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Stability of Hippocampal Place Cell Activity Across the Rat Estrous Cycle

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ABSTRACT: Findings from both in vitro and in vivo studies have shown that estrogen exerts pronounced effects on hippocampal morphology and physiology. The degree to which these molecular findings influence hippocampal processing in freely behaving animals is unclear. The present study assessed the effect of the estrous cycle on hippocampal place cells in naturally cycling rats during two behavioral states. Female Sprague-Dawley rats were trained to alternate on a U-shaped runway for food reinforcement. Single-unit recordings of hippocampal CA1 cells were conducted under two conditions: (1) at rest on a holder, and (2) running on the maze. Spatial firing characteristics of the cells were examined at different stages of the estrous cycle (i.e., diestrus, proestrus, and estrus). Specifically, information was collected on (1) mean firing rates; (2) basic place field parameters; and (3) changes in the firing dynamics of these cells (e.g., burst properties). The findings showed a decrease in mean firing rate on the maze during proestrus. However, other basic measures of spatial tuning and burst properties were unchanged. The current study suggests that there is relative stability of hippocampal place cells across the estrous cycle during a well-trained task. © **2004 Wiley-Liss, Inc.**

KEY WORDS: hippocampus; estrous cycle; place cells; estrogen; physiology

INTRODUCTION

The hippocampus is important for processing the spatial layout and configural representation of an environment both in rodents (e.g., O'Keefe and Nadel, 1978; O'Keefe and Speakman, 1987; Sutherland and Rudy, 1989; Jarrard, 1993) and in humans (Maguire et al., 2000). Further support has come from extracellular recordings of hippocampal pyramidal cells both in rodents (O'Keefe and Dostrovsky, 1971; O'Keefe and Speakman, 1987) and in humans (Ekstrom et al., 2003). These "place cells" show spatial tuning and fire strongly when an animal is in a certain location or place in the environment (O'Keefe and Speakman, 1987).

Hippocampus-dependent behaviors are affected by estrogen (e.g., Frye, 1995; Markus and Zecevic, 1997; Daniel et al., 1999; Tropp and Markus, 2001a). In addition, it has been shown that hippocampal CA1 dendritic morphology fluctuates across the rat estrous cycle (Woolley et al., 1990; Woolley and McEwen, 1992). Synaptic density is higher during proestrus (high levels of estrogen) compared with estrus (low estrogen), with a 30% decrease in synaptic density on the CA1 apical dendrites during estrus (Woolley and McEwen, 1992).

Both in vivo (e.g., Terasawa and Timiras, 1968; Warren et al., 1995; Córdoba Montoya and Carrer, 1997; Good et al., 1999) and in vitro (e.g., Wong and Moss, 1992; Murphy and Segal, 1996; Woolley et al., 1997) work has explored the effects of fluctuations in ovarian steroids (e.g., estrogen) on hippocampal physiology. High levels of estrogen have been associated with an increase in hippocampal plasticity and long-term potentiation (LTP). Specifically, estrogen decreases the threshold for LTP induction both in naturally cycling anesthetized animals (Warren et al., 1995; Good et al., 1999) and in awake ovariectomized rats (Córdoba Montoya and Carrer, 1997).

Overall, anatomical and electrophysiological studies show that high levels of estrogen (both natural and artificial replacement) are associated with (1) an increase in spine and synapse density, (2) an increase in LTP induction, and (3) a decrease in seizure threshold (for review, see Woolley, 1998). Presumably, these changes should affect the manner in which the hippocampus processes information. Specifically, these changes should influence the tuning characteristics of the CA1 pyramidal neurons (place cells). Yet despite all the previous research on the effects of estrogen, there are no studies that have examined the effects of the estrous cycle on hippocampal place cell representation of the environment.

There is extensive research on the properties of hippocampal place cells. Place cells are sensitive to the location, speed, direction, and turning angle of the animal (see Redish, 1999). Place cells are also sensitive to different aspects of the environment, such as distal landmarks (e.g., O'Keefe and Speakman, 1987; Knierim et al., 1995; Redish, 1999). In addition, Mehta et al. (1997, 2000) showed experience-dependent asymmetric expansion of hippocampal place cells (i.e., an increase in mean place field size and a shift in the location of the field).

When examining hippocampal place cells one must take into account that there are two major electroencephalographic (EEG) states in the rat hippocampus: theta (when the animal is active) and sharp waves (when the animal is at rest) (Ranck, 1973; Buzsáki, 1996). An increase in population bursts occurs during the sharp wave state, while theta activity is prominent during ex-

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ploratory behaviors. Changes in synaptic density and plasticity could potentially cause a change in the burst properties of these cells. These include changes in the tendency to burst, average number of spikes in a burst, and the duration of bursts. A general feature of extracellularly recorded action potentials of hippocampal pyramidal cells is an activity-dependent attenuation in amplitude of spikes within the burst (Ranck, 1973; Quirk and Wilson, 1999; Quirk et al., 2001). A reduction in spike amplitude has been related to a decrease in the ability of somatic spikes to back-propagate into the dendrites of hippocampal cells (Buzsáki et al., 1996). Hippocampal slice work suggests that back-propagating action potentials are important for certain types of synaptic plasticity (e.g., LTP) and may play a role in learning and memory (e.g., Magee and Johnston, 1997).

Findings from both in vitro and in vivo studies have shown the effects that alterations in estrogen levels have on hippocampal morphology and physiology. However, a change at the molecular level may not be reflected at other levels. This is especially true when examining awake freely behaving animals, in which many modulatory processes are active and can interact with hippocampal processing. The present experiment examined the degree to which the firing characteristics of hippocampal cells would be altered over the natural estrous cycle in freely moving rats. Specifically, this research examined (1) changes in basic firing properties of hippocampal cells (e.g., firing rate, specificity, number of place fields and field size), and (2) changes in the firing dynamics of hippocampal cells (e.g., burst characteristics, spike amplitude attenuation, and place field expansion). Since the anatomical changes (e.g., spine and synapse density) are found in CA1 (Woolley and McEwen, 1992), but not CA3 (Woolley et al., 1990), both cells layers were examined.

MATERIALS AND METHODS

Subjects

Fourteen female Sprague-Dawley rats (6–8 months of age; Harlan Sprague-Dawley, IN) were used in this experiment. Rats were singly housed in transparent plastic tubs, in a room with a 12:12-h light/dark cycle. Male rats were kept in the same colony room to promote cyclicity (Vandenbergh, 1983). All animals were weighed daily and handled extensively before any behavioral training.

Estrous Cycle Verification

The females received daily vaginal lavages ~4–5 h before lights turned off to assess cycle status. The lavages were examined under a light microscope to identify the proportion of cornified epithelial cells, nucleated epithelial cells, and leukocytes. Animals that displayed vaginal smears that contained predominately leukocytes (≥60%) were classified as diestrus I or II. Smears that contained primarily nucleated epithelial cells (≥60%) and no leukocytes (≤10%) were classified as proestrus. Smears that contained primarily cornified cells (≥90%) were classified as estrus. This is a highly reliable method to determine the estrous cycle status of each animal (Schwartz and Hoffman, 1972). An animal's cycle was considered to be irregular if it showed persistent days of only estrus or diestrus, or skipped stages of the cycle (see Tropp and Markus, 2001b). An experimenter blind to the recording data verified when the animal was cycling regularly. Only animals that displayed a regular estrous cycle were included in the experiment. Cycle status was assessed both before and after hippocampal recordings to verify that the animals remained in the same stage of the cycle. The data were discarded from any animal that changed estrous cycle stage during recordings.

Apparatus and Training Procedure

All animals were food deprived to 90–95% of their ad libitum body weights. This level of food deprivation does not interfere with the maintenance of the rat estrous cycle (Tropp and Markus, 2001b). The testing environment consisted of a small room (2.1 × 2.1 m), which was dimly lit by lights from an open doorway. A U-shaped runway was located in the center of the room and was raised 96 cm off the floor. The runway consisted of three black Plexiglas arms (width = 10 cm). The total path length of the runway was (228 cm). Rats were pre-trained to alternate on the U-shaped runway for food reinforcement (BioServe Pellets) on an automated system (custom written software by A. Kuzin). The feeders dispensed a single food pellet when the animal passed through photoelectric beams that were located on both sides of the apparatus (Fig. 1). The rats were given a 30-min session each day to learn to alternate in the apparatus (from Feeder A to Feeder B). The animals were trained until they reached a criterion of 80 alternations for at least 4 days of training.

Surgery

After reaching criterion levels of performance, the animals received surgical implantation of an electrode microdrive for single-unit recordings. Animals were anesthetized with a 4-ml/kg dose

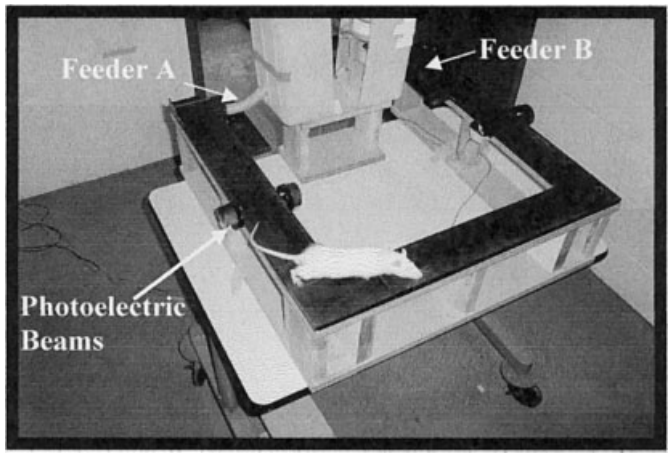

FIGURE 1. Behavioral apparatus and training procedure. Animals were trained to alternate (from Feeder A to Feeder B) on a U-shaped runway to receive a food reward. The animal made a total of 40 alternations during a recording session, usually within 15 min.

(intramuscular) of ketamine cocktail, consisting of ketamine (12.4 mg/ml), acepromazine (0.1 mg/ml), and xylazine (1.27 mg/ml). Each animal was placed in a stereotaxic apparatus (ASI Instruments, Warren, MI) and implanted with a miniature microdrive (O'Keefe and Recce, 1993; Wilson and McNaughton, 1993; Oler and Markus, 2000) containing four independently movable tetrode recording probes. Two small holes were drilled into the skull bilaterally over the dorsal hippocampus (3.2 mm posterior to bregma and 2.2 mm lateral from the midline). The microdrive device was mounted onto the skull by small anchor screws and dental acrylic. Each tetrode was composed of four twisted polyamide-insulated, 14-μm nichrome wire (H.P. Ried, Palm Coast, FL). The distance between the wire tips was ~10 μm, such that local neuronal activity was reflected as a slightly different signal on each wire. A comparison of the recording signal from each electrode allowed for differentiation of the firing of one cell from another. The tips of the tetrode were gold plated with an impedance of ~300–500 kΩ. After surgery, all animals were given an injection (intramusular) of a 0.25-ml/kg dose of Buprenex (0.03 mg/ml) (Reckitt and Colman, Richmond, VA) to minimize pain. The animals were allowed several days to recover before retraining started. In addition, food deprivation was resumed over the course of 1–2 weeks after surgery.

Recordings

The animals wore a multichannel headstage device that contained two arrays of infrared light emitting diodes (Multichannel Concepts, Gaithersburg, MD). An overhead video tracking system provided information about the rat's location and head direction (Dragon Tracker SA-3; Boulder, CO). During the recording session, the animals were connected to the recording apparatus by a multiwire cable that was attached to a pulley system in the ceiling to counterbalance the weight of the headstage. This design allowed for the animal to move freely on the apparatus. Rack-mounted amplifiers (Assembly Hunter: Neuralynx, Tucson, AZ) were used to detect the signals from the head stage. The signal was amplified up to 5,000 times and was filtered at 300 Hz to 6 kHz, which was sent to a PC-based analog-to-digital signal-capture board (Data Translation, Marlboro, MA). Firing and positional data were time-stamped by a synchronization clock board (ComputerBoards, Mansfield, MA). A 1.0-ms sample of data was acquired at a rate of 25 kHz. An analysis of the multi-single-unit recordings from each probe was conducted off-line, using a spike parameter clustering method (McNaughton et al., 1989; Mizumori et al., 1989; Markus et al., 1994) on a Sun Blade 100 computer workstation (Sun Microsystems, Palo Alto, CA). The clustering was based on the relative amplitudes of the signals and the spike durations (see Wilson and McNaughton, 1993). Data collection and analysis were carried out with software written by M. Wilson, L. Frank, and M. Quirk (MIT, Cambridge, MA) and W.E. Skaggs (University of Pittsburgh, Pittsburgh, PA).

Experimental Procedure

After recovery from surgery, animals were retrained on the alternation task described above. The electrodes were slowly lowered into the CA1 region of the dorsal hippocampus. The average latency to complete one alternation (i.e., from Feeder A to Feeder B) provided an index of the animals' activity/motivational level.

Data were collected in two stages:

1. *Recordings on holder*: ~10 min of recordings of cells were obtained while the animal sat quietly on a small platform (diameter = 19 cm) outside the maze room.
2. *Recordings on maze*: recordings of cells as the animal performed 40 alternations on the maze (20 in each direction). The maze recording session lasted about 15 min.

Recordings of cells were obtained during different phases of the estrous cycle: diestrus, proestrus, and estrus. This design allowed for comparisons of the firing characteristics of the cells under high levels of estrogen (i.e., proestrus) as well as during low levels of estrogen (i.e., estrus and diestrus). While in some cases the same cells were recorded across days, most of the time they were different cells.

Data Analysis

Basic firing properties

The firing rates, number of fields, place field size, and specificity (information per spike) were measured during maze recording (see Oler and Markus, 2000). Firing rate maps were prepared for each cell by dividing the recording environment into a 64 × 64-bin array, consisting of 0.5 × 0.5-cm squares. The mean firing rate for each bin was calculated for each cell by dividing the number of spikes by the time spent in that bin. An adaptive smoothing method was used to create firing rate maps (Skaggs and McNaughton, 1998). A velocity filter was used to collect data only if the animal moved faster than 2.0 cm/s. This allowed for the analysis of data only when the rat was moving (i.e., in theta) and not in sharp wave states (e.g., Chrobak and Buzsáki, 1998).

Place fields were designated as an area of 15–200 bins sharing adjacent edges, with a firing rate per bin greater than two standard deviations above the mean firing rate for the cell on the entire apparatus (Muller and Kubie, 1987). Place field size was defined as the number of bins sharing adjacent edges that comprised the place field.

Specificity of the place field was calculated in terms of the amount of spatial information content (in bits) that a single spike conveyed about the animal's location. Spatial information content of spike discharge was calculated using the formula:

$$\text{Information content} = \sum P_i(R_i/R)\log_2(R_i/R)$$

where i is the bin number, P_i is the probability for occupancy of bin i, R_i is the mean firing rate for bin i, and R is the overall mean firing rate (see Skaggs et al., 1993).

Classification of cells

Cells recorded on maze. Cells were classified by their mean firing rate and/or spike "width" (duration from maximum to minimum voltage) and were designated as complex spike cells (putative pyramidal cells) or theta cells (presumably interneurons) (Markus et al., 1994). Complex spikes cells recorded on

the maze were classified by a mean firing rate of ≤2.5 Hz and a valid place field. Theta cells were classified by mean firing rate of >2.5 Hz. Additionally, theta cells were classified by a narrow spike width (spike width < 288 μs, mean width = 214.7 ± 4.74). Because most of the parameters examined are affected by low firing rate, cells that had a very low firing rate (firing rate < 0.1 Hz) were not analyzed.

Cells recorded on holder. Cells recorded on the holder were defined by their spike width and designated as complex spikes or theta cells. Complex spike cells were classified by a spike width ≥ 288 μs (mean spike width = 353.95 ± 3.99). Theta cells were classified by a spike width < 288 μs (mean width = 219.43 ± 4.38). If the cell was also recorded on the maze then information on firing rate and place field information was also taken into account. Low rate cells (<100 spikes) were not analyzed.

Burst properties

Pyramidal cells can display complex spike bursts (e.g., Ranck, 1973). Data were obtained regarding the properties of these bursts for the holder and maze recordings. A burst was defined as having an interspike interval of <10 ms. The average number of spikes in a burst, mean burst duration (ms), and "burstiness" (number of burst spikes divided by total spikes) were examined.

Spike amplitude attenuation

A general feature of extracellularly recorded action potentials of hippocampal pyramidal cells is an activity-dependent attenuation in amplitude (Quirk and Wilson, 1999). This effect has been observed in pyramidal cells that display complex spike bursts (Ranck, 1973; Quirk and Wilson, 1999; Quirk et al., 2001). To assess spike amplitude attenuation, we compared the amplitude of the first and the third spike for bursts that had 3 spikes or more (Quirk et al., 2001).

$$\text{Attenuation of the burst} = \frac{\text{amplitude of third spike}}{\text{amplitude of first spike}}$$

Place field expansion

Mehta et al. (1997) showed evidence of experience-dependent asymmetric expansion of hippocampal place cells. To assess place field expansion, we calculated the center of mass for each place field (centroid) and the size of each field during the early trials (first four runs) to later trials (last four runs) on the alternation task. Analyses were conducted on the field movement of the centroid and the difference in field size from later trials to early trials (late field size–early field size).

Histology

After completion of all recording sessions, animals were euthanized with CO_2 and perfused intracardially with a 4% para-

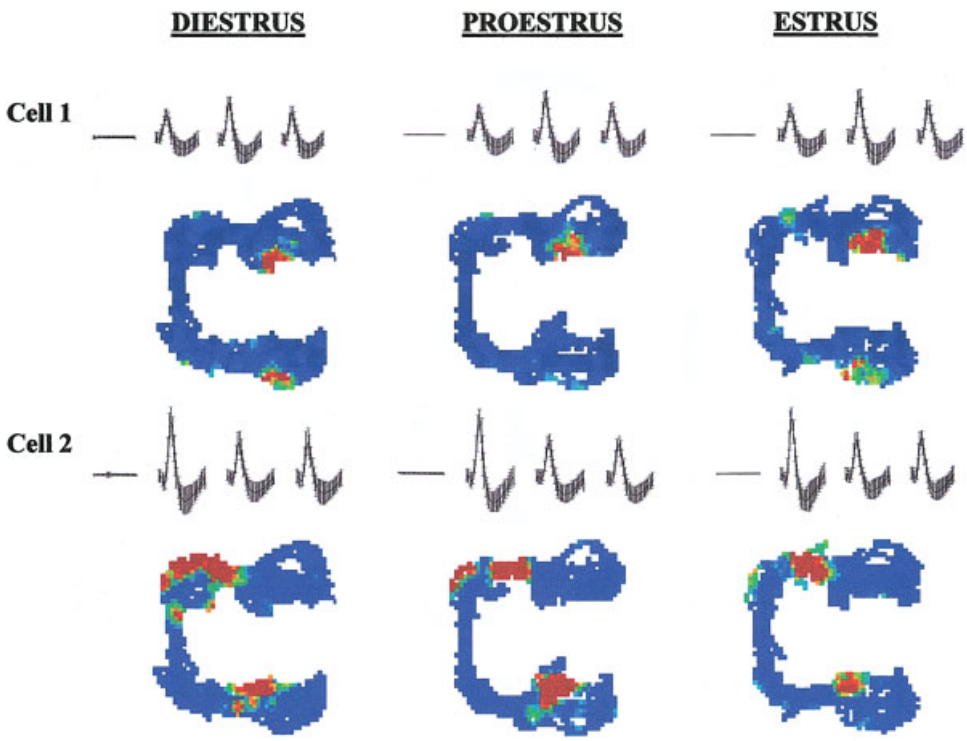

FIGURE 2. Place fields across the estrous cycle. Example of two simultaneously recorded hippocampal place cells from a rat running on the U-shaped task during different stages of the estrous cycle. Note the similarity in the average waveforms and firing rate maps across the cycle. Maximum firing rate is indicated by red and occupancy with no firing by blue. The firing rate scale was held constant for each cell across the cycle (cell 1, red = 10 Hz; cell 2, red = 5 Hz). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

formaldehyde solution. The electrodes were raised and the brains were removed and placed in paraformaldehyde for 48 h. Coronal sections (50 μm) were sliced using a cryostat or a vibratome. The slices were mounted and Nissl stained and examined under a microscope. Assessment of recording location was based on the histology (electrode tracks) and/or physiological markers (cell layers, sharp waves, and ripples) and/or the amount the electrode was advanced. It should be noted that assessment of electrode location allowed for a differentiation between CA1 and deeper layers. However, it did not provide enough information to distinguish dentate gyrus and CA3 cells.

RESULTS

Hippocampal cells were recorded across the estrous cycle (Fig. 2). The complex spike cells and theta cells were identified as CA1 or CA3-DG (dentate gyrus) cells. Separate analyses were con-

TABLE 1.

Total Number of Cells From Each Individual Animal for Complex Spike Cells (CS) and Theta Cells (θ) Recorded Across the Estrous Cycle on the Holder and Maze

	Holder cells											
	CA1 CS			CA1 θ			CA3-DG CS			CA3-DG θ		
Rat ID	D	P	E	D	P	E	D	P	E	D	P	E
253	2	—	—	1	—	—	—	—	—	—	—	—
254	5	1	2	1	—	—	—	—	—	—	—	—
361	4	5	2	1	—	—	2	4	1	1	1	2
362	1	1	2	—	—	—	—	—	—	—	—	—
460	3	—	1	—	—	1	9	9	6	—	—	—
485	1	—	—	2	—	—	—	—	—	—	—	—
548	—	—	—	—	—	—	7	9	3	1	—	—
683	5	—	3	—	—	—	5	8	4	1	1	—
685	4	4	—	3	2	3	1	—	—	—	—	—
696	1	1	—	—	—	1	—	—	—	—	—	—
698	—	1	1	—	1	—	—	—	—	—	—	—
701	—	4	3	—	2	1	2	3	—	—	1	2
763	11	8	8	—	1	3	1	3	3	—	—	—
765	—	—	—	—	1	1	—	—	—	—	—	—
Total:	37	25	22	8	7	10	27	36	17	3	3	4

	Maze cells											
	CA1 CS			CA1 θ			CA3-DG CS			CA3-DG θ		
Rat ID	D	P	E	D	P	E	D	P	E	D	P	E
253	—	—	—	—	—	—	—	—	—	—	—	—
254	7	1	3	1	—	—	—	—	—	—	—	—
361	—	—	—	—	—	—	—	—	—	—	—	—
362	1	1	—	—	—	—	—	—	—	—	—	—
460	4	—	3	—	—	1	8	10	8	1	—	—
485	—	—	—	1	—	—	—	—	—	—	—	—
548	—	1	1	—	—	—	6	9	7	1	—	—
683	3	1	6	—	—	—	6	6	9	1	1	—
685	5	3	—	3	2	3	1	—	—	—	—	—
696	—	1	—	—	—	1	—	—	—	—	—	—
698	—	2	3	—	1	—	—	—	—	—	—	—
701	—	5	2	—	1	1	1	3	—	1	—	2
763	11	9	9	1	1	4	—	3	4	—	—	—
765	—	—	—	1	1	1	—	—	—	—	—	—
Total:	31	24	27	7	6	11	22	31	28	4	1	2

D, diestrus; P, proestrus; E, estrus.

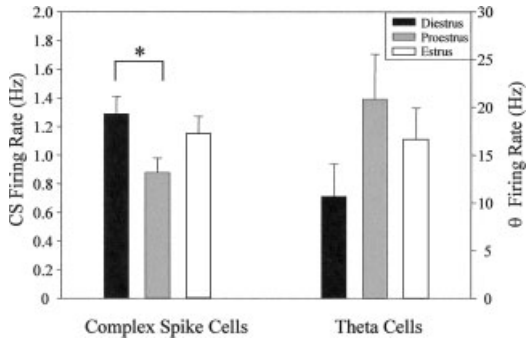

FIGURE 3. Firing rate of hippocampal CA1 cells. Mean firing rate was analyzed for complex spike cells and theta cells as the animals ran the U-shaped maze. There was a significant difference in mean firing rate, for complex spike cells, across the estrous cycle. Firing rate was lowest during proestrus and significantly different from the diestrous females (Scheffe, $P < 0.05$). There were no significant differences in mean firing rate across the estrous cycle for theta cells. Note the scale difference for firing rate for the two types of cells.

ducted for cells recorded on the maze and cells recorded on the holder (Table 1).

Behavioral Activity

Analysis of variance (ANOVA) ($F_{(2,101)} = 0.41$, $P > 0.1$) revealed no significant differences in maze run latency across the estrous cycle (diestrus: mean = 19.30 ± 1.57s; proestrus: mean = 21.52 ± 2.49s; estrus: mean = 21.94 ± 2.81s). These data suggest that there were no gross differences in the rats' activity levels on the maze across the estrous cycle.

Place Field Characteristics

Complex spike cells on the maze

ANOVA revealed a significant difference in mean firing rate for CA1 cells across the estrous cycle ($F_{(2,81)} = 3.27$, $P < 0.05$) (Fig. 3). Post hoc analysis of firing rate revealed a significant difference between proestrous and diestrous females (Scheffe, $P < 0.05$). However, there were no significant differences across the estrous cycle for other place field measures: mean specificity ($F_{(2,81)} = 0.65$, $P > 0.1$), infield firing rate ($F_{(2,81)} = 1.73$, $P > 0.1$), number of fields ($F_{(2,81)} = 0.04$, $P > 0.1$), and field size ($F_{(2,81)} = 1.43$, $P > 0.1$) (Table 2). In addition, an analysis of the complex spike cells broken down by direction (running clockwise or counterclockwise on the maze) provided similar results (firing rate, $P < 0.05$; all other measures, $P > 0.1$).

Theta cells on the maze

ANOVA conducted on CA1 theta cells revealed no significant differences across the estrous cycle for mean firing rate ($F_{(2,23)} = 1.56$, $P > 0.1$) (see Fig. 3) or specificity (information per spike) ($F_{(2,23)} = 0.72$, $P > 0.1$) (Table 3).

Theta and complex spike cells on the holder

ANOVA revealed no significant estrous cycle differences in firing rate for CA1 complex spike cells recorded ($F_{(2,83)} = 0.07$, $P > 0.1$) on the holder. Similarly, for CA1 theta cells, there were no differences in firing rate across the estrous cycle ($F_{(2,24)} = 2.19$, $P > 0.1$) (Table 4).

Burst Properties of Hippocampal Cells

CA1 pyramidal cells that displayed complex spike bursts were examined over the estrous cycle. Burst properties included: the average number of spikes in a burst, mean burst duration (ms) and "burstiness" (number of burst spikes divided by total spikes).

A two-way ANOVA (cycle × task) was conducted for burst properties. As expected, cells tended to be more active on the holder (when sharp waves were predominant) than on the maze (during theta). There were on average more spikes in a burst ($F_{(1,158)} = 12.97$, $P < 0.001$), a longer mean burst duration ($F_{(1,158)} = 4.02$, $P < 0.05$), and increased burstiness ($F_{(1,158)} = 10.56$, $P < 0.01$) for cells recorded on the holder (Fig. 4). However, there were no differences in these burst properties across the estrous cycle and no interactions (all, $P > 0.1$) (Table 5).

Spike Amplitude Attenuation

A general property of pyramidal cells is an activity-dependent attenuation in amplitude (Quirk and Wilson, 1999). Dividing the amplitude of the third spike by the amplitude of the first spike assessed the amount of attenuation of a burst. A two-way ANOVA (cycle × task) was conducted for spike amplitude attenuation

TABLE 2.

Place Field Characteristics of CA1 Complex Spike Cells (Mean ± SEM)

	Diestrus (n = 31)	Proestrus (n = 24)	Estrus (n = 27)
Firing rate (Hz)[a]	1.29 ± 0.12	0.88 ± 0.10	1.15 ± 0.12
Specificity[b]	0.67 ± 0.06	0.72 ± 0.07	0.77 ± 0.08
Infield firing rate (Hz)	4.95 ± 0.56	3.74 ± 0.35	5.12 ± 0.64
No. of fields	1.87 ± 0.26	1.92 ± 0.26	1.96 ± 0.24
Field size	48.52 ± 4.54	55.08 ± 7.04	42.48 ± 3.66

[a]Firing rate ($P < 0.05$).
[b]Information (in bits) that a single spike conveys about animal's location.

TABLE 3.

Firing Characteristics of CA1 Theta Cells on the Maze (Mean ± SEM)

	Diestrus (n = 7)	Proestrus (n = 6)	Estrus (n = 11)
Firing rate (Hz)	10.70 ± 3.37	20.86 ± 4.69	16.66 ± 3.26
Specificity	0.30 ± 0.12	0.11 ± 0.04	0.23 ± 0.10

TABLE 4.

Firing Rate of CA1 Cells Recorded on the Holder (Mean ± SEM)

Firing rate (Hz)	Diestrus	Proestrus	Estrus
Complex spikes	1.49 ± 0.35 (n = 37)	1.58 ± 0.22 (n = 25)	1.40 ± 0.27 (n = 22)
Theta cells	5.83 ± 1.29 (n = 8)	10.48 ± 3.09 (n = 7)	11.88 ± 2.03 (n = 10)

(Table 6). There was an increase in spike amplitude attenuation for CA1 complex spike cells recorded on the holder versus on the maze ($F_{(1,142)} = 7.94$, $P < 0.01$). However, there was no effect of cycle on spike amplitude attenuation ($F_{(2,142)} = 0.64$, $P > 0.1$) and there were no interactions ($F_{(2,142)} = 0.26$, $P > 0.1$).

Place Field Expansion

Mehta et al. (1997, 2000) showed evidence of experience-dependent asymmetric expansion of hippocampal place cells. Their findings revealed that with experience: (1) there was an increase in mean place field size, and (2) the location of the place field shifted backwards in the direction opposite to the direction of the route.

ANOVA showed no differences across the estrous cycle for field size ($F_{(2,45)} = 0.89$, $P > 0.1$) or field centroid movement ($F_{(2,45)} = 0.15$, $P > 0.1$) (Table 7).

Firing Properties of CA3-DG Cells

Anatomical changes across the estrous cycle are found in CA1 but not in CA3 (Woolley et al., 1990; Woolley and McEwen, 1992). Therefore, firing properties were examined in both cell layers. There were no significant effects of the estrous cycle on any of the basic place field properties of CA3-DG cells (ANOVA, mean rate ($F_{(2,80)} = 2.56$, $P = 0.08$), specificity ($F_{(2,80)} = 1.44$, $P > 0.1$), infield firing rate ($F_{(2,80)} = 2.26$, $P > 0.1$), number of fields ($F_{(2,80)} = 1.93$, $P > 0.1$), and field size ($F_{(2,80)} = 0.97$, $P > 0.1$) (Table 8a). There were not enough CA3-DG theta cells recorded on the maze (n = 7) to conduct an analysis of firing rate or specificity. In addition, there were no significant estrous cycle differences in firing rate for complex spike cells recorded on the holder (ANOVA, $F_{(2,79)} = 0.83$, $P > 0.1$). Similarly, for theta cells, there were no differences in firing rate across the estrous cycle on the holder ($F_{(2,9)} = 1.49$, $P > 0.1$) (Table 8b).

There were no significant cycle effects in burst properties for CA3-DG cells (average number of spikes in burst ($F_{(2,145)} = 1.46$, $P > 0.1$); mean burst duration, ($F_{(2,145)} = 1.39$, $P > 0.1$; burstiness ($F_{(2,145)} = 0.14$, $P > 0.1$) (Table 8c). Lastly, there were no effects of cycle ($F_{(2,125)} = 1.83$, $P > 0.1$) in spike amplitude attenuation for CA3-DG cells (Table 8d).

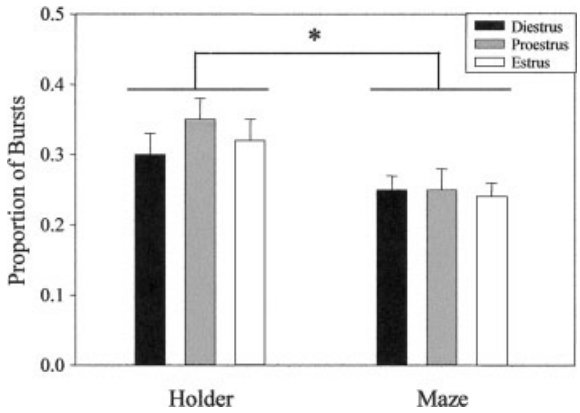

FIGURE 4. Proportion of bursts at rest and on the maze. The proportion of bursts ("burstiness") was measured by dividing the number of burst spikes by the total number of spikes. There were a greater proportion of bursts for cells recorded on the holder compared with cells recorded on the maze ($P < 0.01$). Burstiness did not vary across the estrous cycle for either condition.

DISCUSSION

The current study showed a relative stability of hippocampal place cell firing characteristics across the estrous cycle. The one exception was mean firing rate, in which firing rate was lowest during proestrus. However, spatial tuning characteristics such as specificity, infield firing rate, number of place fields, and place field size did not vary across the cycle. Additionally, cell dynamics such as spike amplitude attenuation, field expansion, and burst properties were unchanged across the cycle. Similar findings of stability were seen when individual cells were recorded across days of the

TABLE 5.

Burst Properties for CA1 Cells Recorded on the Holder and Maze

Mean ± SEM			
Holder	Diestrus (n = 37)	Proestrus (n = 25)	Estrus (n = 22)
Avg. spikes in burst	2.38 ± 0.03	2.38 ± 0.03	2.41 ± 0.05
Mean burst duration (ms)	7.8 ± 0.22	8.0 ± 0.24	8.3 ± 0.28
Burstiness	0.30 ± 0.03	0.35 ± 0.03	0.32 ± 0.03
Maze	Diestrus (n = 28)	Proestrus (n = 21)	Estrus (n = 25)
Avg. spikes in burst	2.29 ± 0.04	2.29 ± 0.04	2.24 ± 0.03
Mean burst duration (ms)	7.6 ± 0.36	7.4 ± 0.18	7.9 ± 0.20
Burstiness	0.25 ± 0.02	0.25 ± 0.03	0.24 ± 0.02

TABLE 6.

Spike Amplitude Attenuation for CA1 Cells Recorded on the Holder and Maze

Mean ± SEM	Diestrus	Proestrus	Estrus
Holder	0.93 ± 0.01 (n = 34)	0.93 ± 0.01 (n = 23)	0.94 ± 0.02 (n = 22)
Maze	0.98 ± 0.02 (n = 25)	0.95 ± 0.02 (n = 18)	0.97 ± 0.01 (n = 20)

estrous cycle. However, the small sample size precluded a formal analysis of these data.

When animals are running on the maze the hippocampus displays theta activity that is accompanied by increased inhibition and a reduction in the firing rate of the pyramidal cells (i.e., complex spike cells) (Ranck, 1973; Buzsáki, 1996). In contrast, when the animals are at rest, sharp wave activity is prominent, resulting in a reduction of inhibition and increased firing of the complex spike cells. As expected, in the current study theta cells (putative interneurons; Ranck, 1973) fired more when the animals ran on the maze compared with when they rested on the holder (mean firing rate theta cells: maze = 15.97 ± 2.19; holder = 9.58 ± 1.32; $P < 0.05$). Similarly, complex spike cells fired in bursts significantly more on the holder than on the maze (see Fig. 4). However, these effects did not interact with the cycle.

Stability of Hippocampal Firing Properties

Estrogen increases spine and synapse density on hippocampal CA1 pyramidal cells (Woolley et al., 1990; Woolley and McEwen, 1992; Woolley, 1998). These findings are important since dendritic spines are thought to be the postsynaptic sites of excitatory input to hippocampal pyramidal cells (Woolley, 1998). The degree to which these morphological changes affect the functional physiology of the cell was a major goal of the current study. To date there are no other studies linking changes in dendritic morphology across the estrous cycle to changes in hippocampal cell firing characteristics during behavior.

In the present study, most firing characteristics remained stable across the estrous cycle. These included basic measures of spatial tuning, as well as burst properties. This result may seem unexpected given the findings of estrogen effects on dendritic spine regulation through inhibitory and excitatory processes (e.g., Murphy et al., 1998; Rudick and Woolley, 2001; Segal and Murphy, 2001). However, it should be noted that most of those studies examined the effects of estrogen on structural changes rather than the functional physiology of the cell as a whole. Furthermore, in vitro studies do not allow for the possibility of indirect effects from extrahippocampal afferents. Many other brain regions are affected by changes in estrogen levels and may modulate hippocampal activity. These include the hypothalamus (e.g., Carrer and Aoki, 1982; Frankfurt et al., 1990), cortical areas such as the prefrontal cortex (e.g., Kolb and Stewart, 1991) and subcortical areas such as the medial septum diagonal band of Broca (MSDB), median raphe, and supramammillary area (e.g., Leranth et al., 2000; Lam and Leranth, 2003; Prange-Kiel et al., 2004). For instance, subcortical areas have been shown to play a role in the regulation of hippocampal synaptic plasticity. These findings are significant since these other areas can directly or indirectly regulate hippocampal plasticity (Leranth et al., 2000).

In addition, during a rat's natural 4- to 5-day estrous cycle, there are fluctuations in other blood hormone levels including gonadotropin-releasing hormone (GnRN), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (Butcher et al., 1974; Nelson, 2000). For example, during proestrus both estradiol and progesterone rise, corresponding to a peak in spine and synapse density followed by a rapid decrease during estrus (Woolley and McEwen, 1993). Treatment of animals with the progesterone antagonist RU 486 during proestrus blocks the drop in spine density observed during estrus (Woolley and McEwen, 1993). Progesterone plays a biphasic effect on spine density and is thought to enhance the effects of estrogen during proestrus (Woolley and McEwen, 1993). Consequently, in vitro studies may not allow for examination of the full array of hormone interactions and interactions between brain regions.

In vivo studies have in fact suggested stability in the basic physiology of hippocampal pyramidal cells. In awake rats, there was no effect of estrogen replacement on the Schaffer collateral–CA1 baseline input/output curves or on the paired pulse responses (Córdoba Montoya and Carrer, 1997). Similarly, no changes in the input/output curves have been observed in anesthetized naturally cycling rats (Warren et al., 1995; Good et al., 1999). Taken together, previous research and the current results indicate that despite morphological changes basic firing properties remain stable across the cycle.

Changes in Firing Rate Across the Estrous Cycle

In the present study, the only effect of cycle found was during proestrus, which was associated with decreased firing rate of CA1 complex spike cells on the maze. No differences were found in the "infield" firing rate of these cells or field size, indicating that the source of the reduction was in the spontaneous or "out-of-field"

TABLE 7.

Place Field Expansion for CA1 Cells

Mean ± SEM	Diestrus (n = 20)	Proestrus (n = 9)	Estrus (n = 17)
Field movement	2.04 ± 3.99	4.80 ± 3.35	4.26 ± 3.32
Field size	2.20 ± 4.05	4.78 ± 5.05	−3.00 ± 2.87

firing rate of the cells. In other words, the complex spike cells were more inhibited in proestrous animals. It should be noted that during proestrus there was also a tendency for an increase in firing of theta cells on the maze (Table 3). The fact that so few theta cells were recorded precludes any definitive conclusions and this issue should be addressed in future studies. This decreased firing of complex spike cells during proestrus was found only as animals ran on the maze (i.e., in theta) and was not seen when the animals were at rest on the holder.

Because the present experiment is the only one to examine hippocampal firing properties in animals both running on a maze and at rest, it is difficult to relate the findings to previous research. The only other study to examine awake rats was by Córdoba Montoya and Carrer (1997), who examined the effects of estrogen replacement on synaptic plasticity. Their animals were limited to movement in a small isolated chamber (20 × 22 cm) and the recordings were conducted only when the animal was still. Thus, their recording conditions were comparable to our holder situation. Similar to the current finding of stability in firing rate on the holder, they found no effect of estrogen replacement on input/output curves (Córdoba Montoya and Carrer, 1997).

The present results indicate a difference between recordings conducted on the holder and those conducted as animals traversed the maze. It is well known that the hippocampal EEG state is substantially different when the animal is still (i.e., resting) versus running on the maze (e.g., Buzsáki, 1996). The present results indicate an interaction between the estrous cycle and the behavioral task demands imposed on the animal (holder versus maze conditions). Similarly, when baseline recordings are conducted, there are no cycle effects on input/output curves. However, when the system is manipulated (i.e., high frequency stimulation) estrous cycle effects on hippocampal plasticity are found (seizure threshold: Terasawa and Timiras, 1968; LTP: Warren, et al., 1995; Cór-

TABLE 8.

Firing Properties of CA3-DG Cells

a. Place field characteristics of complex spike cells CA3-DG (mean ± SEM)

	Complex spike cells CA3-DG		
	Diestrus (n = 22)	Proestrus (n = 31)	Estrus (n = 28)
Firing rate (Hz)	0.91 ± 0.12	1.18 ± 0.11	0.88 ± 0.10
Specificity	0.71 ± 0.06	0.88 ± 0.07	0.90 ± 0.10
Infield firing rate (Hz)	3.74 ± 0.42	5.68 ± 0.59	5.30 ± 0.80
No. of fields	2.23 ± 0.32	1.58 ± 0.13	2.07 ± 0.30
Field size	56.09 ± 6.59	46.26 ± 4.44	54.39 ± 5.62

b. Firing rate of CA3-DG cells recorded on the holder (mean ± SEM)

Firing rate (Hz)	Diestrus	Proestrus	Estrus
Complex spikes	0.79 ± 0.12 (n = 27)	0.91 ± 0.12 (n = 36)	1.08 ± 0.21 (n = 17)
Theta cells	20.93 ± 11.41 (n = 3)	8.27 ± 4.15 (n = 3)	6.32 ± 2.41 (n = 4)

c. Burst properties for CA3-DG cells recorded on the holder and maze

Mean ± SEM			
Holder	Diestrus (n = 27)	Proestrus (n = 36)	Estrus (n = 17)
Avg. spikes in burst	2.36 ± 0.05	2.41 ± 0.05	2.42 ± 0.08
Mean burst duration (ms)	7.6 ± 0.32	7.8 ± 0.22	8.2 ± 0.48
Burstiness	0.32 ± 0.02	0.30 ± 0.03	0.32 ± 0.05
Maze	Diestrus (n = 18)	Proestrus (n = 28)	Estrus (n = 19)
Avg. spikes in burst	2.26 ± 0.03	2.32 ± 0.03	2.38 ± 0.07
Mean burst duration (ms)	7.7 ± 0.21	7.8 ± 0.20	8.3 ± 0.53
Burstiness	0.27 ± 0.02	0.32 ± 0.02	0.30 ± 0.04

d. Spike amplitude attenuation for CA3-DG cells recorded on holder and maze

Mean ± SEM	Diestrus	Proestrus	Estrus
Holder	0.90 ± 0.02 (n = 23)	0.92 ± 0.01 (n = 31)	0.94 ± 0.01 (n = 13)
Maze	0.96 ± 0.01 (n = 16)	0.91 ± 0.01 (n = 25)	0.95 ± 0.02 (n = 17)

doba Montoya and Carrer, 1997; Good et al., 1999). The results of the present study indicate that these previous findings of changes in excitability and plasticity do not produce a robust difference in baseline firing properties, during a well-trained task in a familiar environment. Similar to the LTP results, it is possible that these types of plasticity effects would be manifested when the animal is first exposed to a new learning task.

Possible Confounds

A potential confound in this study was the possibility of estrous cycle related differences in activity levels (e.g., Wade, 1972; Tropp and Markus, 2001a). The possibility of a change in activity needed to be addressed since animal velocity can affect place cell firing characteristics (McNaughton et al., 1983). To prevent this possible confound, the animals were over-trained on the task before recordings began. This overtraining resulted in a consistent rate of running as indicated by the fact that there were no estrous cycle differences in latency to run the maze.

The current task was not hippocampus dependent. Many single-unit experiments have used non-hippocampus-dependent tasks, such as foraging for food in a cylinder, to examine place field characteristics (e.g., Ranck, 1973; Muller and Kubie, 1987). Notably, even on non-hippocampal tasks, hippocampal place cells are shown to re-map (e.g., Markus et al., 1995) and show correlates to non-hippocampal learning such as delayed-eye blink conditioning (e.g., Berger and Thompson, 1978). Even a direct comparison of a non-hippocampal task (forced-choice task) to a hippocampal task (spatial memory task) found no differences in measures of reliability and specificity in CA1 cells of young rats (Mizumori et al., 1996: see Fig. 4; however, note the aging effects). Taken together, these findings suggest that hippocampal cells reflect the ongoing behavior of the animal regardless of whether the behavior is dependent on the hippocampus.

Relationship Between Anatomy and Physiology

One of the important results of the current study is the lack of a direct relationship between changes at a molecular level and function of the system. This lack of correlation between levels has been found previously. For example, during aging, the hippocampus undergoes substantial anatomical and physiological changes (e.g., Barnes and McNaughton, 1985; Geinisman et al., 1995). However, only minimal differences are found in the basic firing properties of hippocampal place cells (e.g., Markus et al., 1995; Barnes, 1998; Oler and Markus, 2000; however see, Mizumori et al., 1996). There were no age differences of place field measures of reliability, specificity, or selectivity found (Oler and Markus, 2000). Thus, changes at the anatomical level may not be directly reflected at the single-unit level of examination.

Estrogen Effects on Hippocampal Function

One might predict that an increase in spines and plasticity would enhance hippocampal function. In fact in ovariectomized animals, estrogen replacement substantially improves spatial ability. For instance, ovariectomized rats with estrogen replacement show better hippocampal performance on radial maze tasks than rats without estrogen replacement (e.g., Daniel et al., 1997; Luine et al., 1998; Fader et al., 1999; Korol and Kolo, 2002). This estrogen enhancement has also been shown for the water maze (e.g., Packard and Teather, 1997). However, when examining naturally cycling animals the behavioral studies have not been consistent. Proestrous rats (high estrogen levels) have shown impairment on hippocampal tasks, such as contextual fear conditioning (Markus and Zecevic, 1997), the water maze (Frye, 1995; Warren and Juraska, 1997; however, see Berry et al., 1997), and object exploration (Tropp and Markus, 2001a). Conversely, in the radial arm maze, high levels of estrogen do not seem to be detrimental for performance (Stackman et al., 1997) and may change the way animals navigate (Korol et al., 2004). These data indicate that changes in anatomy and LTP are not directly expressed as increased hippocampal function. Rather the relationship is complex and is dependent upon task demands and the involvement of other brain areas (Desmond and Levy, 1997; Markus and Zecevic, 1997; Tropp and Markus, 2001a).

In summary, the current study indicated that the molecular changes that occur over the estrous cycle are not directly reflected at the single-unit level. The results suggest that the degree of effect may correspond to the behavioral demands put on the hippocampus. Moreover, as the behavioral findings indicate, the requirements of a task may interact with the hippocampus in different ways.

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