Medial Prefrontal Cortex Cells Show Dynamic Modulation With the Hippocampal Theta Rhythm Dependent on Behavior

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ABSTRACT: Both the hippocampus and the medial prefrontal cortex are essential for successful performance in learning- and memory-related tasks. Within the hippocampus the theta rhythm plays an integral role in the timing of action potentials of hippocampal neurons responding to elements of any given task. Medial prefrontal cortex (mPFC) neurons display firing rate changes to specific facets of behavioral tasks (Jung et al., 1998. Cereb Cortex 8:437–450). We recorded units in the mPFC and field potentials in the hippocampus to determine whether behaviorally correlated mPFC cells fired with phase relationships to the hippocampal theta rhythm. In two different behavioral tasks (running a linear track and foraging in two distinct environments) we found mPFC cells that alternated between theta entrained firing and nonphasic firing depending on the ongoing behavior, while other cells were modulated during all conditions in both tasks. The majority of the mPFC cells with a significant correlation of firing rate changes with behavior were entrained to hippocampal theta. Cells that fired to specific events during only one direction of running were predisposed to theta modulation only in that direction. mPFC neurons have the capability to respond to behaviorally relevant elements by dynamically alternating between hippocampal theta entrained and nonphasic firing. © 2005 Wiley-Liss, Inc.

KEY WORDS: medial prefrontal cortex; hippocampus; theta; phase; memory; modulation

INTRODUCTION

The role of the hippocampus during behavior has been thoroughly researched and great progress has been made into unraveling how the hippocampus plays its central role in memory tasks (Eichenbaum, 2004). The prefrontal cortex has been shown to be involved in a range of complex tasks in primates and rodents, including rule learning, decision making, working memory, and other high-level cognitive tasks (Miller et al., 2002). In many imaging studies, both the hippocampus and prefrontal cortex are selectively activated during memory demanding tasks (Brewer et al., 1998; Stern et al., 2001; Schon et al., 2004; Karlsgodt et al., 2005). Without question these two areas are inherently involved in the completion of most memory tasks; however, very little is known about how these two areas work together during active processing tasks despite the large anatomical distance.

Rodent research has begun to develop the argument that the rat medial prefrontal cortex is homologous to the monkey and human dorsolateral prefrontal cortex (Groenewegen, 1988; Brown and Bowman, 2002; Uylings et al., 2003). Lesions of the medial prefrontal cortex (mPFC) in rat impair performance on goal- and delay-based tasks, such as the Morris-water maze (de Bruin et al., 1994), radial-arm maze (Kesner, 1989), spatial alternation (Grannon et al., 1994), and cause difficulties accommodating new starting positions in a modified water maze (Grannon and Poucet, 1995). Floresco et al. (1997) used temporary inactivations to selectively impair subicular outputs to the medial prefrontal cortex during radial-arm maze performance and found deficits during delayed tasks. Anatomical connections of the rat medial prefrontal cortex are extremely similar to those found in primate dorsolateral prefrontal cortex, including dense reciprocal connections with major sensory association cortices, the striatum, the hippocampus, and dorsomedial thalamic nuclei (which contain projections from the rhinal cortex, hippocampus, and subcortical structures) (Groenewegen and Uylings, 2000; McKenna and Vertes, 2004). Prefrontal cortex single-unit studies in rats (Schoenbaum and Eichenbaum, 1995; Jung et al., 1998; Baeg et al., 2003; Mulder et al., 2003; Hok et al., 2005) during goal-related behaviors found behavioral correlates for almost every aspect of the animal's behavior during a specific task including directional specificity, turn specificity, trial onset and offset. Cells also showed firing changes depending on the location of goals, the location where searching for reinforcements took place, and during delay periods.

Hippocampal principal cells, recorded in awake animals, fire with fixed relationships to the theta oscillation in the hippocampal field potential (Fox et al., 1986; O'Keefe and Recce, 1993; Skaggs et al., 1996; Csisvari, 1999). Neurons recorded outside the hippocampus are phasically modulated by the hippocampal theta rhythm, including neurons in the entorhinal cortex (EC) (Dickson et al., 1995), the striatum (Berke et al., 2004), the cingulate cortex (Colom et al., 1988), the amygdala (Pare and Gaudreau, 1996), and

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the nucleus accumbens (Tabuchi et al., 2000). A recently published report (Siapas et al., 2005) showed medial prefrontal cortex neurons can and do fire with specific phase relationships to the hippocampal theta rhythm while the animal performed various behavioral tasks.

Given the propensity of mPFC neurons to encode goal information and various other behavioral aspects of tasks, along with the capability of mPFC neurons to fire in phase with the ongoing hippocampal theta rhythm, mPFC neurons that are behaviorally correlated should be more likely to be entrained to the hippocampal theta rhythm. To examine this possibility, we recorded single units from the medial prefrontal cortex of rats and field potentials in the hippocampus as the animals performed two simple behavioral tasks. In one task, the animal would forage for a set of reinforcements in two separate open environments, and the other task involved running on a linear track. In our data, we found cells in both tasks that would alternate dynamically between hippocampal theta-entrained firing and nonphasic firing. Like previous studies, we found a number of prefrontal cortex cells had responded to clear behavioral correlates in the linear track task and hippocampal theta phase analysis revealed a majority of these cells fired with a significant phase relationship. Furthermore, of the cells with clear unidirectional behavioral correlates and unidirectionally significant theta phase modulation, the vast majority were phase modulated during the same directional run as their behavioral correlate.

METHODS

Subjects

Four adult male Long-Evans rats, obtained from Charles River Laboratories, Inc. (Wilmington, MA), were used. The animals were individually housed and kept in a 12 h light–dark cycle with water available ad libitum. During training and after recovery from surgery, animals were maintained at 90% free feeding weight.

Surgery

Animals were deeply anesthetized under halothane gas and pretreated with atropine (0.1 mg/kg) to prevent excessive salivation. Rats were placed in a David Kopf (Tujunga, CA) stereotaxic frame, and holes were drilled into the skull using a David Kopf stereotaxic drill. Three tetrode bundles (25 μm formvar coated tungsten wire) in a moveable drive mechanism were implanted into medial prefrontal cortex (3.4 mm AP; 0.5 mm ML). Four bundled single electrodes (40 μm coated tungsten wire) in a moveable drive mechanism were implanted into the hippocampus (−3.6 mm AP; 2.5 ML). The tips of these wires were at vertical intervals of about 330 μm each. The two drivers were mounted to skull screws using dental acrylic and then shielded in plastic sheeting. The recording wires were connected to a 20 pin Microtech™ plug, which was affixed to the

plastic shielding. After surgery, animals were given nonsteroidal oral antibiotics and analgesics as needed. Animals were given at least 1 week of recovery before recordings.

Electrophysiology

The tungsten recording wires passed the signal through pre-amplifier FETs (NB Labs, Denison, TX) and an amplifier (Neuralynx Inc., Tucson, AZ) before being passed to a Data Translation (Marlboro, MA) A/D board for storage by Sciworks software (from DataWave Technologies, Boulder, CO) running on a PC workstation. Unit recordings were filtered between 600 and 60 kHz and field channels were filtered between 1 and 475 Hz. After recovery from surgery, hippocampal wires were slowly lowered until hippocampal ripples were present. Then the wires were slowly lowered further until the theta rhythm across the four channels showed a clear 180° phase reversal indicative of wires centered around the hippocampal fissure (Buzsaki, 1986). Multiunit files were sorted with cluster-cutting techniques, using Offline Sorter (Plexon Inc., Dallas, TX). If clusters were impossible to disambiguate into individual cells, the data were not included in any further analysis. LED's were mounted to the headstage cable and position data were recorded for offline analysis, using Sciworks software (from DataWave Technologies, Boulder, CO).

Behavior

Linear track

The linear track was a 70″ × 3″ elevated platform. Animals had to run from one goal location (at one end of the platform) to another goal location (at the opposite end of the platform) to receive reinforcements. After the animal had arrived at one goal location and began to consume reinforcement, the other goal location was baited.

Foraging tasks

Animals were run in two environments (environment 1 was a 29″ × 25″ elevated platform; environment 2 was a 65″ × 7″ elevated platform) for 20–30 min on the same day. The order of environments was counterbalanced across days. Reinforcements were small pieces of Froot Loops (Battle Creek, MI) that were placed throughout the environments (about 10–15 pieces). After the animal had recovered all the reinforcements, a new set was placed in the environment. This process was repeated for the entire session.

Data Analysis

Position data

Recorded position data was transferred to Neuroexplorer (Plexon Inc., Dallas, TX), where the data were smoothed to correct false position markings. The data from linear track recordings were further analyzed to select trips from goal A to goal B and separately select trips from B to A. Every animal's

behavior in the task was extremely consistent, in that the animal generally turned from one goal to the next in the same direction, immediately after consuming the reward. Because of this, we were able to separate out A–B trials as starting at the point the animal turned his head after eating a reinforcement to begin the journey to goal B and ending after the reinforcement consumption at B. The same held true for B–A trials. All spatial analyses of unit activity in both tasks were done using Neuroexplorer.

Theta Analysis and Phase Estimations

Spike times from individual units and raw local field potential data were read into Matlab (Mathworks, Natick, MA) for further analysis. Theta periods were identified by first filtering the EEG record between 3 and 10 Hz using a Butterworth filter. Local minima and maxima were identified in the EEG. A theta period was selected as a period of three consecutive trough/peak pairs occurring with amplitude greater than negative/positive thresholds (which were set by eye for each session), within the theta frequency. Phases relative to the theta oscillation were estimated linearly between zero crossings in the EEG (e.g., the time-point halfway between two zero crossings was considered 180°). Phase information for individual units in the linear track task was analyzed for three separate periods: (1) runs from goal A to goal B; (2) runs from goal B to goal A; and (3) overall or entire session.

A cell was considered modulated if it showed a unimodal distribution of its spikes' phases relative to hippocampal theta rhythm. Nonuniformity was tested by using the Rayleigh's statistic, $Z = nR^2$, where n equals the number of spikes and R is the mean resultant length (the magnitude of the vector that results when each spike is represented as a vector on the unit circle whose angle relative to some fixed point is given by the spike's phase and the vectors are all summed together). Stephens (1969) showed that when $n > 25$, Z is distributed approximately as the chi-squared distribution with 2 degrees of freedom, thus a cell was considered modulated if its Z was greater than 6 ($P < 0.05$).

Unit Classification for Behavioral Correlates

Cells recorded in the linear track were analyzed for behaviorally correlated changes in firing rate during the performance of the task. After positional analysis was completed, allowing the determination of the beginning of each run from one goal to the next, perievent histograms were constructed around these timestamps. To determine significance of directionally specific firing rate changes, single factor ANOVA's were performed comparing both of the directional runs on the linear track. Firing rates were accrued in 250 ms bins and these bins were compared for 3-s blocks surrounding the beginning of one trial and the end of the previous trial. The point at which a trial begins is time N. Since all animals completed the task in a continuous fashion, time N (zero in Fig. 1), reflected the end of one trial and the beginning of the subsequent trial. Because of the relative consistency of each animal's behavior in this task,
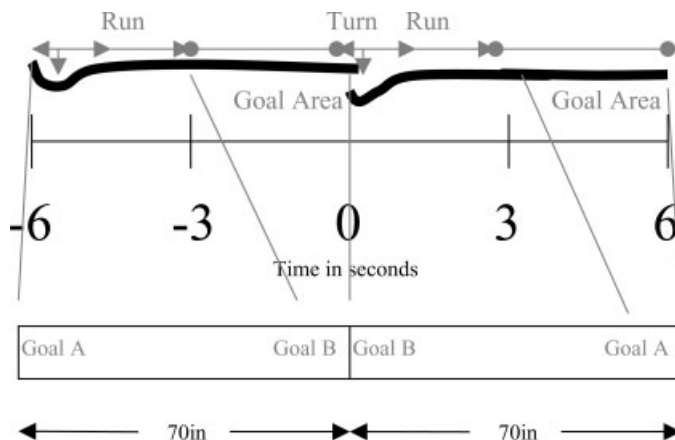

FIGURE 1. Diagram of the time-course of events during running of the linear track task. The top black lines are the averaged position data from a typical session for A–B and B–A runs. The positions are relative to the time spent (middle) in that location as seen in the X-axis of the plot for $N = -6$ s to $N = +6$ s. The gray arrows above the animal's path show the direction of movement, and the circles mark start and end of time spent without movement at the reinforcement area. Since the animals were involved in a relatively continuous behavior, it was necessary to assign trial start and stop times based on the time the animal lifted its head after consuming reinforcement and turned to begin the trip to the opposite goal.

we were able to classify cell types by comparisons of two consecutive 3-s blocks before trial start and two consecutive 3-s blocks after trial start. The 3-s blocks before trial start reflect behavior for the opposite direction from the 3-s blocks after time N (see Fig. 1 for relationship between animal position and time).

Behavioral correlations were categorized as follows: cells that exhibited significant firing rate changes for the period $N - 6$ s to $N - 3$ s were classified as responding to reinforcement approach and consumption (goal area). Cells that had significant firing rate changes for the period $N - 3$ s to time N were classified as responding to the either reinforcement consumption (goal area) or the turn that began a new trial (turn cells). Turn cells reflect firing while the animal lifted its head after reinforcement and turned to begin the trip to the next goal location. The distinction between goal area and turn-cell classification was determined by visual analysis of the perievent histograms and perievent rasters and in all cases dissociation between these two classifications was readily visually apparent (see Fig. 2 for representative examples). In some cases this significant firing rate change persisted for the first couple of seconds after time N, and in these cases significant firing rate changes in the opposite direction were required for turn-cell classification. To further distinguish turn cells from goal cells, visual analysis of the perievent histograms and perievent rasters were performed while taking the animals' average trial time into consideration. Cells with significant firing rate changes for the period N to $N + 3$ s and/or $N + 3$ s to $N + 6$ s were classified as run cells.

In a small number of sessions in which the animal displayed slower trial times, the periods of $N - 9$ s to $N - 6$ s and $N +$
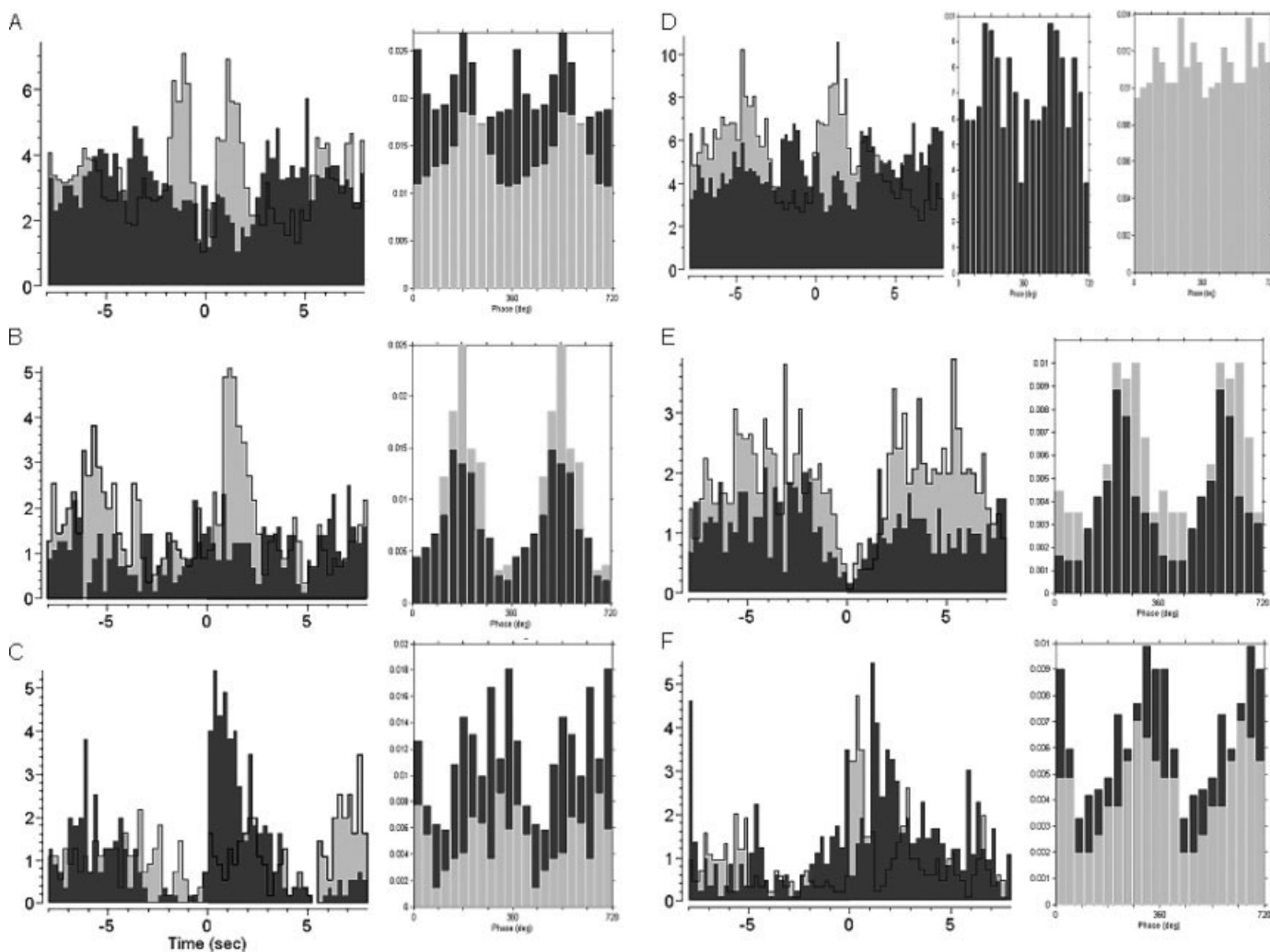
FIGURE 2.   Examples of medial prefrontal cells recorded during performance of the linear track task. In all plots: dark gray = goal A to goal B runs, light gray = goal B to goal A runs; all plots show the end of one directional trial for times before 0 and the start of a run in the same direction after time 0; on the right are phase histograms for all corresponding trips in the given direction. In all plots the Y-axis for phase histograms are spikes per theta cycle, X-axis degrees of the theta wave; the Y-axis in all rate histograms is firing rate in Hz. A: this cell responded in goal area B and during the B–A run. This cell is significantly hippocampal theta modulated only during B–A trials and fires nonphasically during A–B runs (A–B $Z = 3.12$; $P = 0.21$; B–A $Z = 20.32$; $P = 3.86E-05$). Thus it is only entrained during the period of lower firing rates. This cell exhibits large firing rate increases while the animal is consuming reward in goal area B, then is inhibited while the animal turns to begin the trip from B–A, and has another strong firing rate increase as the animal runs from goal B to goal. B: this cell responded during the B–A run. It is significantly modulated in both directions (A–B $Z = 46.37$; $P = 1.36E-10$; B–A $Z = 114.76$; $P = 5.93E-11$). Notice, how the firing rate increase does not appear until a couple seconds after the animal has begun to run from goal B to goal A. C: this is a A–B turn cell that is significantly modulated in both directions (A–B $Z = 24.35$; $P = 5.14E-06$; B–A $Z = 21.37$; $P = 2.28E-05$). The firing rate increase is no longer significantly different from B–A trips when the animal is running to the goal, and is inhibited while the animal is at both goals. D: this cell responds in a single goal area (B) and during the opposite direction (A–B) turn. This cell responds at goal B (as can be seen in the dark gray plot in the perievent histogram before

time 0) and then there is another significant firing rate increase as the animal turns and leaves goal B heading back to goal A. This increase persists throughout the run to goal A (seen in the light gray plot in the perievent histogram for the period after time 0). This cell was significantly modulated only during runs from goal A to goal B (which includes time spent at goal B), as shown in the dark gray phase histogram ($Z = 13.31$; $P = 0.0,013$) and was not modulated during runs from B–A ($Z = 0.64$; $P = 0.73$). Even though this cell responded during both directional trips (while in goal B and when running from goal B), only the trip from A–B is significantly modulated, which could be indicative of a dual coding process by mPFC neurons (separate coding by both firing rate and hippocampal theta modulation). E: this cell is a B–A run and goal area cell that is significantly modulated for runs in both directions (A–B $Z = 59.28$; $P = 3.48E-11$; B–A $Z = 27.6$; $P = 1.01E-06$). In the perievent histogram an increase is seen in the light gray trace before and after the animal turned from goal B to goal A (around time 0). This cell was inhibited during turns in both directions. F: this cell is a B–A turn and A–B run cell that was significantly modulated in both directions (A–B $Z = 20.85$; $P = 2.97E-05$; B–A $Z = 26.03$; $P = 2.22E-06$). In the perievent histogram the light gray trace shows an increase in firing rate just before and right at time 0, and in the dark gray plot the increase is only seen a second after time 0. This cell responded as the animal ran from goal A to goal B and then showed a decreased firing rate as the animal approached goal B and consumed the reinforcement. When the animal lifted his head to turn and begin the run from goal B back to goal A the cell responded again and was inhibited during the run from B–A.

6 s to $N + 9$ s were analyzed for dissociation of run and reinforcement correlates. In a subset of these slow running time sessions, the animal consistently delayed at the end of each arm of the maze after consuming reinforcement before beginning the next trip. For these sessions, the period during which the animal lifted its head after reinforcement consumption was used as the end of that trial, and the time when the animal began locomoting to the other goal location was the start of the next trial.

Cells that were correlated to one direction run or another were readily classified into ten categories: (1) single direction whole trip (significant firing rate changes for the turn, run, and reinforcement periods in one direction); (2) single direction run (significant firing rate changes for N to $N + 3$ s, and/or significant firing rate changes for $N + 3$ s to $N + 6$ s in one direction); (3) single direction turn (significant firing rate changes during $N - 3$ s to N in one direction, and in some cases significant firing rate changes in the other direction for N to $N + 3$ s); (4) single direction turn and run (significant firing rate changes for $N - 3$ s to N/N to $N + 3$ s (turn) and N to $N + 3$ s/6 s (run) in one direction); (5) single direction turn and

opposite direction run (significant firing rate changes for $N - 3$ s to N/N to $N + 3$ s (turn) and N to $N + 3$ s/6 s (run) in the opposite direction, note for this cell category that N refers to two separate ends of the track); (6) single direction turn and goal area (significant firing rate changes in one direction during $N - 6$ s to $N - 3$ s or $N - 3$ s to N (goal area) and significant firing rate changes during N to $N + 3$ s (turn) in the same direction); (7) single direction turn and opposite goal area (sig-
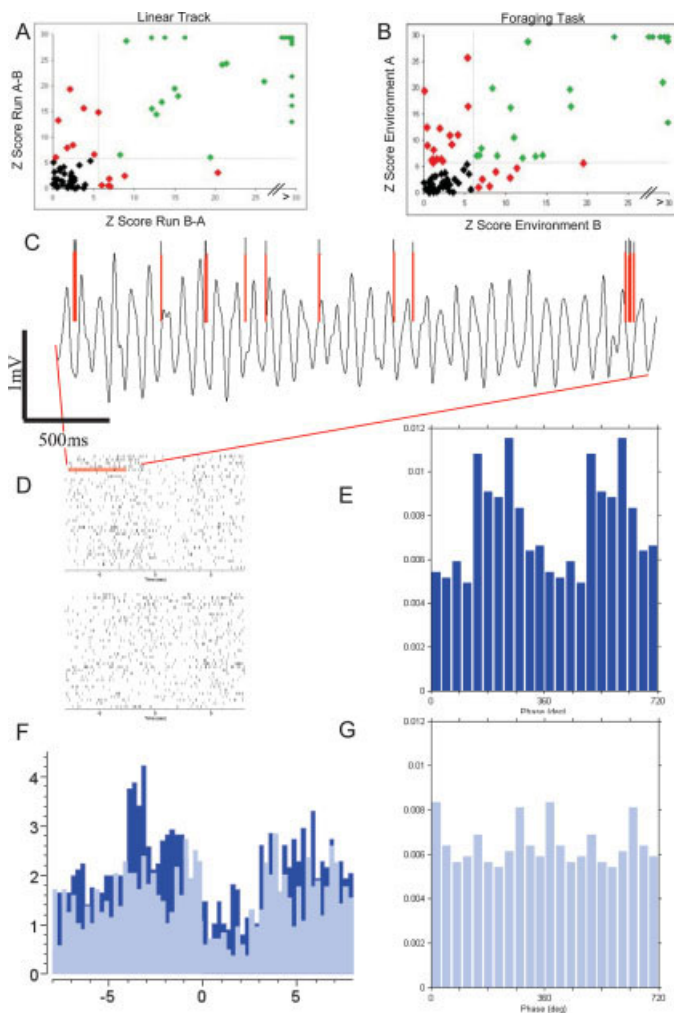

FIGURE 3. Rayleigh's statistic for each condition of both tasks and representative example of unidirectional entrained and behaviorally correlated unit. A, the *X*-axis is Rayleigh's statistic for A–B runs, and the *Y*-axis is Rayleigh's for B–A runs. The dotted lines are at $Z \geq 6$ (the critical value for *Z*; $P < 0.05$). The red data points are cells that were unidirectionally theta entrained; the green data points were entrained during runs in both directions; and black data points were not significant for either run. B, the *X*-axis is Rayleigh's for foraging sessions in environment A, and the *Y*-axis is Rayleigh's for foraging sessions in environment B. The dotted lines are Z critical ($Z \geq 6$; $P < 0.05$). In red are data points that were significant in only one environment, green are data points significant in both environments, and in black are data points that were not significant in either environment. In both plots large Rayleigh's scores ($Z \geq 30$) are plotted on the edge of the graph to improve visual presentation. C–F, a representative example of a goal area and opposite turn cell that responded for B–A trips only and was phasically modulated for B–A trips only. In C is a trace of hippocampal theta rhythm and on top of that are the spikes for this prefrontal unit. The red markers show a clear consistent phase relationship for this unit to the theta rhythm in the hippocampus (this cell fired on the trough during B–A runs). The trace in C is taken from the highlighted area in the top perievent rasterplot shown in D, which occurred during the animal's approach to the reinforcement in that trial. The top plot in D is centered on the beginning of A–B runs as time 0, and the bottom plot is centered on the beginning of B–A runs. Goal area B firing changes can be seen in the top plot before time 0 and in the bottom plot at 6–9s after time 0. The increase in firing in the bottom plot just before and at time 0 represents firing during the animals turn from goal B beginning the trip toward goal A. This cell was also inhibited during both runs. These rasterplots show a consistent increase in spikes while the animal was at goal area B and when the animal left goal area A to head to goal area B. E and G are the two phase histograms for all trips in each different direction in the entire session. In dark blue (E) are A–B trips and in light blue (G) are B–A trips (*Y*-axis is spikes per theta cycle; *X*-axis is degrees of the theta cycle). The bimodal shape of the dark blue plot shows a clear phase relationship with hippocampal theta rhythm ($Z = 19.41$, $P = 6.11E-5$), while the flat plot in light blue shows no such relationship ($Z = 2.09$, $P = 0.35$). In F are the two perievent histograms plotted on the same axis (*X*-axis: time in seconds, centered on trial start; *y*-axis: firing rate). In dark blue are A–B trips and in light blue are B–A trips. For the dark blue plot, datapoints before time 0 are from the end of all A–B trials and points after time 0 are from the start of all A–B trials (the same is true for the light blue plot and B–A trials). The increase sseen before time 0, in the dark blue dataset, corresponds with a firing rate increase in goal area B. This increase is statistically significant for both the $N - 6$s to $N - 3$s block and the $N - 3$s to N block, though as the plot shows during B–A trips the cell was inhibited for the turn from goal B heading toward goal A. In the light blue plot the peak at times $N - 2$ to N represent firing as the animal turned from goal A and headed toward goal B.

nificant firing rate changes in one direction during $N - 3$ s to N (goal area) and significant firing rate changes during N to $N + 3$ s (turn) for trips in the opposite direction, note for this cell category that N refers to two separate ends of the track); (8) single direction run and goal area (significant firing rate changes for N to $N + 3$ s and for $N + 3$ s to $N + 6$ s in one direction); (9) single goal area and run in opposite direction (significant firing rates changes during $N - 6$ s to $N - 3$ s in one direction and corresponding firing rate change during $N + 3$ s to $N + 6$ s in the same direction, note for this cell category that N refers to two separate ends of the track); and (10) single direction reinforcement or goal area (significant firing rate changes during $N + 3$ s to $N + 6$ s in one direction).

To classify cells that responded to common behavioral events in both directions, single factor ANOVA's were performed on firing rate changes in sequential time blocks (the same time blocks were used as in single direction classification). Common event cells (both directions) were classified into three categories: (1) turns in both directions; (2) runs in both directions; and (3) goal area firing in both directions.

Histology

After each animal had completed the experiment, they were deeply anesthetized under halothane gas and were electrolytically lesioned at each electrode location. They were then perfused with a solution containing 25 parts 10% buffered formalin, 1 part glacial acetic acid, and 10 g of potassium ferrocyanide. This solution causes a Prussian blue reaction, which marks the location of the iron particles deposited by the electrode lesion in blue. The brains were then removed and stored in a 10% buffered formalin solution for about 1–2 weeks. After this time, the brains were then sliced, mounted, and stained with neutral red to determine precise electrode locations. In all cases, hippocampal implant tracks went into CA1 and terminated near the hippocampal fissure. Since multiple sessions were recorded from individual animals, the precise recording locations could not be derived from the prefrontal cortex tetrodes, but all electrode tracks were in the medial prefrontal cortex. Using records of driver lowerings we were able to confirm that most recordings reported here were made in prelimbic areas.

RESULTS

Linear Track

Phase modulation

A total of 74 clearly isolated units from four animals were recorded while the animals performed the linear track task. All animals performed very well on the task with an average trial time of (6.48 s) and showed very little variability in trial time. Of the 74 cells recorded during this task, 41 cells showed statistically significant phasic modulation ($P < 0.05$) to the hippocampal theta rhythm in at least one aspect of the task (A–B

runs, B–A runs, or overall)(see Fig. 3A for Rayleigh's scores of the entire population). Only one cell recorded in this task was a fast spiking cell (>10 Hz firing rate) and this cell had neither a behavioral correlate nor a significant phase relationship. In this task, the majority of the phase-modulated units were modulated in both directions or for the entire session, as determined by a significant Rayleigh's statistic for both A–B runs, B–A runs, and overall ($n = 24$) or for only overall ($n = 2$). Fourteen cells showed significant phase modulation to hippocampal theta rhythm in only one direction, which was determined by a significant Rayleigh's statistic in one direction alone ($n = 7$) or a significant Rayleigh's statistic in one direction and a significant Rayleigh's statistic overall ($n = 7$) (for an example see Fig. 3).

Behavioral correlates

Fifty five of the cells recorded in the task showed significant firing rate changes correlated with specific behavioral periods (see Table 1). Fourteen of these cells were classified as responding to common events in both directions. Forty one cells were classified as responding to events in only a single direction (examples of phase modulated single direction event cells are shown in Figs. 2 and 3).

Many of the cells recorded would go through periods of inhibition that generally preceded or followed the behavioral event that correlated with increased firing rate (examples can be seen in Fig. 2E–F). These periods of inhibition can potentially convey as much information as the firing rate increases and in previous reports both excitatory and inhibitory changes have been used to classify medial prefrontal units' behavioral correlates (Jung et al., 1998; Mulder et al., 2003). Since the overwhelming majority of the cells recorded in this task that exhibited periods of inhibition also had period of significantly heightened firing rates, we classified these cells based on the periods of firing rate increases, because periods of inhibition make the analysis of theta entrainment difficult. Cells that only showed clear periods of inhibition to a behavioral correlate were classified for that specific correlate.

Phase modulation of behaviorally correlated units

Of the 55 cells which had clear statistically significant behavioral correlates, 31 were significantly phasically modulated by the hippocampal theta rhythm either in both directions or in a single direction or for the entire session ($P < 0.05$). Of the 15 cells that were classified as behaviorally correlated with common events (both directions), six were entrained to the hippocampal theta rhythm (all in both directions).

Of the 40 cells classified as responding to behavioral events on runs in only one direction, 25 were entrained to the hippocampal theta rhythm. Fifteen were entrained in both directions and overall, and nine were entrained in one direction (four in one direction and overall, and five in only one direction). For the cells that were modulated in only direction and showed a clear behavioral correlate for event/s in one direction, eight out

Number of Cells Classified in Each Category of Behavioral Correlation and Significant Hippocampal Theta Rhythm Phase Modulation for Cells Recorded During Both the Linear Track and Muli-Environment Foraging Task

	Total no. of cells	No. of Hippocampal theta modulated cells in both directions/ environments	No. of Hippocampal theta modulated cells in a single direction/ environment
Cells recorded in alleyway task	74	27	14
Cells with significant behavioral correlates	55	21	9
Turns in both directions	10	5	0
Runs in both directions	2	0	0
Goal area firing in both directions	3	1	0
Single direction entire trip	5	2	0
Single direction run	8	1	3
Single direction turn	6	2	0
Single direction turn and run	1	1	0
Single direction turn and opposite direction run	1	1	0
Single direction turn and goal area	2	1	1
Single turn and opposite direction goal area	2	0	1
Single direction run and goal area	6	2	3
Single direction run and opposite goal area	1	1	0
Single goal area	8	3	1
Cells recorded in foraging task	81	23	21

of 9 were phase modulated for the direction of that behavioral correlate (this is discussed further below). An example of one cell that was correlated to approach and consumption at a single goal area is shown in Figure 3. This unit was phasically modulated only during the directional runs in which it showed a clear behavioral correlate.

Each of the four animals had mPFC cells that were only phase modulated during one run (rat 1:$n = 3$; rat 2:$n = 3$; rat 3:$n = 1$; and rat 4:$n = 7$). Every animal that had more than one single-direction modulated cell (all except rat 3) had cells that were phase-locked to different single directions. Altogether, 14 unidirectional theta-entrained neurons were recorded, 8 were entrained only during A–B runs and 6 were entrained only during B–A runs (see Fig. 3). For example, in one session three such cells were recorded from rat 4. Cell 1 was significantly theta-phase-locked during runs from goal A to goal B ($Z = 8.4$; $P = 0.014$; mean phase angle = 195°) and not during B–A runs ($Z = 2.46$; $P = 0.29$); cells 2 and 3, recorded from that same tetrode in the same session were not modulated during A–B runs (2. $Z = 0.64$; $P = 0.73$; 3. $Z = 0.51$; $P = 0.77$ and were theta phase locked during B–A runs (2. $Z = 5.99$; $P = 0.049$); (mean phase angle = 3.38°) 3. $Z = 6.8$; $P = 0.032$; mean phase angle = 334°). Of these three neurons, two were found to have significant behavioral correlates. Cell 1 was classifed as a A–B turn and run cell, and this cell's firing was theta phase locked during these A–B runs; cell 2 had no significant behavioral correlate using our criteria; and cell 3 fired at significantly elevated rates during runs from goal B to goal A (B–A run cell) and this cell was only phase-locked to theta during B–A trips.

Testing of theta phase modulation for cells with unidirectional behavioral correlates

Cells that had significant behavioral correlates in a single direction and were theta entrained in only one direction were grouped by the direction of behavioral correlate. Then a single factor ANOVA was performed on all the cells' Rayleigh's statistics comparing runs in the behaviorally correlated direction vs. the noncorrelated direction. The results of this analysis ($F = 7.25$; $P < 0.05$) show a predisposition for unidirectional theta entrained cells with behavioral correlates to have the entrainment and behaviorally modulated firing rate changes during runs in the same direction (8/9 cells showed this).

Testing of behavioral correlations for cells with significant theta phase modulation

A total of 41 cells recorded during linear track running were entrained to hippocampal theta rhythm either for both directional runs or in only one direction. Of these entrained cells, 76% (31/41) had significant behavioral correlates during the running of the task. Of the cells recorded, a clear majority of entrained units were also behaviorally correlated, showing a predilection for theta-entrained mPFC units to respond to behavioral events.

Distribution of mean phase angles for adjacent neurons

The mean phase angles were analyzed for all significantly entrained neurons recorded with the same hippocampal elec-
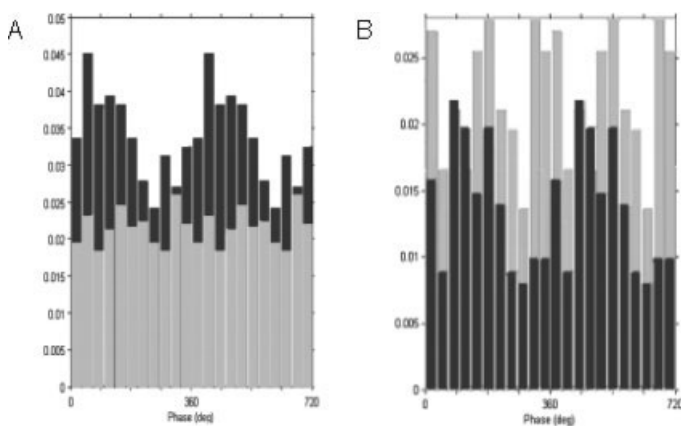
FIGURE 4. Representative phase histograms from two mPFC cells recorded during foraging in both environments. For each cell, both sessions were recorded on the same day consecutively. A, the dark gray plot represents the phase histogram for this cell during the foraging task in environment 1, and the light gray plot is the histogram for environment 2. The phasic shape of the distribution from environment 1 shows entrainment to hippocampal theta rhythm ($Z = 19.37$, $P = 6.21E-005$), while the distribution from environment 2 is flat ($Z = 0.075$, $P = 0.956$). B, the dark gray distribution is from a foraging session in environment 1 and has the phasic shape of a modulated histogram ($Z = 9.65$, $P = 0.008$). The light gray distribution is from a session in environment 2 and has no significant phasic properties in the distribution ($Z = 0.68$, $P = 0.738$). In this example this cell had a reduced firing rate in the environment in which it was phasically modulated to the hippocampal theta rhythm.

trode position. Medial prefrontal units recorded from the same tetrode often had unique phase preferences and overall the variance of mean phase for modulated cells from the same hippocampal recording position was greater than a single 180° phase (mean range = 260°). In the example cited above from a session with rat 3, the 3 cells were all phase-locked to different points of the recorded hippocampal theta wave. This example is indicative of the relationships of the mean phase angles from neurons recorded with the same hippocampal recording position. Further overall phase dependence analysis was not possible for the current data set, because of a lack of recording data of the true hippocampal theta phase (usually obtained from multiple recording sites surrounding the hippocampal fissure or inferred from populations of hippocampal pyramidal cells (Skaggs et al., 1996)).

Foraging Task

A total of 81 cells were recorded from four animals performing the foraging task. Two of the 81 cells recorded in this task could be classified as fast spiking cells (>10 Hz firing rate) and both were significantly phase modulated in both environments. Of all the recorded and separable cells, 44 fired with a statistically significant ($P < 0.05$) phase relationship to the hippocampal theta rhythm. Of these cells, 23 were phase modulated in both environments when run consecutively on the same day.

Twenty one cells were phase modulated only in one environment when run consecutively on the same day (see Fig. 3B). Examples of two cells that were only phase modulated in a single foraging environment are shown in Figure 4. Each of the four animals recorded from displayed multiple cells that were phase locked in only a single environment, and all had examples of single environment phase locking cells for both environments.

DISCUSSION

Medial prefrontal cortex cells recorded in two different behavioral tasks alternated between hippocampal theta rhythm entrained firing and nonphasic firing based on different behavioral and environmental factors. A majority of theta entrained cells had clear behavioral correlates (31/41), and those cells that were unidirectionally phase modulated in the linear track task had behavioral correlates in the same direction (8/9). Cells that responded to common events during both directional trips and were phase modulated were theta entrained during both trips (6/6). The capability of medial prefrontal units to fire with a phase relationship to hippocampal theta rhythm could serve as a mechanism allowing these two distant yet inherently related areas to work together during active processing tasks.

Jung et al. (1998) recorded from cells in the medial prefrontal cortex while the animals performed an array of behavioral tasks including the eight-arm radial maze. In the eight-arm radial maze, they found 74% of the recorded medial prefrontal cells had clear behavioral correlates, including direction of running on arms, goal approach, continuous firing at both specific and all goal locations, and during turns. Similar behavioral correlates of mPFC cells were found in other reports (Baeg et al., 2003; Mulder et al., 2003; Hok et al., 2005). In the current report, we found similar task requirements correlated with firing rate changes for medial prefrontal units. In the linear track task, a substantial majority of the cells recorded that had clear behavioral correlates to changes in their firing rates were cells that were modulated by the hippocampal theta rhythm (31 cells modulated out of 55 behaviorally correlated cells). These cells responded to a range of different behavioral events that together represent all facets of this specific task (see Fig. 2 for examples of a range of cells that had different behavioral correlates and were theta entrained). Cells responding vigorously to specific events in the linear track task tended to be phasically modulated (63% of recorded prefrontal cells). More specifically units with unidirectional theta entrainment and behavior correlates exhibited both responses during runs in the same direction (8 out of 9 cells). Also, of the units responding to common behavioral events in both directions 6 out of 6 theta entrained cells were entrained during trips in both directions. In the foraging task, a much higher percentage of theta-entrained neurons switched dynamically between phasic and nonphasic firing (21/44 total modulated). This may be due to the difference in environ-

ments being greater than the difference between directional runs on the linear track. Taken together, the results from both of these tasks reveal a tendency of medial prefrontal cells to not only respond to behavioral events with changes in firing rate but also by dynamically alternating between hippocampal theta entrainment and nonphasic firing. It is possible that a task that demands more involvement of medial prefrontal cortex and a task that would allow for finer analysis of more discreet behavioral events could reveal an even larger percentage of specific behaviorally correlated cells displaying behaviorally specific modulatory changes in firing to the hippocampal theta rhythm.

The majority of the behaviorally correlated cells responded while the animal was at one of the goal areas (27 out of 55; including single goal areas, both goal areas, single entire trips, runs and goal area, and turn and goal area). Two-thirds of these goal-related cells were phasically modulated by hippocampal theta. A similar preponderance of goal-related mPFC cell firing has been found in previous studies (Baeg et al., 2003; Hok et al., 2005), and the present results suggest these goal responding neurons are involved in hippocampal-mPFC interactions.

Each of the four animals in this study had cells that were phase locked in only a single direction and other cells that were phase locked in only a single environment. In the three animals that had multiple single direction phase locking cells, each animal had cells phase locked in both directions individually (the same held true for all four animals in the multi-environment foraging task). Given that cells from the same animal were phase locked for only a single direction and that others from that animal in the same session were phase locked for only the other direction, it makes it unlikely that gross behavioral differences (such as differing running speeds, or paths, or some other unaccounted for variable) between trial types or environments could account for the differential phase locking. Rather we believe the phase locking is indicative of a behaviorally related interaction between the hippocampus and the mPFC. This effect is clear in the nine cells that showed unidirectional theta entrainment on the linear track and also had unidirectional behavioral correlates during runs in the same direction (8/9), and also in the six theta entrained cells that responded to common events (those occurring during both directional runs) that were all theta entrained during runs in both directions.

It is interesting to consider how medial prefrontal cortex cells come to fire to the hippocampal theta rhythm in spite of a lack of strong and consistent theta recorded in medial prefrontal field potentials (Siapas et al., 2005; data not reported from the current study). Siapas et al. suggested this entrainment might arrive via excitatory input to the medial prefrontal areas from the monosynaptic pathway from ventral CA1 and subiculum directly to medial prefrontal areas (Swanson, 1981). If hippocampal principal units in ventral CA1 and the subiculum are driving medial prefrontal unit theta entrainment, it would be suspected that medial prefrontal units would fire to only a small range of hippocampal theta, just like hippocampal pyramidal cells (Skaggs et al.,

1996). However medial prefrontal units recorded from the same tetrode often had unique phase preferences and overall the variance of mean phase for modulated cells from the same hippocampal recording position was greater than a single 180° phase (mean range = 260°). We believe these results suggest that another means of modulation could be affecting these medial prefrontal cells. For the mPFC cells recorded for this study, the probability of a cell to be phase modulated was much higher if another cell from that same tetrode was also phase modulated. This increased probability could be attributable to the organization of the prefrontal cortex into hypercolumns. It is possible that each hypercolumn could perform a different function as has been shown in other cortical association areas, such as high-level association cortices (for review see Kaas and Collins, 2001) and proposed in models of prefrontal cortex (Hasselmo, 2005; Koene and Hasselmo, 2005). Within the mPFC, some hypercolumns might be involved in the interaction with the hippocampus (in an efferent or afferent fashion), whereas others are involved with processes independent of the hippocampus. It is also possible that the same hypercolumn could switch between interacting with the hippocampus (seen during periods of hippocampal phase modulated firing) and periods when hippocampal interacting does not take place. It is possible that the hippocampal modulation of a hypercolumn could arise from direct excitatory input via the ventral CA1/subicular afferents or from another source such as the dense reciprocal mPFC-hippocampal connections through the nucleus reuniens of the thalamus, or possibly from an entirely different source such as the cingulate cortex or the striatum (McKenna and Vertes, 2004). With the complex array of connections into and out of the medial prefrontal cortex and given the variety of phase preferences of individual units from the same tetrode, it is probable there are multiple mechanisms that enable medial prefrontal hippocampal theta entrainment.

The direction of the mPFC-hippocampal interactions also has considerable functional relevance. In the neural network model presented by Koene et al. (2003), trial specific goal information from mPFC neurons had to arrive with a specific phase relationship with respect to the ongoing hippocampal theta rhythm. When this input did not arrive at the appropriate phase, performance of the virtual rat decreased. The ability of mPFC neurons to sometimes fire in phase with theta and at other times not depending on the animals current behavior suggests that mPFC neurons may be performing a similar function to the modeled neurons (Koene et al., 2003). It is difficult to understand why mPFC neurons would switch between theta entrained and nonphasic firing if they were being driven by hippocampal afferents. If mPFC neurons that respond to behavioral correlates are driven by hippocampal afferents, it would be expected that all mPFC cells with directionally specific behavioral correlates would also have directionally specific theta entrainment, due to hippocampal principal cells unfaltering theta entrained firing. However sixteen out of the 41 mPFC cells with directionally specific firing rate changes were

modulated during trips in both directions, suggesting these changes in firing rate are not attributable to hippocampal afferent input.

In this study, we present data that demonstrate the capability of mPFC cells to change dynamically between hippocampal theta entrained firing and nonphasic firing, as the continuing behavior is changing both in respect to various aspects of the linear track task and in respect to the environment the foraging task was performed within. The data also show that mPFC cells that are actively involved in behavioral tasks are predisposed to fire entrained to the hippocampal theta rhythm. The results provide a new level of insight into the mechanisms by which the mPFC processes information internally and interacts with the hippocampus and other limbic structures. Our data suggest mPFC neurons may code some behaviorally relevant information with increases in firing rate that are hippocampal theta modulated, while the same neuron can show increases in firing rate to other elements of the task that are not phasically modulated. Further studies may demonstrate that hippocampal theta phase relationships of mPFC neurons can provide an additional variable along with firing rate changes, which mPFC cells can use to code behaviorally relevant events or stimuli, as hippocampal pyramidal cells do (Huxter et al., 2003). These findings reveal a functional correspondence of two areas involved in the active processing of complex cognitive and behavioral tasks and help to elucidate the nature of this interaction. Future studies of the interactions of medial prefrontal cells and the hippocampal theta rhythm in tasks that are dependent on both areas should continue to reveal the nature of the cooperative processing that is occurring in both areas during such tasks.

REFERENCES

Baeg EH, Kim YB, Huh K, Mook-Jung I, Kim HT, Jung MW. 2003. Dynamics of population code for working memory in the prefrontal cortex. Neuron 40:177–188.

Berke JD, Okatan M, Skurski J, Eichenbaum HB. 2004. Oscillatory entrainment of striatal neurons in freely moving rats. Neuron 43:883–896.

Brewer JB, Zhao Z, Desmond JE, Glover GH, Gabrieli JD. 1998. Making memories: brain activity that predicts how well visual experience will be remembered. Science 281:1185–1187.

Brown VJ, Bowman EM. 2002. Rodent models of prefrontal cortical function. Trends Neurosci 25:340–343.

Buzsaki G. 1986. Generation of hippocampal EEG patterns. In: Isaacson RL, Pibrim, KH editors. The hippocampus. New York: Plenum Press. p 137–167.

Colom LV, Christie BR, Bland BH. 1988. Cingulate cell discharge patterns related to hippocampal EEG, their modulation by muscarinic and nicotinic agents. Brain Res 460:329–338.

Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsaki G. 1999. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. J Neurosci 19:274–287.

de Bruin JP, Sanchez-Santed F, Heinsbroek RP, Donker A, Postmes P. 1994. A behavioural analysis of rats with damage to the medial prefrontal cortex using the Morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. Brain Res 652:323–333.

Dickson CT, Kirk IJ, Oddie SD, Bland BH. 1995. Classification of theta-related cells in the entorhinal cortex: cell discharges are controlled by the ascending brainstem synchronizing pathway in parallel with hippocampal theta-related cells. Hippocampus 5:306–319.

Eichenbaum H. 2004. Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44:109–120.

Floresco SB, Seamans JK, Phillips AG. 1997. Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. J Neurosci 17:1880–1890.

Fox SE, Wolfson S, Ranck JB Jr. 1986. Hippocampal theta rhythm and the firing of neurons in walking and urethane anesthetized rats. Exp Brain Res 62:495–508.

Grannon S, Poucet B. 1995. Medial prefrontal lesions in the rat and spatial navigation: evidence for impaired planning. Behav Neurosci 109:474–484.

Grannon S, Vidal C, Thinus-Blanc C, Changeux JP, Poucet B. 1994. Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. Behav Neurosci 108:883–891.

Groenewegen HJ. 1988. Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography. Neuroscience 24:379–431.

Groenewegen HJ, Uylings HB. 2000. The prefrontal cortex and the integration of sensory, limbic and autonomic information. Prog Brain Res 126:3–28.

Hasselmo ME. 2005. A model of prefrontal cortical mechanisms or goal directed behavior. J Cogn Neurosci (in press).

Hok V, Save E, Lenck-Santini PP, Poucet B. 2005. Coding for spatial goals in the prelimbic/infralimbic area of the rat frontal cortex. Proc Natl Acad Sci USA 102:4602–4607.

Huxter J, Burgess N, O'Keefe J. 2003. Independent rate and temporal coding in hippocampal pyramidal cells. Nature 425:828–832.

Jung MW, Qin Y, McNaughton BL, Barnes CA. 1998. Firing characteristics of deep layer neurons in prefrontal cortex in rats performing spatial working memory tasks. Cereb Cortex 8:437–450.

Kaas JH, Collins CE. 2001. The organization of the sensory cortex. Curr Opin Neurobiol 11:498–504.

Karlsgodt KH, Shirinyan D, van Erp TG, Cohen MS, Cannon TD. 2005. Hippocampal activations during encoding and retrieval in a verbal working memory paradigm. Neuroimage 25:1224–1231.

Kesner RP. 1989. Retrospective and prospective coding of information: role of the medial prefrontal cortex. Exp Brain Res 74:163–167.

Koene RA, Hasselmo ME. 2005. An integrate-and-fire model of prefrontal cortex neuronal activity during performance of goal-directed decision making. Cereb Cortex (in press).

Koene RA, Gorchetchnikov A, Cannon RC, Hasselmo ME. 2003. Modeling goal-directed spatial navigation in the rat based on physiological data from the hippocampal formation. Neural Netw 16:577–584.

McKenna JT, Vertes RP. 2004. Afferent projections to nucleus reuniens of the thalamus. J Comp Neurol 480:115–142.

Miller EK, Freedman DJ, Wallis JD. 2002. The prefrontal cortex: categories, concepts and cognition. Philos Trans R Soc Lond B Biol Sci 357:1123–1136.

Mulder AB, Nordquist RE, Orgut O, Pennartz CM. 2003. Learning-related changes in response patterns of prefrontal neurons during instrumental conditioning. Behav Brain Res 146:77–88.

O'Keefe J, Recce ML. 1993. Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus 3:317–330.

Pare D, Gaudreau H. 1996. Projection cells and interneurons of the lateral and basolateral amygdala: distinct firing patterns and differential relation to theta and delta rhythms in conscious cats. J Neurosci 16:3334–3350.

Schoenbaum G, Eichenbaum H. 1995. Information coding in the rodent prefrontal cortex. II. Ensemble activity in orbitofrontal cortex. J Neurophysiol 74:751–762.

Schon K, Hasselmo ME, Lopresti ML, Tricarico MD, Stern CE. 2004. Persistence of parahippocampal representation in the absence

of stimulus input enhances long-term encoding: a functional magnetic resonance imaging study of subsequent memory after a delayed match-to-sample task. J Neurosci 24:11088–11097.

Siapas AG, Lubenov EV, Wilson MA. 2005. Prefrontal phase locking to hippocampal theta oscillations. Neuron 46:141–151.

Skaggs WE, McNaughton BL, Wilson MA, Barnes CA. 1996. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. Hippocampus 6:149–172.

Stephens MA. 1974. EDF Statistics for goodness of fit and some comparisons. J Am Stat Assoc 69:730–737.

Stern CE, Sherman SJ, Kirchoff BA, Hasselmo ME. 2001. Medial temporal and prefrontal contributions to working memory tasks with novel and familiar stimuli. Hippocampus 11:337–346.

Swanson LW. 1981. A direct projection from Ammon's horn to prefrontal cortex in the rat. Brain Res 217:150–154.

Tabuchi ET, Mulder AB, Wiener SI. 2000. Position and behavioral modulation of synchronization of hippocampal and accumbens neuronal discharges in freely moving rats. Hippocampus 10:717–728.

Uylings HB, Groenewegen HJ, Kolb B. 2003. Do rats have a prefrontal cortex? Behav Brain Res 146:3–17.