. This was subtracted from the frequency per trial in Figures 1b,c, and 3b.

Because of technical problems, EEG data were unavailable for Rat 3 on day 4. Missing values for these trials were replaced by the average over the same trials on days 2, 3, and 5 (day 1
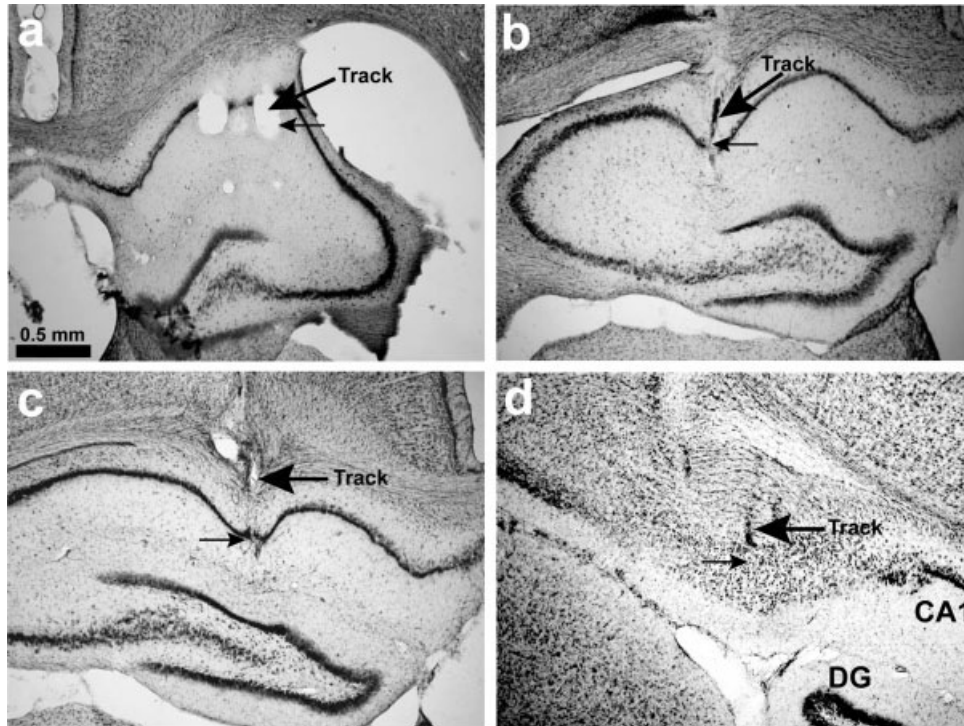
FIGURE 2. Examples of EEG-electrode recording sites in anterodorsal CA1 (a–c) and dorsal subiculum (d), showing photomicrographs for specific rats (see Figs. 3a and 4a). Thick arrows point to tissue damage and other indicators of the track made by a given tetrode. Thin arrows point to likely recording locations. The scale bar in A applies to all photomicrographs, and does not take into account tissue shrinkage. (a) Location of electrode used in Rat 1 (throughout the experiment; CA1 stratum radiatum). (b) Location of electrode used in Rat 2 (CA1 in/around pyramidal layer). (c) Location of electrode used in Rat 3 (Days 1–4; CA1 in/around pyramidal layer). (d) Location of electrode used in Rat 3 (Days 5, 1*–3*; deep subiculum).

was not included, since theta frequency was much lower on this day compared with the others). Hence, all four rats were included in the ANOVA showing the effect of familiarity in days 1–5 see below. (The significant effect of day in this ANOVA remains significant if data from Rat 3 are excluded.) In addition, the test trial series for Rat 1 was terminated at the end of change day 1*, thus data for days 2* and 3* are missing for this rat. As a result, all ANOVAs involving effects over days 1*–3* only include data from the remaining 3 rats. See Figure 4 for all data.

RESULTS

During the first 5 days of foraging in the brown square, environment "a," we found a progressive increase in theta frequency with increasing familiarity across days of experience (day (1–5) $\times$ trial (1–4) repeated measures ANOVA, effect of day: $F_{4,12} = 24.82$, $P < 0.001$; effect of trial: n.s., $P = 0.26$; day $\times$ trial interaction n.s., $P = 0.39$), see Figure 1. There was a nonsignificant hint of an increase in theta frequency across trials within a day (Fig. 1b) due largely to a strong effect in one of the rats (see Fig. 4, Rat 2). In the second phase of the experiment (days 1*,2*,3*, see Fig. 1), during trials in which new Environments

"b" and "c" were substituted for the expected brown square, there was a sharp decrease in theta frequency in the new environments, which lessened over days (environment (new/old) $\times$ day (1*–3*) ANOVA, effect of environment: $F_{1,2} = 62.10$, $P = 0.016$; effect of day: n.s., $F_{2,4} = 6.51$, $P = 0.055$; day $\times$ environment interaction: $F_{2,4} = 10.27$, $P = 0.027$). The rate at which theta approached baseline in the new environments was significantly greater than that associated with the first ever trials in the brown square, with theta frequency increasing with days of exposure in both the initial Environment "a" and the new one "b," but more so in "b" (Environment (b vs. a) $\times$ day (1*–3* in b, 1–3 in a) $\times$ trial (1–2) ANOVA, effect of environment: $F_{1,2} = 30.00$, $P = 0.032$; effect of day: $F_{2,4} = 38.94$, $P = 0.002$; effect of trial n.s., $P = 0.147$; environment $\times$ day interaction: $F_{2,4} = 9.00$, $P = 0.033$).

Thus the effect of unexpectedly experiencing Environments "b" or "c" in place of Environment "a" produced a greater reduction than the initial exposure to Environment "a," when compared with baseline, and the interaction indicates attenuation of this differential effect over days (see Fig. 1b). This may reflect the need for a stronger contextual novelty signal when existing environmental representations need to be changed, i.e., when a prior expectation is violated, as compared with the effect of absolute novelty, when an environmental representation needs to be set up
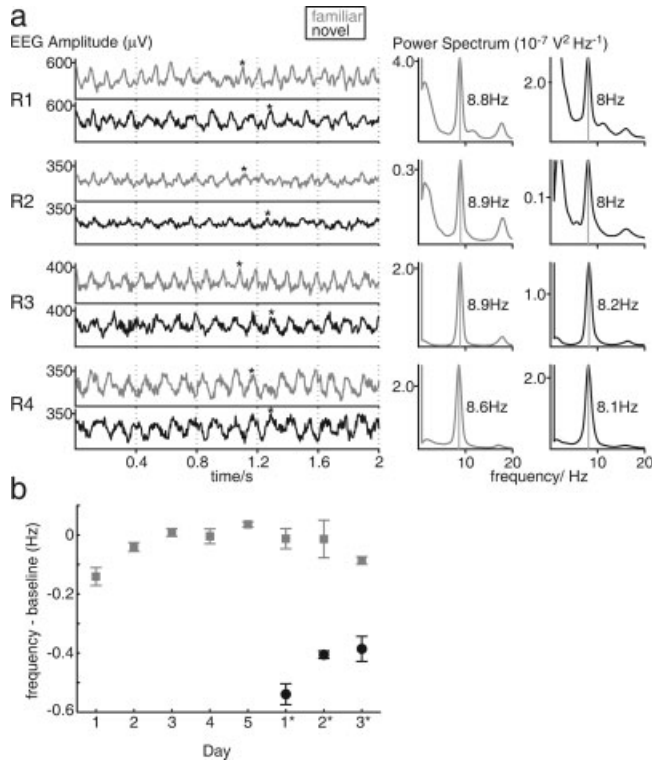
FIGURE 3. Effect of novelty on theta frequency, no effect of running speed. (a) Theta is visibly faster in novel environment "b" (trial 3, black lines) than familiar environment "a" (trial 2, grey lines) on day 1*: individual traces from each rat (left, asterisk marks 10th cycle); power spectra for whole trial (familiar: right; novel: far right). (b) The effect of novelty on theta frequency does not reflect variations in running speed. Data from each trial were subsampled to remove any differences in median speed across trials in the subsampled data. Mean theta frequency is shown versus days of experience in familiar environment "a" (grey points) or novel environments "b" and "c" (black points) in subsampled data for which median speed is constant across trials. Points show the mean across rats, error bars show s.e.m.

for the first time. Although not the focus of this article, CA1 place cell responses were recorded from three of the rats on the manipulation days 1*–3*, and showed complete remapping caused by the transition from Environment "a" to Environments "b" and "c," consistent with previous reports of remapping when such large changes are made to the rat's environment (Wills et al., 2005; Fyhn et al., 2007).

The effect of environmental novelty on change days 1*–3* was visible in the raw EEG traces and immediately apparent in comparing power spectra from trials in new and familiar environments, Figure 3a (see also Fig. 4). We only included EEG recorded while rats were actively foraging (moving faster than 5 cm/s), and the frequencies found were consistent with movement-related theta (8–9 Hz, see Figure 4). However, our results might still reflect novelty-related changes in running speed (which has occasionally been reported to affect theta frequency, and which increases across the experiment, see Fig. 4b). To test for any indirect effects of the average running speed in a trial on theta frequency, such as might be mediated by body tem-

perature (Whishaw and Vanderwolf, 1971), we regressed median speed per trial against frequency across the entire dataset for each rat. We found nonsignificant effects of speed on frequency of variable strength across rats ($r^2 = 0.006$–0.097; $P = 0.06$–0.65 see Table 1 for details). To control for any potential effects of running speed in our analysis of the effect of novelty, we subtracted the linear effect of speed on frequency indicated by the regression analysis, and repeated the above ANOVAs on the residuals. The effect of days of experience over days 1–5 ($F_{4,12} = 28.82$, $P < 0.001$) and of environmental novelty on days 1*–3* ($F_{1,2} = 61.35$, $P = 0.016$) remained effectively unchanged. The greater effect of unexpected Environment "b" on days 1*–3* compared to novel Environment "a" on days 1–3 increased slightly and attenuated more slowly over days (effect of environment: $F_{1,2} = 145.01$, $P = 0.007$; effect of day: $F_{2,4} = 12.44$, $P = 0.019$; environment × day interaction: $F_{2,4} = 2.56$, $P = 0.193$; effect of Trial: n.s., $P = 0.101$).

We also calculated the theta frequency in subsets of data from each trial, chosen so that the median running speed was constant in each trial for a given rat (see Materials and Meth-
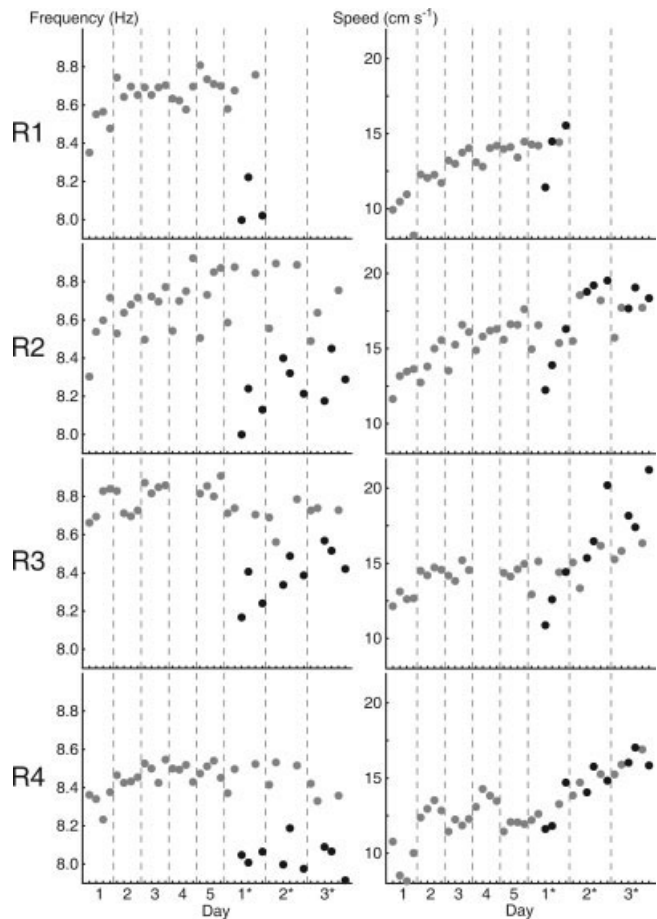
FIGURE 4. Theta frequency (a) and median running speed (b) for individual rats and trials. Data from the familiar environment "a" shown in grey, data from the unexpected novel environments "b" and "c" shown in black. See Figure 1 for details of the environments used in each trial.

Correlation Between Median Running Speed and Theta Frequency Per Trial Per Rat

	r^2	P	n (trials)
Rat 1	0.097	0.12	26
Rat 2	0.096	0.06	38
Rat 3	0.006	0.65	34
Rat 4	0.012	0.51	38

ods for more details), to control for any immediate effects of running speed on the concurrent theta frequency. These sub-sampled data are shown in Figure 3b. Similarly to the above analysis, the effect of days of experience over days 1–5 ($F_{4,12} = 12.44$, $P < 0.001$), and of environmental novelty on days 1*–3* ($F_{1,2} = 73.00$; $P = 0.013$) remained, with the effect of initial novelty in Environment "a" being slightly reduced, and the greater effect of unexpected Environment "b" on days 1*–3* slightly increased ($F_{1,2} = 124.83$, $P = 0.008$) and showing less attenuation over days (environment × day interaction: $F_{2,4} = 0.33$, $P = 0.738$; effect of Trial: n.s., $P = 0.30$; no other significant interactions).

Overall, controlling for variation in running speed slightly weakened the increase in theta frequency seen over days-of-experience within a given environment and slightly strengthened the theta frequency reduction on encountering the unexpected environments on change days 1*–3*. Stress has been also reported to affect theta frequency, but tends to increase it (Fontani et al., 1984), consistent with the effects of anxiolytics in decreasing theta frequency (Gray and McNaughton, 2000), so that any novelty-induced stress could only have weakened the novelty-related decrease in frequency reported here.

DISCUSSION

Our results suggest that environmental novelty is signaled by a reduced HF theta frequency (compared with that in a familiar environment), with unexpected change to a familiar environment within a common arena setting causing a greater reduction than first exposure to an entirely novel setting. As well as suggesting a new mechanism for signaling novelty, our results support the suggestion that the hippocampus performs a comparator function in detecting where experience (in this case, environmental layout) differs from expectation, rather than detecting absolute novelty *per se* (Sokolov, 1963; Hasselmo et al., 1996; Honey et al., 1998; Aggleton and Brown, 1999; Strange et al., 1999; Gray and McNaughton, 2000; Lisman and Otmakhova, 2001; Vinogradova, 2001; Kohler et al., 2005; Kumaran and Maguire, 2006). The specific mechanism found, theta frequency reduction, has significant implications

for understanding the oscillatory coding found within the hippocampal system (O'Keefe and Recce, 1993; Skaggs et al., 1996; Lisman and Otmakhova, 2001; Harris et al., 2002; Mehta et al., 2002; Huxter et al., 2003; Hasselmo, 2006; Burgess et al., 2007). In this context, we note that, although theta phase can vary with location across the HF, theta frequency is constant within HF (Buzsaki, 2002) and thus suitable for mediating a generic signal such as contextual novelty. Since gamma power is higher during theta-associated behaviors, relates to hippocampal memory function, is strongly modulated by theta phase in the HF and neocortex in rats and humans, and theta frequency and gamma frequency covary (Bragin et al., 1995; Chrobak and Buzsaki, 1998; Canolty et al., 2006; Montgomery and Buzsaki, 2007; Jensen and Colgin, 2007) our findings have implications for models of oscillatory encoding in memory in both the theta and gamma ranges (Lisman and Idiart, 1995; Lisman and Otmakhova, 2001; Sederberg et al., 2003; Mormann et al., 2005; Jacobs et al., 2006; Osipova et al., 2006; Rizzuto et al., 2006).

We can only speculate as to the mechanism for the theta frequency reduction, however there appear to be two components to HF theta: the movement-related cholinergically independent theta with frequency 8–9 Hz, and a lower frequency atropine-sensitive (i.e., cholinergically mediated) frequency of around 6–7 Hz (Kramis et al., 1975; Klink and Alonso, 1997). The medial septum sets the pace of HF theta and provides both GABAergic and cholinergic inputs, see (Buzsaki, 2002; O'Keefe, 2006) for reviews. Increase in cholinergic input to the hippocampus, as seen during exploration of a novel environment (Thiel et al., 1998; Giovannini et al., 2001), results in a reduction of hippocampal theta frequency (Givens and Olton, 1995). Our results support the idea that the GABAergic input sets baseline theta frequency during motion, with increased cholinergic input mediating a reduction in overall frequency in response to novelty. This would be consistent with the rapid and slow synaptic actions of GABA and acetylcholine, respectively, and with the proposed involvement of acetylcholine in signaling novelty (Carlton, 1968; Hasselmo et al., 1995; Meeter et al., 2004; Hasselmo, 2006). This interpretation is less obviously consistent with a proposed role of acetylcholine in "expected uncertainty," as compared with "unexpected uncertainty" (Yu and Dayan, 2005), but our random foraging task precludes any direct correspondence to this proposal. The trigger for increased cholinergic innervation from the medial septum might arise from the HF itself, on the basis of the comparison of the stored representation of an environment with the altered perceptual input (Sokolov, 1963; O'Keefe and Nadel, 1978; Hasselmo et al., 1996; Myers et al., 1996; Gray and McNaughton, 2000; Borisyuk et al., 2001; Lisman and Otmakhova, 2001; Vinogradova, 2001; Meeter et al., 2004).

An electrophysiological signature of contextual novelty has been identified in the human hippocampus (e.g., the 'MTL-P300' Halgren et al., 1980; Rugg and Coles, 1995; Knight, 1996; Grunwald et al., 1998; Grunwald and Kurthen, 2006). The cause of these event-related potentials is the subject of much speculation: as well as synaptic/neuronal activity triggered

by the novel event, further suggested contributions include non-time-locked "induced" activity, event-triggered phase alignment of ongoing oscillations and increased power at specific frequencies (Makeig et al., 2002; Rizzuto et al., 2003; Fell et al., 2004; McCartney et al., 2004; Duzel et al., 2005; Axmacher et al., 2006). Our results imply that a fourth mechanism may play a role: namely stimulus-induced changes in characteristic frequencies such as theta.

The proposed relationship between novelty and theta frequency has implications for, but is not necessarily incompatible with, current models in which theta provides separate phases for encoding and retrieval (Borisyuk et al., 2001; Hasselmo et al., 2002; Meeter et al., 2004; Hasselmo, 2006), interacts with gamma frequency oscillations to organize memory for sequences (Lisman and Idiart, 1995; Lisman and Otmakhova, 2001; Jensen and Colgin, 2007), or provides a clock signal against which information is encoded by firing phase in hippocampus (O'Keefe and Recce, 1993; Burgess et al., 1994; Skaggs et al., 1996) or entorhinal cortex (Burgess et al., 2007). More specifically, our results support the oscillatory interference model (Burgess et al., 2007) of the firing of medial entorhinal grid cells (Hafting et al., 2005), which predicts that a change in the frequency of movement-related theta underlies the increase in the spatial scale of the firing pattern of grid cells seen in novel environments (Fyhn et al., 2006). This in turn might contribute to changes in place cell firing when the rat experiences a novel environment ("remapping" Bostock et al., 1991), by causing a mismatch between the grid cell inputs to place cells and environmental sensory information from lateral entorhinal cortex (Burgess et al., 2007). This model requires further experimental investigation to determine its relationship to mechanisms supporting the effects of novelty, e.g., the potential roles acetylcholine and atropine-sensitive theta, but predicted the result presented here and suggests a new mechanism by which environmental novelty might trigger the formation of new hippocampal representations.

In conclusion, we have demonstrated a new potential mechanism for signaling of environmental novelty: a reduction in the theta frequency generated by HF. Our results have important implications for the interpretation of event-related potentials, and support the idea that the HF plays a greater role in "contextual novelty" or "comparator" processing than absolute novelty *per se*. In addition, they support the hypothesis of a cholinergically mediated mechanism for novelty detection, and provide indirect support for the oscillatory interference model of grid cell firing.

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