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Research report

Tagging items in spatial working memory: A unit-recording study in the rat medial prefrontal cortex

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ABSTRACT

The rat medial prefrontal cortex has been suggested to be involved in executive functions and, more specifically, in working memory and response selection. Here, we looked for prefrontal neural correlates as rats performed a modified radial arm maze task that taxed such functions. Rats had to learn the position of four rewarded arms among eight, and visit each rewarded arm only once, thus avoiding repeated visits. In addition, rats were left on the maze after the four successful visits to baited arms until they had visited all the arms twice. Prefrontal neural activity was examined during choice periods, i.e. 2 s before the rat entered the arms. We found that a substantial proportion of recorded medial prefrontal neurons were selectively activated before either the first or second visit to the arms irrespective of their reward status, thereby tagging already visited arms. These behavioral correlates show that, within the rodent medial prefrontal cortex, neuronal populations demonstrate behavioral correlates suggestive of its role in guiding prospective search behavior and thus executive functions.

1. Introduction

Executive functions refer to a set of cognitive processes that are required to perform flexible and voluntary goal-directed behaviors based on stored information in accordance with the context [30]. Within these processes, working memory is a register that temporally organizes and stores information so that it can be internally manipulated and ultimately guide response selection. Although executive functions require the prefrontal cortex in both human and non-human primates [1,8], much less is known about their anatomical substrate in the rat. Recent evidence, however, points to the medial prefrontal cortex (mPFC) as a candidate structure [27]. Although lesion evidence suggests a critical involvement of mPFC in executive functions [10], the neural and cellular underpinnings of these functions are still poorly understood [33].

One of the most widely used tasks to assess executive functions and working memory in the rat is the eight-arm radial maze, which requires the animal to visit each maze arm only once [19]. Given the strong working memory component and the complex spatial response selection mechanisms necessary to solve this task, one would expect that neurons in the mPFC modulate their activity as the rat performs the task, i.e., as the number of items (arms) stored in the working memory buffer gradually increases. Contrasting with this prediction, however, Jung et al. [16] found little evidence that mPFC neurons discharge across successive arm visits. Rather, prefrontal neuronal activity was correlated with specific behaviors or multiple components of the task (e.g. approaching or leaving the goal, going to the centre, etc.; see also [9,12,16,22]).

In order to investigate more thoroughly the prefrontal representation during a working memory task, the present study employed a modified version of the eight-arm maze, in which only four arms were rewarded. Furthermore, after successful completion of visits to the baited arms, the rat was left in the maze until it had visited each arm twice, thus allowing us to examine experience-dependent firing modulations. We reasoned that working memory of visited arms during task performance was essential when the rat actually had to select the arm to be visited next. Accordingly we focused our analysis of single-unit discharge modulations during arm choice periods. This procedure made it possible to identify a neural representation of the rewarding properties of arms, but most importantly, it also allowed us to examine whether mPFC cell discharge carries a temporal memory code, i.e. varies across successive arm visits within a trial. Our findings strongly suggest that, during decision making in the radial maze task, medial prefrontal neurons exhibit discharge correlates indicating that specific arms are tagged according to both previous choices and expectancies.

2. Materials and methods

Many of the procedures used here are described elsewhere. For further details, the reader is referred to Hok et al. [12]. All procedures complied with both European

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(European Community Council Directive 86/609/EEC) and French (Council directive no. 87848, permission no. 13.76 to BP) institutional guidelines.

2.1. Subjects

Four Long Evans black hooded male rats (R. Janvier, St.-Berthevin, France) weighing 300–350 g were housed one per cage (40 cm × 26 cm × 16 cm in height) at 20 ± 2°, under a 12 h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.). They had free access to water and were food deprived to 85% of their ad lib body weight.

2.2. Apparatus

The apparatus consisted of an elevated (50 cm above the floor) radial maze constructed from stainless steel. The maze comprised an octagonal central platform (23 cm on each side) and eight arms (50 cm in length, 9 cm in width) radiating from the centre, and bounded by 2-cm raised edges. The maze was placed in an experimental room (3 m × 3 m) providing numerous environmental cues. No attempt was made to attenuate such cues. A food cups was placed 1 cm from the distal portion of each arm. Each cup had a 3 cm high edge that prevented the rat from seeing its content until it had reached the arm end. Only four out of the eight cups were baited with three 45 mg sucrose odorless pellets. The four correct arms were randomly chosen for each subject from a set of eight possible patterns [23]. A typical pattern of baited arms was 1-3-5-6, and other patterns obtained by rotation of the typical pattern (e.g., 2-4-6-7 or 3-5-7-8). For a given rat, the pattern of baited arms remained consistent during the whole experiment.

2.3. Pre-operative training

Preliminary training consisted of handling the animals 10 min/day for 10 days. During this period, they were food deprived to reduce their body weight to 85% of their initial weight, and accustomed to eat 45-mg sucrose pellets. Each rat was then placed on the maze with sucrose pellets spread over the central platform and arms of the maze, and was allowed to freely move and eat on the maze for five 10-min daily sessions.

During pre-operative training, each rat was given two trials per day with an intertrial interval of about 90 min. Before each trial, the four "positive" cups were baited with three 45-mg pellets. A trial consisted of placing the animal in the maze where it remained until (1) all four reinforcements had been collected, (2) 16 choices had been made, or (3) 5 min had elapsed, whichever occurred first. Detailed records were taken of the arms visited and total running times. The criterion for entering an arm was the animal placing its four paws beyond a line 5 cm from the central platform. This distance was chosen as rats were never observed to return to the central platform beyond it. Training continued for 6 weeks (72 trials) before animals were surgically implanted with an electrode headstage.

2.4. Electrode implantation

At the end of training, surgery to implant a driveable bundle of 16 formvar-insulated 25-μm nichrome electrodes [17] was completed under general anesthesia (ketamine/xylazine, 0.88 ml/kg i.m.). The central tip of the electrode bundle was implanted above the prelimbic area of the medial prefrontal cortex (3.5 mm A, 0.5 mm L to Bregma, 2.5 mm DV to dura) [20].

Upon completion of the experiment, the final position of the electrode array was marked by passing anodal current (15 μA for 30 s) through one of the wires. Under deep anesthesia, the rats were perfused transcardially with saline followed by 10% formalin, and their brains were removed, marked by the Prussian blue reaction, sectioned at 40-μm intervals, and stained with cresyl violet for verification of electrode placements.

2.5. Recording methods

Screening and recording were done with a cable attached at one end to a commutator that allowed the rat to turn freely. The other end of the cable was connected to a light emitting diode (LED), a headstage with a unity gain operational amplifier (op-amp) for each wire and finally a connector that mated with the rat's electrode connector. The LED, which was used for tracking the rat's head position, was positioned on the midline about 1 cm above the head and somewhat forward of the rat's eyes. The op-amps were used to rectify signals before they were led to the commutator via the cable. The fixed side of the commutator was connected to a distribution panel. From the panel, the desired signals were further amplified (gain: 10,000), band-pass filtered (0.3–10 kHz), digitized (32 kHz) to be stored by a Datawave Discovery system (Longmont, CO). Before a recording session, spike discharges were separated using Datawave on-line clustering software to simplify later off-line separation. The LED, which was tracked with an overhead TV camera connected to a digital spot follower, was detected in a grid of square regions (pixels), permitting a resolution of 2.5 cm for head position.

2.6. Post-operative trials

Commencing 1 week after surgery, the rat underwent post-operative recovery trials, in which testing was conducted as during pre-operative training. Simultaneously, the activity from each microwire was screened daily. If no waveform of sufficient amplitude (>100 μV, i.e. three times larger than background noise of ca. 30 μV) was found, the electrodes were lowered 25–50 μm after screening sessions to look for action potentials sufficiently reliable to be successfully recorded as the rat performed the radial maze task.

Once a unit, or set of units, was isolated, it was recorded for several recording trials. The single modification brought in the course of recording trials compared to post-operative recovery trials was that, instead of being removed after successful completion of visits to the baited set of arms, the rat was left in the apparatus until it had visited *all* arms of the maze *at least twice* or after 15 min had elapsed, whichever occurred first. Thus, a recording trial involved at least 16 arm visits (two visits to each of the four-baited arms and two visits to each of the four non-baited arms), and two rounds of visits were defined for each arm separately. While arms in the baited set contained a food reward on the first round of visits, no food reward was available on the second round since food cups were not replenished in the course of a recording trial. In contrast, arms in the non-baited set never contained food rewards. An error was scored each time the rat repeated a visit to a given arm before all arms were visited within a given round of visits. When possible, rats were subjected to several (≤3) recording trials within the same day (between-trial interval > 1 h). During recording trials, in addition to electrophysiological unit activity and position data that were automatically collected, several events were time-stamped by the experimenter by pressing appropriate keys on the computer keyboard. Such events included entry to each arm, reaching arm end, and returning to the central platform. Arm entries were used to count correct choices and errors (returning to a previously visited arm within a given visit round). Furthermore, although the four-baited arm version of the radial maze does not encourage algorithmic responding, it is necessary to discard such responding as an explanation of performance. Therefore, the order of successive arm entries within a trial was further analyzed to determine if rats had developed a stereotyped strategy across trials (e.g., always starting the trial by visiting the same arm and then visiting remaining arms in the same order over successive trials; rotated versions of such regular patterns were also examined).

2.7. Electrophysiological analyses

The first step in off-line analyses was to refine boundaries for waveform clusters that were defined before recording. Candidate waveforms were discriminated using Datawave sorting software, which allows separating waveforms based on at most eight characteristic features including spike amplitude, spike duration, maximum and minimum spike voltage, time of occurrence of maximum and minimum spike voltages, voltage at experimenter-defined points of the waveforms. Waveforms were then processed with Plexon offline sorter (Dallas, TX), which permits additional refinement of cluster boundaries and provide autocorrelation functions. Inter-spike interval histograms were built for each unit and the whole unit was removed from analysis if the autocorrelogram revealed the existence of inter-spike intervals <2 ms (refractory period), inconsistent with good isolation.

Once single units were well separated, firing rate maps were constructed at a pixel resolution of 2 cm according to the auto-scaling scheme of Muller et al. [18]. The pixels with non-zero spike rates are ordered by rate and divided into intervals such that each interval contains 4/5ths the number of pixels as the one before with the smallest interval representing pixels of the highest rate. In order of increasing rate, pixels from consecutive intervals are colored orange, red, green, blue and purple. Zero rate pixels are yellow.

Raster plots of firing activity were made for the period from −4 s to 0 s (500 ms-bins) before arm entry by using the event flags entered by the experimenter each time the rat entered a maze arm. Within this time window, the period extending from −4 s to −2 s before arm entry was considered as the baseline, while the period extending from −2 s to arm entry was the period of interest as the rat likely made its choice at this time. These raster plots were accumulated to produce corresponding peri-event time histograms (PETH) with Neuroexplorer (Plexon). To obtain more reliable estimates of activity changes, multiple trials for a single cell were cumulated whenever possible. Four PETHs were built for each cell to compare unit activity during entries in baited arms during either the first or the second round of visits, and during entries to non-baited arms during either the first or the second round of visits. Significance of changes in firing rates was assessed by looking for 500 ms bins deviating either positively (activation) or negatively (inhibition) from the baseline in each normalized PETH [32]. Each peri-event bin was expressed as a *z* score based on the mean and SD of the firing rate during the respective basal period of this event. Bins with *z* scores >1.64 SDs (95% confidence interval) away from the baseline mean firing rate were considered a significant response.

3. Results

3.1. Behavior

Successful unit recordings from mPFC neurons were obtained from four rats performing the task reliably in 40 recording sessions.

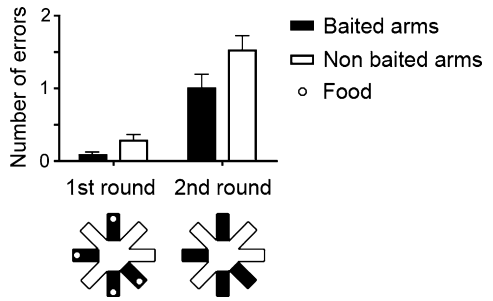

Fig. 1. Error scores across the two rounds of visits. Data are shown as means ± S.E.M. Rats made fewer errors for baited arms than for non-baited arms on each round ($p < 0.05$). Furthermore, the number of errors to each arm set increased considerably from the first to the second round ($p \ll 0.001$ both for baited and non-baited arms).

In the first round of visits, rats consistently visited the baited arms first, and only then visited the non-baited arms. Rats made very few errors (i.e. repeated arm entries) and did not use algorithmic responding (i.e. there was no stereotypic organization of visit order within either the baited or the non-baited set; see Section 2). In the second round, exhaustive exploration of the maze was resumed, although visits to the baited and non-baited arms were less orderly organized and rats made more errors. Overall the data indicate that rats optimized their performance during the first round of visits, but much less so during the second round.

This general description of the behavior was confirmed by several analyses. In the first round of arm visits, rats consistently visited the baited arms before the non-baited arms, as shown by the number of visits to baited arms in the first four choices (3.05 ± 0.10), which was far greater than expected by chance ($t(39) = 10.4$, $p \ll 0.001$; student's t test). In the second round, the animals also tended to visit the (previously) baited arms before the non-baited arms in the first four choices [2.37 ± 0.16; $t(39) = 2.36$, $p < 0.05$], a trend however less pronounced than in the first round [$t(39) = 3.54$, $p < 0.01$], showing less well organized choice behavior. This finding suggests that the rats' strategy was different on the two rounds of visits, which therefore were well discriminated by the animals. This hypothesis was further supported by the observation that mean durations of arm visits were shorter in the first round than in the second round [1st round: 12.4 ± 5.4 s per arm; 2nd round: 22.3 ± 10.7 per arm; $t(39) = 7.72$, $p \ll 0.001$].

The number of errors for each arm set and visit round is displayed in Fig. 1. Rats made less errors (i.e. repeated arm entries) for baited arms than for non-baited arms on each round [1st round: $t(39) = 2.24$, $p < 0.05$; 2nd round: $t(39) = 2.59$, $p < 0.05$]. Furthermore, the number of errors to each arm set increased considerably from the first to the second round [baited arm set: $t(39) = 4.54$, $p \ll 0.001$; non-baited arm set: $t(39) = 5.34$, $p \ll 0.001$]. These variations in working memory performance grossly parallel the organization of choice behavior described above. Although memory load increased as the rat makes more arm visits, the near optimal performance in the first round and clearly non-linear increase of errors in the second round suggests that rats in the second round knew that all arms already had been visited. Since food was no longer available, they simply explored the maze, thereby demonstrating their memory of previous choices.

3.2. Electrophysiological

We recorded 131 neurons in 40 behavioral sessions. Putative locations of recorded units were reconstructed based on the initial electrode placement and experimenter's daily record of electrode number and depth. Fig. 2 shows the area in which neurons were recorded. With two exceptional cells recorded in the ventral part of the dorsal anterior cingulate cortex, all cells were

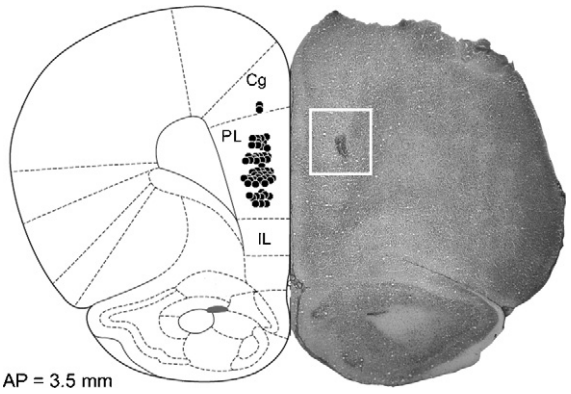

Fig. 2. Coronal representation of the brain showing recording locations within the mPFC areas. Most recorded neurons were located in the prelimbic area. The microphotograph shows the marked location of electrode tip in one rat. Abbreviations—Cg: cingulate area; IL: infralimbic area; PL: prelimbic area. From [20].

from the prelimbic area. Within this area, there was no clear relationship between the anatomical locus of cells and the responses described below. The mean discharge rate of the recorded neurons was 4.46 ± 0.96 Hz, slightly below that reported by Poucet [22] in an open-field (7.99 Hz), above that reported by Jung et al. [16] in a radial maze (2.21 Hz) and comparable to that reported by Pratt and Mizumori [24] also in a radial maze (5.96 Hz). Based on their electrophysiological characteristics, most recorded units (75%) were pyramidal neurons with long duration negative phases

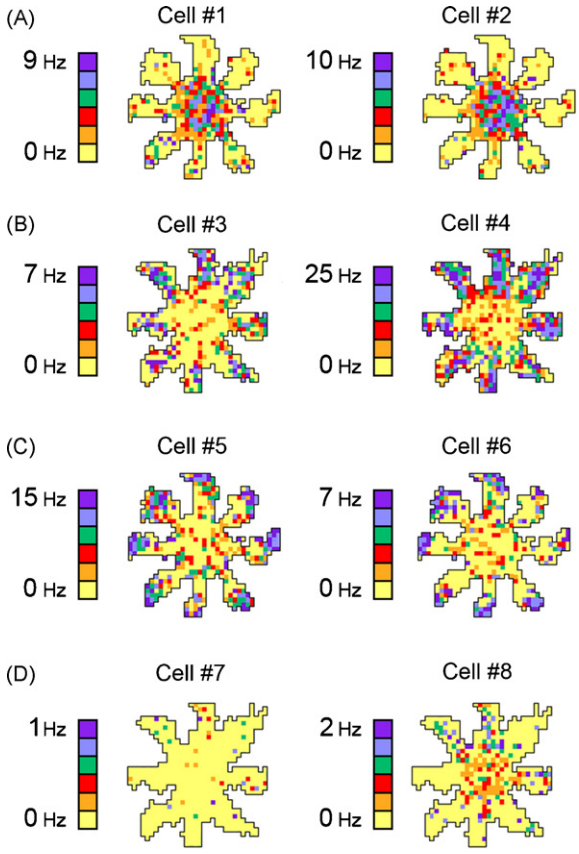

Fig. 3. Firing rate maps of representative cells firing in the centre platform of the radial maze (A), along the maze arms (B), at the end of the maze arms (C), or showing no strong spatial selectivity (D). Each firing rate map was built using the data from the entire recording session. In all maps, a bar shows the color code for the pixels in the map. Yellow indicates no firing, and purple indicates maximum firing. Orange, red, green and blue indicate intermediary firing from low to high.

(>0.35 ms). 0nly a minority of neurons (8%) were clearly identified as interneurons (waveform negative phase <0.3 ms), with the remaining 17% cells being more ambiguous (negative phases in the range 0.3–0.35 ms). The very small proportion of recorded interneurons precluded any further analysis of response type as a function of cell type.

As reported in previous work [16,24], many neurons fired either prior to arm choice while the rat was on the central platform, after arm choice (i.e., on the arms themselves) or at the arm ends (see spatial firing rate maps in Fig. 3). Since we were interested in how cell activity was modulated at choice time, we focused our analysis on the changes in unit activity occurring before arm entries, as it is most likely during this period that the rat made a prospective choice about which arm to choose next. Although firing modulations at the central choice point were easily observable in the spatial firing rate maps (see cells 1 and 2 in Fig. 3A), this representation was not appropriate to reveal if they were selective to specific arm choices. Peri-event time histograms (PETHs) were used to derive this information.

To build the PETHs, the time interval from −4 s to 0 s (500 ms-bins) before each arm entry was divided into two periods, from −4 s to −2 s and from −2 s to 0 s before arm entry. Activity in the first period (−4 s to −2 s) was used as the baseline. The second period, which immediately preceded arm choice (−2 s to 0 s), was used to calculate choice-related activity, which was compared to baseline activity (see Section 2). The duration of the choice period (2 s) was chosen based on the median time spent by rats on the central platform during task performance (2.11 ± 0.24 s) even though, as suggested below, the rat actual decision likely occurred in the last second preceding arm entry. We reasoned that if cell firing was to code significant events at choice time, this coding would most likely be seen as a variation in discharge rate during the −2 s to 0 s period relative to baseline discharge during the −4 s to −2 s period, and this would happen even if behavior during the baseline period was relatively undetermined and could vary to some extent.

For each cell, four PETHs were built for choice periods preceding either the first or the second visits to baited arms, and choice periods preceding either the first or the second visits to non-baited arms (see Section 2). These PETHS made it possible to identify and unambiguously dissociate either firing modulations by expected reward (first vs. second visits to baited arms) or firing modulations related to visit round (first vs. second visits to non-baited arms).

Looking at significant changes during the choice period (−2 s to 0 s) relative to the baseline (−4 s to −2 s) revealed that, of 131 recorded neurons, 51 (38.9%) had a significant firing modulation during the choice period. Of the remaining 80 neurons, 31 had no significant firing modulation during choice periods, 26 displayed complex and unreliable firing changes, and 23 modulated their discharge selectively as the rat was about to enter either baited or non-baited arms irrespective of visit round ($n = 8$) or according to

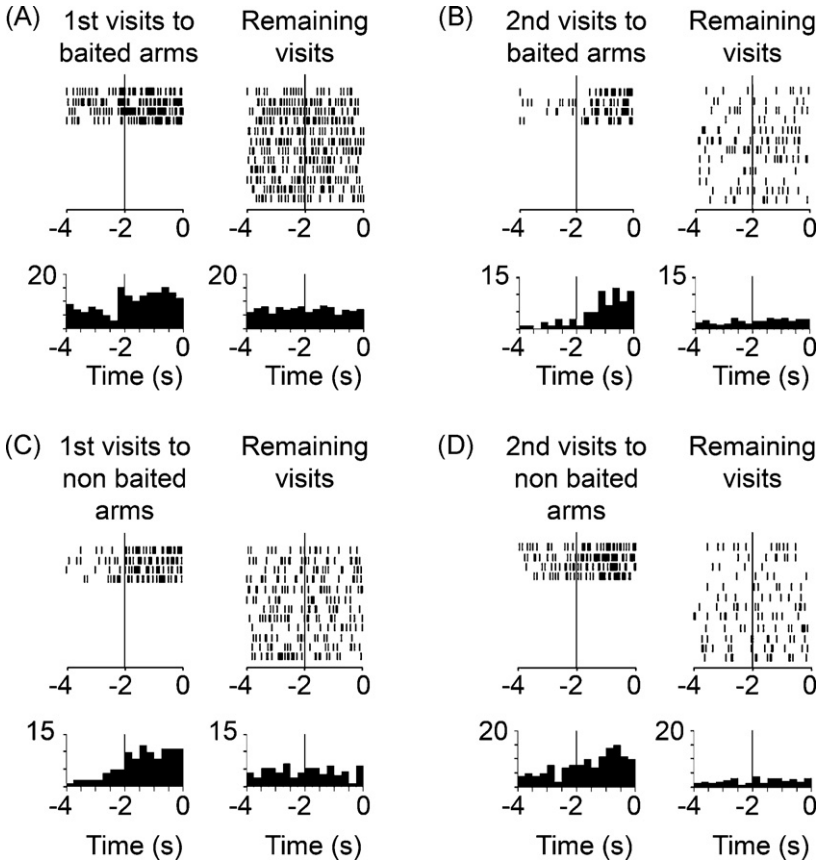

Fig. 4. Temporal modulation of example cells. (A and B) Raster plots and peri-event time histograms for 2 cells whose activity was modulated before the first (A) or second (B) visits to the baited arm set. (C and D) Raster plot and peri-event time histogram for 2 cells whose activity was modulated before the first (C) or second (D) visits to the non-baited arm set. Each panel shows 4 s of data across multiple choices for a single cell during a single trial in the radial maze. Each row in raster plots corresponds to one arm visit and is aligned to arm entry at $t = 0$ s. Continuous vertical lines the separate reference period (−4 s to −2 s) and choice period (−2 s to 0 s). Tick marks along a row indicate the time of action potentials. The time scale in the corresponding peri-event histograms is the same as for the raster plot. Discharge activity (in spikes/s) is accumulated across trials (250-ms bins) and show on the *y*-axis. In each panel, the left raster and peri-event time histogram show activity for the choice periods preceding specific arm visits while the right raster and peri-event time histogram ("remaining visits") show activity for the remaining 12 arm choices. For example, in panel A, remaining visits include both the four 1st visits to non-baited arms and the eight 2nd visits to all arms. The absence of discharge modulation for remaining visits attests to the selectivity of the observed effects.

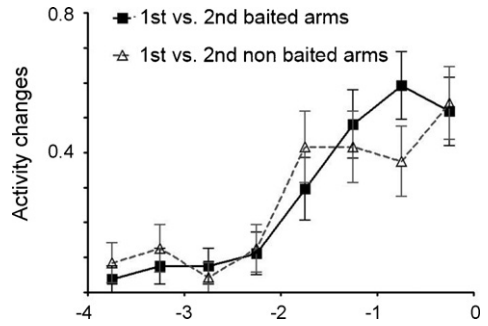

Fig. 5. Dynamics of activity changes. Aggregate temporal modulation for the first vs. second round of choices of baited arms ($n = 27$ cells) and for the first vs. second round of non-baited arms ($n = 24$ cells). See text for explanations.

the choice round irrespective of the baited status of arms ($n = 15$). Because the interpretation of such modulations was ambiguous, no further analysis was carried out on these latter neurons.

The raster plots and PETHs in Fig. 4 illustrate the most common discharge patterns in the 51 cells showing unambiguous firing modulations during choice periods. Although all statistical analyses were conducted by comparing four-choice PETHS (i.e., 1st non-baited visits vs. 2nd non-baited visits; 1st baited visits vs. 2nd baited visits), the right PETH in each panel of Fig. 4 shows firing for the 12 choices that did not result in firing changes, so that it is easy to see the selectivity of the modulation displayed in the four-choice PETH displayed on the left. 27 cells were selectively modulated during choice periods that preceded visits to baited arms (13 cells during first visits and 14 cells during second visits; Fig. 4A and B, respectively). These cells displayed a firing pattern that uniquely anticipated the presence (or absence) of a reward in the arms across the two rounds of choices. Similarly, 24 other cells were active during choice periods that preceded visits to non-baited arms (9 cells during first visits and 15 cells during second visits; Fig. 4C and D, respectively). Since these firing modulations concerned arms that were never baited, this discrimination could not result from a change in the rewarding properties of the arms. Therefore, these cells had a firing pattern that uniquely distinguished the two rounds of choices.

The time-course of firing modulations for each category of response during choice periods is shown in Fig. 5. The aggregate response for a given choice category was obtained by pooling together the neurons that had a significant modulation for that category. For each cell, a value of +1 was assigned to bins exhibiting a significant modulation in firing and a value of 0 to bins not showing such modulation. The aggregate response change was obtained by averaging these values across all cells in the same category. This analysis, which provides a summary description of the activity of all cells within an arm choice category, revealed a gradual build-up of the overall pattern throughout the choice period, peaking in the last second preceding arm choice. That the modulations were maximal during such a late and narrow time window before arm choice implies that they were directly related to actual choice behavior rather than to some uncontrolled artifactual behavior, as the range of possible behaviors during the 1 s period preceding choice was considerably restricted. Lastly, no strong difference in the time-course of firing modulations across categories was observed.

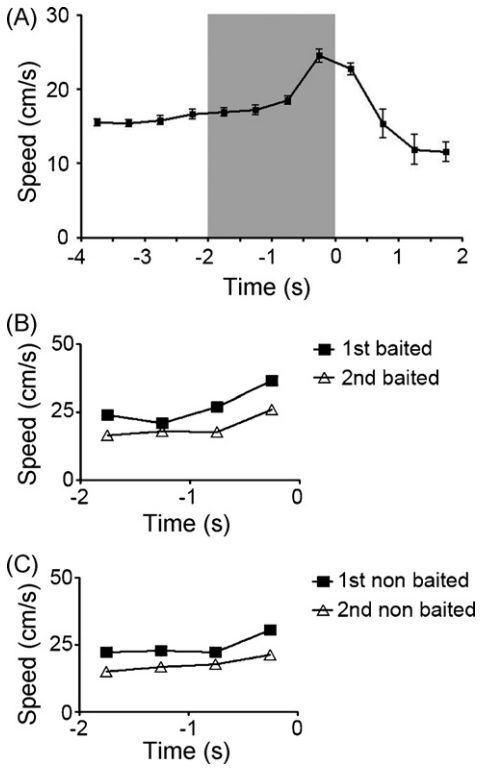

Fig. 6. Speed profiles during radial maze performance. (A) Mean speed profile shown for the period extending from −4 s before rat entry into the arms to +2 s after arm entry. The general pattern seen in all rats was characterized by a sudden acceleration just before the rat enters the arm (in the last 0.5 s) followed by deceleration as the rat reaches the arm end. The gray area shows the choice period used for detailed analysis of speed profiles. (B and C) Detailed speed profiles for restricted sets of arm choices. The main effects for these speed profiles are reported in Table 1.

This was confirmed by an ANOVA with response category as the between factor and time interval as the within factor which reveal no significant effect of response category and no interaction response category × time interval ($F < 1$).

To examine whether the dynamics of firing modulations could be explained by gross changes in rats' motor behavior, we computed speed profiles during choice periods for all trials and all choices. For each animal, the mean speed was calculated over 500 ms intervals starting 4 s before and ending 2 s after the rat entered the arms. The resulting speed profiles show a sudden acceleration just before the rat enters the arm followed by deceleration when the rat reaches the arm end (Fig. 6A). To discard the possibility that the observed changes in neural activity were directly linked to a change in speed during choice periods, we looked at the 2 s choice period in greater detail (Fig. 6B and C). Several ANOVAs analyses of variance were conducted with 500 ms time intervals as the repeated measures and the appropriate category of arm choices as the between measure. These analyses revealed a non-significant tendency of rats to move faster during the first round of choices

Table 1
Analysis of locomotor behavior during choice periods.

	Choice	Time	Choice × time
1st vs. 2nd baited arm choices	$F(1,6) = 2.96$, $p = 0.14$, ns	$F(3,18) = 14.63$, $p \ll 0.0001$	$F(3,18) = 1.36$, $p = 0.29$, ns
1st vs. 2nd non-baited choices	$F(1,6) = 6.13$, $p = 0.048$	$F(3,18) = 14.53$, $p \ll 0.0001$	$F(3,18) = 1.35$, $p = 0.29$, ns

A summary of the repeated measures ANOVAs conducted on the speed profiles of each rat during choice periods for various categories of maze arms. The table displays the main effects of arm choice (choice) and time interval (time) and their interaction (choice × time).

than during the second round of choices and a significant effect of time interval simply reflecting the acceleration at the end of the 2 s period. More importantly, no significant interaction time × choice was observed for any comparison (Table 1). Thus the changes in neural activity across different categories of arm choices can hardly be attributed to a direct motor effect, since these analyses failed to distinguish significant differences in the time course of speed profiles during the choice periods.

4. Discussion

The main finding of this study is that mPFC neuronal activity recorded from rats performing the radial maze task reflects the status of maze arms in various ways, with a substantial fraction of cells specifically modulating their activity at choice time, i.e. as the rat was about to enter arms. By leaving the rat on the maze after exploration of the baited arms, so that all arms were eventually visited twice, we found interesting behavioral correlates for these mPFC choice cells. Approximately half the cells fired differentially before the rats entered non-baited arms across successive visits. Because these arms were never baited, this modulation most likely reflects the memory that they were recently visited (i.e., working memory), and not a change in their reward status. Furthermore, this modulation cannot be merely explained by changes in motivational or attentional state between successive visits, since this hypothesis would predict firing modulations for *both* baited and non-baited arms, but *not for non-baited arms only*. In other words, modulation of firing during successive visits to non-baited arms appears to reflect a pure working code for visited arms. Another half of choice cells fired differentially before the rats entered the baited arm set across successive visits, thus possibly representing the expectation of reward in the arms at choice time. As above, changes in motivation or attention can be ruled out as a general explanation since they would predict non-specific effects.

Although differential firing to non-baited arms across successive visits provides support for a pure working memory code in the mPFC, how this working memory is implemented appears to be different from previous suggestions. Rather than a memory code provided by continuous, accumulating activity as the rat successively enters the arms and stores these items in memory [16] or by persistent discharge increases associated with specific items ("delay cells") [2,3,5,25], our study revealed a phasic discharge before arm choice. This discharge occurs at a strategic time during task performance since changes in cell activity in relation to recently visited arms at choice time provides information directly useful to impact the rat's choices. In other words, each arm would be marked with a memory tag indicating that it has been recently visited. By sorting out the significant aspects of recent experience, these tags would allow for planning the next choices.

The interpretation of cells seen to discriminate successive visits to baited arms is slightly more difficult, given that these arms were not re-baited after the first visit. Therefore, it is possible that these cells reflected the rat's expectation of reward rather than visit round (see [13] for a similar finding for hippocampal place cells). Since reward expectation was strongly influenced by visit round, however, we argue that, whichever of these two aspects was coded by their activity, these cells are endowed with properties allowing the rat to perform efficient choices based on its previous behavior, a capability that defines executive functions [30].

How are working memory tags observed in mPFC neurons generated? A tentative explanation rests on recent demonstrations of highly correlated activity in mPFC and hippocampus [15,21,29], suggesting a strong functional interplay relationship between the two structures. Furthermore, lesion evidence indicates that ventral hippocampal lesions strongly alter anticipatory mPFC activity [4] as well as temporal order memory [14]. Therefore, an interesting possibility is that modulations of mPFC activity could actually result from different firing patterns generated in the hippocampus across successive visits to the same location.

Whether mPFC working memory tags are based on a sense of familiarity or on an active recollection process (in which the detail and context of specific experience to be retrieved is coded) is also an important question. Interestingly, recent work strongly supports the idea that the mPFC is involved in recollection [6]. Using a non-match-to-sample task, the authors found that mPFC damage severely reduced recollection-based performance, while sparing familiarity. It is therefore tempting to speculate that the observed modulations of neuronal discharge in the present study might be the implementation of such recognition memory.

Finally, our study shows that a simple variant of the radial maze task can reveal the neural underpinnings of complex processes by building on the rat's natural behavior to patrol its environment [28]. Although food searching forms the basis of radial maze performance, patrolling refers to the tendency of rats to resume exploration of a familiar environment from time to time to check its stability and has developed as a result of the rapidly changing distribution of food resources [28]. In the present study, we exploited this ability and reasoned that upon completion of visits to baited arms (food searching), the rat would visit non-baited arms in an optimal way, avoiding already visited arms in both arm sets (patrolling). We found that the first round of visits was organized very orderly with few errors. Even though the second round was much less orderly, it was not random and resulted in reasonably efficient and exhaustive maze patrolling. Thus, the data suggest that rats knew which arms they had visited in both rounds and that they used this knowledge to determine prospectively their future choices. In conjunction with the discharge properties of mPFC neurons, behavioral evidence therefore shows that the organization of natural behavior, such as food searching and patrolling, relies on efficient mechanisms that reflect the implementation of executive functions.

In conclusion, our findings suggest that mPFC neurons in the rat may carry out operations usually thought to tax executive functions. Previous reports based on lesion evidence in the radial maze task strongly suggest a role of mPFC in organizing recently acquired spatial information [26], spatial working memory [31], and spatial temporal order memory [11]. The present evidence might reveal the neural counterpart of these different processes, which all converge to endow the rat medial prefrontal cortex with a function in guiding prospective search behavior [7].

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