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# Prelimbic Prefrontal Cortical Encoding of Reward Predictive Cues

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## Abstract

Animals appoint incentive value and learn to approach otherwise behaviorally inert stimuli if these stimuli come to predict the delivery of reward. Interestingly, this adaptive Pavlovian learning process has been implicated in behavioral control disorders, such as drug addiction. One brain region implicated in directing conditioned approach behavior is the prelimbic region of the prefrontal cortex. The present study employed in vivo electrophysiology in the prelimbic cortex to characterize the distribution of neural responses to the presence of a cue that had

acquired incentive value after being associated with a primary reward. Male rats were trained in a Pavlovian autoshaping task in which a lever was presented prior to reward delivery. Following repeated pairings of lever availability and reward delivery, rats pressed the lever even though reward delivery was not contingent on any interaction with the lever. Neurons in the prelimbic cortex selectively encoded the presentation of the reward-predicting lever. Although the response was heterogeneous, most responsive neurons decreased their firing rate in response to the presence of the lever. These findings characterize the varied responses of prelimbic cortical neurons to reward cues and are consistent with evidence that the role of the prelimbic cortex in reward learning depends on the downstream target.

## 1 INTRODUCTION

An environmental cue that predicts the availability of a pleasurable reward can become a powerful incentive unto itself. The process by which this occurs is important to characterize not just because it is essential for normal behavior, but also because it may be involved in impulse control disorders and addiction (Colaizzi et al., [2020](#); Tomie et al., [2016](#)). One useful model for the study of acquired incentive is conditioned approach (i.e., Pavlovian autoshaping; Brown & Jenkins, [1968](#)), which assesses an animal's tendency to approach an otherwise motivationally neutral cue that predicts a rewarding outcome (Flagel & Robinson, [2017](#)).

Incentive learning involves many structures implicated in general reward learning, including the medial prefrontal cortex (mPFC). Many experiments have demonstrated a role for the mPFC in behaviors that require the use of cues to pursue specific rewards (Balleine & Dickinson, [1998](#); Killcross & Coutureau, [2003](#); Mulder et al., [2003](#); Otis et al., [2017](#)), and neurons in the mPFC encode cue-evoked reward-seeking behaviors (Homayoun & Moghaddam, [2009](#); Horst & Laubach, [2013](#); Petykó et al., [2015](#)). However, the relationship between mPFC activity and Pavlovian autoshaping is complex. Although Pavlovian autoshaping induces glutamate, norepinephrine, and serotonin release in the mPFC (Batten et al., [2018](#); Tomie et al., [2014](#)), and lesions of the mPFC reduce cue approach behavior (Serrano-Barroso et al., [2019](#)), there is little evidence that the mPFC encodes such Pavlovian cues.

In the present experiment, we characterized the mPFC encoding of a cue that had acquired incentive value. Using in vivo electrophysiological techniques, we recorded single unit activity in the prelimbic mPFC during a Pavlovian autoshaping task and describe cue-selective activity patterns.

## 2 MATERIALS AND METHODS

Subjects: Male, Sprague Dawley rats ( $n = 16$ ; Envigo, Indianapolis, IN) weighing 300–350 g were individually housed with a 12:12 hr light:dark cycle. Body weights were maintained at 90% of free feeding weight during testing. All procedures were approved by the Marquette University Institutional Animal Care and Use Committee.

Autoshaping: Pavlovian autoshaping occurred in Plexiglass operant chambers (MED-Associates; St. Albans, VT). Two retractable levers flanked a centrally located, recessed, food cup. Cue lights were located above each lever. Daily 1-hr training sessions comprised 25 CS+ trials and 25 CS- trials. During CS+ trials, the lever and light on one side of the food hopper were extended and illuminated, respectively, for 10 s, after which a sucrose pellet (45 mg; Bio-Serv) was delivered to the food cup. During CS- trials the lever and light on the other side were presented in the same manner but were not followed by sucrose delivery. Because our goal was to determine if mPFC neurons encoded the presentation of a cue that had acquired incentive value, criterion for inclusion was the acquisition of selective CS+ approach in the autoshaping task. This was determined by the demonstration of CS+ approach probability >80% on Day 10 of conditioning. Six rats failed to achieve this criterion. Of these rats, four exhibited a behavioral profile more akin to goal tracking, whereas two showed little goal tracking.

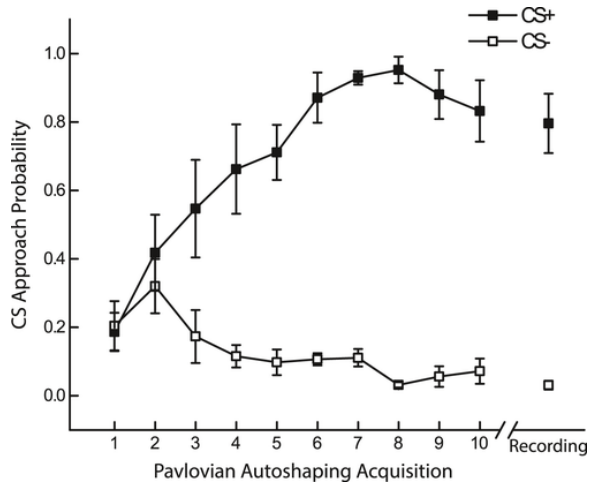
Electrophysiology: The 10 remaining subjects received electrode implantation surgery following conditioning. Under isoflurane anesthesia, 8-wire stainless steel microelectrode arrays (NB Labs; Denison, TX) were implanted bilaterally in the prelimbic PFC (PL) at AP: +3.0 mm, ML:  $\pm 0.6$  mm @ 0°;  $\pm 1.6$  mm @ 15°, DV: -4.0 mm @ 0°; -4.1 mm @ 15°. After recovery (12–19 days), recordings were conducted using a neurophysiological system (Plexon; Dallas TX), a commutator (Crist Instruments; Hagerstown, MD), and unit isolation software described previously (Wheeler et al., [2015](#)). Animals received an additional training session to verify recovery from surgery. Electrophysiological recordings occurred over 2 days of autoshaping training, with units recorded on the day with the most robust signal used for analysis.

Data Analysis: After testing, subjects were euthanized and microwire placements were verified as described previously (Wheeler et al., [2015](#)). Units recorded from wires outside of the PL were excluded from analysis. Firing rates of individual cells were aligned to CS+ and CS- onset. Spike histograms (1s bins) were created. Phasic cells were identified using ANOVA ( $\alpha = 0.05$ ) with the following levels: 10 s pre-CS period, 5 s early CS period, and 5 s late CS period. Differences were used to identify phasic responses. Histogram bins were normalized to the area under the receiver operating characteristic (auROC). The firing rates within each time bin across trials were compared to the firing rates throughout the baseline. A receiver operating characteristic was created from this comparison by plotting the probability of the firing rate during the window of interest exceeding a given value against the probability that the baseline firing rate exceeded that same value. This comparison was made for the range of values from zero through the maximum firing rate of a given unit. Unit auROC normalizations were also used for assessing the magnitude of unit responses by calculating the absolute deviation from 0.5 for each bin of the effect period and further calculating the area under the resulting curve.

Comparisons of signal intensity or behavior were made using ANOVA, *t* tests, or nonparametric tests ( $\alpha = 0.05$ ) using Python and R. In the event of sphericity violations in repeated measures ANOVA, the Greenhouse–Geisser corrected *p* value is reported.

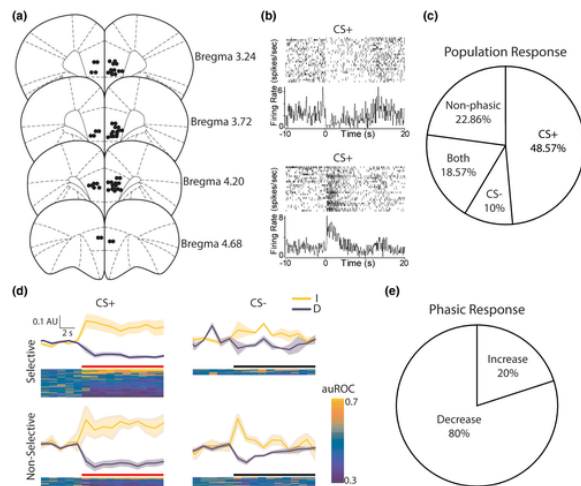
### 3 RESULTS AND DISCUSSION

To study the prelimbic encoding of reward learning in a Pavlovian autoshaping paradigm, animals were trained to discriminate between two compound lever/light cues that either predicted noncontingent sugar pellet delivery (CS+) or did not (CS-; Figure 1). A within-subjects 2 (CS type)  $\times$  10 (Day) ANOVA on CS approach found an interaction between CS type and Day,  $F(9, 81) = 15.76, p = 1.95e-8$ . Planned contrasts between the first day of training and the last day of training found that while CS+ approach increased,  $t(9) = 5.65, p = .0002$ , CS- approach fell,  $t(9) = 2.9, p = .015$ .



**FIGURE 1** Autoshaping behavior. Approach probability (mean  $\pm$  SEM) for the CS+ (filled squares) and CS- (open squares) across training. Rats interacted with the CS+ on more trials on Day 10 relative to Day 1 ( $p < .001$ ) but interacted with CS- on fewer trials ( $p = .015$ ). X-axis break indicates microelectrode surgery

Single unit activity in the prelimbic mPFC (Figure 2a) was recorded during a conditioning session following acquisition. The majority of neurons responded to the CS+ with a decrease or increase in firing rate (Figure 2b) (45/70), with a plurality doing so selectively (31/45). Only 11% (8/70) of units significantly altered their firing rate during CS- presentation (Figure 2c), demonstrating predominantly selective encoding of the reward-predicting cue. Consistent with this, units that responded to both the CS+ and CS- (14/70) did not do so equally (Figure 2d): CS- responses were significantly weaker than CS+ responses (ANOVA on areas under the auROC during the effect;  $F(1, 13) = 17.164, p = .01046$ ).



**FIGURE 2** Electrophysiology recordings. (a) Electrode placements in the prelimbic mPFC. (b) Peri-event rasters depicting one decreasing (top) and one increasing (bottom) unit response to CS+ onset (0 s) (c) Response distribution of PFC neurons to the CS+ and CS-. (d) auROC (mean  $\pm$  SEM) for selective and nonselective units that increased (I) or decreased (D) firing rate in response to the CS. Horizontal line indicates CS duration. Colorplot: individual unit auROC normalizations for CS-responsive units. Deviations above and below 0.5 depict increased and decreased firing rates. (e) Distribution of CS+ responsive neurons

In addition to characterizing the selectivity of the prelimbic response to a salient CS+, a directionality in the encoding was observed (Figure 2e). Of the neurons that encoded the CS+, a large majority (36/45) showed a firing rate reduction. Only 20% (9/45) exhibited an increase in activity when the reward-predicting cue was presented. These results demonstrate that prelimbic mPFC neurons selectively encode reward-predictive cues, and a plurality of these units encode these cues with a reduction in firing rate.

These results contribute to our understanding of the mPFC's role in encoding reward learning. In an anticipatory licking paradigm, Otis et al. (2017) observed overwhelmingly excitatory encoding of a reward cue in the mPFC. This contrasts with the predominantly inhibitory encoding reported here. Interestingly, Otis et al. found a predominantly inhibitory encoding profile specifically in neurons that project from the mPFC to the paraventricular thalamus (PVT). Taken together, one possible interpretation of these findings is that the two different learning paradigms result in fundamentally distinct mPFC encoding. Alternatively, it is possible that autoshaping preferentially engages the mPFC–PVT pathway. Interestingly, the PVT has been implicated in the pursuit of the primary reward rather than the predictive cue (Campus et al., 2019; Haight et al., 2015). If mPFC glutamatergic projections reduce downstream drive during CS+ presentations, animals may be more likely to attend to the cue rather than the primary goal. While in vivo electrophysiology does not easily allow for the distinction of efferent pathways, ongoing experiments are examining this possibility. Such studies will identify the contributions of specific PFC output pathways in approach motivation, as well as determine whether activity in these circuits reflect any sex differences in the behavior (King et al., 2016; Pitchers et al., 2015).

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## CONFLICT OF INTEREST

The authors declare no competing financial interest.

## AUTHOR CONTRIBUTIONS

MGS, DSW, and RAW designed the experiments. MGS, KRS, and DSW performed the surgeries, conducted the experiments, and analyzed the data. MGS, DSW, and RAW wrote the manuscript.

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