Firing Patterns of Accumbal Neurons During a Pavlovian-Conditioned Approach Task

Xun Wan¹ and Laura L. Peoples¹,²

¹Department of Psychology, University of Pennsylvania, and ²Neuroscience Graduate Group, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

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Wan, Xun and Laura L. Peoples. Firing patterns of accumbal neurons during a Pavlovian-conditioned approach task. J Neurophysiol 96: 652–660, 2006. First published April 26, 2006; doi:10.1152/jn.00068.2006. The nucleus accumbens (NAc) is necessary for the expression of Pavlovian-conditioned approach behavior but not for the expression of instrumental behavior conditioned in sessions that set a low response requirement. Although numerous studies have characterized firing patterns of NAc neurons in relation to instrumental behavior, very little is known about how NAc neurons encode information in Pavlovian tasks. In the present study, recordings of accumbal firing patterns were made during sessions in which rats performed a Pavlovian-conditioned approach task. Most of the recorded neurons (74/83, 89%) exhibited significant responses during the conditioned stimulus (CS) presentation and/or the reward exposure. The reward responses were prevalent, predominantly inhibitory, and comparable to reward responses observed in various types of behavioral paradigms, including instrumental tasks. The CS responses could be segregated into multiple subtypes on the basis of directionality, onset latency, and duration. Several characteristics of the CS firing patterns were unique relative to cue responses observed previously during alternative types of conditioning sessions. It is possible that the novel firing patterns correspond to the differential contributions of the accumbens to Pavlovian-conditioned approach behavior and instrumentally conditioned behavior. Regardless, the novel patterns of firing add to existing evidence that characterization of accumbal firing patterns in Pavlovian tasks may provide additional information about the neurophysiological mechanisms that mediate accumbal contributions to behavior.

INTRODUCTION

The nucleus accumbens (NAc) is necessary for the expression of Pavlovian-conditioned approach behavior and for the invigorating effects of Pavlovian-conditioned stimuli on instrumental behavior (Cardinal et al. 2002a,b; Dalley et al. 2005; de Borchgrave et al. 2002; Di Ciano et al. 2001; Eyny and Horvitz 2003; Hall et al. 2001; Parkinson et al. 1999, 2002). The accumbens also facilitates the maintenance of instrumental behavior when reinforcement is delayed, response requirements are high, and the behavior is maintained by a conditioned reinforcer (Cardinal and Everitt 2004; Di Ciano and Everitt 2001; Parkinson et al. 1999; Salamone et al. 2005; Taylor and Robbins 1986). However, the neurophysiological mechanisms by which the accumbens contributes to these behaviors are still poorly understood.

Numerous studies have characterized firing patterns of NAc neurons in relation to instrumental behavior in rats and primates (e.g., Apicella et al. 1991; Bowman et al. 1996; Carelli and Deadwyler 1994; Nicola et al. 2004a, b; Peoples and West 1996; Roop et al. 2002; Setlow et al. 2003; Schultz et al. 1992; Williams et al. 1993; Wilson and Bowman 2005). The instrumental tasks used in most of these studies set a low response requirement (typically fixed ratio 1, FR1). Although the firing patterns observed during these sessions are of interest, they may not be sufficient to fully reveal how the NAc contributes to behavior because a number of studies have indicated that the NAc is not necessary for the expression of instrumental behavior conditioned with a low response requirement (Balleine and Killcross 1994; Cardinal et al. 2002a, b; de Borchgrave et al. 2002). Therefore it seems necessary to characterize firing patterns of the NAc during alternative behaviors that depend more strongly on its function to provide additional information regarding the neurophysiological mechanisms that underlie accumbal contributions to behavior. Because it is well established that the NAc is critically involved in the acquisition and expression of Pavlovian-conditioned approach behavior, the present study was designed to conduct an initial electrophysiological characterization of accumbal firing patterns during such a behavioral task.

During daily sessions, animals were exposed to presentations of a light conditioned stimulus (CS) followed by delivery of a sucrose unconditioned stimulus (US). Electrophysiological recordings were made on the 10th day of conditioning. A high proportion of neurons responded during the CS presentation and/or the reward exposure. Responses during the reward exposure were quite comparable to those observed in various types of behavioral situations, including instrumental sessions. In contrast, the accumbal responses during the CS presentation showed a number of characteristics that were distinct relative to previously characterized accumbal responses to reward-predictive cues. The origin of these differences may or may not correspond to unique contributions of the NAc to Pavlovian-conditioned approach behavior. Regardless, the present findings, combined with previous studies of different types of instrumental tasks, highlight the potential utility of characterizing accumbal firing patterns in a variety of behavioral paradigms that are linked to accumbal function.

METHODS

Subjects

The subjects of the present study were five male Long-Evans rats (Charles River, Wilmington, MA). Animals were individually housed on a reversed 12-h light-dark cycle in a ventilation-controlled envi-
rnenment. Rats had ad lib access to water throughout the study, except during the brief daily experimental sessions, and had ad lib access to food until 1-wk post surgery. Thereafter, food was restricted (~20 g/day) to maintain their body weight at 350 g. All animal care and protocols were in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. Public Health Service and approved by the Animal Care and Use Committee of the University of Pennsylvania.

Surgical procedures

Rats were initially anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). Anesthesia was maintained by subsequent ketamine injections (50 mg/kg). One microwire recording array consisting of 16 quad-Teflon-coated stainless steel wires was implanted unilaterally into the NAc (AP, +0.5 to +3.0 mm from bregma; ML, ±0.5 to ±1.5 mm from midline; DV, −6.8 mm from skull) (Paxinos and Watson 1998). A separate ground wire was placed under the skull. The electrode headstage was fixed to the skull with acrylic dental cement secured with stainless steel bone screws. A more detailed description of the surgical procedures was provided in another report (Peoples 2003).

Apparatus

All training and recordings were conducted in 30 × 35 × 30-cm Plexiglas experimental chambers (Med Associates, St. Albans, VT). The chambers were housed in larger sound-attenuating and ventilated cubicles. Each chamber was equipped with a food trough built into one wall. A small well was located at the base of the trough. The well was attached via tubing to a motorized syringe pump that was used to infuse sucrose solution. The pump was located several feet outside of the chamber and was attached via tubing to a motorized syringe pump that was used to infuse sucrose solution. A stimulus light (1 cm diam) was immediately preceded or followed the US onset and lasted well beyond the 5-s US period. This long-lasting trough entry, referred to as the US trough-in period, firing rate was calculated for the 500 ms that immediately preceded or followed the US onset and lasted well beyond the 5-s US period. This long-lasting trough entry, referred to as the US trough entry, corresponded to the period of reward exposure.

Recording session and electrophysiological recording procedures

On the last day of Pavlovian conditioning (i.e., day 10), a recording session was conducted. The events of the session were identical to the preceding nine conditioning sessions except that animals were attached to the electrophysiological recording system. The electrode headstage on the animal was attached via a flexible recording cable to an electronic swivel. The swivel was attached to filtering and amplification equipment outside the cubicle. Neuronal signals were led to the computer running the Multichannel Acquisition Processor (MAP, Plexon, Dallas, TX). On-line isolation of waveforms was accomplished using the MAP system. All waveforms that exceeded an amplitude threshold were time-stamped and saved for subsequent off-line analysis. After the recording session, Off-Line Sorter (Plexon) was used to further discriminate neural spike waveforms. Briefly, all recorded waveforms were plotted in several two- and three-dimensional scatter plots. Waveforms that shared similar principal component values formed distinct clusters and were defined as single units. Additionally, each unit was subjected to an interspike interval criterion (<3% of interspike intervals were <1,900 μs) to minimize the possibility that more than one neuron contributed spikes to the unit, and a signal-to-noise criterion (≥2X noise band in amplitude) to further minimize the possibility that noise was infiltrating the unit discrimination. Units that could not be separated from each other or from noise were discarded.

During the recording session, both the frequency (i.e., the number per second) and duration (i.e., percent time) of trough entry were measured. To characterize the frequency, the pre-CS, CS, and US periods were analyzed on a basis of 1-s time bins. To characterize changes in the duration, the 10-s US period was divided into three shorter intervals: early (0–3 s from CS onset), middle (3–7 s from CS onset), and late (7–10 s from CS onset).

Analysis of firing patterns

Each isolated unit was examined for two basic types of responses: a CS response and a US trough entry (reward) response. The test for each response consisted of a Wilcoxon test that compared a baseline firing rate with the firing rate during the period of interest (α = 0.01). In testing for CS responses, the firing rate during the 10-s US period was compared with that during the 10-s pre-CS. A similar comparison was made between the 2 s pre- and post-CS. In testing for responses to the US trough entry, the first 10 s after the entry was compared with the 10-s pre-CS. Based on the direction of changes in firing, each unit was assigned into a category (i.e., excitatory or inhibitory). Units were further subtyped based on the time course of the firing pattern (i.e., the onset latency and the duration of the firing pattern).

Population peri-event histograms were constructed to show the average firing pattern of all neurons that exhibited the same type of firing. Repeated-measures ANOVA were conducted to confirm that the group of neurons showed significant changes in firing that were consistent with individual neurons included in that group (α = 0.05). To normalize firing rates, all individual firing rates were subjected to a transformation of log(n + 1) prior to repeated-measures ANOVA.

Analysis of firing related to trough entry behavior

For some neurons, comparisons of firing rate were made between periods in which the animal was engaged in trough entry (referred to as trough-in period) versus periods in which the animal was not engaged in that behavior (referred to as trough-out period). Operationally, the trough-out period was defined as periods that were bracketed by ≥1 s of no trough entry behavior. Its onset was defined as the median time point of that inter-trough-entry-interval. For the trough-in period, firing rate was calculated for the 500 ms that followed the onset of the trough entry. For the trough-out stage, firing
rate was calculated for the 500 ms after the onset of the trough-out period.

Histology

After all experiments were done, rats were injected with a lethal dose of ketamine. Anodal current (50 μA for 4 s) was passed through each microwire. Then animals were perfused with 4% paraformaldehyde. Coronal sections (30 μm) were mounted on slides and incubated in a solution of 5% potassium ferricyanide and 10% HCl to stain the iron deposits left by the recording tip. The tissue was counterstained with 0.2% solution of Neutral Red. Histological analyses were used to confirm that wire tips were located within the nucleus accumbens. The neurons that could be reliably identified as core versus shell, rather than core/shell border, were too few in number to conduct subregional comparisons. All neural data were therefore combined.

RESULTS

Behavior

ACQUISITION. Across the nine conditioning sessions prior to electrophysiological recordings, rats showed an increase in the probability of trough entry during the CS period relative to the pre-CS and US periods (Fig. 1A). These changes were statistically evaluated using repeated-measures ANOVA with trial period and day as factors. Trial periods included the pre-CS, CS, and US periods. The day factor included days 1, 3, 5, 7, and 9. The analysis showed that there was a significant interaction between trial period and day [F(8,32) = 4.03, P < 0.01]. Further comparisons showed that there was a significant between-day increase in the probability of trough entry during the CS period [F(4,16) = 7.62, P < 0.001] but not during either the pre-CS period [F(4,16) = 2.63, P > 0.05] or the US period [F(4,16) = 0.99, P > 0.4].

RECORDING SESSION. During the recording session (i.e., day 10), rats showed differential probabilities of trough entry in the three trial periods [F(2,8) = 45.95, P < 0.001, see Fig. 1A]. The probability of trough entry was greater during the CS period than during either the pre-CS period (Tukey tests, P < 0.001) or the US period (P < 0.001). There was no difference in the probability of trough entry between the pre-CS and US periods (P > 0.3). A comparison between days 9 and 10 showed that the probability of trough entry during the various trial periods was comparable between the two days. The two-factor repeated-measures ANOVA with day and trial period as factors showed that there was no effect of day [F(1,4) = 0.37, P > 0.5] and no significant interaction between trial period and day [F(2,8) = 1.43, P > 0.2]. Therefore behavior was not affected by the recording procedures.

A more detailed analysis of trough entry on the recording day showed that the average latency to the first trough entry equaled 4.2 s post-CS onset. Moreover, the frequency of trough entry increased progressively over the course of the CS (1-s resolution) and then declined rapidly within the first 2 s of the US period (Fig. 1C). Although the frequency of trough entry decreased during the US period, the duration increased. Typically, a trough entry was shorter than 1 s if it occurred during the pre-, early- or middle-CS periods. By contrast, it could be >15 s if occurring around US delivery (i.e., the late-CS or US periods).

The variations in the frequency and duration of trough entry across the trial periods were associated with a progressive net increase in the percent time that rats spent in the trough. A one-factor repeated-measures ANOVA showed that there was a significant effect of trial period [F(4,16) = 93.43, P < 0.001]. Post hoc tests indicated that the percent time did not significantly change between the pre-CS and early-CS periods but increased significantly and successively during the subsequent middle-CS, late-CS, and US periods (Fig. 1B).

Firing patterns

ACCUMBAL RESPONSES TO REWARD EXPOSURE. The majority of all neurons (63/83, 76%) showed a significant change in firing during the first 10 s of the US trough entry relative to the 10-s pre-CS period. For most responsive neurons, the change was an inhibition (inhibitions: 50/63, 79%; excitations: 13/63, 21%).
The time course of the change in firing was quite uniform among neurons. Figure 2A displays an example neuron showing an inhibitory reward response and 2B displays an example neuron showing an excitatory response. For both excitatory and inhibitory responses, the change was stable for the duration of the US trough entry but reversed rapidly as rats exited the trough. The average firing rate of all neurons that exhibited either excitatory or inhibitory responses was subjected to separate repeated-measures ANOVA with trial period as the factor, which included the 10-s pre-CS, the first 10 s of the US trough entry, and the first 10 s following the offset of the entry (referred to as posttough exit). Figure 3A displays the average firing pattern of the inhibitory neurons. The statistical analysis applied to them showed that there was a significant effect of trial period \[F(2,24) = 42.98, P < 0.001\] and that firing during the US trough entry was significantly reduced relative to both the pre-CS \((P < 0.001)\) and posttough exit periods \((P < 0.001)\). Firing during the latter two periods did not differ significantly \((P > 0.4)\). The average firing pattern of the excitatory neurons is depicted in Fig. 3B. The statistical analysis of these neurons showed that there was also a significant effect of trial period \[F(2,24) = 8.48, P < 0.002\]. Firing during the US trough entry was greater than that during either the pre-CS period \((P < 0.002)\) or the posttough exit period \((P < 0.01)\). Firing during the pre-CS and posttough exit periods did not differ significantly \((P > 0.8)\).

Inhibitory CS responses followed any of three time courses. For the largest group of neurons, the inhibition was delayed in onset and developed progressively as the CS interval elapsed (referred to as delayed-onset CS responses, Fig. 4A, top). The firing rate of these neurons did not differ between the 2-s pre- and post-CS but differed significantly between the 10-s pre- and post-CS. For a second group of neurons, the inhibition occurred immediately after CS onset and was maintained for the duration of the CS (referred to as rapid-onset sustained CS responses, Fig. 4A, middle). The firing rate of these neurons differed significantly between the 2-s pre- and post-CS as well as between the 10-s pre- and post-CS. For a third group of cells, the inhibitory response was rapid in onset but short-lived in duration (referred to as rapid-onset transient CS responses, Fig. 4A, bottom). The firing rate of these neurons differed significantly between the 2-s pre- and post-CS but not between

### TABLE 1. Prevalence of CS responses sub-typed according to directionality and time course

<table>
<thead>
<tr>
<th>Subtype</th>
<th>n</th>
<th>Percentage of Total</th>
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<tbody>
<tr>
<td>Delayed-onset (inhibitory)</td>
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<td>26.5</td>
</tr>
<tr>
<td>Delayed-onset (excitatory)</td>
<td>10</td>
<td>12.1</td>
</tr>
<tr>
<td>Rapid-onset sustained (inhibitory)</td>
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<td>13.1</td>
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<tr>
<td>Rapid-onset sustained (excitatory)</td>
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<td>Rapid-onset transient (inhibitory)</td>
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<td>3.6</td>
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<tr>
<td>Rapid-onset transient (excitatory)</td>
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<td>4.8</td>
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<tr>
<td>Rapid-onset responses with longer duration (excitatory)</td>
<td>4</td>
<td>4.8</td>
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CS, conditioned stimulus.
the 10-s pre- and post-CS. Figure 5A shows that the population histogram for all inhibitory CS responsive neurons appeared most consistent with the predominant firing pattern (i.e., progressive inhibitions) except that the onset of inhibitions was not dramatically delayed. Repeated-measures ANOVA showed that the average firing rate of all inhibitory CS-responsive neurons was different during both the 10-s pre- and post-CS [$F(1,35) = 51.11, P < 0.001$] and the 2-s pre- and post-CS [$F(1,35) = 15.41, P < 0.001$].

Excitatory CS responses were similar to inhibitory ones with two exceptions (Fig. 4B). First, excitatory responses with a delayed onset did not exhibit progressive changes (Fig. 4B, top). Second, there was a fourth subtype of excitatory CS responses that was similar to a transient CS response but the duration was extended by 1–3 s (not shown). The population histogram for all excitatory CS-responsive neurons showed a sustained change that had a rapid-onset (Fig. 5B). Repeated measures ANOVA showed that the average firing rate of all excitatory CS-responsive neurons was significantly different between the 10-s pre- and post-CS [$F(1,19) = 41.92, P < 0.001$] as well as between the 2-s pre- and post-CS [$F(1,19) = 19.17, P < 0.001$].

COMBINED ACCUMBAL RESPONSES TO THE CS PRESENTATION AND REWARD EXPOSURE. Almost all the neurons (74/83, 89%) showed a response to at least one experimental event (i.e., the CS presentation or the reward exposure). About half the neurons showed combined responses to both events (45/83, 54%). The most common combined response consisted of inhibitions during both the CS period and the US trough entry (29/45, 64%). This subtype of neurons accounted for more than a third of all neurons. Eighteen percent (8/45) of the combined responsive neurons showed excitations in both periods, whereas the remaining 18% showed opposite responses in the two periods. The prevalence of neurons responding to combinations of events is presented in Table 2.

When the CS responses were segregated by both directionality and onset latency, various types of CS responses exhibited different relationships with the reward responses. Specifically, the rapid-onset CS responses tended to occur with inhibitory reward responses regardless of whether they were excitatory or inhibitory.

![Fig. 5](image1.png)

**FIG. 5.** Average responses of neurons that exhibited CS responses. A: population histogram showing the average response of all neurons ($n = 36$, 43.4% of total) that exhibited inhibitory responses during the CS period. B: population histogram showing the average response of all neurons ($n = 20$, 24.1%) that exhibited excitatory responses during the CS period. In each histogram average firing rate (Hz per 0.25 s) is plotted for the 10-s pre-CS and the 10-s CS periods.

<table>
<thead>
<tr>
<th>Type</th>
<th>$n$</th>
<th>Percentage of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS excitation only</td>
<td>6</td>
<td>7.2</td>
</tr>
<tr>
<td>CS inhibition only</td>
<td>5</td>
<td>6.0</td>
</tr>
<tr>
<td>Reward excitation only</td>
<td>3</td>
<td>3.6</td>
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<tr>
<td>Reward inhibition only</td>
<td>15</td>
<td>18.1</td>
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<tr>
<td>CS excitation with reward excitation</td>
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<td>9.6</td>
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<td>CS inhibition with reward inhibition</td>
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<td>34.9</td>
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<tr>
<td>CS excitation with reward inhibition</td>
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<td>7.2</td>
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<tr>
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<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>No response</td>
<td>9</td>
<td>10.8</td>
</tr>
</tbody>
</table>
inhibitory. In contrast, inhibitory delayed-onset CS responses occurred primarily with inhibitory reward responses, and excitatory delayed-onset CS responses occurred predominantly with excitatory reward responses. As a consequence, the delayed-onset CS responses were more likely than the rapid-onset CS responses to occur with a directionally consistent reward response. These relationships are depicted in Table 3.

**RELATION OF PROGRESSIVE CS RESPONSES TO TROUGH ENTRY.** A comparison between the frequency of trough entry (see Fig. 1C) and the patterns of neural firing (see Figs. 2 and 4) showed that none of the subtypes of the firing patterns were consistently related to the frequency of trough entry. However, the inhibitory delayed-onset CS responses, which most typically occurred with a sustained inhibitory response during the US trough entry, closely mirrored changes in the percent time that rats spent in the trough (see Figs. 1B and 6A). This relationship suggested that this subtype of CS responses might be caused by stimuli or behaviors that occurred during trough entry rather than by caused by the CS.

To test this interpretation, the relationship between the firing rate of the inhibitory delayed-onset neurons and trough entry behavior was evaluated in more detail. Average firing was calculated separately for periods in which the animal was in the trough (referred to as trough-in period) and during periods in which the animal was not in the trough (referred to as trough-out period). Firing rates during these two behavioral stages were compared across the various trial periods (i.e., the pre-, early-, middle-, and late-CS periods; Fig. 6B). Repeated-measures ANOVA with trough period and trial period as factors showed that there was a significant effect of trough period \(F(1,21) = 22.65, P < 0.001\) and trial period \(F(3,63) = 9.34, P < 0.001\), but no significant interaction between them \(F(3,63) = 0.34, P > 0.7\). The fact that the average firing during trough-in periods was consistently lower than that during trough-out periods suggests that events associated with trough entry contributed to the change in firing. On the other hand, the significant change in firing across the various trial periods (especially in trough-out periods) indicated that the change was not determined exclusively by trough-entry-associated events but was potentially related in part to the CS. Taken together, these observations indicated that the inhibitory delayed-onset CS responses were not specifically related to trough entry behavior but were determined by both trough entry behavior and the presentation of the CS.

**DISCUSSION**

The present study demonstrates that in a Pavlovian-conditioned approach task, accumbal neurons in rats showed robust responses during the CS and the US trough entry periods. The firing patterns are summarized in the following text in the context of comparisons to both instrumental conditioning studies, and the two other studies of Pavlovian conditioning that have been described thus far (Day et al. 2006; Roitman et al. 2005).

**Responses to reward**

**FIRING PATTERNS.** In the present study 76% of all recorded neurons showed a response during the period in which animals had access to reward. The majority (79%) of the reward responses were inhibitory. These findings are consistent with the one other Pavlovian-conditioning study that examined accumbal responses to reward exposure (Roitman et al. 2005).

The reward responses in the Pavlovian sessions are also consistent with observations made during most instrumental conditioning studies, in which responses to reward exposure were predominantly inhibitory, and in some cases quite prevalent (e.g., 40–90% of all recorded neurons) (Nicola and Deadwyler 2000; Nicola et al. 2004b; Peoples and West 1996; Taha and Fields 2005; Wilson and Bowman 2005). There are some reports that during instrumental sessions responses to reward were few in number and predominantly excitatory (e.g., Apicella et al. 1991; Bowman et al. 1996; Carelli and Deadwyler et al. 1991; Apicella et al. 1991; Bowman et al. 1996; Carelli and Deadwyler 1996; Taha and Fields 2005; Wilson and Bowman 2005).

**TABLE 3. Relation to the reward responses when the CS responses were segregated by both directionality and time course**

<table>
<thead>
<tr>
<th></th>
<th>Inhibitory Reward Responses</th>
<th>Excitatory Reward Responses</th>
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</thead>
<tbody>
<tr>
<td>Inhibitory delayed-onset CS responses</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Excitatory delayed-onset CS responses</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Inhibitory rapid-onset CS responses</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Excitatory rapid-onset CS responses</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>
FIRING PATTERNS. In the present study, 67% of all recorded behavioral sessions suggest that accumbal responses to sensory properties (e.g., taste or texture) of food and water though prior conditioning during natural exposure to primary innate, unconditioned responses (Roitman et al. 2005), although prior conditioning during natural exposure to primary sensory properties (e.g., taste or texture) of food and water cannot be excluded. Regardless, in total, the comparisons of reward responses during the various Pavlovian and instrumental conditioning sessions suggest that accumbal responses to reward exposure may be quite uniform across a variety of behavioral conditions.

FUNCTION. Evidence related to the function of accumbal responses occurred with a directionally consistent reward response. However, most of them were inhibitory, progressive, and long in duration. In addition, almost all the delay-onset CS responses were excitatory or inhibitory, they tended to occur with an inhibitory reward response. Forty-three percent of the CS responses in the present study was substantially higher than the prevalence of cue responses observed during instrumental conditioning sessions with low response requirements (i.e., 67 vs. 10–15%); although, it was within the range (i.e., >30 to >70%) observed in more complex operant sessions in which response requirements were high and instrumental behavior was expected to be strongly influenced by conditioned stimuli (Shidara et al. 1998; Wilson and Bowman 2005). The prevalence of inhibitory cue responses was also substantially higher than that typically observed in instrumental sessions. Indeed, cue responses during instrumental sessions are consistently reported to be predominantly excitatory (e.g., Carelli 2000; Nicola et al. 2004a; Peoples et al. 1997, 2004; Schultz et al. 1992; Wilson and Bowman 2005). The delayed-onset and progressive changes in some CS responses observed here were distinct from cue responses typically observed during instrumental sessions. Finally, in the present study, the prevalence of neurons that exhibited directionally consistent responses to the cue and the reward was greater than instrumental conditioning sessions.

There are two other studies that described accumbal firing patterns during Pavlovian conditioning (Day et al. 2006; Roitman et al. 2005). Interestingly, the accumbal CS responses observed in the Roitman et al. (2005) study differed from those of the present study in a variety of ways. In the Roitman et al. (2005) study, >40% of neurons exhibited a response to the cue associated with sucrose; the majority of those firing patterns were excitatory and short-lived, ending prior to the offset of the CS. The majority of neurons that showed a CS response also showed a reward to reward. Inhibitory CS responses occurred almost exclusively with inhibitory reward responses, whereas excitatory CS responses occurred with excitatory (31%), inhibitory (42%), or no (27%) reward response.

The overall prevalence of CS responses observed in the Roitman et al. (2005) study is lower than that of the present study, probably due to the lower prevalence of inhibitory CS responses. The predominance of excitatory cue responses is consistent with findings of instrumental conditioning studies but opposite the findings of the present Pavlovian study. However, the absence of long-lasting cue responses in the Roitman et al. (2005) study is different from cue responses observed in both instrumental studies and the present Pavlovian study.

There are numerous parametric differences that potentially underlie the different findings between the Roitman et al. (2005) study and the present one. The parametric differences include but are not limited to the following. The Roitman et al. (2005) study was conducted during an initial acquisition session that exposed animals to two CS-US associations. In the present study, recordings were made after acquisition of a task in which only a single CS-US association was experienced. In the Roitman et al. (2005) study, but not in the present study, termination of the CS and the onset of US delivery were associated with the presentation of additional cues. Another difference between the two studies is that the CS stimuli in the Roitman et al. (2005) study were not spatially localized and the US was delivered through an intra-oral catheter. In contrast, in the present study, the CS was localized and the US was delivered to a trough. Thus conditioned approach responses occurred in the present study but are not expected to have occurred in the study of Roitman et al. (2005). A potential role for some or all of these parametric factors is supported by comparisons of the present study to the more recent study of

Responses to conditioned stimuli

FIRING PATTERNS. In the present study, 67% of all recorded neurons showed a response during the CS presentation. Based on the onset latency, the CS responses could be segregated into two categories, rapid- and delayed-onset responses. Forty-three percent of the CS responses showed a rapid onset. These firing patterns were predominantly sustained, rather than transient, in duration. Regardless of whether the rapid-onset CS responses were excitatory or inhibitory, they tended to occur with an inhibitory reward response. Fifty-seven percent of the CS responses showed a delayed onset. Some of the delayed-onset CS responses were excitatory and sustained over the duration of the CS. However, most of them were inhibitory, progressive, and long in duration. In addition, almost all the delay-onset CS responses occurred with a directionally consistent reward response.

Overall, the prevalence of the CS responses in the present study was substantially higher than the prevalence of cue responses observed during instrumental conditioning sessions with low response requirements (i.e., 67 vs. 10–15%); although, it was within the range (i.e., >30 to >70%) observed in more complex operant sessions in which response requirements were high and instrumental behavior was expected to be strongly influenced by conditioned stimuli (Shidara et al. 1998; Wilson and Bowman 2005). The prevalence of inhibitory cue responses was also substantially higher than that typically observed in instrumental sessions. Indeed, cue responses during instrumental sessions are consistently reported to be predominantly excitatory (e.g., Carelli 2000; Nicola et al. 2004a; Peoples et al. 1997, 2004; Schultz et al. 1992; Wilson and Bowman 2005). The delayed-onset and progressive changes in some CS responses observed here were distinct from cue responses typically observed during instrumental sessions. Finally, in the present study, the prevalence of neurons that exhibited directionally consistent responses to the cue and the reward was greater than instrumental conditioning sessions.

There are two other studies that described accumbal firing patterns during Pavlovian conditioning (Day et al. 2006; Roitman et al. 2005). Interestingly, the accumbal CS responses observed in the Roitman et al. (2005) study differed from those of the present study in a variety of ways. In the Roitman et al. (2005) study, >40% of neurons exhibited a response to the cue associated with sucrose; the majority of those firing patterns were excitatory and short-lived, ending prior to the offset of the CS. The majority of neurons that showed a CS response also showed a reward to reward. Inhibitory CS responses occurred almost exclusively with inhibitory reward responses, whereas excitatory CS responses occurred with excitatory (31%), inhibitory (42%), or no (27%) reward response.

The overall prevalence of CS responses observed in the Roitman et al. (2005) study is lower than that of the present study, probably due to the lower prevalence of inhibitory CS responses. The predominance of excitatory cue responses is consistent with findings of instrumental conditioning studies but opposite the findings of the present Pavlovian study. However, the absence of long-lasting cue responses in the Roitman et al. (2005) study is different from cue responses observed in both instrumental studies and the present Pavlovian study.

There are numerous parametric differences that potentially underlie the different findings between the Roitman et al. (2005) study and the present one. The parametric differences include but are not limited to the following. The Roitman et al. (2005) study was conducted during an initial acquisition session that exposed animals to two CS-US associations. In the present study, recordings were made after acquisition of a task in which only a single CS-US association was experienced. In the Roitman et al. (2005) study, but not in the present study, termination of the CS and the onset of US delivery were associated with the presentation of additional cues. Another difference between the two studies is that the CS stimuli in the Roitman et al. (2005) study were not spatially localized and the US was delivered through an intra-oral catheter. In contrast, in the present study, the CS was localized and the US was delivered to a trough. Thus conditioned approach responses occurred in the present study but are not expected to have occurred in the study of Roitman et al. (2005). A potential role for some or all of these parametric factors is supported by comparisons of the present study to the more recent study of
Pavlovian conditioning (Day et al. 2006). The latter study was procedurally similar to the present one except that a CS minus was included in the session, the localized CSs were levers inserted into the chamber, and the approach response was measured as presses on the lever. Despite these differences, the CS responses in both studies were predominantly inhibitory and sustained in nature. Although other factors may also be important, the comparisons of the various studies of Pavlovian conditioning suggest that the directionality and time course of accumbal responses to conditioned stimuli are influenced by the type of behavior evoked by the stimuli (e.g., approach vs. no approach).

Finally, compared with both the Roitman et al. (2005) and Day et al. (2006) studies, there are two important new findings in the present study. The delayed onset and progressive changes during the CS presentation observed in the present study were unique. Also, to our knowledge, this is the first time that a high prevalence of directionally consistent responses to the cue and the reward has been reported. These novel observations may provide some additional information about how accumbal neurons mediate Pavlovian-conditioned approach behavior (see following text).

**FUNCTION.** Most research on the function of accumbal cue responses has been conducted in relation to instrumental tasks. A number of previous studies have shown that accumbal neurons rarely respond to unconditioned sensory stimuli (Schultz et al. 1992; Setlow et al. 2003; Shidara et al. 1998). There are also no primary sensory afferents to the NAc (Zahn 2000). It is unlikely therefore that the CS responses in the present study were sensory in nature. Studies of instrumental conditioning show that accumbal responses to various types of cues are impacted by manipulations of the contingency between the cue and the reward. Therefore the cue responses are related to reward expectation (Cromwell and Schultz 2003; Hollerman et al. 1998; Nicola et al. 2004a; Schultz et al. 1992; Setlow et al. 2003; Shidara et al. 1998; Williams et al. 1993). Some accumbal responses to discriminative stimuli are additionally impacted by variations in instrumental behavior (Hollerman et al. 1998; Nicola et al. 2004a; Setlow et al. 2003).

Due to the various differences in cue responses between the present Pavlovian task and instrumental tasks, it is difficult to speculate about the potential functional relation in cue responses between the two conditioning paradigms. However, given the strong association between the CS and US that is acquired during Pavlovian conditioning, it is reasonable to expect that accumbal CS responses are related to reward expectation. There is some evidence that this may be so. In the Roitman et al. (2005) experiment, the CS responses were related to expectation of the specific US, consistent with a possible relation between the CS and reward expectation.

Given the necessary contribution of NAc neurons to the expression of conditioned approach behavior, one might also expect that some CS responses are related to the conditioned approach response. Consistently, the inhibitory delayed-onset CS responses mirrored the percent time spent in the trough, and the magnitude of inhibitions was at least partially determined by the occurrence of the conditioned approach response. The absence of long-lasting CS responses in the Roitman et al. (2005) study, relative to the present Pavlovian study and instrumental studies, adds some additional weight to the proposal that the long-lasting CS responses may contribute to conditioned approach. The fact that certain types of CS responses were modulated by the conditioned approach response seems analogous to the observation that cue responses during instrumental sessions are impacted by variations in operant behavior.

However, in the present study changes in firing related to approach behavior were predominantly inhibitory where as changes in firing time-locked to operant behavior during instrumental sessions are predominantly excitatory (Carelli and Deadwyler 1994; Nicola et al. 2004a; Peoples et al. 1997; Schultz et al. 1992). These disparate observations may reflect the fact that the two types of behaviors are differentially influenced by numerous variables and are also differentially dependent on accumbal function, although both of them could be considered as anticipatory behaviors related to obtaining the reward. Further investigations are required to elucidate how different firing patterns of accumbal neurons contribute to these different behaviors.

One additional but not exclusive interpretation for the CS responses is that they reflect conditioned reward responses. In line with this proposal, we observed a high prevalence of neurons that exhibited directionally consistent cue and reward responses. This proposal is consistent with the expectation that Pavlovian CSs are strongly associated with the primary reward. The proposal is also attractive in that it provides a potential explanation as to how the accumbens might contribute to conditioned behaviors that are like unconditioned responses to reward. For example, cue-evoked inhibitory responses could mimic reward-evoked inhibitory responses that facilitate consummatory behavior. In this case, cue-evoked inhibitions might be expected to influence behavior in the same way as reward-evoked inhibitions of the same neurons.

**Summary and conclusions**

The present phenomenological documentation of accumbal firing patterns during a Pavlovian-conditioned approach task demonstrates that accumbal responses to salient events are prevalent. A comparison of the prevalence and topography of the firing patterns in the present study to those observed in studies of instrumental conditioning showed evidence that the accumbal responses to reward exposure are similar across Pavlovian and instrumental tasks as well as other situations. The observation of similar firing patterns across multiple conditioning paradigms suggests that those firing patterns may mediate a common accumbal contribution to behavior.

A number of differences between the present Pavlovian study and instrumental conditioning sessions were also observed. First, the prevalence of CS responses exceeds the prevalence of cue responses in a simple FR1 task, which is used in many recording studies, but equals the prevalence of cue responses in complex instrumental tasks that use cues to maintain and guide behavior. This observation corresponds well with evidence that the NAc plays a more central role in facilitating behaviors that are cue-dependent. Second, inhibitory CS responses were much more prevalent relative to inhibitory cue responses in instrumental tasks. Finally, directionally consistent CS and reward responses were also much more prevalent in the present study as compared with instrumental tasks. The origin of the differences awaits delineation.
It is possible that they reflect the different roles of the accum-
bens in mediating Pavlovian and instrumental behavior. Take 
together, the present findings demonstrate that further charac-
terization of accumbal firing patterns during Pavlovian-condi-
tioned approach tasks as well as other behaviors that depend
strongly on accumbal function may be useful in understanding
the neurophysiological mechanisms that mediate accumbal
contributions to behavior.

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