

Hippocampal Place Cells Acquire Location-Specific Responses to the Conditioned Stimulus during Auditory Fear Conditioning

Marta A.P. Moita,¹ Svetlana Rosis,² Yu Zhou,²
Joseph E. LeDoux,² and Hugh T. Blair^{3,*}

¹Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

²Center for Neural Science
New York University
New York, New York 10003

³Department of Psychology
University of California, Los Angeles
Los Angeles, California 90095

Summary

We recorded neurons from the hippocampus of freely behaving rats during an auditory fear conditioning task. Rats received either paired or unpaired presentations of an auditory conditioned stimulus (CS) and an electric shock unconditioned stimulus (US). Hippocampal neurons (place and theta cells) acquired responses to the auditory CS in the paired but not in the unpaired group. After CS-US pairing, rhythmic firing of theta cells became synchronized to the onset of the CS. Conditioned responses of place cells were gated by their location-specific firing, so that after CS-US pairing, place cells responded to the CS only when the rat was within the cell's place field. These findings may help to elucidate how the hippocampus contributes to context-specific memory formation during associative learning.

Introduction

Fear conditioning is an associative learning task in which subjects are presented with a neutral CS paired with an aversive US. The subject quickly learns that the CS predicts the US. Consequently, the CS acquires the capacity to elicit behavioral, autonomic, and endocrine responses that are characteristically expressed in the presence of danger (Bolles and Fanselow, 1980; Bouton and Bolles, 1980; Fanselow, 1980; Smith et al., 1980). The amygdala is critical for the acquisition of such conditioned emotional responses during fear conditioning, and evidence indicates that the amygdala is an important site of synaptic plasticity where memories of the CS-US association are stored (Fanselow and LeDoux, 1999; LeDoux, 2000; Maren, 2001; Blair et al., 2001). In contrast to the amygdala, the hippocampus is not necessary for acquiring fear responses to a discrete CS, but evidence indicates that the hippocampus does participate in memory storage for other aspects of the fear conditioning experience. For example, subjects with hippocampal damage fail to acquire fear of the environment, or context, in which the US is presented (Anagnostaras et al., 1999; Kim and Fanselow, 1992; Phillips and LeDoux, 1992). These findings are consistent with the widely accepted view that the hippocam-

pus is critical for storing "declarative" or "episodic" memories, which involves placing events within a spatio-temporal context (Eichenbaum, 2000; Eichenbaum et al., 1999; Nadel and Payne, 2002; Squire and Zola, 1996; Tulving and Markowitsch, 1998).

In a study of human patients, Bechara et al. (1995) demonstrated that the amygdala and hippocampus play distinct roles in storing memories of a fear conditioning experience. Patients were presented with a visual stimulus (CS) that was paired with an aversive loud noise (US). A patient with amygdala damage failed to acquire autonomic fear responses to the CS after it was paired with the US, but this same patient was able to correctly describe, through verbal reports, that the CS had been followed the US, indicating that declarative memory was intact. Conversely, a patient with hippocampal damage acquired normal autonomic fear responses to the CS, but was unable to explicitly remember the training experience during which the CS and US were paired.

These findings strongly suggest that the amygdala and hippocampus store different representations of the same learning experience. Neurophysiological studies have shown that neural activity in both the amygdala and hippocampus strongly correlates with the acquisition of associations between a discrete CS and US (Berger et al., 1980; Best and Best, 1976; Buchel et al., 1999; Buzsáki et al., 1979; Collins and Pare, 2000; Desmedt et al., 1998; Freeman et al., 1997; LaBar et al., 1998; Laroche et al., 1987; McEchron and Disterhoft, 1997; Munera et al., 2001; Olds et al., 1972; Pine et al., 2001; Quirk et al., 1995; Repa et al., 2001; Rogan et al., 1997; Stolar et al., 1989). During aversive classical conditioning, neurons in the amygdala (Collins and Pare, 2000; Quirk et al., 1995; Repa et al., 2001) and hippocampus (McEchron and Disterhoft, 1997; Munera et al., 2001) acquire similar conditioned responses that correlate with the onset of the CS. This pattern of results raises an intriguing question: if the amygdala and hippocampus store different information about the aversive conditioning experience, why does the neurophysiological representation of the CS look similar in these structures after learning?

Since the hippocampus is known to be required for context-specific learning (Anagnostaras et al., 2001; Holland and Bouton, 1999; Nadel and Payne, 2002; Phillips and LeDoux, 1992), it might be expected that the representation of the CS-US association in the hippocampus (unlike in the amygdala) is somehow specific to the context in which that association was learned. Supporting this possibility, the hippocampus contains neurons called *place cells* that fire selectively when a rat visits a particular location in space (O'Keefe and Dostrovsky, 1971). Since spatial location is an important element of context, place cells may provide a substrate for the hippocampal representation of context-specific memories (for a review, see Redish, 2001). However, it is not currently known whether place cells acquire conditioned responses to a CS during aversive conditioning because most previous studies of hippocampal neurons during aversive learning have been performed in restrained animals.

*Correspondence: blair@psych.ucla.edu

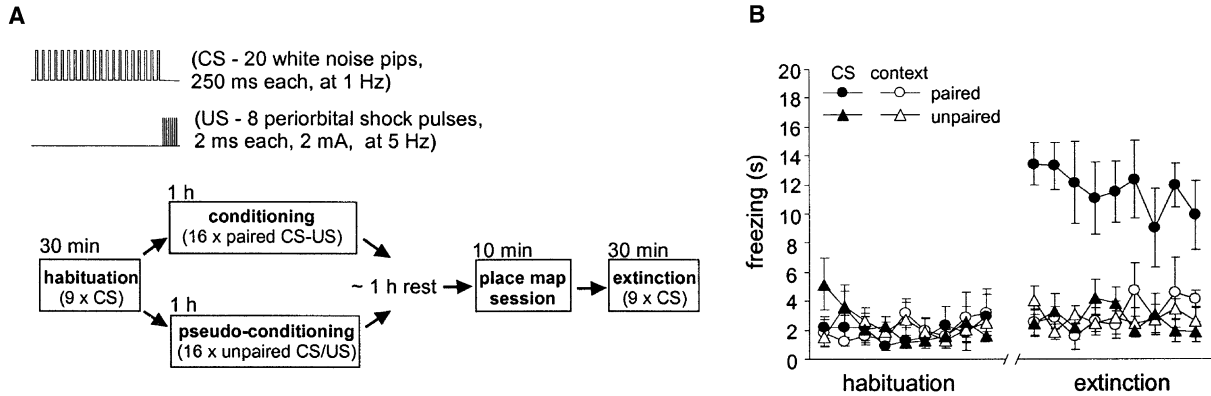


Figure 1. Experimental Design and Conditioned Freezing Behavior

(A) Fear conditioning was conducted using an auditory CS and a periorbital shock US. Rats first received 9 presentations of the CS alone (habituation), followed by 16 paired or unpaired presentations of the CS and US. After a 1 hr break outside of the experimental chamber, rats were returned to the training box for a 10 min place map session, followed by nine test trials in which the CS was presented alone (extinction). Note that habituation, acquisition, and extinction sessions were all conducted in the same training box.

(B) The graph shows amount of time spent freezing to the 20 s auditory CS (filled symbols) versus the 20 s context period prior to CS presentation (open symbols). No freezing is observed during habituation, whereas after conditioning, rats in the paired group (circles) but not the unpaired group (triangles) exhibit robust freezing to the CS, but not to the training context.

In the present study, we investigated whether hippocampal place cells acquire conditioned responses to an auditory CS and whether these responses are place specific. Rats, chronically implanted with hippocampal recording electrodes, were placed in an experimental chamber where they foraged for small food pellets dropped from an overhead dispenser (Muller et al., 1987). While foraging, rats underwent fear conditioning in which the auditory CS was either paired or explicitly unpaired with an electric shock US. The CS was a sequence of white noise pips, which allowed multiple sampling of evoked neural responses to the auditory stimulus during each conditioning trial (Repa et al., 2001; Rogan et al., 1997). The US was a train of brief shock pulses delivered to the eyelid (Figure 1A). Fear learning was assessed by automated scoring of freezing behavior, a standard index of conditioned fear (Bouton and Bolles, 1980; Fanselow, 1980).

Results

Behavior

Fear conditioning was conducted in 16 rats that were implanted with hippocampal recording electrodes. Rats were evenly divided among two groups, one receiving paired ($n = 8$) and the other unpaired ($n = 8$) CS-US presentations. As shown in Figure 1B, rats in the paired group froze during the 20 s auditory CS after (extinction) but not before (habituation) conditioning, whereas rats in the unpaired group froze very little during the CS before or after conditioning [$F(14) = 9.7, p = 0.007$ for group; $F(14) = 44.6, p = 0.00001$ for session; $F(14) = 36.9, p = 0.00002$ for group \times session]. To determine whether the freezing observed in the paired group during extinction in the training context was a response to the auditory CS or to the context, we also analyzed freezing to the training context during the 20 s period immediately preceding each CS presentation. Figure 1B shows that during extinction rats in the paired (but not unpaired)

group showed significantly higher levels of freezing to the auditory CS than to the context during extinction [$F(14) = 19.2, p = 0.0006$ for group; $F(14) = 53.3, p < 0.0001$ for stimulus type; $F(14) = 22.45, p = 0.0003$ for group \times stimulus type]. Thus, paired but not unpaired CS-US presentations caused rats to acquire conditioned freezing responses to the auditory CS.

Because the rats exhibited noticeable head movements during the delivery of the periorbital shock and the rate of firing of hippocampal cells is positively correlated with the speed of certain movements (Eichenbaum et al., 1989; McNaughton et al., 1983), we performed a detailed analysis of head movement responses (Figure 2). Prior to conditioning (habituation), the rats' average head speed during foraging was low (~ 30 cm/s). Movement speed was highest during the shock (~ 70 cm/s). Smaller but significant head movements were also evoked by the onset of the auditory CS. Although these CS-evoked head movements occurred in both groups during all sessions of the experiment, such movements were most pronounced during extinction when they occurred against a background of freezing behavior. Therefore, CS-evoked head movements were more pronounced in the paired group than unpaired group during extinction (since only the paired group froze to the CS during extinction). It will be important to examine whether this difference between the groups in the behavioral response to the CS can account for group differences in CS-evoked neural activity.

Single-Unit Recording

A total of 154 hippocampal cells were recorded during fear conditioning from 16 rats. One rat from the paired group had no cells that met criteria for inclusion in our analysis, but from the remaining 15 rats, 65 cells were recorded that met criteria for inclusion (see Experimental Procedures). Of these 65 included cells, 47 cells (paired group $n = 25$, unpaired group $n = 22$) were classified as complex spike cells, and 28 (paired group $n = 18$,

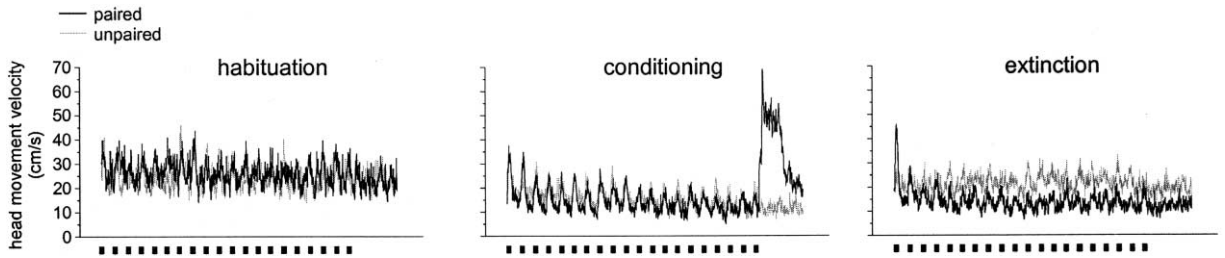


Figure 2. Head Movement

Graphs show the mean head movement speed during a 20 s trial of each experimental session, averaged first across trials within the session, and then across rats. Black bars below each graph indicate the time of each pip presentation. Note that in all sessions, there is a tendency for the rat to perform head movements at the onset of the auditory pips. Also note that rats in the paired group generate fast head movements during shock delivery at the end of conditioning trials. Rats in the unpaired group performed similar fast head movements in response to shock-alone presentations (data not shown).

unpaired group $n = 10$) were classified as theta cells (see Experimental Procedures). Complex spike activity in the hippocampus is generated by excitatory pyramidal neurons, whereas theta activity is characteristic of inhibitory interneurons (Fox and Ranck, 1981; Ranck, 1973). Complex spike cells will henceforth be referred to as “place cells,” since all displayed location-specific firing. The general firing properties of place and theta cells are summarized in Table 1.

Conditioned Auditory Responses of Place Cells

To analyze auditory responses of place cells, we constructed peri-event time histograms (PETHs) of spikes evoked by the white noise pips that comprised the auditory CS (see Experimental Procedures). In addition, to allow parametric comparisons of pip-evoked responses across different populations of recorded cells, the PETH for each cell was normalized by computing Z scores based on the cell’s baseline firing rate during the silence between white noise pips (see Experimental Procedures). To quantify each cell’s pip-evoked response, we averaged the response during the first 150 ms (after stimulus onset) of the normalized PETH, so that a single value representing pip responsiveness was attributed to each cell. Henceforth, we denote a cell’s normalized pip response during habituation as Z_{HAB} and during extinction as Z_{EXT} .

Table 1. Mean and Standard Error for Firing Properties of Place and Theta Cells

	Place Cells	Theta Cells
Peak to peak amplitude (μm) ^a	231 \pm 16	180 \pm 16
Mean firing (Hz) ^a	2.23 \pm 0.21	13.0 \pm 0.18
Peak firing (Hz) ^a	13.0 \pm 2.6	38.0 \pm 3.7
Spatial information content (bits/spike) ^b	1.91 \pm 0.93	0.08 \pm 0.07
Infield peak firing rate (Hz) ^b	8.27 \pm 1.68	–

^aIndicates measures taken from the habituation and extinction sessions.

^bIndicates measures taken from the place map sessions. No significant difference between the paired and unpaired groups was found for any of these measures, and therefore the data relative to cells in both groups were pooled together.

Place cells from the paired (Figure 3A) and unpaired (Figure 3B) groups responded very little to the pips during the habituation session, before conditioning had occurred. Analysis of raw (unnormalized) PETHs for individual place cell responses revealed that only 16% (4/25) of cells in the paired group and 9% (2/22) in the unpaired group exhibited significant pip-evoked responses during the habituation session. Similarly, analysis of normalized PETHs showed that place cells from both groups showed very little pip responsiveness before conditioning (mean $Z_{HAB} = 0.21 \pm 0.08$ paired group; 0.05 ± 0.12 unpaired group).

After conditioning, the proportion of pip-responsive cells increased to 52% (13/25) in the paired group, but decreased slightly to 5% (1/22) in the unpaired group. A chi-square test revealed that the postconditioning increase in the proportion of tone-responsive cells was highly contingent upon group ($\chi^2 = 9.09$; $p < 0.005$). Analysis of normalized PETHs showed that pip responsiveness increased in the paired group (mean $Z_{EXT} = 0.89 \pm 0.20$) but not the unpaired group (mean $Z_{EXT} = 0.10 \pm 0.11$). Most cells in the paired group exhibited some degree of conditioned increase in their pip response, as shown by the fact that the rank-ordered distribution of conditioning-induced changes in pip responses ($Z_{EXT} - Z_{HAB}$) is highly skewed toward positive values for cells from the paired group (Figure 3A). By contrast, the distribution for cells in the unpaired group is symmetrical, showing that these cells had equal probability of increasing or decreasing their pip response after conditioning (Figure 3B). A 2×2 ANOVA indicated that Z_{EXT} was greater than Z_{HAB} for place cells recorded from the paired group, but not the unpaired group [$F(44) = 9.11$, $p = 0.004$ for group; $F(44) = 10.89$, $p = 0.002$ for session; $F(44) = 8.37$, $p = 0.006$ for group \times session]. The baseline firing rate (the between-pip activity to which pip-evoked responses were normalized) did not change significantly after conditioning in the paired or unpaired groups [$F(44) = 2.46$, $p = 0.12$ for group; $F(44) = 0.45$, $p = 0.50$ for session; $F(44) = 0.002$, $p = 0.96$ for group \times session]. Taken together, these data indicate that place cells from the paired group, but not the unpaired group, acquired conditioned responses to the auditory CS. This finding is in agreement with previous studies, showing that aversive conditioning to an auditory CS causes hippocampal neurons to develop

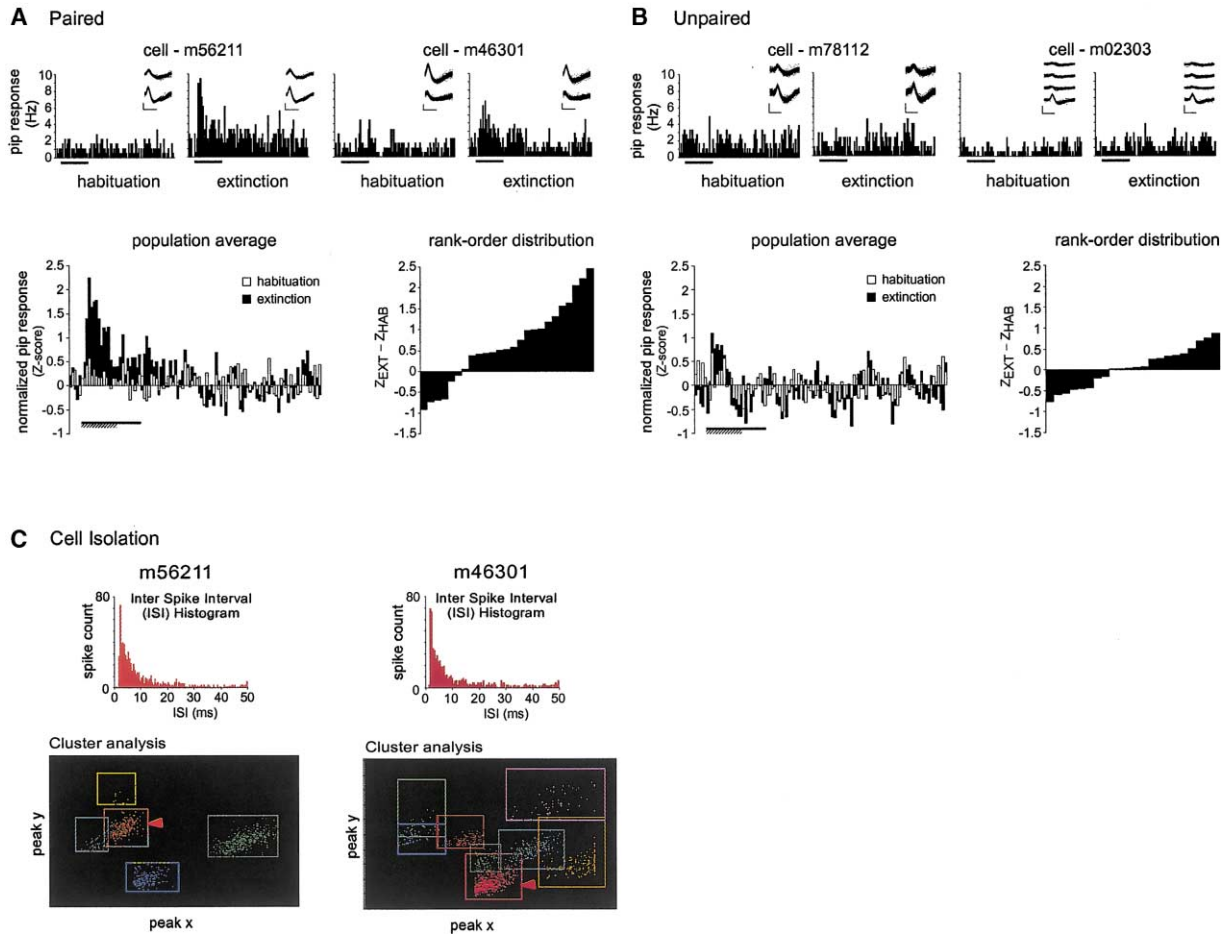


Figure 3. CS-Evoked Responses of Place Cells

(A) Paired Group. Top row shows raw PETHs of responses for two example cells to the CS pips (horizontal black bar below the PETH indicates the 250 ms period during which the pip was presented; insets at upper right show representative spike traces for the corresponding cell and session; vertical scale bar is 200 μ V, horizontal scale bar is 1 ms). Bottom row shows the Z score normalized PETH (left) of the CS response averaged over all recorded place cells. The pip-evoked response was analyzed during the first 150 ms of the pip indicated by the horizontal hatched bar (see Experimental Procedures). On the right, a rank-ordered distribution shows the conditioning-induced change in pip response ($Z_{EXT} - Z_{HAB}$) for each place cell in the study.

(B) Unpaired Group. Same as in (A), but showing the responses to the CS pips of place cells recorded from the unpaired group.

(C) Cell Isolation. Interspike interval histograms (1 ms bins) and cluster diagrams (peak amplitude on x versus y, stereotrodes) for the example cells from the paired group shown in (A). Arrows indicate clusters of the example cells.

evoked responses to that CS (Laroche et al., 1987; McEchron and Disterhoft, 1997; Munera et al., 2001).

Theta Synchronization

In the population-averaged PETHs for place cells shown in Figure 3, there is a clear oscillation of the firing rate above and below baseline with a period of about 150 ms (corresponding to the theta oscillation frequency of 6–7 Hz). Thus, it appears that both before and after conditioning, theta-frequency modulation of place cell firing tended to become synchronized to the onset of the CS pips. To quantify this effect, we performed a Pearson product-moment correlation between the Z scores in each bin of the normalized PETH and a sine wave signal with a period of 150 ms, aligned with the Z score sequence so that the rising phase of the first sine wave cycle began at the bin corresponding to the onset of the auditory pip. We found that the correlation between the PETH and this “theta wave” signal was signifi-

cant for place cells in both groups, both before and after conditioning [$r(98) = 0.20$, $p < .05$ for paired group in habituation; $r(98) = 0.23$, $p < .02$ for paired group in extinction; $r(98) = 0.23$, $p < .02$ for unpaired group in habituation; $r(98) = 0.33$, $p < .001$ for unpaired group in extinction]. Buzsaki et al. (1979) have reported a similar finding that during early trials of an associative learning task, the CS tended to reset the phase of the hippocampal theta EEG.

Control for CS-Evoked Movements

The firing rate of place cells is known to be positively correlated with the movement speed of the rat (Eichenbaum et al., 1989; McNaughton et al., 1983). Since we know that the auditory CS evokes movement responses from the rat (see Figure 2), it is possible that the conditioned neural responses in cells from the paired group might not be auditory responses at all, but may instead reflect a conditioned enhancement of the rat’s CS-

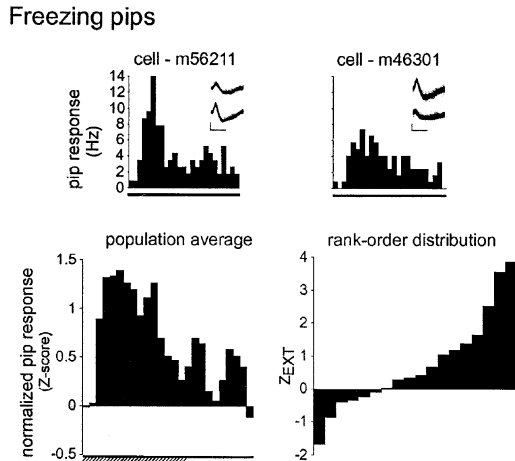


Figure 4. Responses to Freezing Pips of Place Cells in the Paired Group

On the top row, raw PETHs show the response of the example cells to pips during which the rat was freezing during the extinction session (insets show representative spike traces). Below each PETH, a horizontal black bar indicates the 250 ms period during which the pip was presented. The bottom row shows data for the population of all place cells recorded from the paired group. The Z score normalized PETH (left) shows the averaged response of all recorded place cells during the freezing pips. On the right, a rank-ordered distribution shows the response to freezing pips during extinction (Z_{EXT}) for each place cell in the study.

evoked movements. To control for the influence of motor activity on place cell firing, we isolated only those pips during which no detectable head movement occurred (see Experimental Procedures) and analyzed responses to these pips, referred to as freezing pips. The resulting PETHs were normalized to baseline activity recorded during silence between pips when rats were also immobile, so the rats' behavior was similar during both the pips and baseline periods. Figure 4 shows that even during periods of immobility, place cells recorded from the paired group showed clear pip-evoked responses during extinction. The proportion of place cells from the paired group that exhibited significant pip-evoked responses during freezing pips in extinction was 48% (12/25), almost the same as when the rat was moving. Parametric comparisons of freezing pip responses using normalized PETHs was not possible, because rats did not freeze enough prior to conditioning or in the unpaired group to perform such an analysis. Nonetheless, our analysis of freezing pips during extinction in the paired group suggests that after auditory fear conditioning, place cells exhibited auditory-evoked responses to the CS that were not directly related to the rat's execution of motor responses during the CS.

Place Specificity of Conditioned Auditory Responses

The defining characteristic of place cells is their location-specific firing, and yet, we have shown here that place cells can acquire conditioned responses to a non-spatial sensory stimulus, such as an auditory CS (Figures 3 and 4). Does aversive conditioning cause place cells to acquire nonspatial firing properties by inducing a sensory-evoked response to the auditory CS that oc-

curs no matter where the rat is located? Or is the conditioned auditory response of each place cell modulated by that cell's place-specific firing properties, so that sensory responses occur only when the rat is in the cell's preferred firing location? To answer these questions, we examined the interaction between CS-evoked activity and spatially tuned firing of place cells.

To measure the spatial properties of place cells, we recorded cells during a 10 min place map session (see Figure 1) that preceded the extinction session, during which rats foraged for food pellets without any CS or US presentations. Place maps were plotted (Figure 5A) to show each cell's preferred firing location, or place field, in the recording chamber during this session (see Experimental Procedures). The pip response of each place cell in the paired group (which is the only group that showed conditioned pip responses) during extinction was then analyzed separately for pips occurring inside and outside of the cell's place field (see Figures 5A and 5B). To control for sensory-evoked head movements, we analyzed only responses to freezing pips, as above in Figure 4B. To insure sufficient sampling of auditory responses inside of a cell's place field, we required that a minimum of 10 freezing pips had to occur when the rat was located inside of the cell's place field, otherwise the cell was excluded from our analysis of place-specific pip responses (19 of the 25 cells in the paired group met this criterion). Similarly, at least 10 freezing pips had to occur outside of the cell's firing field in order to be included in the analysis of out-of-field pip responses (20 of the 25 cells in the paired group met this criterion).

Analysis of raw (unnormalized) PETHs revealed that 37% (7/19) of cells in the paired group exhibited significant pip-evoked responses when the rat was freezing inside of the cell's place field. This percentage is somewhat lower than that for the place-independent analysis of freezing pips reported above, probably because of poorer sampling (since fewer freezing pips were included in the place-dependent analysis). By comparison, only 1 out of 20 cells in the paired group exhibited significant pip-evoked responses when the rat was located outside of the cell's place field during extinction. Thus, pip-evoked responses seemed to occur mainly when the rat was located inside of a cell's place field. Supporting this, we found that for cells in the paired group, the normalized in-field pip response was significantly larger during extinction than the out-of-field pip response; that is, $Z_{IN} > Z_{OUT}$ [$t(32) = 2.23$, $p = 0.03$]. It should be noted that of the 20 place cells in the paired group that we analyzed for place-specific CS-evoked responses, only 3 cells had a different preferred firing location after versus before conditioning, and one of these was the lone cell that exhibited pip responses outside of its place field. We conclude from these findings that conditioned auditory responses of place cells are not purely sensory-evoked responses, but are instead "gated" by the spatial firing properties of place cells.

Conditioned Auditory Responses of Theta Cells

We next investigated whether theta cells acquired conditioned responses to the auditory CS. To do so, re-

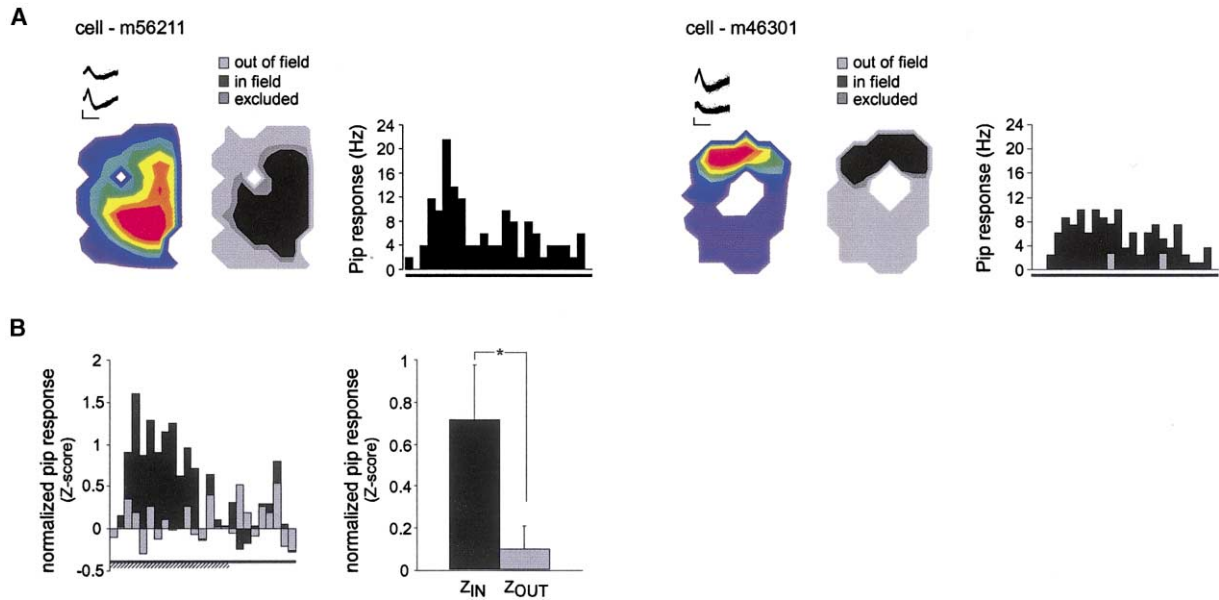


Figure 5. Spatial Selectivity of Conditioned Responses of Place Cells

(A) Example Place Cells. Color-coded place map shows an overhead view of the training box, with hot colors indicating regions where the place cell fired at a high rate, and cool colors showing regions of low firing (white space indicates undersampled regions that were excluded from analysis, see Experimental Procedures). Based on this firing rate map, the box was divided into in-field versus out-of-field regions (grayscale place map). PETH at right shows that the in-field (black bars) pip response was much larger than the out-of-field (gray bars) pip response for these example cells (horizontal black bar indicates the 250 ms period during which the pip was presented). Note that cell m56211 did not respond to the pips occurring outside the cell's place field, hence the lack of gray bars in the PETH.

(B) Z score normalized PETH shows the in-field (black bars) versus out-of-field (gray bars) response averaged over all place cells in the paired group. Bar graph at right shows mean normalized response during the first 150 ms of pips occurring inside (black) versus outside (gray) of a cell's place field, averaged across cells in the paired group.

sponses of theta cells to the auditory CS before and after conditioning were compared. Theta cells from the paired (Figure 6A) and unpaired (Figure 6B) groups responded very little to the pips during the habituation session, before conditioning had occurred. Analysis of raw (unnormalized) PETHs revealed that 17% (3/18) of cells in the paired group and 20% (2/10) of cells in the unpaired group exhibited significant pip-evoked responses during habituation. Similarly, analysis of normalized PETHs showed that place cells from both groups showed little pip responsiveness before conditioning (mean $Z_{HAB} = -0.08 \pm 0.17$ paired group; $Z_{HAB} = 0.54 \pm 0.26$ unpaired group).

After conditioning, the proportion of pip-responsive theta cells increased to 78% (14/18) in the paired group, but decreased to 10% (1/10) in the unpaired group. A chi-square test revealed that the postconditioning increase in the proportion of tone-responsive cells was highly contingent upon group ($\chi^2 = 26.03$; $p < 0.001$). Analysis of normalized PETHs showed that pip responsiveness increased in the paired group (mean $Z_{EXT} = 0.91 \pm 0.20$) but not the unpaired group (mean $Z_{EXT} = 0.16 \pm 0.19$). As for place cells, the rank-ordered distribution of conditioning-induced changes in pip responses ($Z_{EXT} - Z_{HAB}$) is highly skewed toward positive values for cells from the paired group, indicating that most cells increased their pip response after conditioning (Figure 6A). By contrast, the distribution for cells in the unpaired group is skewed to the left, showing that these cells tended to decrease their pip response after

conditioning (Figure 6B). A 2×2 ANOVA indicated that Z_{EXT} was greater than Z_{HAB} for place cells recorded from the paired group, but not the unpaired group [$F(26) = 0.003$, $p = 0.96$ for group; $F(26) = 6.00$, $p = 0.02$ for session; $F(26) = 18.89$, $p = 0.0002$ for group \times session]. The baseline firing rate (to which pip-evoked responses were normalized) did not change significantly after conditioning in the paired or unpaired groups [$F(26) = 0.08$, $p = 0.78$ for group; $F(26) = 3.59$, $p = 0.07$ for session; $F(26) = 0.21$, $p = 0.65$ for group \times session]. These data suggest that, like place cells, theta cells from the paired group (but not the unpaired group) acquired conditioned responses to the auditory CS. This observation differs from previous reports showing that a majority of theta cells become inhibited, rather than excited, by the CS following eyeblink conditioning in restrained rabbits (Berger et al., 1983; McEchron and Disterhoft, 1997). This discrepancy could be due to a number of experimental factors, including the use of restrained rabbits versus unrestrained rats, or the fact that eyeblink conditioning typically involves many training trials presented over several days, whereas our fear conditioning experiment involved a single training session. Our present results are consistent with a previous study showing that place and theta cells show similar firing correlates with sensory stimuli during a delayed-nonmatch-to-sample task (Wiebe and Staubli, 2001).

Theta Synchronization

To examine whether rhythmic firing of theta cells was synchronized to the CS, we correlated the theta-cell

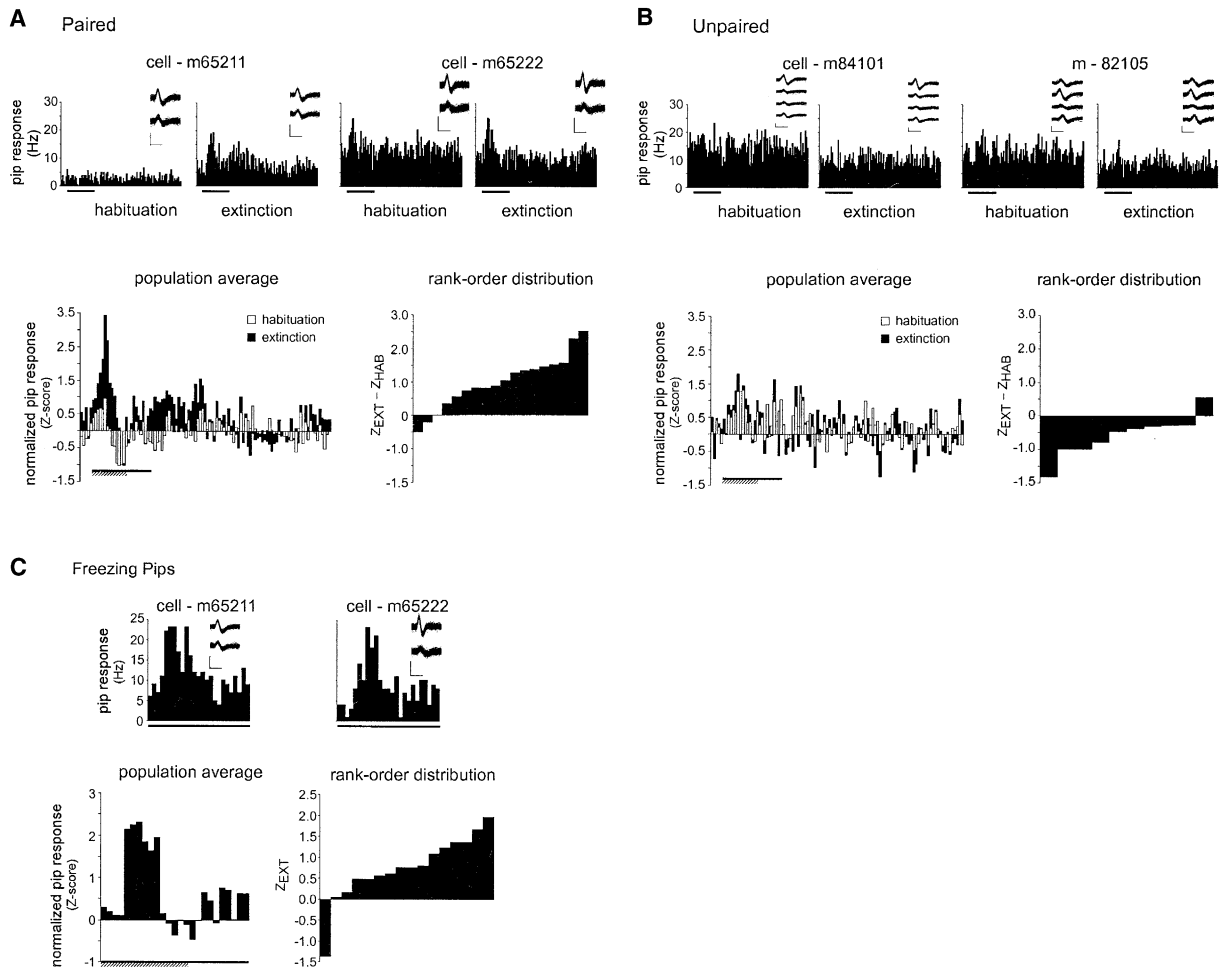


Figure 6. CS-Evoked Responses of Theta Cells

(A) Paired Group. On the top row, raw PETHs show the responses of two example cells to the presentation of CS pips, for both the habituation and extinction sessions (insets show representative spike traces). The bottom row shows data for the population of all theta cells recorded from the paired group. The Z score normalized PETH (left) shows the averaged response of all recorded place cells. On the right, a rank-ordered distribution shows the conditioning-induced change in pip response ($Z_{EXT} - Z_{HAB}$) for each place cell in the study.

(B) Unpaired Group. Same as in (A), but showing the responses of cells recorded from the unpaired group.

(C) Freezing Pips. Responses of theta cells recorded from the paired group to freezing pips (as for place cells in Figure 4). The top row shows data from example cells, and the bottom row shows the data for the population of all place cells recorded from the paired group. At lower right is shown a rank-ordered distribution of the response to freezing pips during extinction (Z_{EXT}).

PETHs with a theta wave signal, as explained above for place cells. We found that this correlation was significant only for theta cells in the paired group after conditioning (but not before conditioning), and not at all in the unpaired group [$r(98) = 0.06$, $p = \text{n.s.}$ for paired group in habituation; $r(98) = 0.27$, $p < .01$ for paired group in extinction; $r(98) = 0.11$, $p = \text{n.s.}$ for unpaired group in habituation; $r(98) = -0.09$, $p = \text{n.s.}$ for unpaired group in extinction]. Thus, the rhythmic firing of theta cells only became synchronized to the CS during extinction in the paired group, exactly the same condition for which place and theta cells exhibited conditioned CS-evoked responses. This raises the intriguing possibility that CS-evoked responses may somehow be related to synchronization of theta cell firing to the CS.

Control for CS-Evoked Movements

Like place cells, theta cells are known to change their firing rate with the rat's movement speed (Buzsaki, 2002;

Vanderwolf, 1969). Therefore, as for place cells (see above), we analyzed responses of theta cells only to freezing pips. Figure 6C shows that even during periods of immobility, theta cells recorded from the paired group showed clear pip-evoked responses during extinction. The proportion of theta cells from the paired group that exhibited significant pip-evoked responses during freezing pips in extinction was 67% (12/18), similar to when the rat was moving. These data suggest that, like place cells, theta cells from the paired group (but not the unpaired group) acquired conditioned responses to the auditory CS, and these responses were not caused by CS-evoked motor activity.

Hippocampal Processing and Behavioral State

Rats in the paired group are in a different behavioral state during extinction (when they fear the CS) than during habituation (when they do not fear the CS). Thus,

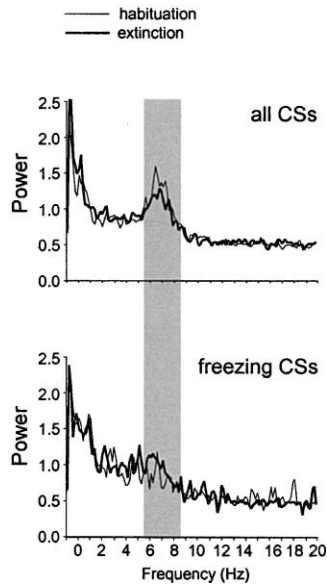


Figure 7. Theta Rhythm

Each graph shows the power spectrum (bin width 0.0256 Hz) of the hippocampal multiunit recording signal during the habituation (thin lines) and extinction (thick lines) sessions. The top graph shows the power spectrum of the cumulative multiunit unit signal from all CS periods of the session. The bottom graph shows the power spectrum of the multiunit signal during only those portions of the CS period when the rats were immobile. Shaded region indicates the theta band (6–8 Hz).

the fact that CS-evoked responses are larger during extinction than habituation in cells from the paired group may not be due to the fact that the rat has learned an association between the CS and US, but simply due to the fact the CS is occurring when the rat is in a fearful state during extinction, as opposed to a nonfearful state during habituation.

To investigate the influence of the rat's behavioral state on hippocampal processing, we compared levels of hippocampal theta rhythm during habituation versus extinction in the paired group. To do this, we performed a power spectrum analysis of a multiunit spike channel recorded from the hippocampus of each rat in the paired group and compared the power spectrum of this multiunit signal during the CS in habituation versus extinction. Figure 7 (top) shows that during both sessions, there was a clear peak of rhythmic oscillation in the theta frequency range of 6–8 Hz during the CS period. The peak is slightly smaller during extinction than habituation, but the difference is not statistically significant [$t(6) = 1.3$, $p = 0.24$]. Since theta rhythm is usually more prevalent during movement than immobility (Buzsaki, 2002; Vanderwolf, 1969), the slight reduction in theta power during extinction may reflect the fact that theta activity is reduced by freezing during extinction. However, it has been reported that during fearful or attentive states, a form of theta rhythm (referred to as “type II” theta) occurs during immobility (Leung and Vanderwolf, 1980; Sainsbury et al., 1987; Sainsbury and Montoya, 1984). To investigate whether freezing was accompanied by type II theta during extinction, we compared the power spectrum of the multiunit signal during periods

of immobility when the CS was present in habituation and extinction. Figure 7 (bottom) shows that when the rat was immobile during the CS, there was little evidence of theta rhythm during habituation or extinction. Thus, we did not observe the emergence of type II theta rhythm during freezing to the CS in extinction.

In summary, we found that rats from the paired group exhibited movement-related theta rhythm during the CS in both the habituation and extinction sessions. Therefore, fearful and nonfearful states did not appear to radically alter the hippocampal processing state, as assessed by theta rhythmicity. However, as noted above, rhythmic firing of theta cells was synchronized to the CS presentation only when the rat was in a fearful state, and not at other times. This synchronization of theta cells may reflect a change in the hippocampal processing state during fear, which could in turn be related to the emergence of CS-evoked responses in place and theta cells after the CS was paired with the US.

Responses to the Shock US

Finally, we examined the responses of place and theta cells to the periorbital shock US. Since the shock US consisted of a train of very brief (2 ms) shock pulses, and since individual shock pulses were delivered 200 ms apart, it was possible to record neural responses to the shock during the ~198 ms intershock interval (note that the first 10 ms of the intershock interval was contaminated by stimulus artifact, and was therefore ignored).

To analyze shock responses of hippocampal cells, we constructed PETHs showing responses evoked by shock pulses. To control for the effects of the rapid head movements during shock delivery on the shock-evoked neural activity (Figure 3), PETHs were normalized to the cell's baseline firing rate during periods when the rat made rapid head movements in the absence of either the CS or US (see Experimental Procedures). Figure 8 shows that both place cells (Figure 8A) and theta cells (Figure 8B) responded robustly to the shock. Analysis of unnormalized PETHs revealed that in the paired group, 48% (12/25) of place cells and 56% (10/18) of theta cells were shock responsive. In the unpaired group, 73% (16/22) of place cells and 70% (7/10) of theta cells were shock responsive. The mean normalized response was slightly larger in the paired group relative to the unpaired group, but this difference was not statistically significant for place [$t(40) = 1.06$, $p = 0.30$] or theta [$t(25) = 1.34$, $p = 0.19$] cells.

It was not possible to examine whether shock responses of place cells were place specific because rats moved very rapidly during the shock. Thus, it was not possible to accurately classify shocks that occurred inside versus outside of a cell's place field.

Discussion

The role of the hippocampus in memory formation has been widely investigated using classical conditioning tasks in rats (reviewed in Anagnostaras et al., 2001; Holland and Bouton, 1999). In agreement with previous studies, the findings reported here show that neurons in the hippocampus acquire conditioned responses to

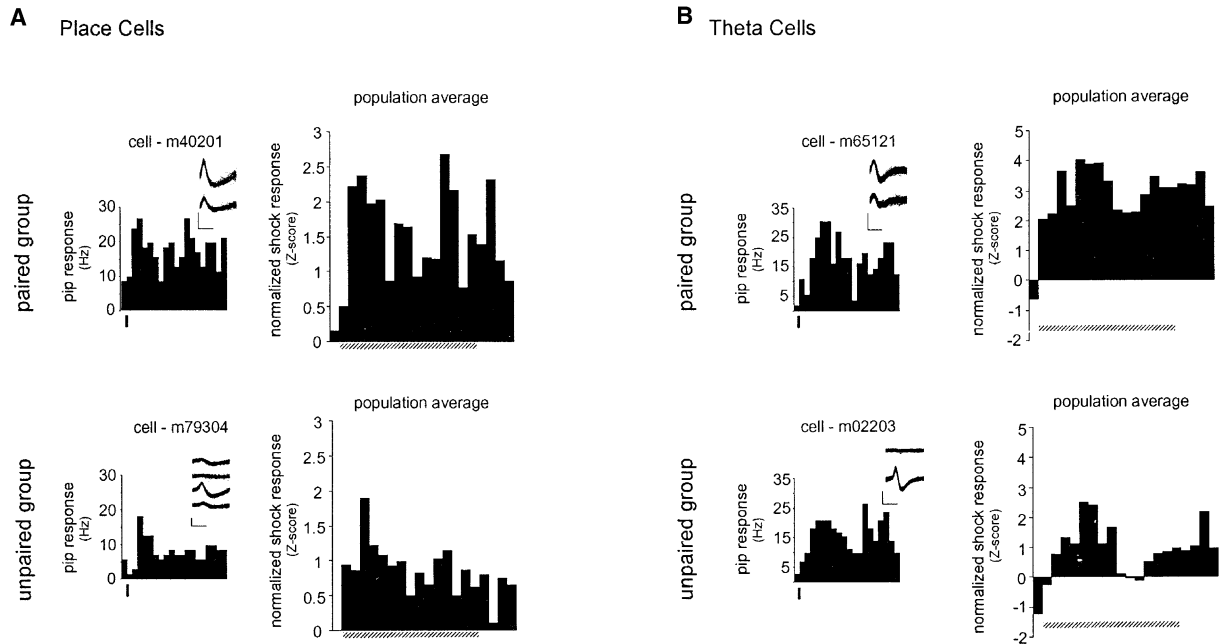


Figure 8. Shock-Evoked Responses of Place and Theta Cells

(A) Place Cells. Top row shows responses of cells from the paired group, bottom row shows unpaired group. Left column shows responses of example cells to shock delivery (hash mark below PETH indicates the shock), right column shows the averaged normalized responses of all place cells (horizontal hatched bar indicates the first 150 ms of the intershock interval, which was used for statistical analysis of shock responses).

(B) Theta Cells. Same as in (A) but showing the shock responses of theta cells.

the auditory CS during aversive conditioning. But importantly, the present study shows that aversive conditioning causes place cells to acquire CS-evoked responses, and that these evoked responses are gated by the cells' place-specific firing. The present study also shows that hippocampal theta cells exhibit enhanced CS-evoked responses following auditory fear conditioning.

We observed that theta-frequency modulation of place cell firing was always well synchronized to the CS pips, regardless of whether the CS had been paired with the US. By contrast, the rhythmic firing of theta cells became synchronized to the presentation of the CS only after the CS had been paired with the US, the same condition under which the CS-evoked responses of place cells and theta cells were enhanced. This suggests that CS-evoked responses may somehow be related to synchronization of theta cell firing to the CS.

Why do CS-evoked responses emerge in the hippocampus following auditory fear conditioning? One possibility is that an associative learning process causes the CS to acquire behavioral significance when it is paired with the US, and this learned association enhances the processing of the CS within the hippocampus. If so, then CS-US convergence within the hippocampus might drive Hebbian synaptic plasticity that is believed to underlie the emergence of CS-evoked responses in the hippocampus after conditioning (Levy and Steward, 1979; McNaughton and Miller, 1986; Murea et al., 2001). Supporting this idea, we found that many hippocampal neurons that acquired responses to the auditory CS were also responsive to the shock US. Thus, it appears that CS and US signals converge on

single cells in the hippocampus, as would be required for Hebbian plasticity. Alternatively, conditioned responses of hippocampal neurons to the CS could result from associative plasticity in other brain structures besides the hippocampus, which subsequently leads to enhancement of auditory inputs to the hippocampus.

It is also possible that in our auditory fear conditioning task, conditioned enhancement of CS responses was not directly related to an associative learning process, but was instead due to the fact that after CS-US pairing, presentation of the CS caused the rat to enter a different behavioral state (i.e., fear). That is, the CS response may have been enhanced during extinction in the paired group because the rat was afraid during the CS presentation after (but not before) conditioning, and not because the CS had become associated with the US per se. Arguing against the possibility that this is the main explanation of our results is the fact that our analysis of theta rhythmicity (Figure 7) did not reveal evidence for a profound change in the hippocampal processing state during the CS after conditioning. However, this does not provide conclusive evidence that the findings are due to associative Hebbian plasticity. Other possibilities are that the enhanced CS-evoked responses reflect changes in attention or motivational state triggered by the CS presentation.

Regardless of whether conditioned CS-evoked responses reflect a mnemonic, attentional, or motivational process, a key finding of the present study is that conditioned CS responses of place cells are gated by the location-specific activity of the cells. There has been considerable debate about whether the rat hippocam-

pus is mainly devoted to spatial and navigational information processing, or whether it is more broadly involved in nonspatial episodic memory processes as well (Eichenbaum et al., 1999; O'Keefe, 1999; Redish, 2001). The latter view is supported by studies showing that hippocampal complex spike cells can respond to nonspatial cues, such as odors (Otto and Eichenbaum, 1992; Wiebe and Staubli, 1999; Wood et al., 1999) and auditory tones (Berger et al., 1976; McEchron and Disterhoft, 1997; Munera et al., 2001; Sakurai, 1994), as well as other nonspatial variables, such as trial type in a nonmatch-to-sample task (Hampson et al., 1999; Wiebe and Staubli, 1999; Wood et al., 1999). However, only recently have studies begun to address the question of whether such nonspatial responses occur independently of the rat's spatial location. These studies have reported that nonspatial responses of complex spike cells are often gated by their spatial firing properties, although some cells respond to nonspatial variables in a location-independent manner (Wiebe and Staubli, 1999; Wood et al., 1999). In the present study we found that place cells exhibit conditioned sensory responses to the auditory CS primarily when the CS occurs while the rat is inside the cells' place fields. This suggests that the primary role of the hippocampus in associative learning may not be to encode that the CS predicts the US, but rather to encode that the CS is followed by the US when the rat is in a specific location or context (Nadel and Payne, 2002). This conclusion is supported by a previous study showing that conditioned multiunit CS responses in the rabbit hippocampus are more pronounced in the spatial context where training occurred than in a novel context (Freeman et al., 1997).

These findings might help to explain why hippocampal damage impairs context specificity in several forms of associative learning, such as contextual fear conditioning (Anagnostaras et al., 2001) and extinction of a discrete CS (Corcoran and Maren, 2001), but does not affect the acquisition of a simple CS-US association when the CS and US overlap in time (Anagnostaras et al., 1999; Kim et al., 1995; Phillips and LeDoux, 1992). Context-independent representations of simple CS-US associations are likely to be stored in brain structures other than the hippocampus, such as the amygdala in the case of fear conditioning (LeDoux, 2000; Maren, 2001) or the cerebellum in the case of eyeblink conditioning (Kim and Thompson, 1997; Medina et al., 2002).

In conclusion, we have shown that during auditory fear conditioning, hippocampal neurons (both place and theta cells) acquire responses to the auditory CS. The CS-evoked responses of place cells are gated by their spatial-specific firing. We have also shown that place and theta cells respond to the shock US, suggesting that CS-US convergence within the hippocampus may drive the acquisition of conditioned responses of hippocampal cells to the auditory CS. These findings support the view that the hippocampus contributes to context-specific memory formation during associative learning.

Experimental Procedures

Subjects and Surgery

Male Sprague-Dawley rats weighing 350–400 g were reduced to 85% of their ad-lib weight through limited daily feeding. Under deep

nembutal anesthesia (40 mg/kg), electrode drives consisting of 6–10 independently movable bundles of two (stereotrode) or four (tetrotrode) formvar-insulated nichrome wires (23 μ m diameter, California Fine Wire Company, Grover Beach, CA) were stereotaxically implanted above the hippocampus (3.3 mm posterior, \pm 2.0 mm lateral, 1.5 mm ventral to bregma). Silver wires (75 μ m diameter, stripped of insulation 2 mm from the tip) were threaded through the skin of the right eyelid for delivery of the periorbital shock US. Postsurgical analgesics (2 mg/kg ketoprofen) were given daily for 3 days after surgery.

Fear Conditioning

One day prior to fear conditioning, rats were preexposed for 15 min to the box where conditioning took place (36 \times 24 \times 44 cm wooden chamber coated with white latex, brown formica floor washed with peppermint soap, enclosed in a bright sound-attenuating chamber with white foam). Rats were also preexposed in the same manner to a second "neutral" chamber as part of a separate experiment (omitted from Figure 1 for clarity). Throughout preexposure and subsequent experimental sessions in the boxes, rats constantly foraged for 20 mg food pellets dropped from an overhead dispenser at \sim 30 s intervals. Fear conditioning was conducted as illustrated in Figure 1 (rats also chased pellets for 10 min in the neutral chamber prior to the habituation and extinction sessions, omitted from Figure 1 for clarity). Habituation and extinction sessions consisted of nine presentations of the CS alone. Acquisition consisted of 16 CS-US presentations that were either paired (first US shock pulse occurring 300 ms after the offset of the last CS noise pip) or unpaired (no shock occurring within 30 s of any pip). Intertrial interval varied pseudorandomly between 95 and 240 s. Rats continued chasing food pellets in the training box for \sim 10 min following the last acquisition trial.

Single-Unit Recording

Beginning 5 days after surgery, daily screening sessions were conducted in which electrode tips were advanced slowly (\sim 80 μ m/day) until complex spike cells and theta cells were encountered in the CA1 layer of the hippocampus, which was identified on the basis of EEG signals and single-unit spike patterns (Buzsaki, 1986; Ranck, 1973). Histological examination verified that all electrode tips were located in CA1. Single-unit spikes were identified using online and offline cluster analysis software (Datawave Technologies, Broomfield, CO), which employed a multidimensional window discriminator to select units based on waveform parameters such as peak-to-peak amplitude, peak-to-baseline amplitude, spike width, latency-to-peak, and latency-to-valley of the spike. Background noise on the electrode channels was approximately \pm 30 μ A from baseline during all recording sessions. Single-units had to meet several criteria for inclusion in the study. First, spike waveforms had to remain stable and well discriminated throughout the experiment. Second, ISI histograms had to exhibit a refractory period of at least 2 ms, so that high-frequency multiunit spike waveforms would not be included in the data set. Third, cells had to fire a minimum of 50 spikes during a given session to be included in the analysis of that particular session.

Data Analysis

Position and Speed Tracking

The rat's spatial position was monitored by a video tracking system, which sampled (at a rate of 60 Hz) the location of two light-emitting diodes attached to the animal's headstage. The animal's spatial position was computed as the center point between the two diodes (we discarded sample points during which one of the two diodes was occluded). Anatomically, this midpoint corresponded approximately to the center of the rat's head, midway between the eyes. The animal's head movement speed (in cm/s) at sample time t was computed as $(\alpha/\delta) \times [(x_{t+1} - x_{t-1})^2 + (y_{t+1} - y_{t-1})^2]^{1/2}$, where x_{t-1} is the x coordinate position sample immediately preceding sample t , x_{t+1} is the x coordinate sample immediately following sample t (the y coordinates are similarly notated), $\alpha = 30$ is the number of distance intervals sampled per second (half the position sampling rate), and $\delta = 3.33$ is the resolution (in pixels/cm) of the video tracker.

Freezing

An episode of freezing was defined as a period during which the rat's movement speed was zero (that is, the animal's tracked position did not change) for a period of 1/3 s or more. To obtain the total amount of time the rat spent freezing during a given time span (e.g., the 20 s CS period), the durations of all freezing episodes that occurred during that time span were summed together. Freezing scores obtained by this method were >90% correlated with scores of experienced human observers.

Place Maps

Analyses of place maps included only spikes and position samples from periods when rats were moving at a speed of at least 18 cm/s. Pixels were binned at a resolution of 9 cm², and pixel bins that were undersampled (those with cumulative visit times of <1 s) during the pre- or postconditioning session were not included in the place map for either session. Firing rate in each bin was computed by dividing the number of spikes the cell generated in that bin by the number of position samples the rat spent in that bin. Firing rate maps were smoothed by a single iteration of adjacent pixel averaging. Contour plots were generated using Origin 5.0 (OriginLab, Northampton, MA). Cells were considered to have place-specific activity during a given session when the spatial information content (Skaggs et al., 1993) exceeded 0.2 bits/spike and the peak firing rate exceeded 1.5 Hz. In-field regions of the place map were defined as pixels in which the cell's firing rate exceeded the mean firing rate by at least one standard error, and out-of-field regions as those pixels where the firing rate was at least one standard error below the mean rate. Pixels where the firing rate fell within one standard error of the mean rate were regarded as ambiguous and were therefore excluded from analysis of place-specific CS responses. This method of identifying place fields provided a common normalized standard that controlled for intercell and intersession variability in field sizes and firing rates.

Peri-Event Time Histograms (PETHs)

PETHs of neural responses to the auditory CS and shock US were constructed by cumulatively summing spike occurrences within time bins surrounding the onset of each pip or shock that occurred during a given behavioral condition (e.g., freezing versus moving, in-field versus out-of-field). The bin size was 10 ms for all PETHs. Analysis of sensory-evoked responses was restricted to a time period of 150 ms (approximately one hippocampal theta cycle) following the onset of the sensory stimulus. A cell was considered to exhibit a significant sensory-evoked response if two or more bins (within the designated 150 ms period) showed spike counts that exceeded the expected spike count for a single bin within a 99% confidence interval, assuming that spikes were generated by an independent Poisson process (Abeles, 1982).

Z Score Normalized PETHs

Population-averaged PETHs were generated by normalizing spike counts in each bin of the PETH to a Z score, based on an expected spike count for that bin, and then averaging the normalized PETHs across cells. The auditory pip PETHs for a session (e.g., habituation) were normalized using an expected bin count that was computed as the mean bin count during the 300 ms period of silence preceding the onset of each pip during that session. The same 300 ms prepip period was used to compute the expected bin count for freezing pip PETHs, with the additional constraint that only those pre-CS periods during which the rat's head was motionless were included in the average for the expected bin count. The 300 ms prepip period was also used to normalize the in-field and out-of-field pip response PETHs for place cells, again subject to the freezing constraint, and also with the additional constraint that the expected bin count incorporated only pre-CS periods that occurred when the rat was inside (for in-field PETHs) or outside (for out-of-field PETHs) of the cell's place field. Rats performed fast head movements during the shock US (Figure 2), so shock response PETHs were normalized using an expected bin count that was computed as the mean bin count during periods when the rat's head was moving at a speed ≥ 45 cm/s in the absence of both the shock and auditory CS.

Acknowledgments

This work was conducted in the Center for Neural Science at New York University. Supported by Program PRAXIS XXI/FCT under the

Programa Gulbenkian de Doutorado em Biologia e Medicina to M.A.P.M.; National Institutes of Health Grants MH12341 to H.T.B. and MH38774, MH46516, and MH00956 to J.E.L.; and an Office of Naval Research Grant to New York University.

Received: May 1, 2002

Revised: December 9, 2002

References

- Abeles, M. (1982). Quantification, smoothing, and confidence limits for single-units' histograms. *J. Neurosci. Methods* 5, 317–325.
- Anagnostaras, S.G., Maren, S., and Fanselow, M.S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J. Neurosci.* 19, 1106–1114.
- Anagnostaras, S.G., Gale, G.D., and Fanselow, M.S. (2001). Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus* 11, 8–17.
- Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., and Damasio, A.R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science* 269, 1115–1118.
- Blair, H.T., Schafe, G.E., Bauer, E.P., Rodrigues, S.M., and LeDoux, J.E. (2001). Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn. Mem.* 8, 229–242.
- Berger, T.W., Alger, B., and Thompson, R.F. (1976). Neuronal substrate of classical conditioning in the hippocampus. *Science* 192, 483–485.
- Berger, T.W., Laham, R.I., and Thompson, R.F. (1980). Hippocampal unit-behavior correlations during classical conditioning. *Brain Res.* 193, 229–248.
- Berger, T.W., Rinaldi, P.C., Weisz, D.J., and Thompson, R.F. (1983). Single-unit analysis of different hippocampal cell types during classical conditioning of rabbit nictitating membrane response. *J. Neurophysiol.* 50, 1197–1219.
- Best, M.R., and Best, P.J. (1976). The effects of state of consciousness and latent inhibition on hippocampal unit activity in the rat during conditioning. *Exp. Neurol.* 51, 564–573.
- Bolles, R.C., and Fanselow, M.S. (1980). A perceptual-defensive-recuperative model of fear and pain. *Behav. Brain Sci.* 3, 291–323.
- Bouton, M.E., and Bolles, R.C. (1980). Conditioned fear assessed by freezing and by the suppression of three different baselines. *Anim. Learn. Behav.* 8, 429–434.
- Buchel, C., Dolan, R.J., Armony, J.L., and Friston, K.J. (1999). Amygdala-hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. *J. Neurosci.* 19, 10869–10876.
- Buzsaki, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Res.* 398, 242–252.
- Buzsaki, G. (2002). Theta oscillations in the hippocampus. *Neuron* 33, 325–340.
- Buzsaki, G., Grastryan, E., Tveritskaya, I.N., and Czopf, J. (1979). Hippocampal evoked potentials and EEG changes during classical conditioning in the rat. *Electroencephalogr. Clin. Neurophysiol.* 47, 64–74.
- Collins, D.R., and Pare, D. (2000). Differential fear conditioning induces reciprocal changes in the sensory responses of lateral amygdala neurons to the CS(+) and CS(-). *Learn. Mem.* 7, 97–103.
- Corcoran, K.A., and Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J. Neurosci.* 21, 1720–1726.
- Desmedt, A., Garcia, R., and Jaffard, R. (1998). Differential modulation of changes in hippocampal-septal synaptic excitability by the amygdala as a function of either elemental or contextual fear conditioning in mice. *J. Neurosci.* 18, 480–487.
- Eichenbaum, H. (2000). Hippocampus: mapping or memory? *Curr. Biol.* 10, R785–R787.
- Eichenbaum, H., Wiener, S.I., Shapiro, M.L., and Cohen, N.J. (1989).

- The organization of spatial coding in the hippocampus: a study of neural ensemble activity. *J. Neurosci.* 9, 2764–2775.
- Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., and Tanila, H. (1999). The hippocampus, memory, and place cells: is it spatial memory or a memory space? *Neuron* 23, 209–226.
- Fanselow, M.S. (1980). Conditional and unconditional components of postshock freezing. *Pavlov. J. Biol. Sci.* 15, 177–182.
- Fanselow, M.S., and LeDoux, J.E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* 23, 229–232.
- Fox, S.E., and Ranck, J.B., Jr. (1981). Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. *Exp. Brain Res.* 41, 399–410.
- Freeman, J.H., Jr., Weible, A., Rossi, J., and Gabriel, M. (1997). Lesions of the entorhinal cortex disrupt behavioral and neuronal responses to context change during extinction of discriminative avoidance behavior. *Exp. Brain Res.* 115, 445–457.
- Hampson, R.E., Simeral, J.D., and Deadwyler, S.A. (1999). Distribution of spatial and nonspatial information in dorsal hippocampus. *Nature* 402, 610–614.
- Holland, P.C., and Bouton, M.E. (1999). Hippocampus and context in classical conditioning. *Curr. Opin. Neurobiol.* 9, 195–202.
- Kim, J.J., and Fanselow, M.S. (1992). Modality-specific retrograde amnesia of fear. *Science* 256, 675–677.
- Kim, J.J., and Thompson, R.F. (1997). Cerebellar circuits and synaptic mechanisms involved in classical eyeblink conditioning. *Trends Neurosci.* 20, 177–181.
- Kim, J.J., Clark, R.E., and Thompson, R.F. (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behav. Neurosci.* 109, 195–203.
- LaBar, K.S., Gatenby, J.C., Gore, J.C., LeDoux, J.E., and Phelps, E.A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron* 20, 937–945.
- Laroche, S., Neuenchwander-el Massioui, N., Edeline, J.M., and Dutrioux, G. (1987). Hippocampal associative cellular responses: dissociation with behavioral responses revealed by a transfer-of-control technique. *Behav. Neural Biol.* 47, 356–368.
- LeDoux, J.E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184.
- Leung, L.S., and Vanderwolf, C.H. (1980). Behavior-dependent evoked potentials in the hippocampus CA1 region of the rat. II. Effect of eserine, atropine, ether and pentobarbital. *Brain Res.* 198, 119–133.
- Levy, W.B., and Steward, O. (1979). Synapses as associative memory elements in the hippocampal formation. *Brain Res.* 175, 233–245.
- Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annu. Rev. Neurosci.* 24, 897–931.
- McEchron, M.D., and Disterhoft, J.F. (1997). Sequence of single neuron changes in CA1 hippocampus of rabbits during acquisition of trace eyeblink conditioned responses. *J. Neurophysiol.* 78, 1030–1044.
- McNaughton, N., and Miller, J.J. (1986). Collateral specific long term potentiation of the output of field CA3 of the hippocampus of the rat. *Exp. Brain Res.* 62, 250–258.
- McNaughton, B.L., Barnes, C.A., and O'Keefe, J. (1983). The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. *Exp. Brain Res.* 52, 41–49.
- Medina, J.F., Christopher Repa, J., Mauk, M.D., and LeDoux, J.E. (2002). Parallels between cerebellum- and amygdala-dependent conditioning. *Nat. Rev. Neurosci.* 3, 122–131.
- Muller, R.U., Kubie, J.L., and Ranck, J.B., Jr. (1987). Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. *J. Neurosci.* 7, 1935–1950.
- Munera, A., Gruart, A., Munoz, M.D., Fernandez-Mas, R., and Delgado-Garcia, J.M. (2001). Hippocampal pyramidal cell activity encodes conditioned stimulus predictive value during classical conditioning in alert cats. *J. Neurophysiol.* 86, 2571–2582.
- Nadel, L., and Payne, J.D. (2002). The hippocampus, wayfinding and episodic memory. In *The Neural Basis of Navigation*, P.E. Sharp, ed. (Boston: Kluwer Academic Publishers), pp. 235–247.
- O'Keefe, J. (1999). Do hippocampal pyramidal cells signal non-spatial as well as spatial information? *Hippocampus* 9, 352–364.
- O'Keefe, J., and Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* 34, 171–175.
- Olds, J., Disterhoft, J.F., Segal, M., Kornblith, C.L., and Hirsh, R. (1972). Learning centers of rat brain mapped by measuring latencies of conditioned unit responses. *J. Neurophysiol.* 35, 202–219.
- Otto, T., and Eichenbaum, H. (1992). Neuronal activity in the hippocampus during delayed non-match to sample performance in rats: evidence for hippocampal processing in recognition memory. *Hippocampus* 2, 323–334.
- Phillips, R.G., and LeDoux, J.E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* 106, 274–285.
- Pine, D.S., Fyer, A., Grun, J., Phelps, E.A., Szeszko, P.R., Koda, V., Li, W., Ardekani, B., Maguire, E.A., Burgess, N., and Bilder, R.M. (2001). Methods for developmental studies of fear conditioning circuitry. *Biol. Psychiatry* 50, 225–228.
- Quirk, G.J., Repa, C., and LeDoux, J.E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron* 15, 1029–1039.
- Ranck, J.B., Jr. (1973). Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral correlates and firing repertoires. *Exp. Neurol.* 41, 461–531.
- Redish, A.D. (2001). The hippocampal debate: are we asking the right questions? *Behav. Brain Res.* 127, 81–98.
- Repa, J.C., Muller, J., Apergis, J., Desrochers, T.M., Zhou, Y., and LeDoux, J.E. (2001). Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nat. Neurosci.* 4, 724–731.
- Rogan, M.T., Staubli, U.V., and LeDoux, J.E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 390, 604–607.
- Sainsbury, R.S., and Montoya, C.P. (1984). The relationship between type 2 theta and behavior. *Physiol. Behav.* 33, 621–626.
- Sainsbury, R.S., Heynen, A., and Montoya, C.P. (1987). Behavioral correlates of hippocampal type 2 theta in the rat. *Physiol. Behav.* 39, 513–519.
- Sakurai, Y. (1994). Involvement of auditory cortical and hippocampal neurons in auditory working memory and reference memory in the rat. *J. Neurosci.* 14, 2606–2623.
- Skaggs, W.E., McNaughton, B.L., Gothard, K., and Markus, E.J. (1993). An information-theoretic approach to deciphering the hippocampal code. In *Advances in Neural Information Processing*, S.J. Hanson, J.D. Cowan, and C.L. Giles, eds. (San Mateo, CA: Morgan Kaufman), pp. 1030–1037.
- Smith, O.A., Astley, C.A., Devito, J.L., Stein, J.M., and Walsh, R.E. (1980). Functional analysis of hypothalamic control of the cardiovascular responses accompanying emotional behavior. *Fed. Proc.* 39, 2487–2494.
- Squire, L.R., and Zola, S.M. (1996). Structure and function of declarative and nondeclarative memory systems. *Proc. Natl. Acad. Sci. USA* 93, 13515–13522.
- Stolar, N., Sparenborg, S., Donchin, E., and Gabriel, M. (1989). Conditional stimulus probability and activity of hippocampal, cingulate cortical, and limbic thalamic neurons during avoidance conditioning in rabbits. *Behav. Neurosci.* 103, 919–934.
- Tulving, E., and Markowitsch, H.J. (1998). Episodic and declarative memory: role of the hippocampus. *Hippocampus* 8, 198–204.
- Vanderwolf, C.H. (1969). Hippocampal electrical activity and volun-

tary movement in the rat. *Electroencephalogr. Clin. Neurophysiol.* *26*, 407–418.

Wiebe, S.P., and Staubli, U.V. (1999). Dynamic filtering of recognition memory codes in the hippocampus. *J. Neurosci.* *19*, 10562–10574.

Wiebe, S.P., and Staubli, U.V. (2001). Recognition memory correlates of hippocampal theta cells. *J. Neurosci.* *21*, 3955–3967.

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