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Research report

Neurons in rat medial prefrontal cortex show anticipatory rate changes to predictable differential rewards in a spatial memory task

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Abstract

The present study electrophysiologically examined the contribution of prelimbic and infralimbic neurons in the medial prefrontal cortex (mPFC) to integration of reward and spatial information while rats performed multiple memory trials on a differentially rewarded eight arm radial maze. Alternate arms consistently held one of two different reward amounts. Similar to previous examinations of the rat mPFC, few cells showed discrete place fields or altered firing during a delay period. The most common behavioral correlate was a change in neuronal firing rate prior to reward acquisition at arm ends. A small number of reward-related cells differentiated between high and low reward arms. The presence of neurons that anticipate expected reward consequences based on information about the spatial environment is consistent with the hypothesis that the mPFC is part of a neural system which merges spatial information with its motivational significance.

Keywords: Spatial navigation; Neural systems; Reward; Prefrontal cortex; Amygdala; Medial ventral striatum; Electrophysiology

1. Introduction

Since the seminal studies of Mogenson and co-workers, the medial ventral striatum (mVS) has been viewed as one potential region where traditionally defined memory structures such as the hippocampus and amygdala may impact motor structures via pallidal efferents (for review see Ref. [42]). Stimulation of either the hippocampus or amygdala of the rat invokes firing rate changes within the mVS, and chemical excitation of these regions cause behavioral changes that are mVS-dependent [43,47,75–77]. This suggests that perhaps one means for the hippocampus to impact behavior is via its projections (from the subiculum) to the mVS [35]. The hippocampus itself has been long implicated as an important structure for rat navigation, as its removal results in drastic impairments in spatial tasks (for review see Ref. [2]) and "place cells" (neurons which fire when a rat occupies a discrete location in its spatial environment) are found in the rat hippocampus [48,49,57]. However, despite the further defining of necessary sensory and mnemonic components that drive hippocampal representations of space, the precise way that these signals affect behavior remains to be determined.

To begin to address this issue, Lavoie and Mizumori [29] recorded from mVS neurons in freely moving rats during performance of a win-shift navigation task, to determine what neuronal correlates might be found in a motor structure that receives afferent information from the hippocampus. Recordings revealed that the nucleus accumbens and surrounding striatum encoded not only spatial information, but also motivationally relevant information about the expectation and presence of reward within the environment. Furthermore, a subset of

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reward-related neurons distinguished between arms that held high and low reward amounts. A recent report by Martin and Ono [32] in a different spatial task (foraging for brain stimulation) has confirmed the presence of location- and reward-related cells within the mVS. These data suggest that the mVS may be an important site for the integration of motivational information with spatial representations. Given that a likely source of afferent spatial information is the hippocampal complex, and that hippocampal place fields appear to be independent of reward placement in rich spatial environments [68], it was of interest to determine what brain regions forward reward-related information to be integrated within the mVS. Pratt and Mizumori [54] subsequently recorded from the basolateral nuclei of the amygdala (BLA) and demonstrated that they are a possible source of reward and reward expectancy information during performance of the win-shift spatial task. The medial prefrontal cortex (mPFC) is also a possible candidate for providing the mVS with spatially relevant reward information [65]. The prelimbic (PL) and, to a lesser extent, the infralimbic (IL) regions of the mPFC in the rat are densely innervated by the ventral subiculum and the CA1 region of hippocampus [4,5,19,69]. Given the known role of these latter regions in spatial processing, the mPFC itself may have access to spatial information. The mPFC also receives afferents from several brain regions that process the significance of rewards and the cues that predict rewards. In particular, the PL and IL areas of mPFC receive projections from the medial posterior regions of the BLA of the rat, as well as extensive input from the ventral tegmental area [17,25,33,34,46,70]. Individual neurons within the amygdala and tegmentum have been shown to encode rewards and the cues that predict them [28,44,45,60,62].

Given the anatomical convergence of spatial and reward systems, there is a potentially important role of the mPFC in the rat for encoding relevant reward locations within a spatial environment. Consistent with other brain regions associated with reward systems, much of the prefrontal cortex of the rat supports electrical self-stimulation (for review see Ref. [58]). When anatomically disconnected from lateral regions of the rat prefrontal cortex, stimulation of the medial PFC induces place preferences, but not taste preferences (sulcal prefrontal cortex stimulation produces taste preferences under the same experimental condition, [59]). This suggests a functionally specific role for medial regions of the prefrontal cortex for reward-place associations. Additional support is granted by multiple experiments in which rats with mPFC lesions are impaired at acquiring spatial tasks that involve learning locations of rewards [7,11,18,27,30,51,53,67,74]. Such impairments are similar to those found in studies that lesion the medial striatum [1,10], and are consistent with the view that the mPFC and the mVS are functionally linked. Additionally, recordings from individual units within the mPFC have reported only small numbers of cells with location-specific firing [21,52]. Instead, correlated neurons within the mPFC appear sensitive to specific behaviors during goal-oriented tasks. Recordings of the rat mPFC to date, however, have not examined this region's possible contribution for associations between reward and spatial information.

If the mPFC is important for learning about rewards within a spatial context, individual neurons should alter their activity relative to the presence of predictable rewards in a spatial task. Moreover, if such representations are important for the planning of spatial movements based on different reward contingencies, some neurons should distinguish between different reward amounts reliably located within the environment. The current experiment set out to specifically examine the firing patterns of rat mPFC neurons during a spatial memory task involving differential rewards found in predictable locations. Furthermore, it was of interest to be able to directly compare the correlates of the mPFC with previous reports of recorded cells from mVS and the BLA across studies using comparable analyses. Comparisons of the types and relative number of different behavioral correlates may provide further insight into the representations of reward within a spatial context, and help to define the individual roles of each region within a broader system that may serve to integrate spatial and reward contingencies to ultimately direct behavior.

2. Materials and methods

2.1. Subjects

Subjects consisted of four implanted male Long-Evans rats, obtained from Simonsens Laboratories and housed in the laboratory. Rats were given 2–3 days to acclimate to individual housing before being reduced to and maintained at 80% ad lib body weight. Handling was done daily for no fewer than 7 days prior to beginning behavioral training. Water was available at all times. Rats were maintained on a 12-h light–dark cycle (lights on at 7 a.m.) in a controlled temperature environment of 21°C. All behavioral testing was done during the light phase.

2.2. Apparatus

A semi-automated eight arm radial maze was used for this experiment, consisting of eight black Plexiglas runways (58 × 5.5 cm) radiating from a center platform (19.5 cm diameter) and supported 79 cm from the floor.

The internal segments of each arm could be raised or lowered by remote control to provide access to the arms from the maze center.

The experiment was conducted within an environment that consisted of an ≈ 1.6 m $\times$ 1.6 m square by 3 m deep space enclosed by black drapes. On these drapes were several distinct visual cues. The room was lit by four 15 W bulbs located at the corners of the room.

2.3. Behavioral training

Upon reaching 80% body weight, the rats were placed on the maze and given access to all eight arms with an abundant supply of chocolate milk on each arm. Once the rat had visited and consumed reward on all eight arms, they were gradually shaped to receive reward only at the end of each arm. Maximum time per day on the maze was 1 h.

Once it was established that the rat would visit each arm, they were given trials in which all eight arms were presented. The rats were allowed to visit each arm for chocolate milk reward (five drops). As the rats left each arm, the arm was lowered, and the rat was allowed to visit each radial location only once per trial. One trial consisted of a visit to all eight rewarded locations. The intertrial interval was 2 min. This training continued until eight such trials were completed within a 1 h period.

When this criterion was met, the rats were trained to perform a partial forced-choice memory task. Beginning with this training, all arms were baited prior to each trial with large or small rewards (5 drops or 1 drop, respectively) on odd or even arms. Determination of whether large reward was placed on odd or even arms was randomly determined for each rat, and remained consistent throughout the experiment unless otherwise noted. During the study portion of each trial, four arms were raised in an order determined from a table of random numbers. As the rat visited each arm, the next arm in the sequence was raised to allow access after the rat returned to the maze center. Once an arm had been visited, it was lowered to prevent possible re-entry until the memory phase of the task.

After presentation of the fourth arm, all arms were raised. The memory phase of the task required the rats to continue running the maze until they visited the remaining four arms that had chocolate milk. During this phase, the arms were not lowered as rats left the arms, and any return entries to previously visited arms were recorded as errors. The trial ended once all eight arms were visited. Rats were trained to run 15 such trials in an $\approx$1-h period. Three rats were trained with a 1-min delay between the study and memory portions of the task for five of the 15 daily trials (typically trials 6–10). The delay was accomplished by confining the animal on the center platform without access to any of the radial maze arms. Visual cues in the environment remained visible during the delay. After rats performed 15 such trials for seven consecutive days, training ceased and electrodes were surgically implanted.

2.4. Electrode construction

The stereotrode and microdrive design of McNaughton and co-workers [36,37] was adopted for this experiment. Two lacquer-coated tungsten wires twisted together and coated with epoxilite were threaded through a 30 gauge cannula, leaving 1–2 mm of wire exposed at the bottom. Two or three such cannulas were placed ≈ 1 mm apart on each microdrive. One microdrive was implanted over each hemisphere. Prior to surgery, the stereotrode tips were cut at an angle of 45° and gold plated to an impedance of 50–150 kΩ (tested at 1 kHz). Reference electrodes were constructed from 114 μm teflon-coated stainless steel wire. Two ground leads of 250 μm teflon-coated stainless steel wire were soldered to a jeweler's screw. Amphenol pins were crimped onto the stripped ends of recording electrodes and ground wires. During surgery, pins were inserted into two plastic nine-pin connectors (Science Technology Center, Carleton University, Canada), one per hemisphere.

2.5. Surgical procedure

Following 24 h of food and water deprivation, rats were anesthetized with sodium pentobarbital (40 mg/kg initial dose with 0.05 cm^3 supplements given as needed) and secured in a rat stereotaxic apparatus (Kopf). Atropine sulfate was administered (0.2 cm^3 per rat) to minimize respiratory distress. Burr holes were drilled through the skull and two or three stereotrodes per hemisphere were implanted above the mPFC ($+3.2$ to $+4.2$ mm from bregma, 0.7–0.9 mm lateral, 1.2 mm ventral to brain surface). Two reference electrodes were placed in an accessible quiet region of the brain, and two ground screws were anchored in the skull. Rats were injected with 0.2 cm^3 Bicillin® L-A intramuscularly following surgery to guard against infection. Buprenorphine was available in the event that post-surgical analgesic was required. Rats were allowed 1 week of recovery, at which time they were retrained to the criterion outlined above.

2.6. Cell recording and behavioral tracking

Once rats were running at criterion performance levels, the stereotrodes were checked daily (up to 6 days per week) for spontaneous cellular activity on the recording channels. The stereotrodes were lowered in 21.8 μm increments, up to 175 μm per day, or until

isolated unit activity was encountered. Rats were connected to recording equipment by a preamplification headstage consisting of seven high input impedance field effect transistors (Newark Electronics) and a light-emitting diode. Both hemispheres were checked serially.

Electrophysiological data were recorded and analyzed on a DataWave Neuroscience Workstation (DataWave Technologies, Longmont, CO). Incoming signals were amplified 1000–10 000 times, and filtered at 600 Hz (high pass) and 6 kHz (low pass). Signals were then passed through a window discriminator that initiated a 1 ms sampling period when an impulse from either channel passed a user defined threshold. The entire waveform was recorded by DataWave's Discovery software package. Units were isolated using an interactive cluster-cutting routine, which processed waveforms using eight spike parameters (four for each recording channel), including the maximum and minimum voltages of the sampled waveform, and the latencies of these values from the onset of the sampling period. Following recording, additional parameters were used to further isolate waveforms of units, including a template matching algorithm that was able to distinguish unique waveform shapes. Once units were isolated, they were subject to analysis for behavioral correlates.

The rat's position was monitored and recorded by an automatic tracking system (Dragon Tracker, model SA-2, Boulder, CO) that sampled the position of the diode at a frequency of 20 Hz (resolution 1.5–2.0 cm). The time of each position sample and unit event was logged by the DataWave Neuroscience Workstation.

2.7. Data analysis

Various analysis routines (DataWave Technologies, as well as courtesy of B.L. McNaughton, C.A. Barnes, and S. Leutgeb) were used to analyze unit characteristics and behavioral data. Mean spike amplitude, width, and rate across the entire recording session was calculated for each cell.

To determine behavioral correlates, position data were viewed off-line. Flags were entered into the data at times during which rats performed specific behaviors: reaching the ends of arms, turning on the arm ends, and initiation of inbound movement on the arms. Behavior on odd and even arms were also distinguished, so that differences between high and low reward arms could be observed. Any time during the trial in which the rat stopped for 1 s or greater on the center platform was flagged to provide a comparison for similar behaviors on arm ends. Peri-event time histograms (PETHs) were created to display the change in firing rate in relation to behavior. These histograms plotted the firing rate of a cell 2.5 s prior to and after the flagged event (reaching arm ends, turns, and inbound movement). Flags also marked the beginning and end of each trial, as well as transitions between the study and test phases of each trial. Delay periods were flagged when appropriate. Entire delay periods were plotted by PETHs, and were compared to histograms composed from the same amount of time during the intertrial intervals.

In order for a neuron to be considered to have a behavioral correlate, two criterion were required to be met. First, the firing rate of the cell was required to differ by a factor of two (i.e., either an increase of 200% or a decrease of 50%) during a 500 ms bin proximal to the flagged behavior. This criterion was maintained to provide consistency for cell classification with previous studies published from our lab, and allow subsequent comparisons across different neuronal structures during the current task [29,54]. Second, to verify that cells classified by the above criterion were due to reliable changes in cell activity during the occurrence of specific behaviors of the rat on the maze (rather than spurious changes in activity for a limited number of occurrences of a given behavior), repeated-measure multivariate ANOVAs were performed. Significant results ($P < 0.05$) of this statistic reflected consistent changes in a neuron's activity in the 2.5 s surrounding a particular behavioral event. Both of the above criteria were satisfied for all the behavioral correlates reported in this paper.

To determine whether firing rates differed at reward encounter on high and low reward arms, independent-groups t-tests were then run between high and low reward arm visits for the 500 ms bin that showed the maximal effect size. In cases, where a cell showed both anticipatory and consummatory correlates, one t-test was performed for each component of the behavior.

Onset and offset of reward effects were determined by comparing the mean discharge rate of the reward-correlated cell across 200-ms bins. For cells that were excitatory, the beginning of the effect was defined as when the rate increased to 125% above baseline and remained above this threshold for at least two bins (to avoid classifying a transient change in firing as the onset of an effect). Offset was defined as the time after which the firing rate fell back below 125% of baseline for two 200-ms bins. Onset and offset of inhibitory effects was similarly determined, using a threshold of 75% baseline. These times of onset and offset of effect were determined individually for each neuron on both high and low reward arms. Because of the potentially low resolution of this analysis, high and low reward arms were only considered different if their onset–offset time of effect differed by 400 ms or more. This analysis was done to determine whether reward-related effects could be explained by the differing size of the reward. Past work with reward correlates of amygdala neurons has shown that this method of categorizing reward effects is sufficiently sensitive to detect reward magnitude differences [54].

Analyses were also computed for each cell in order to determine a possible spatial correlate. One analysis compared rates of firing on the outbound and inbound components of each arm (16 rates total). The specificity of each cell was determined by dividing the highest rate by the average of the 15 other rates. Furthermore, the reliability of firing across trials on the highest arm was determined as the proportion of trials in which the cell fired at its highest rate on that arm. Similar to past studies [41,54] that examined the location selectivity of neurons, cells that displayed a specificity score of 2.0 or greater with a reliability of at least 33% of behavioral trials were considered spatial.

Other analyses were run on special cases. These are described in detail in Section 3.

2.8. Histology

Once the electrodes were lowered through the depths of the mPFC, rats were deeply anesthetized with sodium pentobarbital and perfused through the heart with a 0.9% buffered NaCl solution, followed by 10% formalin. Electrodes were retracted and the brain was removed and allowed to sink in a 30% sucrose formalin solution. Forty micrometer frozen sections were then sliced through the penetrated area with a cryostat. Sections were stained with Cresyl violet, and electrode tracks were histologically verified by comparing depth measurements at the time of recording with an electrode track reconstruction derived from examinations of the serial sections for each hemisphere.

3. Results

3.1. Behavioral results

Rats were trained to criterion performance in an average of 26 days (range 16–42 days). They committed few errors, even when subjected to a 1-min delay between the study and test phase of each trial. During the recording of 29 prefrontal data sets from three rats trained with the delay, rats averaged 0.23 errors per trial during non-delay trials, and 1.1 errors per trial with the delay. This error difference was statistically significant ($t_{28} = 6.87$, $P < 0.001$) between delay and non-delay trials, suggesting poorer performance after a delay, despite obvious savings of pre-delay information.

Rats reliably discriminated between high reward arms and low reward arms during the test phase of each trial. A one factor ANOVA for repeated measures was run on the number of entries to high reward arms during the rats' first four choices in the test phases of each 15-trial baseline recording session in which a prefrontal neuron was recorded. This test was significant ($F(3,167) = 345.6$, $P < 0.0001$). As shown in Fig. 1, rats visited high reward arms consistently during the beginning of the test phase, and then progressed to low reward arms.

3.2. Electrophysiological results

Sixty-one neurons were recorded from the PL and IL regions of the prefrontal cortex ($n = 4$ rats). Firing rates ranged from 0.04 to 45.6 Hz, with a mean rate of 5.96 Hz (median = 2.76 Hz). This value is consistent with previous reports as it falls between the mean values for Jung et al.'s [21] regular and fast spiking cells reported from this region and is slightly lower than the mean reported by Poucet [52]. No differences were observed between firing rates, spike widths, or spike amplitude across neurons of different correlate types (see below). Utilizing the criteria explained above, 30 neurons of the 61 total (49.2%) were classified as having behavioral correlates (Table 1). It should be noted that, in the case of significant effects surrounding flagged behaviors, the criterion requiring the cell to vary by a factor of two from its mean rate (Section 2) was more conservative than the multivariate ANOVA test. All cells which satisfied the first criterion also demonstrated statistical significance ($P < 0.05$). The inverse, however, was not true. All neurons were tested by a multivariate ANOVA centered on when the rat reached the reward. Twenty neurons showed significance on the statistic that did not satisfy other behavioral criteria. Visual examination of these data did not show convincing correlates; often there were small changes in firing rate that showed a general increase or decrease in firing rate across the time examined, but that was not always centered on the behavior of interest. All correlated neurons described below fulfilled both criterion.

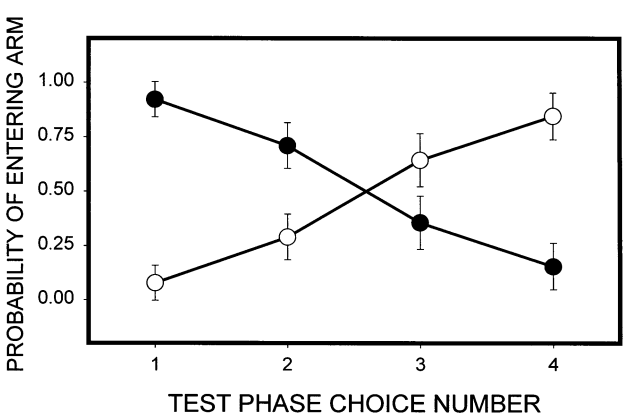

Fig. 1. Behavioral demonstration of the reward arm type that rats visited during the first four choices of the test phase for each trial. Shown is the probability of the rats entering a high reward arm (●) or low reward arm (○) as the test phase progressed. Rats began the test phase of each trial by visiting arms with higher reward magnitude, and subsequently retrieved reward from arms with less chocolate milk.

Table 1
General breakdown of mPFC neuronal correlates

Type of correlate	Number observed
Total number of cells with reward correlates:	19
Total number of cells with movement correlates:	10
Total number of cells with spatial correlates:	6
Reward-related neurons (only)	
Inhibitory neurons	9
Inhibition at encounter (3)	
Anticipatory inhibition (6)	
Excitatory neurons	5
Excitation at encounter (2)	
Anticipatory excitation (3)	
Biphasic	1
Spatial neurons (only)	4
Movement neurons (only)	6
Multiple correlate cells	
Reward and movement	3
Reward and spatial location	1
Movement and spatial location	1
Total cells with correlates	30
Total cells recorded	61

3.3. Baseline properties of mPFC neurons

3.3.1. Reward neurons

Fifteen neurons showed changes in firing rate that were exclusively centered upon reaching the end of the maze arms, without showing similar changes at movement stops on the center platform of the maze. These were classified as reward neurons.

Nine cells of the 15 reward-related neurons inhibited firing immediately prior to or after reaching the arm ends, but did not show a similar pattern during non-rewarded spontaneous stops (defined in Section 2). Six of these were *anticipatory inhibition* neurons that showed a significant decline in the firing rate more than 200 ms prior to the rat's reaching of both high and low reward arm ends, and that continued during the consummation of the reward (Fig. 2(A)). Three of the nine inhibitory neurons decreased firing upon reaching the arm ends, and were classified as *inhibition at encounter* neurons (Fig. 2(B)).

Another class of cells ($n = 5$) increased their firing rate surrounding reward encounter, and was considered *excitatory*. Three of these neurons began their bursting prior to the acquisition of the reward (*anticipatory excitation* neurons, Fig. 3(A)). Two showed a significant change within 200 ms of reward consumption (*excitation at encounter*, Fig. 3(B)).

The remaining reward-related cell showed a biphasic pattern of activity to the acquisition of the reward. Immediately prior to reward acquisition, the cell demonstrated a rise in its firing rate that reversed to an inhibitory pattern once the reward was reached.

3.3.2. Spatial neurons

Similar to previous reports [21,52], only a small number of mPFC neurons displayed spatial correlates. Four cells were exclusively location-specific, and classified as spatial neurons. Graphic illustration of these neurons' location selectivity are shown in Fig. 4, panels B–E. The average specificity and reliability (see Section 2 for calculations) was 2.57 and 33%, respectively; the mean rate was 3.56 Hz. Previous recordings of hippocampal place cells in our laboratory (utilizing the same criterion for place unit activity) show typical specificity scores of 5.0–8.0, and reliability scores between 40–50% [38]. Note the generally broader nature of mPFC fields relative to hippocampal place cells. Also unlike their counterparts in hippocampus, mPFC spatial neurons did not show evidence of complex-spike or "burst" behavior (data not shown).

3.3.3. Movement neurons

Six neurons showed general movement-sensitive firing only. Two neurons changed their firing rate as a function of forward movement by the animal; one inhibited to forward movement, while the other increased its rate. Three neurons doubled their firing rate during turning movements; one of these additionally inhibited once the turn was complete and the rat began its inbound journey to the maze center. The sixth neuron showed the latter pattern only, inhibiting during inbound forward movements.

3.3.4. Cells with multiple correlates

Five additional neurons had significant firing rate changes in response to both reward and movement ($N = 3$), movement and spatial location ($N = 1$), or reward and spatial location ($N = 1$). All three neurons which were sensitive to reward and movement showed anticipatory inhibition prior to the encounter of the reward, combined with a significant increase in firing rate during the onset of turning behaviors at the end of the arms. The one neuron that was sensitive to both movement and spatial location only fired during the turns on specific arms (Fig. 4(A), see further description below). Similarly, one anticipatory burst reward neuron showed its highest firing in anticipation of reward when encountered on one quadrant of the maze.

3.4. Mnemonic properties of prefrontal neurons

3.4.1. Differentiation of reward magnitude

Of the 19 neurons that showed reward-related activity (15 cells with reward correlates only + 4 cells with multiple correlates including reward-related activity), 14 showed changes in firing rate that anticipated the onset of the reward. The remaining five cells altered their activity in response to the start of reward consumption. Generally, rats were run with a constant pattern of high

and low values of reward on specific arms. Each reward neuron was tested to determine if there was a difference in reward-related activity between different valence arms by comparing firing rates between high and low rewarded arms during the 500 ms bin during which the rate change was greatest using an independent samples *t*-test. For neurons that showed significant firing changes during both anticipatory and consummatory phases of reward acquisition, tests were run on both phases of the behavior.

Two of the 14 neurons with reward-related anticipatory activity distinguished between high and low reward arms. Both of these neurons inhibited their firing prior to reward acquisition. This suggests that within the population of mPFC neurons that anticipate the onset of reward there may be a subpopulation of anticipatory cells that distinguish between the relative value of the reward to be gained. To further examine this possibility, one neuron was tested during five trials when the reward valence of the arms was reversed (previously highly rewarded arms received low rewards, and vice versa). As shown in Fig. 5, during the trials when the reward was reversed, the neuron still showed a significantly greater anticipatory inhibition *on the arms which the rat normally received higher reward.*

In addition to suggesting that such neurons are able to encode expected reward contingencies at specific spatial locations, the above manipulation implies that pre-encounter firing of reward-related neurons is not the result of specific visual or odor cues, which might provide information about upcoming reward. To determine whether reward-anticipation responses of neurons

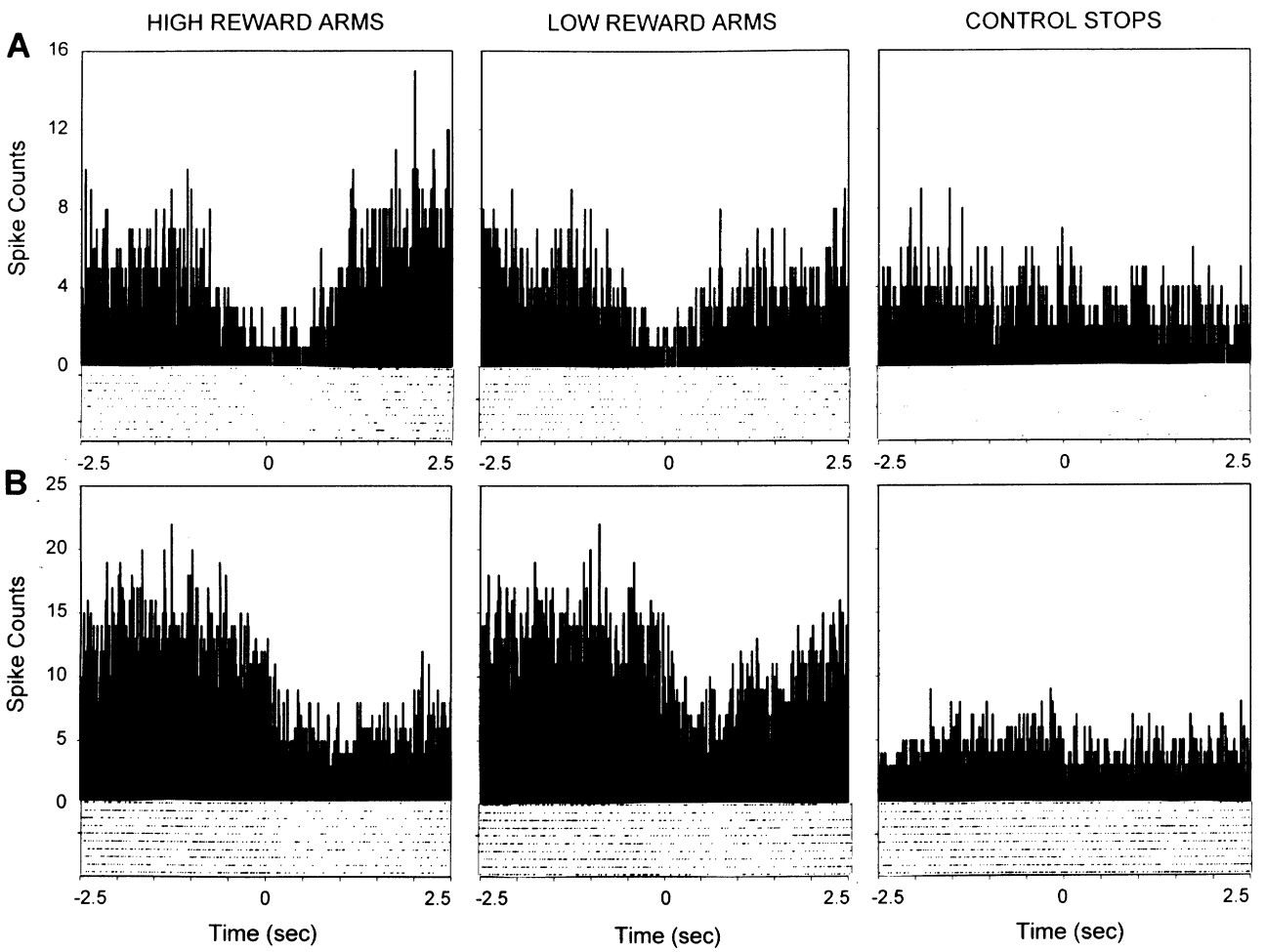

Fig. 2. Examples of the two classes of inhibitory neurons encountered. Each PETH is centered on the time that the animal encountered the reward at the end of each arm. The bin width for each bar is 10 ms. Raster plots beneath each PETH show the spiking activity of each neuron during multiple individual encounters. For arm end visits, PETHs are based on 60 encounters (15 win-shift trials) except as noted in this and subsequent figures. (A) *Anticipatory inhibition* neurons demonstrated a decline in firing rate by at least 50% prior to encountering the chocolate milk reward. For this neuron, the inhibitory effect continued into the consummatory phase of the behavior. (B) *Inhibition at encounter* neurons showed a similar decrease that occurred only once the reward was encountered. For both neurons, the effect at the ends of the arms was compared to the firing patterns when the rats stopped on the center platform (control stops). Note the lack of effect for these control conditions, suggesting that these neurons are not movement-related. Control stops PETHs presented in this manuscript are derived from an average of 20 center platform stops (range 17–26) over the course of 15 trials.

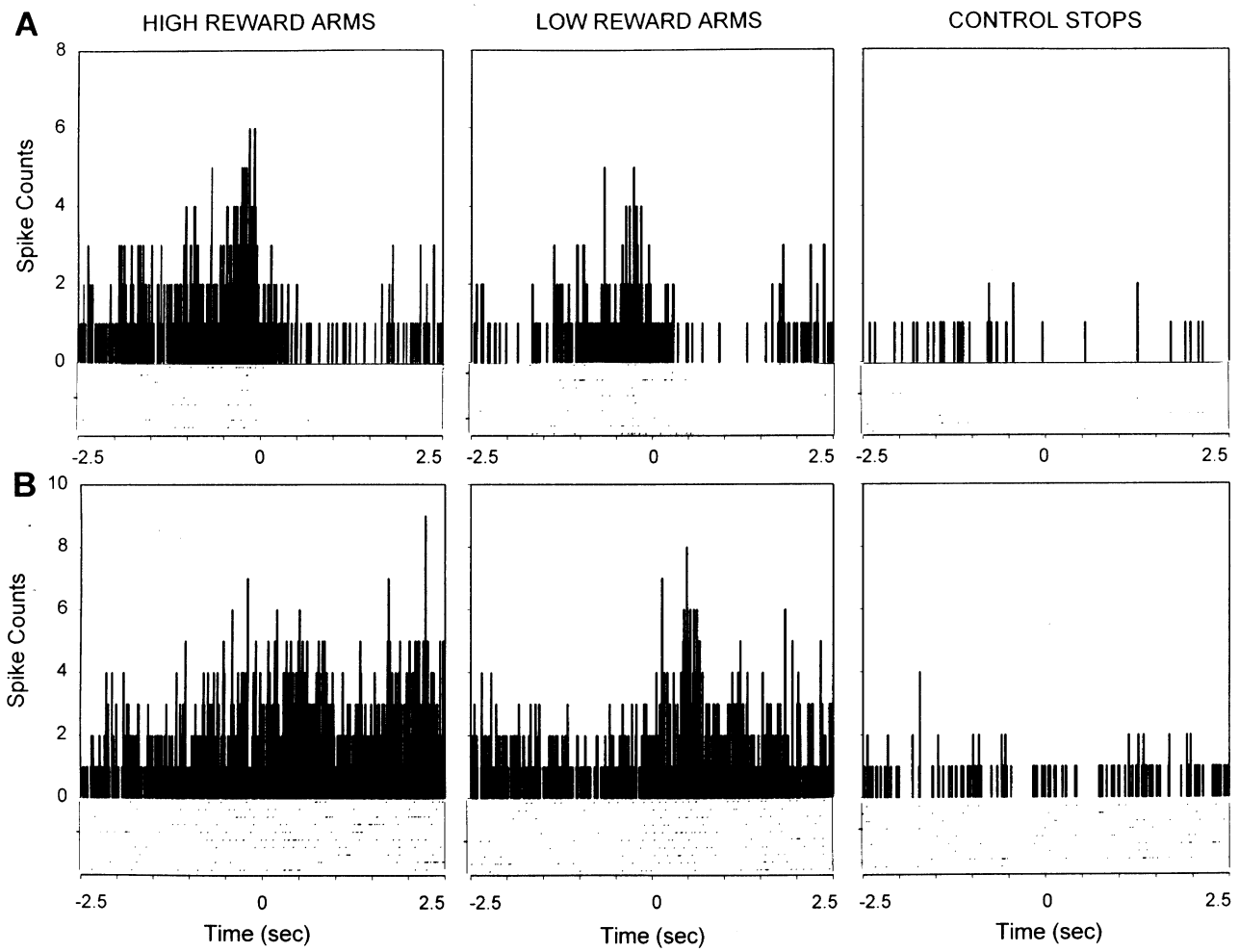

Fig. 3. Examples of excitatory neurons. (A) An *anticipatory excitation* neuron. Note that in this case, the excitatory activity precedes the acquisition of the reward. The difference between high and low reward arms did not meet statistical significance ($t_{118} = 1.497$, $P = 0.137$) and is therefore not further considered. (B) An *excitation at encounter* neuron. A significant rise in the firing rate occurs only after reward is encountered on low reward arms. High reward arms also showed significant rate change only during the 500 ms following reward consumption. Note: cell B was classified conservatively. According to criterion, increased activity began prior to reward acquisition, but ONLY on high reward arms. All cells classified as *anticipatory* showed pre-consummatory activity on both high and low reward arms.

were directly dependent on reward presence, error arms (i.e., when the rat proceeded down arms that they had previously visited) were flagged for additional analyses. Seven neurons were recorded during maze sessions in which the animal made sufficient errors (typically four or greater) to characterize the cells' response to reaching the end of previously visited arms. All seven neurons maintained an anticipatory change in firing rate that was consistent with their change of activity on rewarded arms, passing our criterion for the onset of an effect (Section 2). This further suggests that reward anticipation of these neurons is not dependent on the physical presence of the reward itself (Fig. 6). It should be noted, however, that in all seven cases, the size of the firing rate change from baseline decreased relative to correct arm choices during the task. This decrease in effect compared to correct choices was significant ($t_6 = -4.37$, $P = 0.005$). This finding, while preliminary, is strikingly similar to decreases in correlated neural activity in monkey PFC during delay tasks when errors are committed [8,72,73], and may further support a mnemonic role for the mPFC of the rat in this task.

Six neurons distinguished between high and low reward arms during the consummatory phase of behavior (Two anticipatory inhibition and one biphasic neuron that had effects which lasted into the post-encounter phase, as well as two inhibition at encounter and one excitation at encounter neuron). Close examination of their responses suggest that the significant difference, at least in part, may be due to a prolonged post-encounter effect on high reward arms related to motor or sensory aspects of consummation of the reward. Fig. 7 shows one such response for a cell during a reward switch condition. The stronger and prolonged effect followed the reward during this manipulation, suggesting that this *inhibition at encounter* neuron was sensitive to the

current (not expected) characteristics of the reward. Additionally, no inhibition was seen for this cell on arms where the rat made errors.

Consistent with this finding, only three neurons of our reward-related sample showed a longer effect on low reward arms that exceeded our 200 ms difference criterion. A regression analysis on the remaining excitatory and inhibitory type reward neurons (all but one of which included post-encounter rate changes) revealed a significant correlation of effect offset between high and low arms ($F(1,13) = 6.6$, $P < 0.05$). The slope of the regression line was 0.16, close to an expected slope of 0.2 that would result from assuming that the high reward (5 drops) takes five times longer to consume than the low reward (1 drop). Thus, while anticipatory responses of reward-related neurons appear to be dependent upon reward expectation, most post-encounter neuronal firing rate changes appear to depend on reward presentation.

Although post-encounter neuronal changes appear to be dependent on sensory or motor consequences of reward consummation, it is important to note that these signals may distinguish meaningful differences. For example, one anticipatory inhibition neuron that showed both anticipatory and post-encounter inhibition to reward presentation (Fig. 8(A)) was subjected to a test during which the reward was changed from chocolate to strawberry milk (Fig. 8(B)). Although the anticipatory inhibition remained prior to consumption of the new reward (consistent with above findings), when the rat encountered the new reward there was a significant *increase* in the activity of the cell. This distinction between familiar and novel reward, in addition to reward magnitude detection by some cells (described above), suggests that mPFC neurons may have multiple roles in distinguishing different rewards.

3.4.2. Visual dependence of spatial neurons

Two of the five neurons with location correlates (one with a center field and one multiple correlate cell with both spatial and turn-related properties) were stable for multiple days and subjected to manipulations to determine their stability across environmental manipulations. When subjected to a series of trials during which lights were on for five trials, followed by five trials of darkness and then a light restoration phase, these two

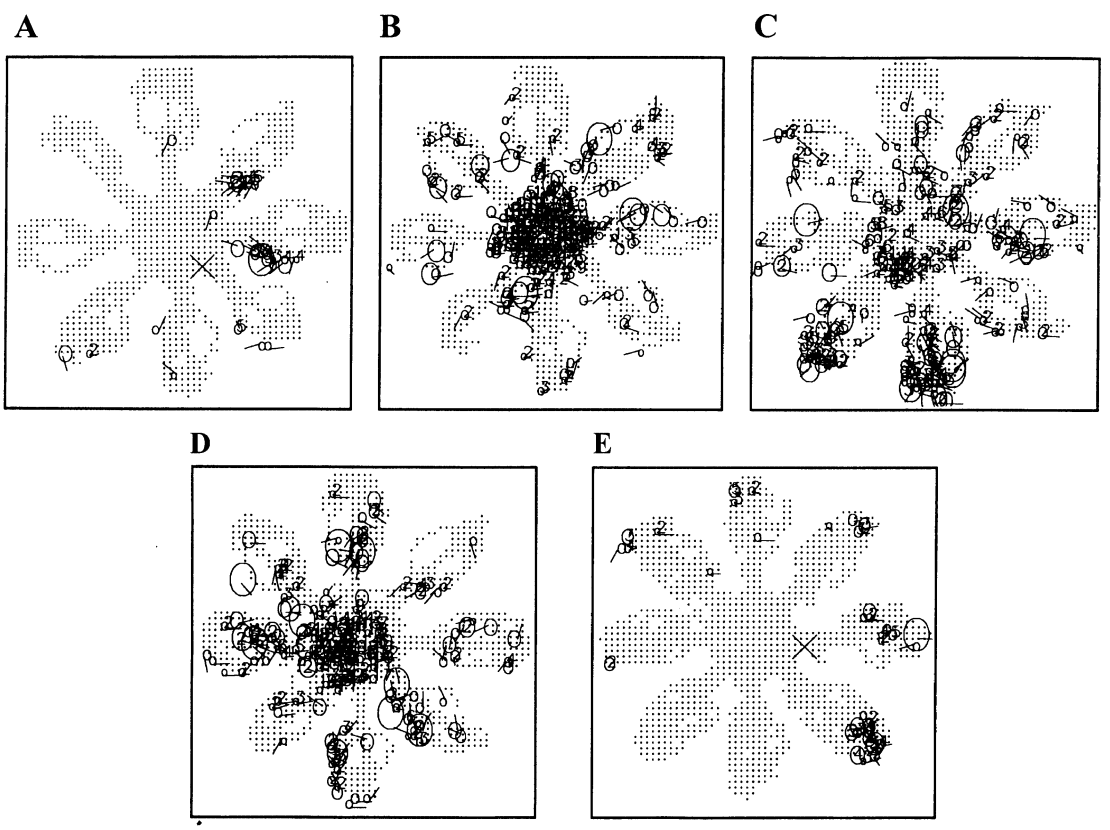

Fig. 4. Spot diagrams indicating spatial firing biases for five neurons. Dots represent pixels (within a 64 × 64 grid centered on the platform) visited by the rat. Circles represent the spatial locations of bursting activity that exceeded 20% of the neuron's maximum rate; circle diameters are linearly related to firing rate. Vector lines projecting from the circles represent the direction the animal was traveling during the action potential discharge. One cell shown had a movement correlate (turn) in addition to its spatial bias (A). Cells B–E demonstrated a spatial correlate only. Note that the spatial nature of these neurons is not as precise as that observed in hippocampus.

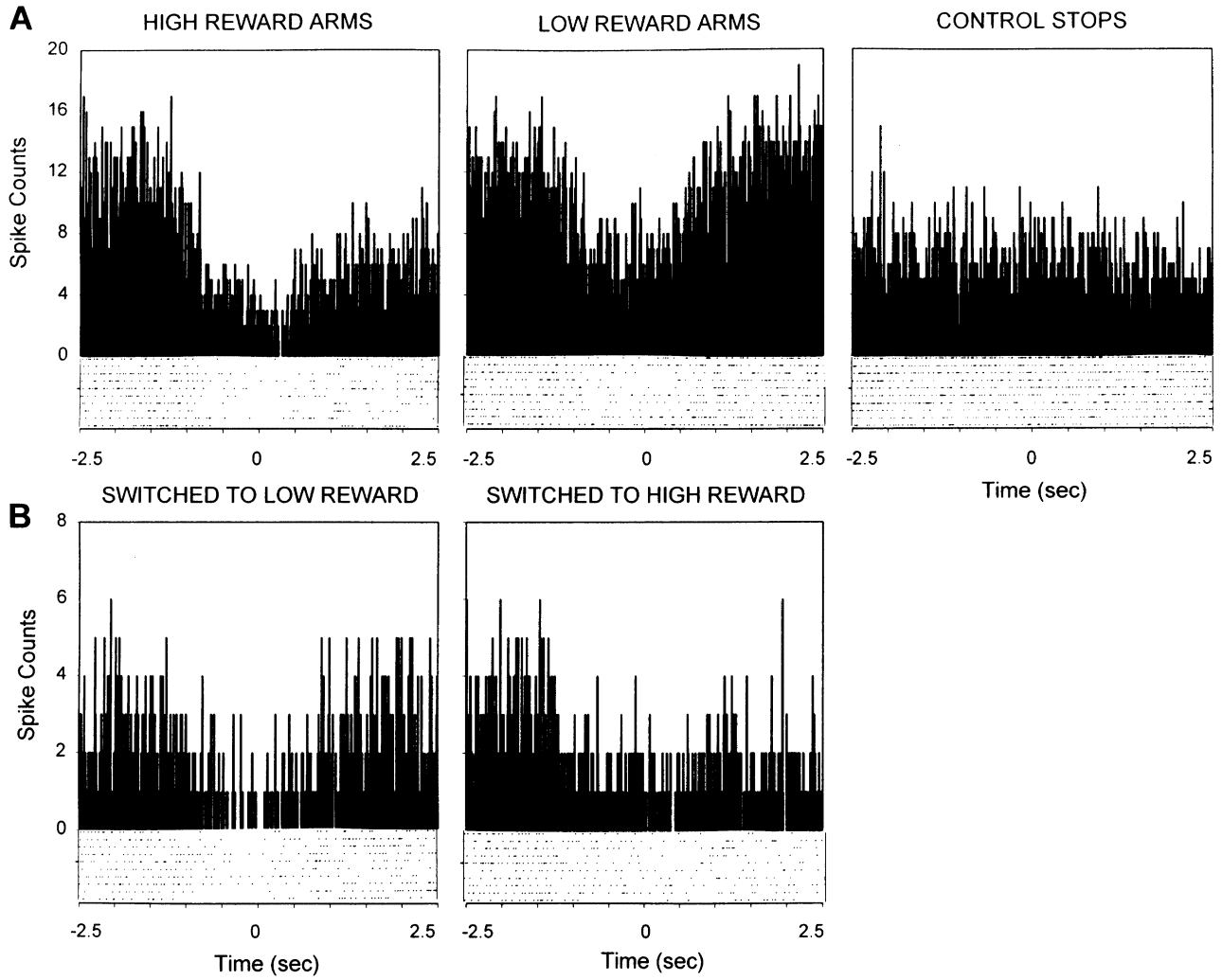

Fig. 5. A differential anticipatory inhibition neuron. Note the more pronounced inhibition in anticipation of reaching the end of the high reward arms when compared to the low reward arms (A). In (B), reward valences were switched between normally high and low reward arms. The stronger inhibition remains significant on the arms, which the rat traditionally received high reward (B, left column), suggesting that this mPFC neuron anticipates different rewards based on previous experience. Additionally, this suggests that anticipatory neurons are not driven by visual/odor qualities of the reward. Scaling differences reflect the higher number of counts from 15 trials ($N = 60$ encounters) for the histograms in (A) versus five trials of data ($N = 20$ encounters) in (B).

spatial neurons both showed an immediate and lasting decrease in their spatial character. As shown in Fig. 9, during the dark phase the spatial fields of these neurons are abolished. Even when lights were turned back on, the two neurons did not show instant or complete recovery across five lit trials. One of these neurons was tested a subsequent day, during which the field returned to its original location. Such slow restoration suggests that it is not strictly visual input per se that is driving these mPFC neurons; rather, past experience with the visual environment may play an important role in the representations that these neurons develop.

Additionally, these two neurons were tested during a phase in which the reward values of the arms were switched (see description above). Both fields remained unaltered, suggesting a functional distinction between spatial and place representations for at least a subpopulation of mPFC neurons. Other neurons with multiple correlates, including one with a reward-related correlate that was restricted to one region of the maze, appear to represent units that integrate information across different information modalities.

3.4.3. Examination of test phase-related activity

Thirty-nine cells were recorded for five trials with a 1-min delay between the study and test phases of each trial. To examine delay-related changes in cellular activity, firing rates were compared between the 1-min delay period and the last minute of the intertrial interval for trials with a delay. A paired t-test showed no tendency for the population to increase or decrease rates due to the delay, $t_{38} = 0.757$, $P > 0.1$. Individual neurons also

showed little evidence of activity changes that were specific to the delay period. Fig. 10 presents one neuron, which did show an elevated discharge at the onset of the delay period; note that a similar increase is also evident during the intertrial interval, a time during which the rat does not have the mnemonic requirement to remember which four arms have been recently visited.

To determine whether reward-related neurons might have neuronal activity specific to either the study phase (the presentation of the first four arms of the trial) or the test phase (once all eight arms are presented) of the task, histograms were generated for arm visits during each phase. These histograms were nearly identical across the two phases for all reward-related neurons. Combined with the data from the delay period, these data do not suggest any phase-dependent activity for rat mPFC neurons during this maze task.

3.5. Histology

Fig. 11 shows the relative locations of the correlated cells that were recorded from during this experiment. Although some similarly correlated cells appeared to cluster together, overall the correlates were generally distributed throughout the entire anterior/posterior extent of the mPFC. This is consistent with a recent report by Jung et al. [20] that has shown that simultaneously recorded neurons in close proximity within mPFC do not appear to reflect common correlates during radial maze or figure-8 track testing paradigms. The apparent lack of correlates in the posterior ventral

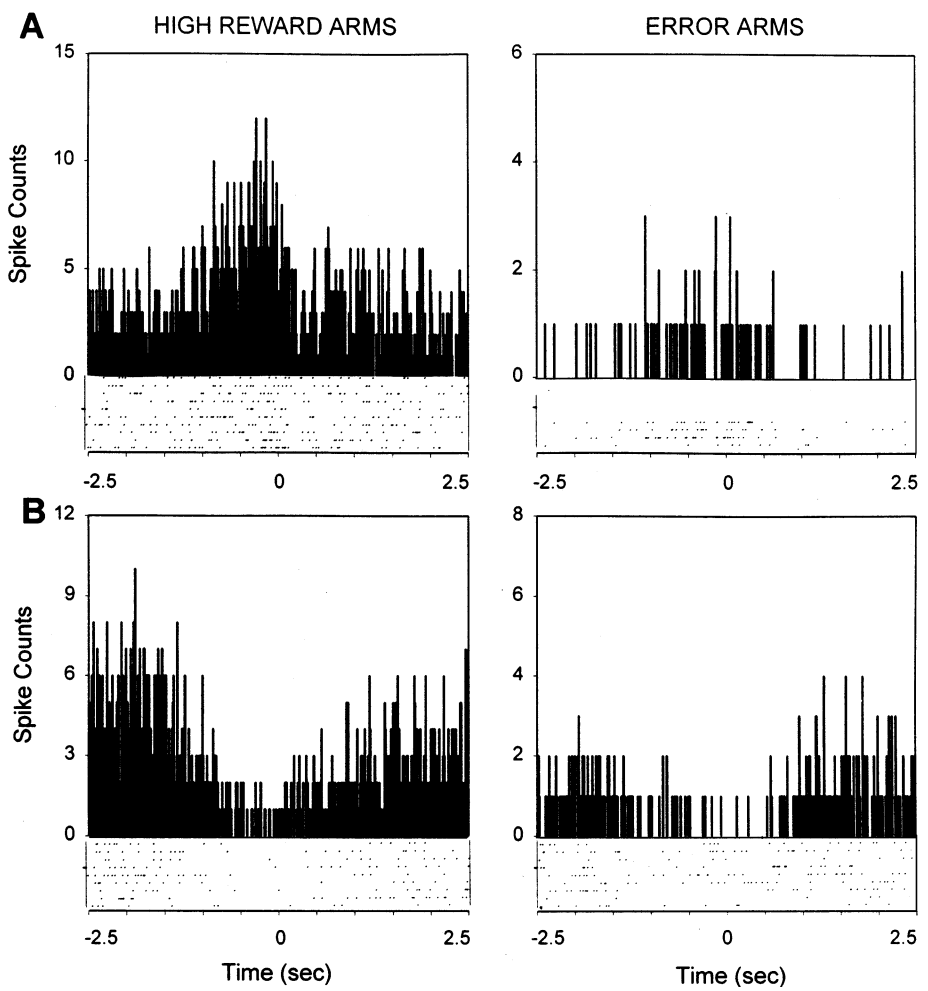

Fig. 6. Examples of anticipatory excitation (A) and anticipatory inhibition (B) neurons that showed similar anticipatory effects when the rat performed errors, at which time no reward was present. This further suggests the use of spatial cues to anticipate the presence of reward by these cells, rather than sensory qualities of the reward itself. PETHs for errors are based on four and 11 error occurrences for (A) and (B), respectively.

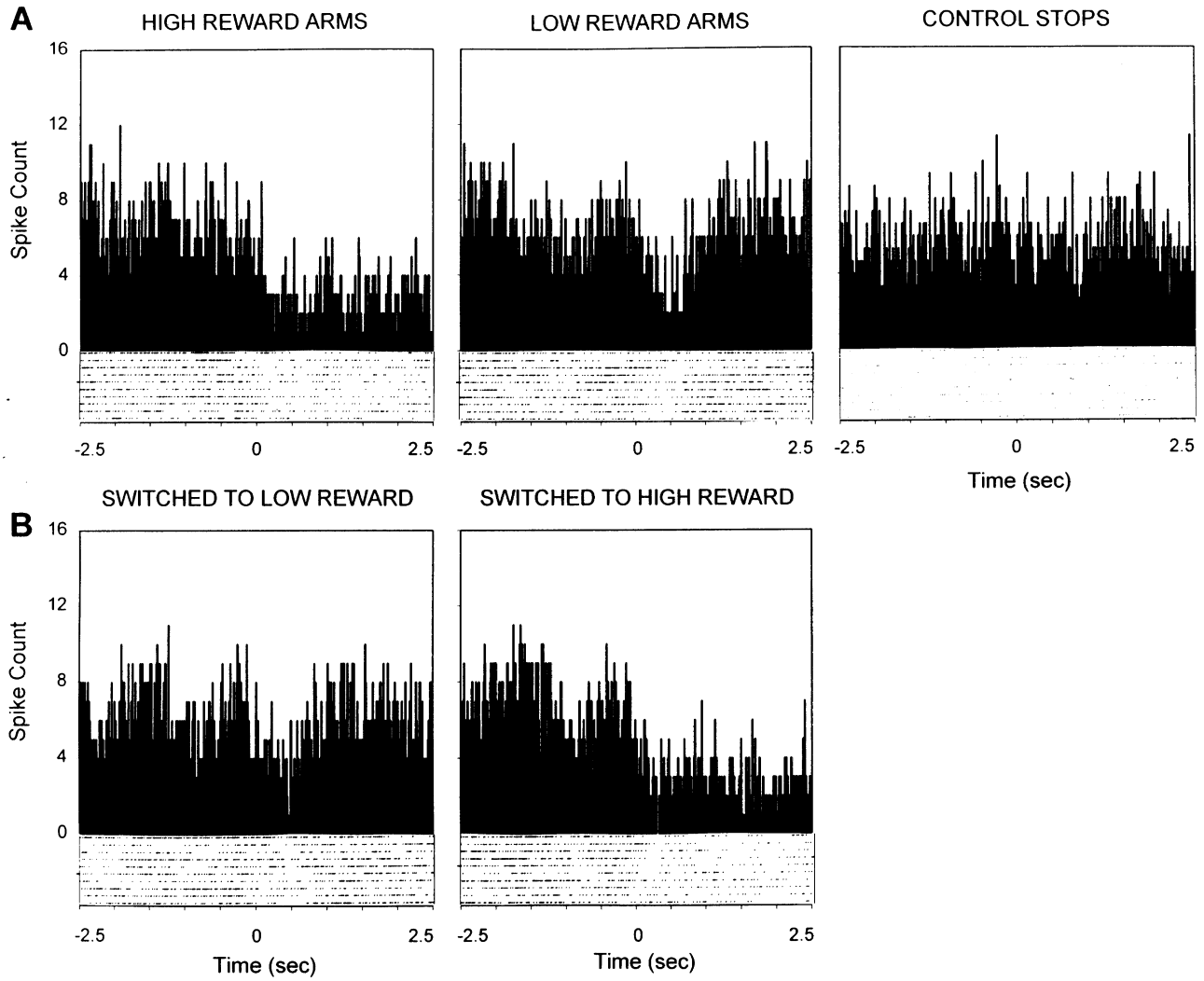

Fig. 7. An example of the reward-dependent nature of the consummatory effect in an inhibition at encounter neuron. The duration of inhibition was greatest on the high reward arms (A). When the level of reward was switched between high and low reward arms on the maze, the effect followed the reward, exhibiting prolonged inhibition on arms that traditionally held low reward (right column, B). When the reward was switched back to the usual arms, the greater response of the neuron again followed the reward (not shown). All histograms in this figure are based on 20 visits to each reward arm type (5 win-shift trials for each reward condition).

regions of the IL cortex in this study may be explained by a relatively low sample of neurons recorded from this region ($N = 7$).

4. Discussion

This experiment sought to define characteristics of mPFC unit firing in relation to the acquisition of differential goal values within the current spatial task. Accordingly, the spatial memory task adopted for this experiment differed from most experiments using win-shift behavior on an eight arm radial maze by including different amounts of reward at predictable locations on the maze. Rats learned the locations of high reward arms, as evidenced by a preference to visit high reward locations during the initial choices of the memory phase of the task. Consistent with Jung et al.'s [21] previous study on rat mPFC neurons, we found neurons that changed firing rate as a result of movement and of approaching or encountering a reward. Additionally, a small number of neurons were found to have a spatial bias.

The most common behavioral correlate of PL and IL neurons was reward-related firing, the majority of which showed changes in firing prior to the acquisition of the reward (Table 1). Anticipatory changes in neuronal firing were not dependent upon the presence of visual information or odor cues associated with the reward itself, as many cells showed similar changes in firing rate when the rat made errors on the arms. Such firing also did not reflect cessation of forward movement since it was not observed when rats paused at non-rewarded locations. Two anticipatory reward cells demonstrated differential firing rates between the high and low reward arms in this task, suggesting that some mPFC neurons discern differ-

entially rewarded locations. Furthermore, one neuron maintained its differential pre-encounter firing when presented with probe trials during which the reward valence of the arms was reversed. This suggests that some mPFC neurons fire in anticipation of specific locations in which particular rewards are expected to be.

Six neurons showed different firing rates after reward was acquired. In most instances, neurons with firing rate changes that began prior to reward consumption continued with the altered rate through the consummatory phase of the behavior. Relatively few cells ($N = 5$) were recorded that initiated firing changes only after the reward was encountered. Post-encounter firing rate changes appeared to depend on reward presence; for most reward neurons the length of the post-encounter effect was shown to be dependent upon the reward amount present. One neuron that inhibited prior to and during normal chocolate milk rewards demonstrated excitatory bursts upon encountering a novel reward, suggesting that the mPFC may distinguish not only different amounts of the same reward but also qualitatively different rewards. The ability of prefrontal units to distinguish between differential reward amounts is consistent with a report by Shapiro et al. [66] that showed differential firing for mPFC neurons to various levels of lateral hypothalamic stimulation (presumably corresponding to different levels of brain stimulation reward). The present report is the first that we are aware of to characterize differential activity for reward in a rat performing a spatial memory task.

Similar to the results reported by Poucet [52] and Jung et al. [21], we recorded few location-specific neurons in this study. Four were determined to be spatially selective, and two additional neurons showed a spatial bias in addition to a movement (turn) or reward-related correlate. One spatial neuron and one spatial/movement

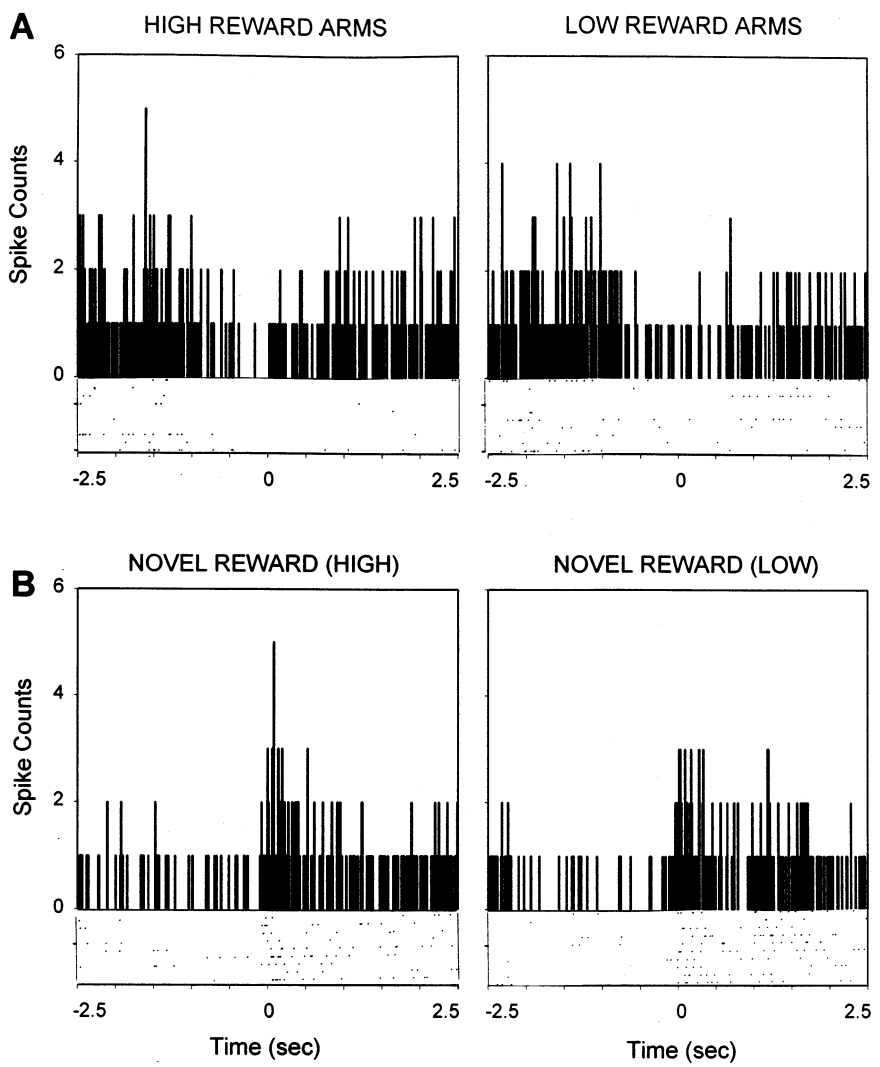

Fig. 8. A cell that showed anticipatory inhibition to chocolate milk reward (A) displayed excitatory activity when presented with a novel, strawberry flavor (B). Such activity suggests that mPFC neurons are capable of distinguishing between different rewards. All histograms in this figure are based on 20 visits (5 win-shift trials) to each reward arm type in each condition.

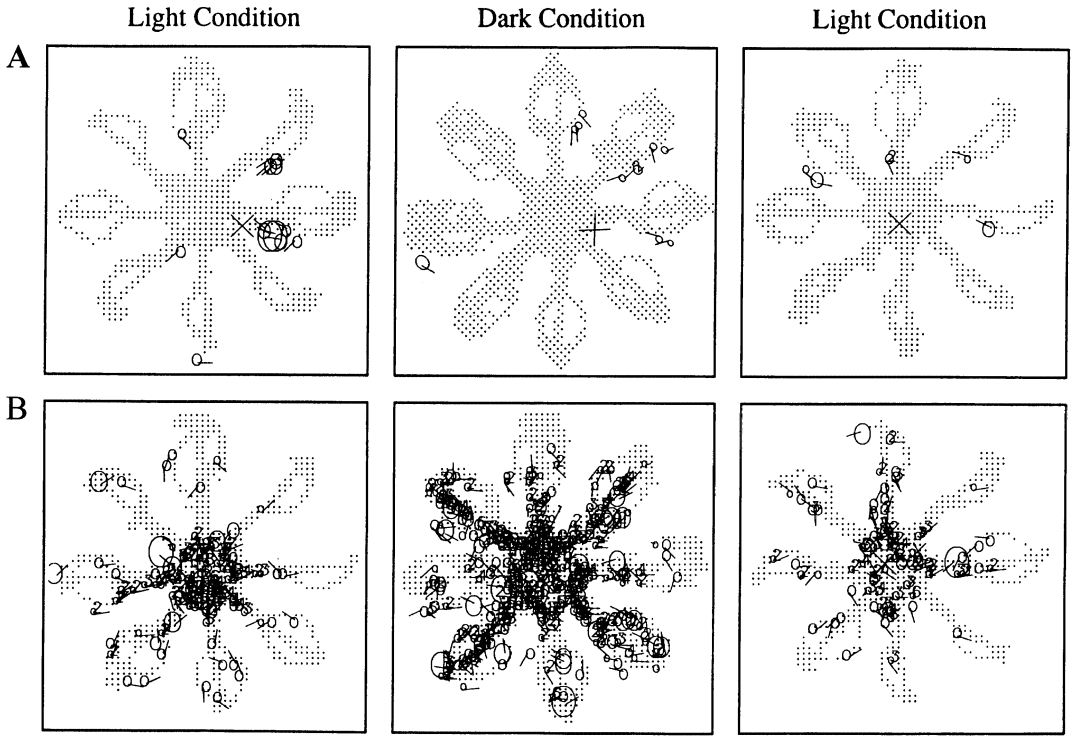

Fig. 9. Spot diagrams for the two spatial neurons shown in Fig. 3(A) and (B) on a subsequent testing day. Both neurons displayed a spatial bias during the initial light trials, but when room lights were turned off, the bias was attenuated. Restoring the light did not restore the field. Cell A was re-tested the following day, at which time the field was restored. This suggests that mPFC spatial responses are modulated by visual experience.

neuron that was stable across several days demonstrated consistent location specificity across multiple recording sessions. This contrasts with cells recorded by Poucet [52], which did not fire in consistent locations across days, and that were subsequently determined to be movement related. The spatial nature of the location-specific cells in this study was dependent on visual experience within the maze environment. The place fields of both neurons were abolished when lights were turned off and remained disrupted even once lights were turned back on. These results differ from recordings of hippocampal place fields, which typically either remain in the dark or re-establish themselves immediately once light is restored [31,40]. The more prolonged attenuation of place fields in mPFC following loss of visual input and its restoration may suggest that the mPFC does not encode a strictly visual sensory-based spatial representation of the environment. Rather, the IL and PL may base spatial representations upon past expectations regarding the environment.

The existence of multiple correlate cells ($N = 6$, 9.8%) within the mPFC indicate that some neurons within the mPFC integrate across spatial, reward, and movement sources of information. This is strikingly similar to recordings from a small number of multiple-correlate neurons from the mVS, which receives projections from these areas [29]. This report is not the first to demonstrate multiple correlates in mPFC neurons. Among the relatively sparse literature with rat mPFC recording during naturally-motivated tasks, a recent study by Gill et al. [12] has shown that PL neurons from rat prefrontal cortex respond to reward as well as movement during an attentional task. However, a previous study by Jung et al. [21] in a spatial maze task similar to the one used in this experiment was unclear regarding whether multiple correlate cells in their sample were of the same modality (e.g., multiple movement correlates) or cross-modal (e.g., movement and goal-related). The presence of cross-modal integration within the mPFC may serve to provide the basis for planning movements to spatial locations, a function that has been attributed to the mPFC by several researchers.

Current speculation about the cognitive function of the prefrontal cortex is diverse. Specifically, behavioral impairments caused by prefrontal lesions have been suggested to be due to impairments in memory for temporal organization [9,22,24,26], attentional deficits [15,50], impairments in strategy switching [56], impairments in working memory [13,14], or disruption of rule-based processes [23]. It should be noted that none of these possibilities are mutually exclusive, and some current views of rat mPFC suggest that it plays a role in more than one of these operations [16,55]. Although the current experiment was not designed to specifically

address the cognitive constructs that such arguments represent, the present study did fail to uncover units that responded to specific mnemonic phases present within the task. None of the 39 units tested with a 1 min delay demonstrated changes in firing rate that were consistent with holding a memory trace across the delay. In addition, no reward unit showed differential activity between the study or test phases of the task. Thus, individual neurons in the rat PL and IL prefrontal cortex do not show direct evidence of contributing to working memory representations across delays in this spatial working memory task, at least as examined by the current analyses. Although this may appear to be in contrast with the obvious presence of delay-type neurons within dorsolateral prefrontal cortex of the monkey, at least two caveats should be made regarding such an interpretation. First, the nature of the current spatial working memory task is more complex than the delayed matching tasks utilized in the monkey literature in that animals are required to hold more than one location in memory at a given time to successfully complete the task. In this manner, the data are not directly comparable. One report that has shown delay-dependant activity of mPFC neurons in rats utilized a delayed match to position task in a Y-maze [3], which more closely approximates the typical behavioral tasks shown to elicit delay-related activity in the monkey dorsolateral prefrontal cortex [13,14]. Additionally, it is possible that individual IL or PL neurons could represent specific sequences of visited arms, and hold such sequences in memory only during trials when that sequence constituted the study phase of the task. Given our random presentation of only five such sequences for any given neuron, our analyses would not be sensitive to neurons that show differential activity to such conditions. The current data, based on a relatively small sample of tested neurons, cannot definitively exclude the rat mPFC from delay- or memory phase-related unit activity during spatial working memory.

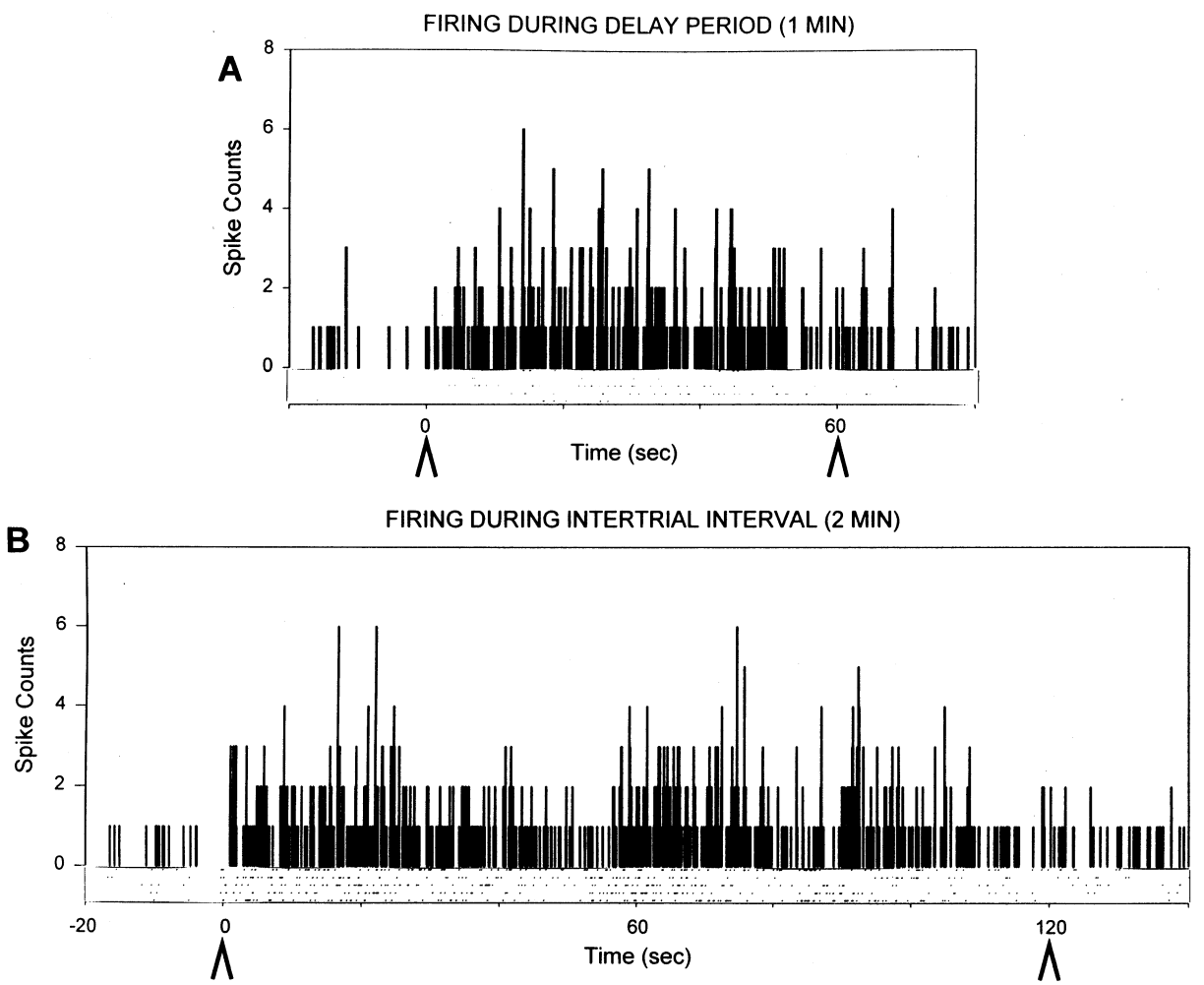

Fig. 10. One neuron that displayed increased activity during the delay period (A). Note, however, that a similar increase was observed during the 2-min intertrial interval (B). This suggests that the increased firing during the delay period was not due to the specific working memory demands of the task. Both histograms represent data from five instances when the rat was confined to the center (either during delay periods or intertrial intervals).

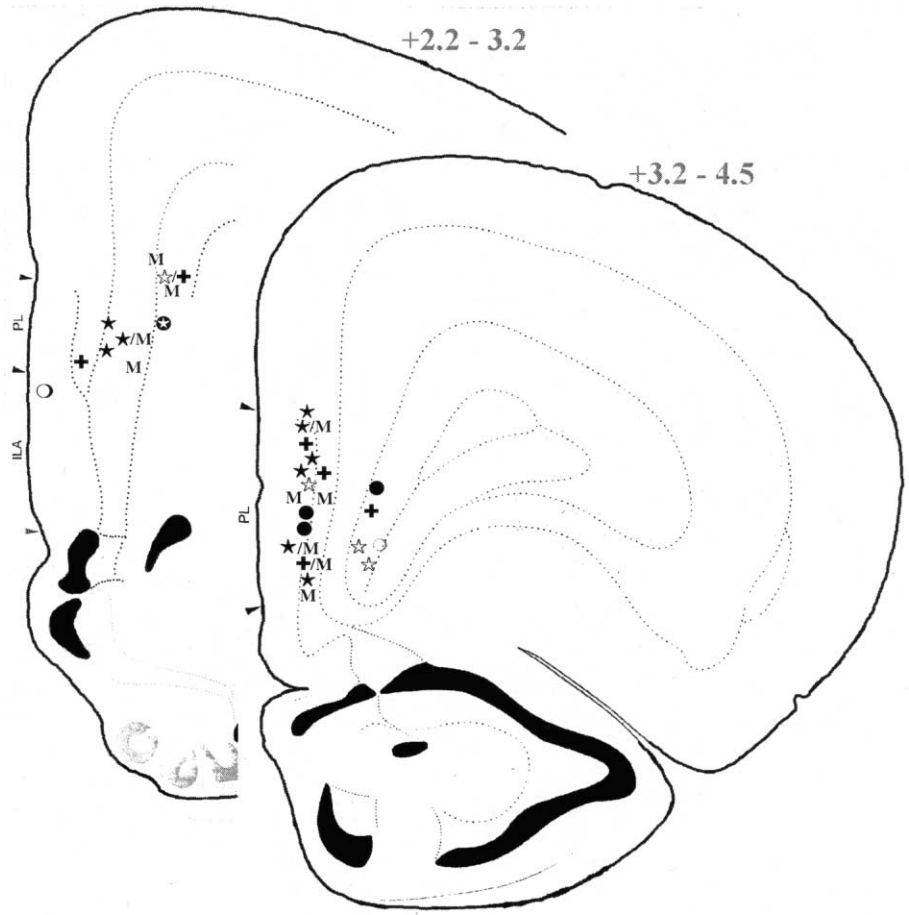

Fig. 11. Location of correlated neurons within the mPFC. Placement measurements are in millimeters anterior to bregma. Overall, correlates were spread throughout the extent of this region. Legend for symbols: (★) Anticipatory inhibition; (☆) Anticipatory excitation; (●) Inhibition at encounter; (○) Excitation at encounter; (+) Spatial; (✪) Biphasic; M Movement. Symbols divided by a slash (/) denote placement of multiple correlate neurons. This figure adapted from Swanson [71].

However, these data are consistent with behavioral evidence that, although the integrity of the rat mPFC is necessary for completion of a win-shift task after a 30-min delay, mPFC activation during the study phase and across the entire delay is not critical [63]. Furthermore, Floresco et al. [6] have shown that it is the interconnection between the PL and the ventral subiculum that mediates normal post-delay performance. Therefore, it is likely that the working memory representations important for this active navigation task are achieved by interactions between at least the mPFC and hippocampus, and may be undetectable by individual unit recordings from any one structure.

The present data suggest that an important property of PL and IL neurons during performance of this spatial memory task is the representation of predictable rewards within a complex environment. Such an interpretation is consistent with reports that mPFC lesions impair the ability of a rat to learn predictable locations of reinforcement (Section 1). The eventual learning of many spatial tasks by mPFC lesioned animals may be mediated by a network of other brain regions also capable of making reward associations. For example, the ventral striatum also receives input from other structures (e.g., the amygdala, see Section 4) that encode cues that predict rewards. However, mPFC lesioned animals show consistent impairments when challenged in tasks where the goal location is varied, suggesting that this region is particularly important when goal locations must be learned quickly [11,53]. In the intact animal, successful learning and recall of place-reward associations is likely to recruit processing within the mPFC. Rats are impaired during reversible inactivation of the mPFC after a 30 min delay on the eight arm radial maze [63]. Inactivation of the mPFC may impair performance during the test phase due to an inability of the rat to predict locations of rewards within a familiar spatial environment. This is entirely compatible with the view that the mPFC is important for prospective planning, as has been suggested by Seamans, Floresco, and Phillips [64]. It is also congruent with other theories of mPFC function, as loss of input regarding reward expectations could be expected to disrupt the function of other systems that depend on

such knowledge (e.g., those of working memory, attention, etc., see above). Furthermore, other regions of prefrontal cortex also encode reward expectancy information. Recordings from orbitofrontal cortex during olfactory discriminations show that sulcal prefrontal neurons are capable of predicting consequences of learned odors [61]. The prefrontal cortex as a whole may serve to predict modality-specific reinforcement contingencies of the external environment.

4.1. The mPFC as part of a spatial context dependent motivation system

In addition to the current recordings from the mPFC, we have previously recorded from the mVS and BLA using the identical task [29,54]. Interesting comparisons can be made across these three studies. As predicted, neurons within both the mPFC and the BLA show reward-related correlates that could relay information to mVS neurons. Reward-related activity was the predominant correlate for both mPFC and BLA, with 31.1% and 30.7% of all neurons recorded expressing goal-related firing, respectively. These two regions had relatively high numbers of cells that fired differentially to high and low rewards (mPFC, 31.6%; BLA, 53.6% of reward-related cells). The mPFC contained the highest percentage of anticipatory reward neurons of any of these three regions (73.7% of reward neurons recorded; BLA, 60.7%, mVS, 43.8%). Although the comparison of individual studies does not allow for a direct test of information flow, these data are consistent with the hypothesis that inputs from the BLA and mPFC influence the smaller number of reward-related neurons within the mVS (15.5% of mVS cells showed reward correlates, 12.5% of these displayed differential activity to reward value).

Despite connections from hippocampal regions to all three structures, the mVS shows the most evidence of location-dependant (18.4% of cells) firing than either the BLA (7.7%) or the mPFC (9.8%). Both the mPFC and the mVS showed a more substantial number of movement-related neurons (16.4% and 20.3%, respectively) than the BLA (2.2%). This is consistent with the proposed functions of these regions for movement preparation. Finally, all three regions had small numbers of neurons that integrated across at least two of the reward, spatial, and movement modalities. This may suggest a functional, as well as anatomical, connection across these three brain areas during the performance of this task.

These data, in addition to experiments published from other laboratories, support our hypothesis that two possible inputs of reward information for the mVS are the BLA and mPFC. Specifically, we suggest that during this task, output from the BLA provide information regarding cues that predict reward, while mPFC predicts reward location based on spatial contingencies in the environment. This motivation-relevant information from the BLA and mPFC is then combined with spatially relevant hippocampal inputs within the mVS. Previously, we have suggested that a possible role for the mVS, and especially the nucleus accumbens, is to monitor the effectiveness of behavioral responses, particularly when the rat is exposed to new spatial contexts [39]. Changes in spatial context could be relayed via subiculum inputs to the mVS, while alterations in reward placement or contingencies could be signaled by mPFC and BLA inputs. In particular, the current experiment suggests an important role for the mPFC in the processing of reward and expected reward outcomes in rats performing goal-oriented spatial navigation.

Acknowledgements

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