

Prefrontal Cortical Cell Firing During Maintenance, Extinction, and Reinstatement of Goal-Directed Behavior for Natural Reward

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KEY WORDS behavior; reward; electrophysiology; water; conditioning

ABSTRACT The prefrontal cortex (PFC) is important for higher cognitive functioning and the processing of reward-related information. Here, electrophysiological recording procedures were used to examine cell firing in the PFC in rats ($n = 12$) during water reinforcement sessions consisting of three phases. In phase one (maintenance), animals pressed a lever (fixed ratio 1) for water reinforcement (0.05 ml/press) paired with an auditory stimulus. Of 62 neurons recorded during maintenance, 39 (63%) exhibited one of three types of patterned discharges relative to the reinforced response for water. Specifically, PFC neurons exhibited increases in firing rate within seconds preceding the response (type PR; $n = 9$ cells) or increases (type RFe; $n = 16$ cells) or decreases (type RFi; $n = 14$ cells) in firing rate immediately following response completion. The remaining neurons did not alter their firing profiles relative to the reinforced response (type nonphasic cells; $n = 23$ cells). In phase two (extinction), lever press responses had no programmed consequences (i.e., water reinforcement and the auditory stimulus were not presented). After 30 min of no responding, phase three (reinstatement) began, during which each lever press response was again associated with water reinforcement paired with the stimulus. Results indicate differential effects of extinction/reinstatement on cell firing rates and patterns dependent on cell type. These findings are discussed with respect to the adaptive nature of PFC activity during goal-directed behaviors for “natural” rewards, and are considered relative to prior studies that examined nucleus accumbens cell firing during a similar behavioral task. **Synapse 56:74–83, 2005.** © 2005 Wiley-Liss, Inc.

INTRODUCTION

Numerous studies have implicated a critical role of the prefrontal cortex (PFC) in mediating instrumental actions (Rolls, 1996; Tremblay and Schultz, 1999, 2000; Balleine and Dickinson, 1998; Watanabe, 1998). PFC lesions lead to deficits in movement initiation, planning, working memory tasks, and attention (Goldman-Rakic, 1987; Bechara, 1998; Fuster, 2001). Electrophysiology studies in primates and rodents have revealed that PFC neurons are responsive during goal-directed behaviors for liquid or food rewards (Niki and Watanabe, 1976, 1979; Watanabe, 1996; Tremblay and Schultz, 1999, 2000; Watanabe et al., 2002; Schoenbaum et al., 1998; Takenouchi et al., 1998; Hikosaka and Watanabe, 2000). Likewise, PFC neurons are activated during the self-administration of drugs of abuse including cocaine and heroin (Chang et al., 1997a,b, 1998; Chang and Woodward, 2000).

The PFC sends extensive afferent projections to the nucleus accumbens (Acb) (Brog et al., 1993; McGeorge and Faull, 1989; Zahm and Brog, 1992), a neural structure that has been extensively studied with respect to its role in mediating goal-directed behaviors. Electrophysiological studies in behaving animals support this view by showing that Acb neurons exhibit patterned discharges during operant responding for natural as well as drug rewards (Carelli, 2004). We have reported that a subset of Acb neurons exhibit patterned discharges (increases or decreases in cell firing) within seconds of the lever press response for water or sucrose

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Received 19 August 2004; Accepted 22 December 2004

DOI 10.1002/syn.20129

Published online in Wiley InterScience (www.interscience.wiley.com).

reinforcement in rodents (Carelli et al., 2000; Roop et al., 2002; Carelli, 2002). Specifically, firing patterns were characterized by increased activity preceding the reinforced response (type preresponse, PR), and either increases (type reinforcement-excitation, RFe) or decreases (type reinforcement-inhibition, RFi) in firing immediately following response completion. Similar types of neuronal firing patterns have been observed in another population of Acb neurons during cocaine self-administration sessions (Carelli and Ijames, 2000).

In our laboratory, additional experiments were completed using extinction and reinstatement procedures to better elucidate factors mediating the patterned discharges of Acb neurons that occur within seconds of the reinforced response for a "natural" (water) reward (Hollander et al., 2002). Acb cells that exhibited an anticipatory increase in firing rate within seconds preceding the water-reinforced response (type PR) showed attenuated activity during extinction and reinstatement phases. Neurons that displayed increased or decreased firing within seconds following water-reinforced responding (type RFe and RFi, respectively) showed attenuated activity during extinction, but exhibited recovery of phasic activity during the reinstatement phase. These findings indicate that particular subsets of Acb neurons are sensitive to disruption of the response-reinforcer contingency associated with responding for a "natural" reward.

The goal of the present study was to expand the aforementioned report by investigating PFC neuronal activity during maintenance, extinction, and reinstatement of operant responding for water reinforcement. Importantly, here we incorporate the same extinction/reinstatement task as used in our prior report that examined Acb cell firing during behavior (Hollander et al., 2002). We hypothesized that: 1) similar types of neuronal firing patterns will be observed in the PFC as in the Acb during goal-directed behavior for water reinforcement (i.e., maintenance phase), and 2) a subset of PFC neurons will be sensitive to extinction/reinstatement procedures in a manner similar to Acb neurons. We believe that similarities may exist in cell firing across the two brain regions since anatomy studies show that the PFC sends extensive afferent projections to the Acb and may therefore influence Acb activity in behaving animals (Brog et al., 1993; McGeorge and Faull, 1989; Zahm and Brog, 1992). As such, these findings will provide insight into the relationship between PFC and Acb cell firing in animals actively engaged in goal-directed behaviors for natural rewards.

MATERIALS AND METHODS

Rats

Male Sprague-Dawley rats ($n = 12$) (Harlan, Indianapolis, IN) weighing 275–350 g were used as subjects. Each animal was individually housed and

weighed daily throughout the duration of the experiment. Two days prior to and for the duration of the experimental sessions, rats were restricted to 10–15 ml/day of water (in addition to 1.0–1.5 ml consumed during each session) but continued to have free access to food (Purina Lab pellets, St. Louis, MO). This regimen maintained body weights at their preoperative levels. Sessions were conducted in a sound-attenuated cubicle (Fibrocrete, Crandall, GA) enclosed in a Plexiglas chamber (43 × 43 × 53, Med Associates, St. Albans, VT). A water receptacle was positioned 2.5 cm from the bottom of the chamber and 7 cm to the right of a retractable lever (Coulbourn Instruments, Allentown, PA).

Water reinforcement task

Animals were initially trained to press a lever for water reinforcement during daily 15-min sessions. The start of the session was indicated by illumination of a cue light (situated 6.5 cm above the lever) and simultaneous extension of the lever. The cue light remained on throughout the session. Rats were trained on a fixed ratio 1 (FR1) schedule of reinforcement for water delivered into a drinking receptacle located next to the lever. Each reinforced lever press response resulted in: 1) the onset of a tone stimulus (80 dB, 800 Hz) for 0.5 sec; 2) simultaneous retraction of the lever (20 sec); and 3) water delivery (0.05 ml). Following the 20-sec postresponse period, the lever was again extended into the chamber and the animal could respond for water reinforcement. Following 3–5 days of stable responding (30–42 lever presses within a 15-min session) surgery for implantation of microelectrode arrays was performed.

Electrophysiology

Rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) and an array of eight microwires (50 μm diameter; tip separation 0.25–0.5 mm; span 1 × 1 mm) was implanted unilaterally in the PFC (AP 2.7 mm, L 0.5–0.8 mm, DV 3–6 mm, relative to bregma). The array (NB Labs, Denison, TX) used for these experiments consisted of eight microwires (50 μm diameter) structured into three rows (2 × 3 × 3) with tip separations of ~ 0.25 mm. A ground wire attached to the array was positioned into the brain at a depth of 3–4 mm and ~ 2 mm away from the recording wires. For a week following surgery, animals were monitored carefully to ensure full and healthy recovery. Presurgical behavioral performance was then reestablished (typically in 1–2 days) and upon consistent lever pressing, electrophysiological recording sessions were completed. Prior to the start of each session the animal was attached to a recording cable, which was connected to a commutator (Med Associates). This equipment allowed the animal free and unrestricted movement within the chamber.

Each recording cable had a headstage containing 16 miniature unity-gain field effect transistors. A reference electrode (inactive; spike absence verified prior to starting the session) was used to record differentially between the remaining microwires in the PFC. Online isolation and discrimination of neuronal activity was accomplished using a neurophysiological system commercially available (MNAP System, Plexon, Dallas, TX). Multiple window-discrimination modules and high-speed analog-to-digital (A/D) signal processing in conjunction with computer software enabled isolation of neuronal signals based on waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal spike events to a Pentium computer. Another computer controlled behavioral events of the experiment (Med Associates) and sent digital outputs corresponding to each event to the MNAP box to be time-stamped along with the neural data. The neurophysiological system has the capability of recording up to four neurons per microwire using real-time discrimination of neuronal action potentials. However, in the present study typically 1–2 neurons were recorded per microwire (Chang et al., 1994; Nicolelis et al., 1997). Criteria for identifying different neurons on a single wire have been described in detail elsewhere (Carelli et al., 1999; Chang et al., 1994; Nicolelis et al., 1997; Nicolelis, 1999). Briefly, discrimination of individual waveforms corresponding to a single cell was accomplished using template analysis procedures to initially isolate individual neurons that were further characterized using the “offline sorter” program provided by the neurophysiological software system (MAP system, Plexon). The template analysis procedure involves taking a “sample” of the waveform and building a template of that extracellular waveform. Subsequent action potentials that “match” this waveform were included as the same cell. The parameters for isolation and discrimination of single unit activity were determined and saved using the neurophysiological software and modified before each session as needed, for example, to discriminate “new” neurons that appeared on a given microwire electrode, or to change the inactive electrode. The Offline Sorter program allows sorting of spike waveforms corresponding to the activity of individual neurons after completion of the experiment. This sophisticated program uses a variety of methods to isolate individual waveforms including manual cluster selection of waveforms in 3D space using principal component projections (Plexon).

PFC recordings during behavior

PFC neurons were recorded during a within-session extinction/reinstatement paradigm completed in three phases: maintenance, extinction, and reinstatement

(Hollander et al., 2002). During the maintenance phase, animal's lever-pressed for water reinforcement (0.05 ml/press) for 15 min as described above. Thereafter, the extinction phase was initiated. During this phase, the cue light remained on, but each lever press resulted in retraction of the lever (20 sec), water was not delivered into the drinking receptacle, and the auditory stimulus was not presented. After a period of 30 min had elapsed without a response, the reinstatement phase began. Specifically, the experimenter administered a “priming” infusion of water (0.05 ml) into the drinking receptacle simultaneously with the onset of the auditory stimulus (80 dB, 800 Hz; 0.5 sec). One to three water/auditory stimulus “primes” were necessary to reestablish lever pressing for all subjects, thereby initiating the reinstatement phase. During reinstatement, each lever press resulted in water reinforcement paired with the auditory stimulus as described for the maintenance phase. The session was ended when the number of responses during reinstatement was similar to that obtained during the maintenance phase for each animal (10–15 min).

Data analysis

Operant responding was assessed via cumulative lever-press records for each session. Off-line peri-event histograms (PEH) and raster displays were generated for each neuron within a 20-sec period that bracketed the reinforced response. PFC discharges were evaluated in four 2.5-sec epochs relative to the reinforced response: 1) baseline firing (–10 to –7.5 sec prior to lever press); 2) response (–2.5 to 0 sec prior to press); 3) reinforcement (0 to +2.5 sec following the response); and 4) recovery (+7.5 to +10 sec following the response). Inspection of PEH/raster displays revealed three general types of neuronal firing patterns similar to those described for nucleus accumbens (Acb) neurons by Carelli et al. (2000). Specifically, criteria for classifying each neuron into one of the three types of patterned discharges were as follows. A neuron was classified as type preresponse (type PR) if it showed a 40% or greater increase in firing rate during at least a 1-sec period of the response epoch only, compared to its respective baseline activity. If a neuron exhibited a 40% increase in activity, which began in the response phase and extended without interruption into the reinforcement phase, it was also classified as a type PR neuron. A neuron was classified as type reinforcement-excitation (type RFe) if it showed a 40% or greater increase in cell firing during at least 1 sec within the 2.5-sec period of the reinforcement phase compared to its respective baseline activity. Finally, neurons were classified as type reinforcement-inhibition (type RFi) if they exhibited a 40% or greater decrease in firing rate during at least a 1-sec period of the response and/or reinforcement epoch, compared to

its respective baseline-firing rate. “Nonphasic” neurons exhibited equivalent firing rates across the four time epochs without the 40% changes in activity characteristic of the three types of patterned discharges described above.

Statistical confirmation of the above cell type classification was accomplished using *t*-test statistics for dependent samples that compared mean peak (types PR, RFe) or trough (type RFi) firing rates for all neurons of a given type to their respective baseline rates. In addition, one-way ANOVAs (followed by post-hoc Bonferroni’s test) were also calculated to: 1) examine changes in peak (type PR or RFe) or trough (type RFi) firing rates during extinction and reinstatement relative to the maintenance phase; and 2) examine baseline firing rates across the test session. All data are presented in the results as mean \pm standard error of the mean.

Histology

Following completion of the experiments, animals were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) and a 13- μ A/5-sec current was passed through all micro-wires. The animal was then perfused with saline followed by 4% paraformaldehyde (PFA). Rat brains were removed from the skull and placed in 4% PFA for further absorption. Serial 40–50 μ m coronal sections were cut throughout the rostrocaudal extent of the PFC using a cryostat. All sections were stained with thionin and counterstained with Prussian blue for electrode tip placement verification with a light microscope.

RESULTS

Behavior

Figure 1 illustrates a cumulative record of behavioral responding for one representative animal during the task. In this case, the animal pressed regularly (39 times) during the maintenance phase (mean latency to lever press following lever extension (LAT) = 0.9 ± 0.2 sec; mean intertrial interval (INT) = 20.5 ± 0.2 sec). Following the onset of extinction (indicated by first arrow), the number of presses during the extinction phase was 123 (mean LAT = 96.5 ± 30.5 sec; mean INT = 116.1 ± 30.5 sec). After 30 min of no responding, the animal was “primed” with an experimenter-delivered water administration paired with the auditory stimulus (indicated by second arrow). Thereafter, the animal completed 20 additional responses with a behavioral pattern similar to that observed during the maintenance phase (mean LAT = 1.3 ± 0.3 sec, mean INT = 34 ± 12.9 sec).

Across all animals ($n = 12$), the average number of presses during the maintenance phase was 41.3 ± 0.6 with a mean INT of 21.3 ± 0.8 sec. The mean latency between lever extension and press was 1.0 ± 0.3 sec.

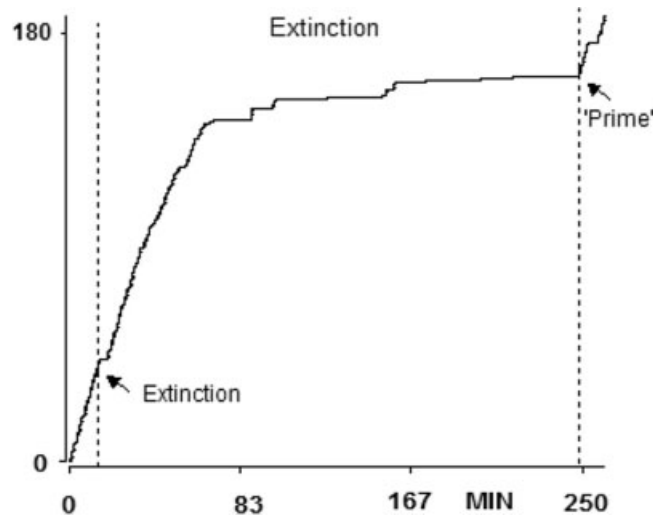


Fig. 1. Cumulative record showing the behavioral (lever press) response pattern of one animal during maintenance, extinction, and reinstatement. Each upward deflection represents one lever press response (FR1). The y-axis is the number of lever presses. Onset of the extinction phase is indicated by “Extinction” at the first arrow. The water prime used to reinstate behavior following extinction is indicated by “Prime” at the second arrow.

During extinction, the animals continued to respond for an extended time but with increased variability and eventually stopped lever pressing. The average number of presses during extinction was 78.8 ± 16.6 sec, with an average INT of 130 ± 45 sec and a mean latency following lever extension of 118.1 ± 49.3 sec. At the completion of a 30-min period without a lever press, behavior was reestablished by response independent water delivery paired with the stimulus (reinstatement phase). Thereafter, animals lever pressed for an additional 10–15 min (mean number of responses = 38.3 ± 2.6 , mean LAT = 3.0 ± 0.8 sec, mean INT = 22.7 ± 2.2 sec). Although the duration of the extinction phase was variable between animals, the behavioral response profiles in all cases were similar.

Prefrontal cortical cell firing during maintenance, extinction, and reinstatement of water-reinforced responding

A total of 62 PFC neurons were recorded during maintenance, extinction, and reinstatement of water-reinforcement sessions in 12 animals. Of 62 neurons, 39 cells (63%) were classified as phasically active during the maintenance phase, showing one of three types of neuronal firing patterns similar to that previously described for Acb neurons (Carelli, 2002). Of these 39 phasically active cells, 9 (23%) neurons exhibited increased firing before the lever press and were classified as type preresponse (PR) cells. Other neurons exhibited an excitation in firing rate following the

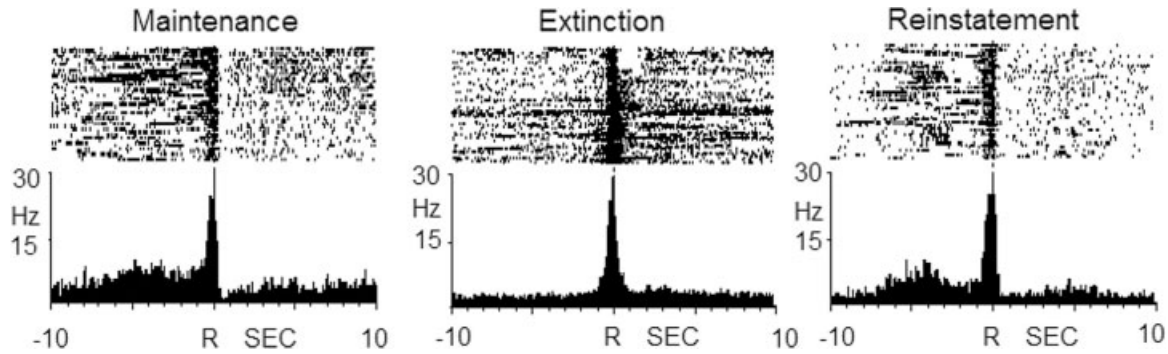


Fig. 2. Activity of a single PFC type PR neuron during maintenance, extinction, and reinstatement of water reinforcement. Left: The raster and PEH indicate that the cell exhibited a slow ramping increase in firing and a second more pronounced peak in activity before the response during the maintenance phase (mean INT = 20.4 ± 0.01 sec). Middle: During extinction, the slow ramping discharge was absent although the peak increase in activity remained

(mean INT = 187.8 ± 38.3 sec). Right: During the reinstatement phase, the slow ramping increase and the peak discharge were again observed (mean INT = 21.2 ± 0.6 sec). The “R” indicates reinforced response here and in subsequent figures. All trials are included in the PEHs and raster displays; Bin size = 80 ms here and in subsequent figures.

lever press during the maintenance phase and were classified as type reinforcement-excitation (RFe) cells ($n = 16$, 41%). Finally, neurons exhibiting an inhibition in activity within seconds before and/or following the lever press were classified as reinforcement-inhibition (RFi) cells ($n = 14$, 36%). The firing rate of the remaining cells ($n = 23$, 37%) did not change prior to or following the water-reinforced response and were therefore classified as type nonphasic. A detailed description of PFC cell firing patterns across the three phases of the session for all types of patterned discharges is described in detail below.

Anticipatory discharge patterns: Type PR neurons

Of 39 phasically active cells, nine neurons exhibited significant anticipatory increases in firing rate within seconds preceding the response for water reinforcement during the maintenance phase, classified as type PR neurons. Across all PR cells, the mean firing rate within 2.5 sec before the lever press (3.1 ± 0.4 Hz) was significantly higher than mean baseline firing rates (1.7 ± 0.8 Hz; $t = 2.4$, $P < 0.05$). A repeated one-way ANOVA revealed no significant overall effect of test phase (maintenance, extinction, reinstatement) on peak firing rates for type PR cells ($F_{2,16} = 0.5$, $P > 0.05$). Specifically, peak firing rates during extinction (2.6 ± 0.6 Hz) were not significantly altered compared to maintenance (3.1 ± 0.4 Hz) or reinstatement (2.7 ± 0.70 Hz). However, closer inspection of the PEHs for a subpopulation of type PR neurons ($n = 7$) during the maintenance phase revealed a second slowly ramping increase in firing rate within 2.5–7.5 sec before the reinforced response but typically peaking between 3.5–5 sec prior to response completion. A repeated one-way ANOVA revealed a significant overall effect of test phase (maintenance, extinction, reinstatement) on this second preresponse

discharge pattern for these type PR cells ($F_{5,41} = 3.02$, $P < 0.05$). Bonferroni’s test revealed a significant increase in the preresponse discharge (2.9 ± 0.8 Hz) compared to baseline (1.6 ± 0.4 Hz) during the maintenance phase ($P < 0.05$). During extinction, the slowly ramping discharge declined (1.92 ± 0.4 Hz) and was not significantly different from baseline during extinction (1.86 ± 0.4 Hz; $P > 0.05$). However, during reinstatement this ramping was again apparent (2.14 ± 0.4 Hz), but was not significantly different from reinstatement baseline rates (1.13 ± 0.18 Hz; $P > 0.05$). Finally, a repeated one-way ANOVA revealed no significant overall effect of test phase on baseline firing rates for type PR cells (baseline extinction: 1.6 ± 0.4 Hz, baseline reinstatement: 1.5 ± 0.6 Hz, $F_{2,16} = 0.1$, $P > 0.05$).

Figure 2 shows the activity of a single type PR neuron during maintenance, extinction, and reinstatement phases. The raster and PEH in the left portion of Figure 2 shows the activity of this cell during the maintenance phase. The neuron had a baseline rate of ~ 3 Hz with a peak increase in activity of ~ 30 Hz immediately prior to the reinforced response. Within 0.5 ms after the response, neural activity declined sharply, then eventually returned to baseline levels. Furthermore, a second slowly ramping increase in firing rate was observed that began ~ 7 sec before the peak discharge. During extinction (middle PEH), the peak discharge remained; however, the slowly ramping increase in activity that preceded it during maintenance was not observed. Following a 30-min period in which no responses were made, the animal was primed with an auditory stimulus paired with water delivery into the drinking receptacle and lever-pressing behavior was reinstated. As illustrated in the raster/PEH in the right portion of Figure 2, the peak discharge of the type PR neuron remained and the smaller preresponse discharge pattern was again observed.

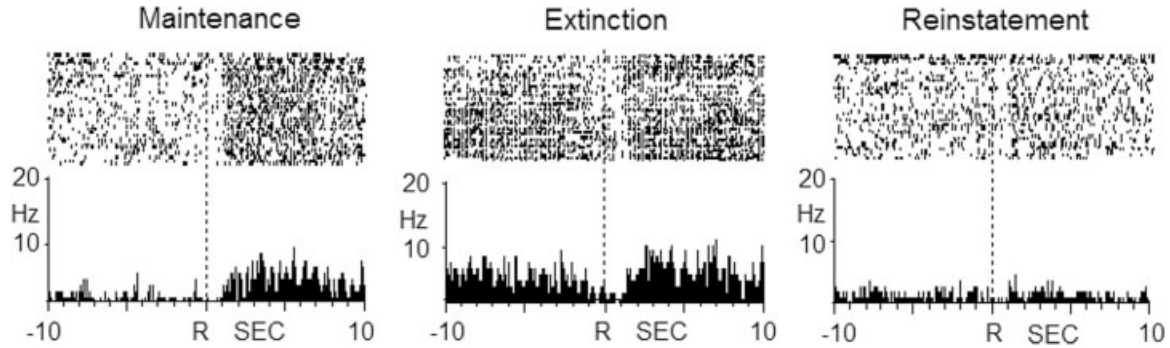


Fig. 3. An example of a type RFe neuron during maintenance, extinction, and reinstatement. Left: The raster and PEH indicate that the cell exhibited a robust increase in firing following the response during the maintenance phase (mean INT = 20.1 ± 0.2 sec). Middle: The same neuron showed an overall increase in activity and a less

pronounced inhibition following response completion (mean INT = 100.4 ± 36.1 sec). Right: During reinstatement, overall firing rates declined however patterned activity was not evident (mean INT = 21.7 ± 0.5 sec).

Postresponse discharge patterns: types RFe and RFi cells

Sixteen PFC neurons exhibited significant increases in firing rate immediately after response completion during the maintenance phase, termed type RFe cells. For these neurons, the mean firing rate within 2.5 sec following the response (3.2 ± 1.1 Hz) was significantly higher than the mean baseline rate (2.3 ± 0.7 Hz; $t_{15} = 3.0$, $P < 0.01$). Although there was a trend toward a decline in mean postresponse firing during extinction (2.8 ± 0.7 Hz) and an increase in postresponse activity during reinstatement (2.9 ± 0.9 Hz), a repeated one-way ANOVA did not reveal a significant overall effect of test phase (maintenance, extinction, reinstatement) on peak firing rates for type RFe cells ($F_{2,30} = 0.4$, $P > 0.05$). Finally, a repeated one-way ANOVA revealed no significant overall effect of test phase on baseline firing rates for type RFe cells ($F_{2,30} = 0.5$, $P > 0.05$), indicating no significant alteration in baseline activity across the test session (extinction baseline = 2.5 ± 0.5 Hz, reinstatement baseline = 2.4 ± 0.7 Hz).

An example of the activity of a single type RFe neuron showing a slightly different firing profile across the three phases of the session is shown in Figure 3. During the maintenance phase, the PFC neuron displayed a pronounced increase in firing from baseline rates of ~ 3 Hz to a peak discharge of ~ 8 Hz beginning 800 ms following the water-reinforced response (left). For this neuron, overall firing rates increased during extinction, and therefore the postresponse activity was not evident. Although overall rates declined during reinstatement (right), the postresponse increase in activity was not reestablished.

The remaining 14 physically active PFC cells were classified as type RFi neurons. These cells had significantly decreased firing rates within 1 sec of the 2.5-sec period before and/or after response completion ($0.9 \pm$

0.3 Hz) compared to baseline activity (2.1 ± 0.4 Hz) during the maintenance phase ($t = 5.3$, $P < 0.0001$). A repeated one-way ANOVA revealed a significant overall effect of test phase on trough firing rates for type RFi cells ($F_{2,26} = 6.0$, $P < 0.01$). The post-hoc test revealed that during extinction, type RFi cells exhibited increased firing at the time of the reinforced response (1.4 ± 0.3 Hz), reflecting a less pronounced type RFi discharge pattern during extinction. This decline in firing was significantly different from mean trough rates during the maintenance phase ($P < 0.01$). During reinstatement, however, the postresponse inhibition in cell firing was again readily apparent for all type RFi cells (1.2 ± 0.3 Hz) and mean postresponse rates were not significantly different from those observed during the maintenance phase ($P > 0.05$). Finally, a repeated one-way ANOVA revealed no significant overall effect of test phase on baseline firing rates for type RFi cells ($F_{2,26} = 1.0$, $P > 0.05$), indicating that baseline rates did not significantly change during extinction (1.9 ± 0.4 Hz) or reinstatement (1.7 ± 0.4 Hz) compared to baseline rates during the maintenance phase.

The PEHs in Figure 4 show the discharge pattern of one RFi cell during maintenance, extinction, and reinstatement. During maintenance (left), the neuron displayed a baseline firing rate of ~ 4 Hz with a decline to 1 Hz following response completion, characteristic of type RFi firing. During extinction (middle), the postresponse inhibition was reduced in duration. During reinstatement (right), the postresponse inhibition was also evident.

The neuronal firing patterns of all PR, RFe and RFi neurons during maintenance, extinction, and reinstatement are presented in Figure 5 as composite PEHs summed over all cells of a specific type. Across all type PR neurons during maintenance (top left), a ramp-like anticipatory increase in cell firing was observed followed by a peak increase in activity at response completion that declined abruptly when

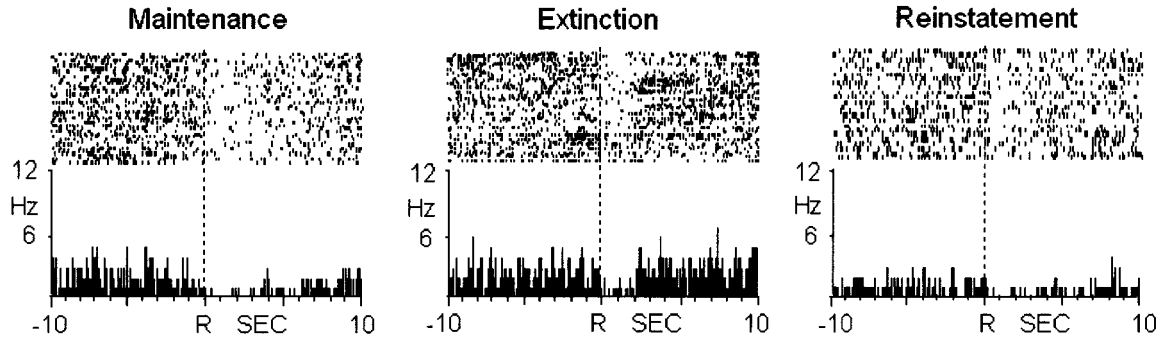


Fig. 4. Raster and PEHs show the activity of a single type RFi neuron during maintenance, extinction, and reinstatement. Left: The cell exhibited a marked decline in cell firing before and immediately following the response, characteristic of RFi firing during maintenance (mean INT = 20.1 ± 0.2 sec). Middle: During extinction, the neuron

increased firing following the reinforced response (mean INT = 100.4 ± 36.1 sec), indicating an attenuation in RFi patterned activity. Right: RFi firing returned during the reinstatement phase (mean INT = 21.7 ± 0.5 sec).

type RFe neurons showed an increase in firing rate (middle left). Likewise, RFi neurons exhibited a marked decline in firing rate at the time of the reinforced response for water (bottom left). These complementary firing patterns suggest that distinct populations of PFC neurons encode different temporal aspects of the same behavioral event. During extinction (middle PEHs), various components of the patterned discharge were attenuated to differing degrees. As shown in the composite PEHs during reinstatement (right) the populations of neurons exhibited phasic activity that was comparable to that observed during the maintenance phase for all cell types.

Histology

Based on visual inspection of serial coronal sections and comparison of each section to the rat brain atlas (Paxinos and Watson, 2005), electrode tip locations included in this study were positioned in the PFC. Here, we define the PFC as including both medial and orbital prefrontal regions consisting of infralimbic and prelimbic areas, as well as all regions of the orbital cortex (Fuster, 1997, 2001; Thierry, 1992; Uylings et al., 2003). Wire placements spanned ~ 4.7 – 2.2 mm rostral-caudal, 0.1 – 2.0 mm medial-lateral, and 3 – 6 mm dorsal-ventral. Of 96 wires implanted in the brain, 15 were located in the infralimbic cortex, 10 in the medial orbital, 27 in the ventral orbital cortex, 30 in the prelimbic region, and 6 were located in the border between the IL and DP regions. Eight wires were located outside the PFC and were not included in the results. Since the array used in the present study consisted of bundles of wires, it was not possible to determine an exact one-to-one correspondence between each wire and cell(s) recorded from them and therefore this information is not included in the present report.

DISCUSSION

The present study examined firing properties of PFC neurons during goal-directed behaviors for a “natural” (water) reinforcer in rodents and if these discharge patterns are altered during a within-session extinction/reinstatement procedure. Three types of patterned discharges were observed within seconds prior to or following completion of the lever-press response, similar to that reported for Acb neurons (Hollander et al., 2002; Carelli et al., 2000). A main finding in this study was that PFC neurons were differentially influenced by extinction procedures, depending on cell type. Thus, PFC neurons that process information about goal-directed behaviors for a natural reward are sensitive to extinction/reinstatement procedures.

PFC cells encode different aspects of instrumental responding for natural reward as evidenced by the phasic excitation or inhibition in cell firing within seconds prior to or following the lever-press response. These findings support the notion that the PFC is essential during the execution of goal-directed behavior (Goldman-Rakic, 1987; Rolls, 1996; Balleine and Dickinson, 1998). Moreover, PFC neuronal discharge patterns during water reinforcement were similar to Acb cell firing patterns observed during an identical behavioral task (Hollander et al., 2002). Interestingly, the percentage of phasically active neurons in the PFC was twice as high compared to Acb neurons (Carelli et al., 2000; Hollander et al., 2002). Nevertheless, the similarity in cell firing patterns across these two brain regions support the view that the PFC may modulate or influence Acb cell firing during goal-directed behavior.

Preresponse discharge patterns

In the present study, a population of PFC neurons exhibited an increase in cell firing within seconds preceding the reinforced response for water (type PR). That is, the neurons displayed an increase in firing

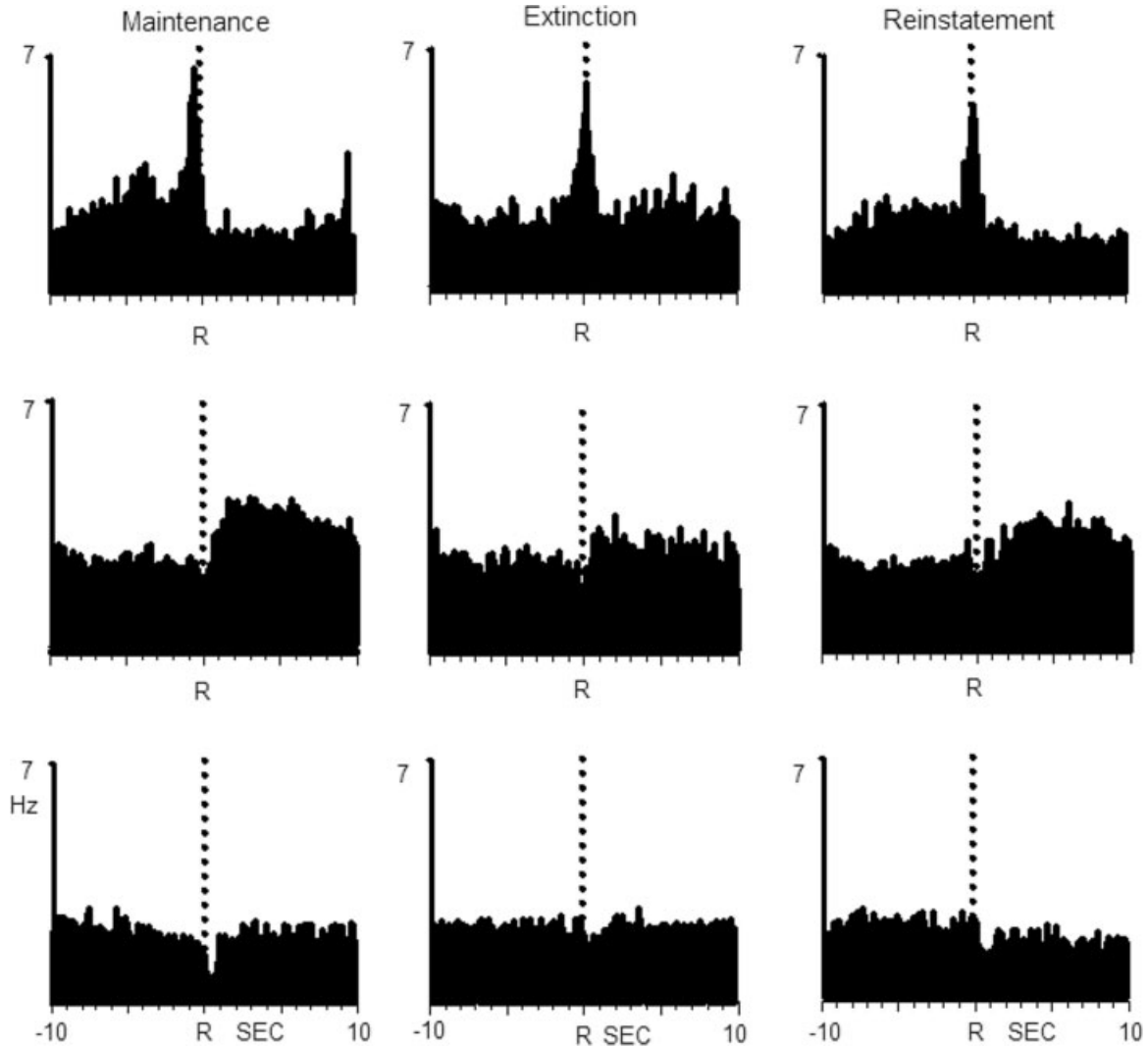


Fig. 5. Composite of summed PEHs of all phasically active PFC neurons during maintenance (left), extinction (middle), and reinstatement (right). Top: Type PR cells ($n = 9$). Middle: Type RFe cells ($n = 16$). Bottom: Type RFi cells ($n = 14$). The complementary nature of the patterned discharges during maintenance is apparent and differentially altered during extinction and reinstatement phases.

within 1 sec prior to the response. Another important characteristic of this cell type was a slow increase in firing starting at ~ 5 – 7 sec prior to the reinforced response. These two different aspects of cell firing suggest that they may reflect different elements of the same instrumental requirement. This possibility was validated, since during extinction the initial component (i.e., slow ramping) disappeared while the rapid increase in cell firing remained present.

The slow ramping preresponse component of PFC neurons may signal “anticipation” of impending reward. Alternatively, in congruence with nonhuman primate studies, this activity may reflect the stimulus-reinforcer relationship, a relationship that has been described as being specific to the PFC (Shidara and Richmond, 2002; Fuster 1997). Furthermore, this

neural signal is reestablished during reinstatement of the behavioral task, thus reflecting the importance of a learned association for this activity.

Interestingly, the dynamics of preresponse PFC firing patterns reported here were similar to that observed for Acb neurons during goal-directed behaviors (Hollander et al., 2002). Specifically, both regions displayed increased firing rate within seconds preceding the reinforced response for water during maintenance. However, in the PFC the anticipatory peak increase in activity immediately preceding the reinforced response did not significantly attenuate during extinction, in contrast to a similar type of preresponse firing in the Acb which did significantly decline during extinction (Hollander et al., 2002). In addition, the present study revealed a second slow ramping increase in PFC activ-

ity which preceded the response and significantly attenuated during extinction. Although not specifically addressed in our prior report, subsequent analysis of Acb cell firing revealed a similar slow ramping pre-response increase in Acb activity which also appeared to decline during extinction. Collectively, these findings reveal that the PFC may indeed be involved in activating some aspects of Acb cell firing during goal-directed behavior but perhaps not all firing characteristics of Acb activity are directly governed by PFC neurons.

Postresponse discharge patterns

A subset of neurons exhibited a significant increase or decrease in firing rate immediately following the reinforced response for water (Figs. 3, 4, maintenance phase). Both excitatory (type RFe) and inhibitory (type RFi) postresponses were altered following the elimination of the natural reward, albeit the reduction in cell firing rate for type RFe cells was not significant. A similar attenuation of phasic firing has been reported in the primate OFC when the expected reward was omitted (Tremblay and Schultz, 2000).

Another set of neurons exhibited a reduction in cell firing following the press (type RFi cells). This type of firing pattern is not unique to water reward, since inhibitory responses following instrumental responding have also been observed in the PFC for rodents self-administering cocaine and heroin (Chang et al., 1997a,b). During extinction, the postresponse inhibition typical of RFi cells was significantly altered. This finding may suggest that type RFi activity may be related to the availability of the reward. Alternatively, the loss of type RFi activity during extinction may reflect a neural responsiveness to the absence of the tone that previously signaled reward availability that is not observed in the other cell types. Importantly, reinstatement of both the reward and tone reestablished the characteristic inhibitory period of RFi cells.

The responsiveness of PFC postresponse neurons during extinction was similar to that observed for Acb cells examined in our prior report (Hollander et al., 2002). That is, both PFC and Acb neurons were influenced by removal of the natural reward during the test session. Thus, the PFC may function to modulate Acb cell firing that is linked to completion of the operant response for reward and the onset of cues associated with that reward.

PFC: One part of a larger brain reward circuit

Although thus far we have considered the role of the PFC in potentially influencing Acb cell firing during behavior, it is important to note that PFC receives numerous inputs from subcortical regions including the amygdala and thalamus (Carr and Sesack, 1996; Sarter and Markowitsch, 1983; Groenewegen and

Uylings, 2000; Beckstead, 1979). Additionally, the PFC receives a dense dopaminergic innervation from the ventral tegmental area (Thierry, 1973). Numerous electrophysiological studies have elucidated the importance of an intact mesocorticolimbic system in mediating reward and reward-related processes (Carelli and Deadwyler, 1994; Carelli and Ijames, 2000, Koob and Nestler, 1997; Wise, 1996) and anatomical studies have provided direct evidence for the close relationship between PFC afferents adjacent to VTA dopaminergic neurons within the Acb (Sesack and Pickel, 1992; Pennartz et al., 1994). The present study clearly shows that a subset of PFC neurons encode the important features of goal-directed behaviors for natural reward; however, future studies are needed to examine the role of dopamine in the PFC in this process.

In this regard, several recent studies have shown that reward-predicting stimuli increase subsecond extracellular DA in the Acb prior to lever pressing for cocaine or sucrose (Roitman et al., 2004; Philips et al., 2003). Since cortical inputs are known to drive burst firing of midbrain DA neurons (Karreman and Moghaddam, 1996; Taber and Fibiger, 1995; Gariano and Groves, 1988) and it has been suggested that they precede VTA activity (Peters et al., 2004), this could potentially lead to the phasic increases in extracellular DA observed in the Acb. Thus, adaptations in cortical cell firing during behavior may have significant effects on DA activity, and consequently the loss or enhancement of DA availability in subcortical areas including the Acb. Therefore, the phasic activity reported in the present study may underlie a bigger functional synchrony between the VTA-Acb-PFC during specific aspects of goal-directed behaviors.

CONCLUSION

In the present study, neuronal firing rates in the PFC were altered by extinction and reinstatement procedures during goal-directed behaviors in rodents, similar to Acb firing properties reported in a prior study (Hollander et al., 2002). These findings may have important implications with respect to our understanding of information processing in the cortex and its potential modulation of subcortical (i.e., Acb) activity during behavior. Firing rates of populations of neurons within the reward circuit are important for response selection. Since cortical neurons guide the activity of subcortical regions, inhibitory patterns of cortical neurons may be important to eliminate competing neurons (e.g., taking neurons off-line) while excitatory neurons may represent a group of cells that are currently "on-line" and actively responding to changes in the environment. Collectively, the dynamic nature of PFC-Acb firing properties revealed during extinction/reinstatement procedures provide

critical insight into neural mechanisms underlying goal-directed behaviors and the plasticity of this signaling in behaving animals.

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