

# Nucleus Accumbens Neurons Are Innately Tuned for Rewarding and Aversive Taste Stimuli, Encode Their Predictors, and Are Linked to Motor Output

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## Summary

The nucleus accumbens (NAc) is a key component of the brain's reward pathway, yet little is known of how NAc cells respond to primary rewarding or aversive stimuli. Here, naive rats received brief intraoral infusions of sucrose and quinine paired with cues in a classical conditioning paradigm while the electrophysiological activity of individual NAc neurons was recorded. NAc neurons (102) were typically inhibited by sucrose (39 of 52, 75%) or excited by quinine (30 of 40, 75%) infusions. Changes in firing rate were correlated with the oromotor response to intraoral infusions. Most taste-responsive neurons responded to only one of the stimuli. NAc neurons developed responses to the cues paired with sucrose and quinine. Thus, NAc neurons are innately tuned to rewarding and aversive stimuli and rapidly develop responses to predictive cues. The results indicate that the output of the NAc is very different when rats taste rewarding versus aversive stimuli.

## Introduction

Successful feeding is accomplished by approach toward and consumption of nutritive substances and avoidance and rejection of potentially dangerous ones. To be successful, some brain systems must be able to differentiate between rewarding and aversive stimuli, as well as incorporate learned associations into their processing to direct appropriate feeding behavior. The nucleus accumbens (NAc) has received extensive attention as a component of the brain's reward system and plays a role in mediating the reinforcing aspects of food. Normal functioning of the NAc is required for various aspects of goal-directed behavior aimed at food (de Borchgrave et al., 2002; Salamone et al., 2003), and feeding can be stimulated by pharmacological manipulation of the NAc (Kelley, 2004; Stratford and Kelley, 1997). The NAc is also important for reward-related learning, as its proper functioning is required for the acquisition of operant responding for food reward (Hernandez et al., 2002; Smith-Roe and Kelley, 2000). In addition, increasing dopamine levels in the NAc with amphetamine promotes Pavlovian to instrumental transfer learning (Wyvell and Berridge, 2001). On a cellular level, individual NAc neurons encode aspects of operant responding (i.e., approach, response, reward) for food reward (Carelli et al., 2000; Yun et al., 2004). While these studies have supported the idea that the NAc plays a vital role in behavior motivated by food, the innate re-

sponsiveness of the NAc to primary rewarding stimuli before any learning has occurred has yet to be characterized.

In spite of its characterization as primarily mediating reward processes, evidence has mounted for a role of the NAc in signaling nonrewarding, salient events, including aversive stimuli. The same pharmacological manipulations that stimulate feeding when performed in the medial NAc shell stimulate defensive behaviors when injections are directed more caudally (Reynolds and Berridge, 2002, 2003). These studies suggest that there are specific circuits within the NAc that process aversive stimuli. It has recently been shown that ventral striatal neurons respond to secondary stimuli that predict aversive consequences in a go/no go behavioral paradigm (Setlow et al., 2003), and the ventral striatum appears to play a role in signaling aversive stimuli in humans as well (Jensen et al., 2003; Seymour et al., 2004). Nevertheless, little is known of how NAc neurons respond to primary aversive stimuli because subjects learn to avoid them.

In this report, we examined the responsiveness of individual NAc neurons to primary rewarding and aversive taste stimuli. This issue is critical in evaluating the NAc as a substrate for reward or for motivational information processing independent of hedonic valence. The challenge in determining the responsiveness of the NAc to aversive as well as rewarding stimuli is that rats will not voluntarily sample aversive stimuli to the same degree as rewarding ones. Here, we have circumvented this challenge by using brief intraoral delivery of rewarding sucrose and aversive quinine solutions. These solutions elicit ingestive and aversive oromotor behavior in the taste reactivity paradigm (Grill and Norgren, 1978). In addition, we paired unique audiovisual cues to each tastant so that rats would learn an association between cue presentation and tastant delivery in a purely Pavlovian paradigm. While firing patterns of NAc neurons have been well characterized for instrumental responding for food in well-trained rats (Carelli, 2002; Carelli et al., 2000; Roop et al., 2002), the role of individual NAc neurons in the acquisition of Pavlovian associations for rewarding and aversive stimuli has yet to be investigated. Both the unconditioned and conditioned behavioral responses in sucrose and quinine trials were assayed by recording the activity of the anterior digastric muscle, whose activity is coupled to licking behavior (Kaplan et al., 1995; Travers and Norgren, 1986). The present study addresses the hypothesis that individual NAc neurons are "hardwired" to respond to primary rewarding and aversive taste stimuli, even in rats without any prior experience with these taste stimuli. Further, NAc responses to cues signaling rewarding and aversive taste stimuli would develop as a function of learning.

## Results

Neural ( $n = 8$  rats) and electromyographic (six of eight rats) recordings were made simultaneously while rats

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were administered a block (30 trials) of cue-sucrose and a block (30 trials) of cue-quinine pairings in a single recording session. On a given trial, a change in lighting coupled with an auditory stimulus (cue) was administered for 6 s. Unique cues were used for each tastant and were counterbalanced across rats. Immediately after cue termination, an infusion pump turned on for 6 s to deliver 200  $\mu$ l of solution directly into the oral cavity of the rat. After each infusion, there was a variable intertrial interval (30–60 s) (see [Experimental Procedures](#) for details). This intraoral delivery permits the characterization of NAc responses to rewarding and aversive taste stimuli without any prior exposure or training. In addition, it permits the administration of equal numbers and durations of exposures to rewarding and aversive stimuli. Finally, since stimuli are delivered to the rats independently of their behavior, no food or water deprivation is required. Thus, rats are tested under homeostatic conditions, and NAc responses to reward and aversion can be assayed without the confound of increased motivation due to food or water restriction.

### Behavioral Responses to Rewarding and Aversive Unconditioned Stimuli

Our first goal was to characterize behavioral responses to the unconditioned stimuli (i.e., sucrose and quinine infusions). Recordings from the anterior digastric muscle showed marked increases in electromyographic (EMG) activity during intraoral infusions. The average ( $n = 6$  rats) unconditioned EMG activity across each block of 30 trials (i.e., sucrose and quinine blocks) is shown in [Figures 1A](#) and [1B](#). The activity during 6 s from  $-12$  s to  $-6$  s relative to pump onset served as baseline. For baseline and infusion periods, EMG activity was binned into 1 s epochs, and a one-way ANOVA with repeated measures was performed. For sucrose infusions ([Figure 1A](#)), there was a significant effect of time on EMG activity [ $F(11,55) = 8.035$ ;  $p < 0.0001$ ]. To determine when EMG activity was first significantly elevated relative to baseline, Student's  $t$  tests using Bonferroni's correction were used to compare each 1 s epoch after pump onset with the first second of baseline. EMG activity was significantly elevated by the third second after pump onset [ $t(5) = 3.82$ ;  $p < 0.05$ ]. For quinine infusions ([Figure 1B](#)), there was also a significant effect of time on EMG activity [ $F(11,55) = 5.243$ ;  $p < 0.0001$ ]. Again, Student's  $t$  tests revealed that EMG activity was significantly higher in the third second after pump onset [ $t(5) = 3.18$ ;  $p < 0.05$ ]. Both baseline [ $t(10) = 2.33$ ;  $p < 0.05$ ] and peak EMG [ $t(10) = 2.99$ ;  $p < 0.05$ ] activity were significantly greater for sucrose infusions compared to quinine.

It is very important to note that while rats respond to intraoral infusions of sucrose almost exclusively with ingestive responses that involve contractions of the anterior digastric muscle (i.e., mouth movements, tongue protrusions, etc.), the response to quinine is typically very different. For the strong concentration of quinine used here, rats typically emit gapes (which require contractions of the anterior digastric muscle) but also engage in a set of body movements, such as chin rubbing to expel the fluid, that do not involve contractions of the anterior digastric ([Grill and Norgren, 1978](#)). Thus,

EMG activity of the anterior digastric alone, although informative, does not give a complete picture of the behavioral response to quinine infusions.

### EMG Activity Reflects Learning of Cue-Tastant Associations

Our second goal was to determine whether behavioral (EMG) responses to the stimuli preceding sucrose and quinine infusions developed—that is, whether these stimuli became conditioned stimuli during this first day of testing. We used several measures to determine whether this conditioning occurred. First, we measured latency to first burst relative to pump onset (see [Experimental Procedures](#) for details). On the first trial in all rats tested, almost no EMG activity was evident during the cue period. Instead, for sucrose and quinine infusions, EMG activity increased in the seconds after the pump turned on to deliver intraoral infusions (see [Figures 1C](#) [top] and [1D](#) [top], raw EMG traces, for examples; [Figures 1E](#) and [1F](#), for averages across all rats). However, with repeated pairings, the timing of EMG activity began to change. For sucrose trials, EMG activity began to consistently invade the cue period, suggesting that rats were learning an association between the cues and impending sucrose delivery ([Figure 1C](#) [bottom], example; [Figure 1E](#), averages across all rats). For quinine, EMG activity was suppressed such that it began later and later relative to pump onset ([Figure 1D](#) [bottom] for example; [Figure 1F](#), for averages across all rats). As can be seen in [Figures 1E](#) and [1F](#), the latency to first burst changed over the course of both sucrose and quinine sessions. Positive values for latency reflect EMG bursts that began during the intraoral infusion, while negative values reflect EMG bursts that began during the cue period before the infusion started. For sucrose, latency rapidly decreased over the first few trials until rats consistently began engaging in oromotor behavior during the cue period by the fourth trial ([Figure 1E](#)). For quinine, the opposite trend emerged. Over the course of the session, rats began suppressing oromotor behavior such that the first burst occurred much later relative to the start of the intraoral infusion. These data support the idea that rats rapidly form an association between cues and sucrose infusions. When quinine data were analyzed using a different method, evidence of a learned association between cues and quinine infusions emerged. As can be seen in [Figure 1G](#), on individual trials in individual rats, the cue paired with quinine evoked EMG activity (i.e., gapes). The total number of 100 ms bins exhibiting EMG activity during the cue period for quinine trials was counted for each trial ([Figure 1H](#)). Using this analysis, we demonstrated that there was increased EMG activity during the cue on the 2nd trial and for several trials thereafter ([Figure 1H](#)). This increase was transient, and by the 10th trial, no increase was observed. These data indicate that the rats did, indeed, make an association between the cue and quinine infusions, but the behavioral expression of it was more subtle and transient than that for sucrose.

### NAc Neurons Respond to Rewarding and Aversive Unconditioned Stimuli

We sought to determine the relationship between NAc cell firing and behavior: (1) during tastant infusion (i.e.,

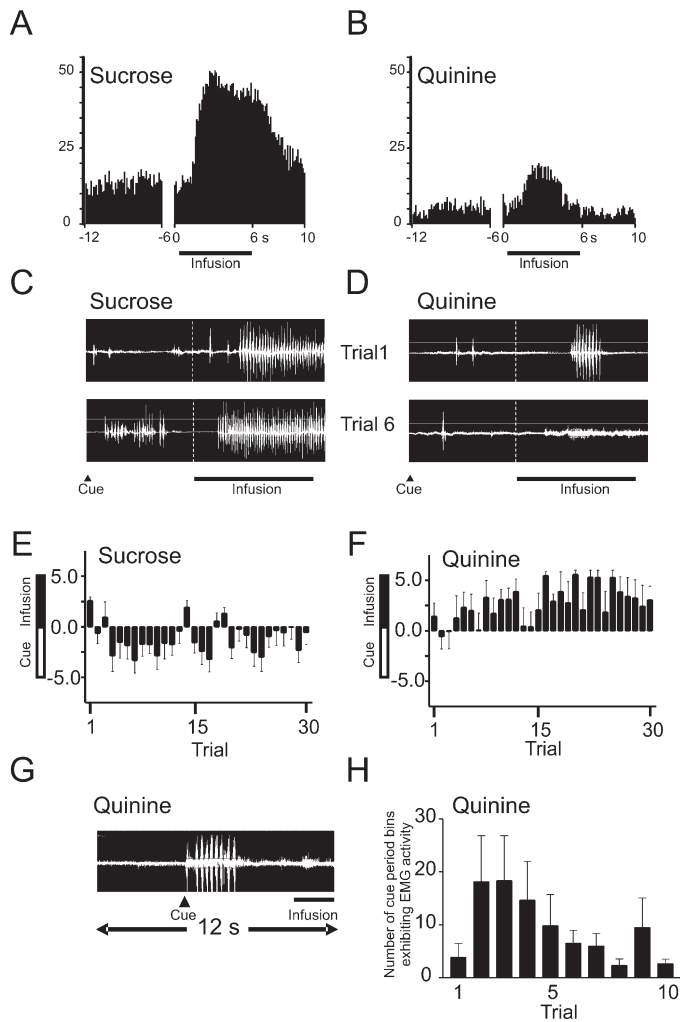


Figure 1. Behavioral Responses to Intraoral Infusions of Taste Stimuli and Cues that Come to Predict Them

EMG activity increases during intraoral infusions of taste stimuli. The mean number of threshold crossings (counts) per each 100 ms bin are plotted for a baseline period (–12 s to –6 s relative to pump onset), as well as for the duration of the intraoral infusion (denoted by the black horizontal bar below the graph) and the 4 s after that duration for 0.3 M sucrose (A) and 0.001 M quinine (B) blocks. EMG activity reflects the learning of cue-tastant associations. (C) Sample EMG traces beginning at cue onset (denoted by arrowhead) and ending just after pump offset for the first cue-sucrose pairing (top) and the 6th cue-sucrose pairing (bottom). The vertical dashed bar denotes pump onset, and the horizontal black bar denotes duration of intraoral infusion. (D) Sample EMG traces beginning at cue onset (denoted by arrowhead) and ending just after pump offset for the first cue-quinine pairing (top) and the 6th cue-quinine pairing (bottom). The vertical dashed bar denotes pump onset and the horizontal black bar denotes duration of intraoral infusion. (E) Average latency to first EMG burst over cue-sucrose trials. Positive latencies are trials in which the first burst, on average, occurred during the intraoral infusion, and negative latencies are trials in which the first burst, on average, occurred during the cue period. Bars represent mean  $\pm$  SEM. (F) Average latency to first EMG burst over cue-quinine trials. (G) Sample EMG trace (12 s) demonstrating quinine-cue-evoked gaps on a single trial in a single rat. Cue onset (denoted by arrowhead) was immediately followed by a set of large-amplitude, long-duration contractions of the anterior digastric muscle—indicative of gapes. (H) Average number of quinine cue-period bins during which EMG activity was present across the first ten cue-quinine pairings across all rats.

unconditioned responses described here) and (2) relative to cues paired with each tastant (conditioned responses explored fully below). This was possible since the electrophysiological activity of individual NAc neurons was recorded during the same classical conditioning sessions as EMG recordings. For sucrose blocks 102 NAc neurons were isolated, and 98 NAc neurons were isolated for quinine blocks. The discrepancy in number comes from loss of isolation of a few neurons between blocks of trials. As can be seen in Figure 2, cells recorded during both sucrose and quinine blocks responded to intraoral infusions with either phasic inhibitions or excitations. Typically, if a cell responded to sucrose, it did not respond in the same way for quinine (Figures 2A and 2B). Likewise, if a cell responded to quinine, it did not respond to sucrose in the same way (Figure 2D). On a few occasions, we recorded cells that had similar responses to both sucrose and quinine (Figure 2C). Relative selectivity of NAc responses will be characterized further below.

The proportions of cells that exhibited inhibitions versus excitations were different between sucrose and quinine. For sucrose, 52 of 102 cells (51%) exhibited a

phasic response during intraoral infusions. Of those cells, 39 (75%) exhibited phasic inhibitions, while 13 (25%) exhibited phasic excitations. For quinine, 40 of the 98 cells (41%) exhibited a phasic response during intraoral infusions. Of those cells, 10 (25%) exhibited inhibitions while 30 (75%) exhibited excitations. The average response to intraoral infusions for all animals across the four classifications of response patterns (sucrose-inhibitory, sucrose-excitatory, quinine-inhibitory, and quinine-excitatory) is shown in Figure 3. For most response types, there was an initial, brief excitatory response that preceded a subsequent response during the intraoral infusion. The initial response is likely due to cue offset rather than the intraoral infusions and is further explored below. Sucrose-inhibitory responses ( $n = 39$ ; Figure 3 [top left]) exhibited a marked decrease in average firing rate in the seconds after the pump turned on [ $F(11, 418) = 7.93$ ;  $p < 0.0001$ ]. Post hoc  $t$  tests with Bonferroni's correction revealed that the average firing rate in the third, fourth, and fifth seconds after pump onset were significantly lower than the average firing rate in the second before the cue onset, which served as baseline (–7 to –6 s relative to

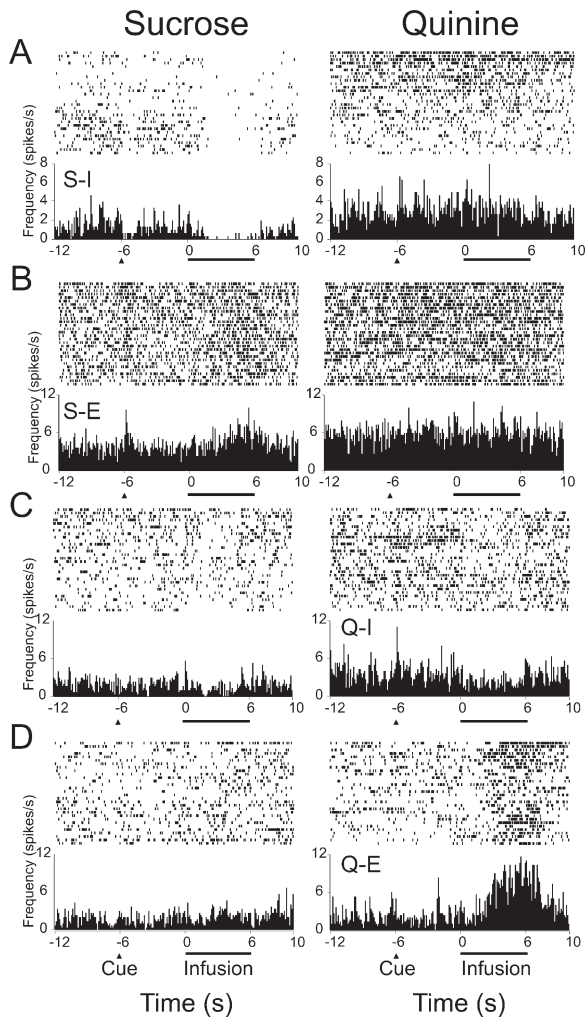


Figure 2. NAc Cells Exhibit Phasic Responses to Intraorally Infused Taste Stimuli

The activity of four representative NAc cells that demonstrate the four classes of responses to intraoral infusions are shown. The raster and peri-event histograms are aligned to the time at which the pump turned on (time 0). The arrowhead denotes the time of cue onset and the horizontal black bar indicates the duration of the intraoral infusion. Note that these representative cells were chosen to demonstrate typical responses to infusions regardless of cue responses. (A) Example of a sucrose-inhibitory cell. The response of this cell to sucrose infusions was a clear inhibition (left). The same cell was unresponsive to quinine infusions (right). (B) Example of a sucrose-excitatory cell. The response of this cell to sucrose infusions was a clear excitation (left). This same cell was unresponsive to quinine infusions (right). (C) Example of a quinine-inhibitory cell. The response of this cell to quinine infusions was a clear inhibition (right). This same cell also exhibited an inhibition to sucrose infusions (left). (D) Example of a quinine-excitatory cell. The response of this cell to quinine infusions was a clear excitation (right). This same cell was unresponsive to sucrose infusions (left).

pump onset) [ $t(38) = 4.21$ ;  $p < 0.001$  for third second]. Sucrose-excitatory responses ( $n = 13$ ; Figure 3 [top right]) showed a gradual increase at around 5–6 s after the start of the infusion. The overall ANOVA for sucrose-excitatory cells was not significant [ $F(11, 132) = 1.75$ ;  $p = 0.07$ ], perhaps due to the small number of cells in this group.

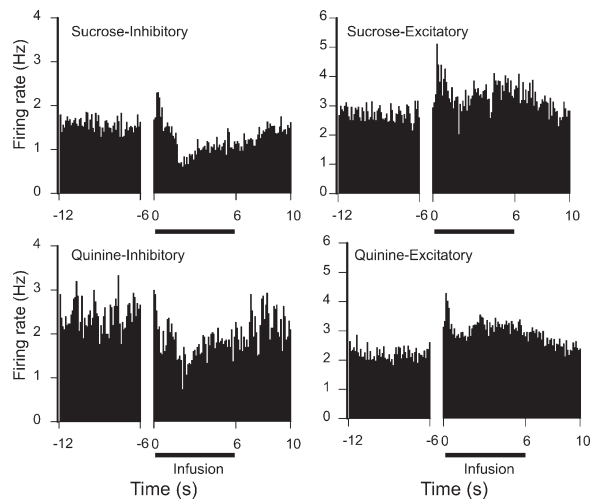


Figure 3. Average Firing Rates of Taste-Responsive NAc Neurons

Composite peri-event histograms display the average firing rate of sucrose- (top) and quinine- (bottom) responsive neurons during baseline (–12 s to –6 s relative to pump onset) and during intraoral infusions (denoted by the black horizontal bar). Data are aligned to the onset of the infusion pump (0 s). Four types of responses were characterized: sucrose-inhibitory (39 of 102 NAc cells, top left); sucrose-excitatory (13 of 102 NAc cells, top right); quinine-inhibitory (10 of 98 NAc cells, bottom left); and quinine-excitatory (30 of 98 NAc cells, bottom right).

Like responses for sucrose, quinine-inhibitory responses ( $n = 10$ ; Figure 3 [bottom left]) exhibited a marked decrease in average firing in the seconds after the pump turned on [ $F(11,99) = 4.61$ ;  $p < 0.0001$ ]. Post hoc  $t$  tests with Bonferroni's correction revealed that the average firing rate in the second through sixth seconds after pump onset was significantly lower than the baseline firing rate [ $t(9) = 4.94$ ;  $p < 0.001$  for third second]. Finally, quinine-excitatory responses ( $n = 30$ ; Figure 3 [bottom right]) exhibited an increase immediately following pump onset and another, more sustained increase in firing rate in the seconds after that [ $F(11, 319) = 10.98$ ;  $p < 0.0001$ ]. Post hoc  $t$  tests with Bonferroni's correction revealed that the average firing rate in the first through sixth seconds after pump onset was significantly higher than the baseline firing rate [ $t(29) = 5.02$ ;  $p < 0.001$  for third second after pump onset].

Responses to taste stimuli were evident in naive rats on the first day of testing. This result suggests that NAc responses to primary rewarding and aversive taste stimuli are unlearned or innate. However, as evidenced in Figure 1, changes in EMG activity with respect to cues occurred rapidly. Thus, to determine if responses to taste stimuli are present before evidence of learning, analysis of neural responses to taste stimuli was restricted to just the first three trials. Signal-to-baseline ratios (peak deviation from baseline/baseline) were obtained for each of the four types of responses observed for the first three trials. If no response was present, then signal-to-baseline ratios should be close to 1. However, if deviations from baseline occurred, then for inhibitory responses, values should be less than 1, and for excitatory responses, values should be greater than 1. We

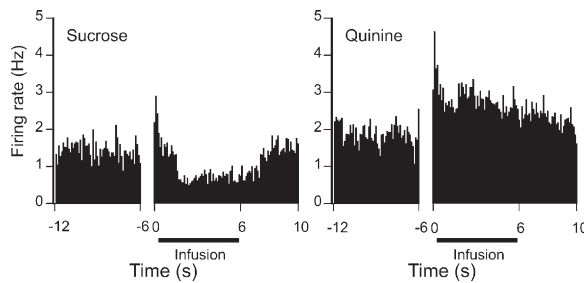


Figure 4. The Same NAc Cells, after an Initial Excitatory Response, Respond in Opposite Directions to Sucrose versus Quinine

The average firing rate for 14 cells that responded with an inhibition to sucrose (left) and an excitation to quinine (right) infusions during baseline (–12 s to –6 s relative to pump onset) and during intraoral infusions (denoted by the black horizontal bar). Data are aligned to the onset of the infusion pump (0 s).

found that neurons that responded with an inhibition for sucrose exhibited a response on the first three trials (average signal-to-baseline ratio = 0.58) as did neurons that exhibited an excitatory response for sucrose (average signal-to-baseline ratio = 1.56). Likewise, neurons that responded with an inhibition for quinine exhibited a response on the first three trials (average signal-to-baseline ratio = 0.59) as did neurons that exhibited an excitatory response for quinine (average signal-to-baseline ratio = 1.91). Thus, NAc neurons were innately responsive for primary rewarding and aversive taste stimuli.

The majority of cells responded for only one tastant. Specifically, 32 of 52 sucrose-responsive cells (62%) responded exclusively for sucrose, while 20 of 40 quinine-responsive cells (50%) responded exclusively for quinine. However, even when neurons responded to both tastants (20 cells), the majority ( $n = 15$ ) responded in the opposite direction. Figure 4 shows 14 cells that, after an initial, brief excitation to pump onset for both sucrose and quinine, responded with an inhibition for sucrose infusions and an excitation for quinine infusions. Again, the initial response is most likely due to cue offset rather than tastant infusion and is explored further below. Together, the results suggest that NAc neurons are relatively selective for rewarding and aversive tastes.

#### Changes in NAc Firing Rates during Intraoral Infusions Occur at the Peak of the EMG Response

Changes in the firing rates of sucrose- and quinine-responsive NAc cells occurred during the intraoral infusions when EMG activity was most pronounced. To determine if there is a relationship between EMG and electrophysiological activity, linear regression analyses were performed for each type of the four classes of responses against EMG activity. Firing rate was plotted against EMG activity for each 100 ms bin of baseline (–12 to –6 s relative to pump onset) and the infusion (0 to 6 s relative to pump onset) and post-infusion periods (6 to 10 s relative to pump onset). Thus, if there is a relationship between phasic changes in NAc cell activity and oromotor output, then as EMG activity increases (i.e., during the infusion), NAc cell activity should

change (for inhibitions, cell activity should decrease, whereas for excitations, cell activity should increase). This was indeed the case. As shown in Figure 5, for all classes of responses there was a strong relationship between EMG and electrophysiological activity. Moreover, changes in firing rate were greatest at the peak of EMG activity. The firing rate of cells that had inhibitory responses to either sucrose (Figure 5A) or quinine (Figure 5C) was negatively correlated with EMG activity. The firing rate of cells that had excitatory responses to either sucrose (Figure 5B) or quinine (Figure 5D) was positively correlated with EMG activity. Together, these results demonstrate that taste-related responses of NAc neurons occur in concert with the motor responses of the rats to those tastants.

#### NAc Responses to Conditioned Stimuli

A final goal of this study was to determine whether the same pool of neurons exhibit phasic changes to audiovisual stimuli paired with each tastant (i.e., conditioned stimuli). As can be seen in Figure 6, cells recorded during both sucrose and quinine blocks responded to cues with either phasic inhibitions or excitations. Typically, if a cell responded to the cue for sucrose, it did not respond in the same way for quinine (Figures 6A and 6B). Likewise, if a cell responded to the cue for quinine, it did not respond to sucrose in the same way (Figures 6C and 6D). We also recorded cells that had similar responses to both sucrose and quinine cues. Relative selectivity of NAc responses to sucrose- and quinine-associated cues will be characterized further below. For sucrose-associated cues, 42 of 102 (41%) cells exhibited a phasic response, while 39 of 98 (40%) cells were phasically activated by quinine-associated cues. Sucrose- and quinine-associated cues evoked a greater proportion of excitations [26 of 42 (62%) and 27 of 39 (69%) for sucrose- and quinine-associated cues, respectively] than inhibitions [16 of 42 (38%) and 12 of 39 (31%) for sucrose- and quinine-associated cues, respectively].

The average perievent histograms for the four response patterns observed (sucrose cue-inhibitory (S-C-I), sucrose cue-excitatory (S-C-E), quinine cue-inhibitory (Q-C-I), and quinine cue-excitatory (Q-C-E)) are shown in Figure 7. S-C-I cells ( $n = 16$ ) exhibited an immediate, brief reduction in firing rate following cue presentation [ $F(11, 165) = 2.94$ ;  $p < 0.01$ ]. Indeed, the average firing rate in the first second after cue presentation was significantly lower than that for the second immediately preceding cue presentation [ $t(15) = 3.23$ ;  $p < 0.01$ ]. For S-C-E cells ( $n = 26$ ), there was a significant effect of time on firing rate [ $F(11, 275) = 6.67$ ;  $p < 0.001$ ] with the first second of average firing rate during the cue being significantly higher than the second immediately preceding the cue [ $t(25) = 6$ ;  $p < 0.001$ ]. With respect to quinine cue responses, the Q-C-I cells ( $n = 12$ ) exhibited decreases in firing rate over time [ $F(11, 121) = 4.16$ ;  $p < 0.0001$ ]. The average firing rate of these cells was significantly lower in the first, third, fourth, fifth, and sixth seconds relative to the second immediately preceding cue presentation [ $t(11) = 5.12$ ;  $p < 0.001$  for first second]. For the 27 Q-C-E cells, increased unit activity during the cue was detected [ $F(11, 286) = 9.70$ ;  $p < 0.0001$ ]. The average firing rate in the first second after cue pre-

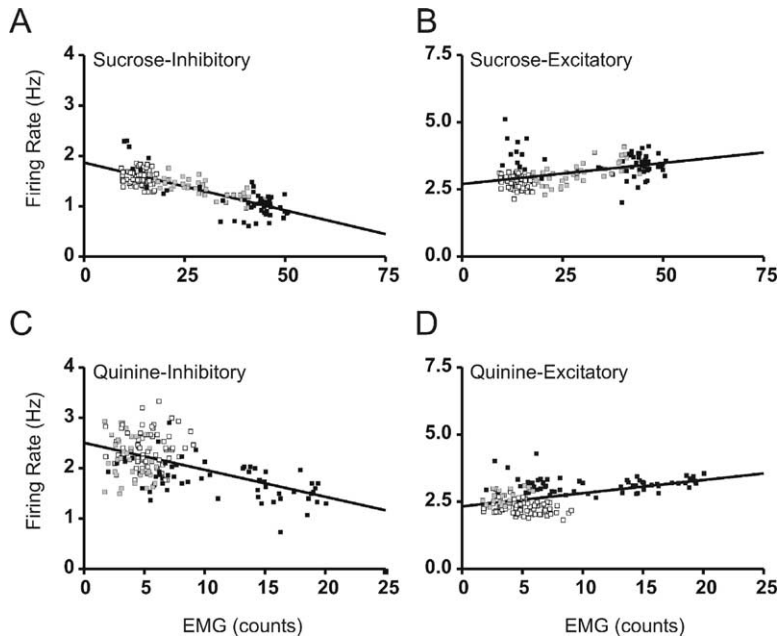


Figure 5. Firing Rate of NAc Cells Is Correlated with EMG Activity

Firing rate (y axis) was plotted against EMG activity (x axis) for each 100 ms bin of baseline (–12 s to –6 s relative to pump onset, points shown in white with outline) and infusion (0 to 6 s relative to pump onset, points shown in black) and post-infusion periods (6 to 10 s relative to pump onset, points shown in light gray). (A) The average firing rate of cells that responded with an inhibition to sucrose was negatively correlated with EMG activity (linear regression analysis:  $r = 0.82$ ;  $p < 0.0001$ ). (B) The average firing rate of cells that responded with an excitation to sucrose was positively correlated with EMG activity (linear regression analysis:  $r = 0.43$ ;  $p < 0.0001$ ). (C) The average firing rate of cells that responded with an inhibition to quinine was negatively correlated with EMG activity (linear regression analysis:  $r = 0.55$ ;  $p < 0.0001$ ). (D) The average firing rate of cells that responded with an excitation to quinine was positively correlated with EMG activity (linear regression analysis:  $r = 0.49$ ;  $p < 0.0001$ ).

sensation was significantly higher than the average firing rate in the second immediately preceding cue presentation [ $t(26) = 7.15$ ;  $p < 0.001$ ]. Note that for both S-C-E and Q-C-E cells the robust, peak increase in firing rate occurred at 200 and 300 ms after cue onset, respectively. There was a second robust increase in firing rate for these cells just shortly after cue offset (400 and 300 ms for S-C-E and Q-C-E cells, respectively; data not shown). This response is actually evident in the average perievent histograms for responses to the intraoral infusions (see Figures 3 and 4). This second increase in firing rate is likely due to cue offset rather than a response to the intraoral infusion, since it occurs so rapidly relative to pump onset and before the infusion-related increase in EMG activity (see Figure 1).

Many of the cells that had responses for cues signaling sucrose delivery also responded to quinine cues. The greatest degree of overlap occurred for cells exhibiting excitatory responses. Of the 26 cells that responded with an increase in firing rate to the cue for sucrose, 13 (50%) responded in a similar manner for the quinine cue (two cells had inhibitory responses, and 11 were nonphasic for the quinine cue). Likewise, of the 27 cells that responded with an increase in firing rate to the quinine cue, 13 (48%) responded in a similar manner for the sucrose cue (one cell had an inhibitory response, and 13 were nonphasic for the sucrose cue). For the cells with similar excitatory responses for sucrose and quinine cues, the magnitude of the response was greater for sucrose cues than quinine cues (average firing rate for baseline:  $2.32 \pm 0.66$  versus  $2.35 \pm 0.71$ , sucrose versus quinine, respectively [mean  $\pm$  SEM]; average firing rate for first second of cue:  $5.22 \pm 1.77$  versus  $4.04 \pm 1.21$ , sucrose versus quinine, respectively) although the difference was not statistically significant.

Many of the NAc cells that responded to cues predicting taste stimuli also responded to the taste stimuli

themselves. Indeed, of the 42 neurons that responded to the cue for sucrose, the overwhelming majority also responded to the sucrose infusion as well. For the 16 S-C-I cells, 14 also responded to the sucrose infusion with an inhibition, whereas one responded with an excitation and one did not respond to the sucrose infusion. For the 26 S-C-E cells, 11 also responded to the sucrose infusion with an inhibition, whereas eight responded with an excitation and seven did not respond to the sucrose infusion. For the 12 Q-C-I cells, four also responded to the quinine infusion with an inhibition, whereas two responded with an excitation and six did not respond to the sucrose infusion. For the 27 Q-C-E cells, one also responded to the quinine infusion with an inhibition, whereas 13 responded with an excitation and 13 did not respond to the quinine infusion.

From inspection of several individual cells that responded to the cue for sucrose, it appeared that changes in cell firing time-locked to the cue were not present on the first few trials but developed over the block (see Figure 6B [left] for example). This would suggest that NAc cells do not innately respond to the sensory features of the cues, but rather to their association with infusions. To determine if cue-evoked changes in NAc cell firing indeed developed over trials, a signal-to-baseline ratio was calculated across the first ten trials for each type of NAc response. To determine signal-to-baseline ratio, the average firing rate in the first second after cue onset was divided by the average firing rate in the second immediately preceding cue onset. As seen in Figure 8 (left), S-C-E cells clearly demonstrated changes in signal-to-baseline ratio over trials. Linear regression confirmed this change, as the slope of the regression line was significantly different from zero (slope =  $0.1059 \pm 0.01653$ ;  $p < 0.001$ ). The other pattern types (S-C-I, Q-C-E, and Q-C-I) did not show this relationship over the first ten trials (data not shown). It is quite possible that quinine cue responses

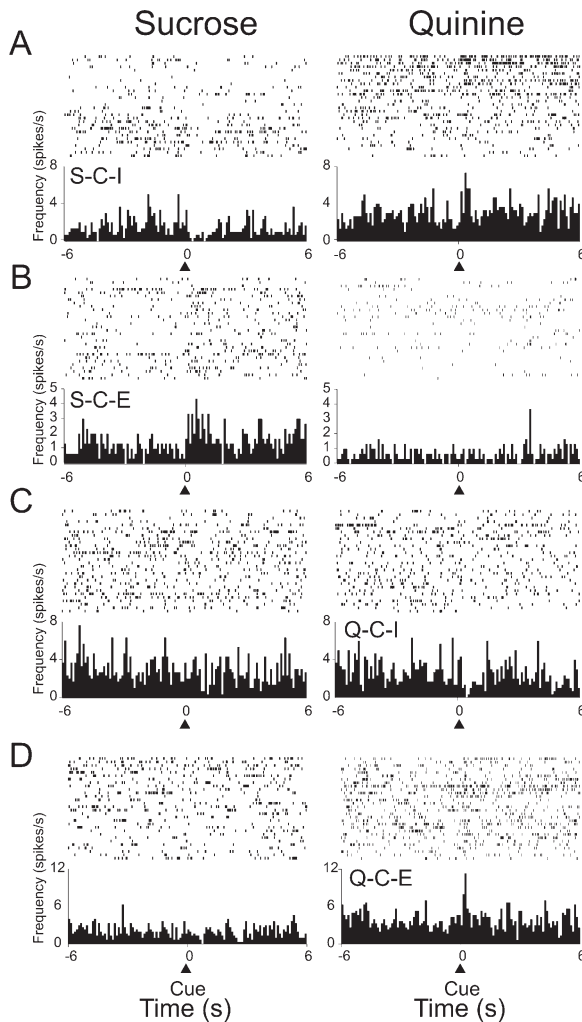


Figure 6. NAc Cells Exhibit Phasic Responses to Cues Predicting Rewarding and Aversive Stimuli

The activity of four representative NAc cells that demonstrate the four classes of responses to cues (conditioned stimuli) are shown. The raster and perievent histograms are aligned to the time at which the cue turned on (time, -6 s relative to pump onset). The arrowhead below the perievent histograms denotes the time of cue onset. (A) Example of a sucrose-cue-inhibitory (S-C-I) cell. The response of this cell to the cue predicting sucrose infusions was a clear inhibition (left). The same cell had an excitatory response to the cue predicting quinine infusions (right). (B) Example of a sucrose-cue-excitatory (S-C-E) cell. The response of this cell to the cue predicting sucrose infusions was a clear excitation (left). This same cell was unresponsive to the cue predicting quinine infusions (right). (C) Example of a quinine-cue-inhibitory (Q-C-I) cell. The response of this cell to the cue predicting quinine infusions was a clear inhibition (right). This same cell was unresponsive to the cue predicting sucrose infusions (left). (D) Example of a quinine-cue-excitatory (Q-C-E) cell. The response of this cell to the cue predicting quinine infusions was a clear excitation (right). This same cell was unresponsive to the cue predicting sucrose infusions (left).

did not show rapid changes in signal-to-baseline ratios because motor responses were suppressed and occurred later and later over trials relative to infusion onset (see Figure 1F).

EMG data strongly support the idea that rats rapidly

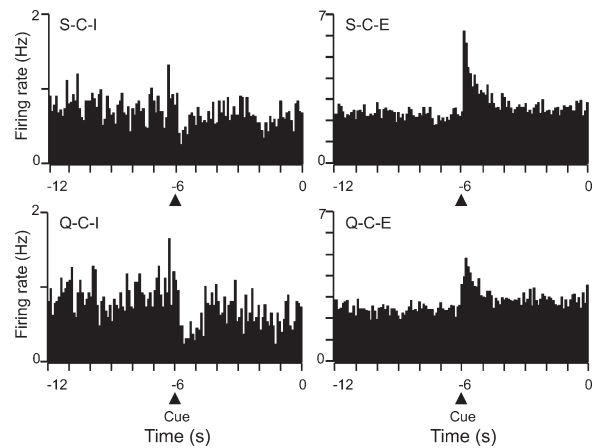


Figure 7. Average Response of Cue-Responsive NAc Neurons

Composite perievent histograms display the average firing rate of sucrose cue- (top) and quinine cue- (bottom) responsive neurons during baseline (-12 s to -6 s relative to pump onset) and during the cue period (-6 s to 0 s relative to pump onset; cue onset denoted by arrowhead). Data are aligned to the onset of the cue (-6 s). Four types of responses were characterized: sucrose-cue-inhibition (S-C-I: 16 of 102 NAc cells, top left); sucrose-cue-excitation (S-C-E: 26 of 102 NAc cells, top right); quinine-cue-inhibition (Q-C-I: 12 of 98 NAc cells, bottom left); and quinine-cue-excitation (Q-C-E: 27 of 98 NAc cells, bottom right).

make an association between cues and sucrose infusions. It is intriguing that both EMG activity and S-C-E neural activity appear to be changing relative to cue presentation over the same subset of trials. To investigate whether the development of S-C-E activity is related to the decreasing latency of first EMG burst, the signal-to-baseline ratio was plotted against latency to first burst for the first ten trials (Figure 8 [right]). There was indeed an inverse relationship between S-C-E signal-to-baseline ratio and latency to first EMG burst (slope =  $-3.630 \pm 1.329$ ;  $p < 0.05$ ). These data support a correlation between NAc cue-evoked phasic activity and the learning of a Pavlovian association.

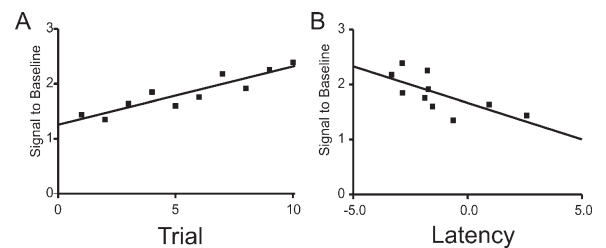


Figure 8. Cue-Evoked Increases in Firing Rate Correlate with Learning

(A) The average signal-to-baseline ratio for cells that responded to the sucrose cue with excitations is plotted across the first 10 cue-sucrose pairings. Linear regression analysis revealed a tight positive correlation ( $r = 0.91$ ;  $p < 0.001$ ).

(B) The average signal-to-baseline ratio for cells that responded to the sucrose cue with excitations is plotted against latency to first burst for the first ten cue-sucrose pairings. Linear regression analysis revealed a negative correlation in that the shorter the latency, the greater the signal-to-baseline ratio ( $r = -0.69$ ;  $p < 0.05$ ).

## Discussion

These data show the relatively selective encoding of rewarding and aversive taste stimuli by individual NAc neurons upon first exposure in naive rats. The changes in firing rate of NAc neurons during tastant infusions were directly correlated with oromotor activity—invoking the ideas of Mogenson that the NAc is a center for converting limbic information into motor commands (Mogenson et al., 1980). The data also demonstrate that, while similar proportions of neurons within the NAc were responsive to aversive as well as rewarding taste stimuli, there was a striking disparity in the direction of firing rate changes. Sucrose infusions evoked primarily inhibitory responses, and quinine infusions evoked primarily excitatory responses. Even when the same NAc cell was responsive to both sucrose and quinine, the direction of the response was typically opposite. These findings, made possible only because rats received identical exposures to rewarding and aversive taste stimuli, suggest that synaptic weights on NAc neurons, as well as the output of the NAc, are very different with respect to hedonic valence. Finally, we have shown here that a subset of NAc neurons develop responses for conditioned stimuli in a purely Pavlovian behavioral paradigm, and these neurons develop responses in concert with behavioral evidence of the learning.

We found that a large proportion of cells in the NAc responded to sucrose (51%) or quinine (41%) on the very first day of testing. The NAc is well positioned to receive taste-related information either from gustatory cortex or the amygdala or from brainstem areas (i.e., nucleus of the solitary tract) (Ricardo and Koh, 1978; Saper, 1982). Most taste-responsive NAc neurons responded to only one of the taste stimuli. This segregation of responses is consistent with a recent report examining the phasic activity of ventral striatal cells during a go/no go odor discrimination task. Rats learned to approach a dish to obtain sucrose or not approach the dish to avoid quinine, on the basis of odor cues (Setlow et al., 2003). Setlow et al. found that many neurons exhibited selective responses for just one of the cues. NAc neurons have also been shown to selectively fire during operant responding for either food or cocaine during a multiple schedule (Carelli et al., 2000). Finally, Hikosaka and colleagues have demonstrated that striatal neurons, which share similar properties to NAc neurons, rapidly change their preferential firing for the direction of eye movements to favor the direction that will earn the subject reward (Kawagoe et al., 1998; Takikawa et al., 2002). An important difference between these studies and the present one is that in the latter, rewarding and aversive stimuli were delivered independently of behavior. Thus, NAc responses in this study are purely reflective of reward and aversion independent of any operant component. Still, NAc neurons were relatively selective.

These findings are consistent with the view that the NAc is organized into functional ensembles (Carelli, 2002; Pennartz et al., 1994)—that separate neural circuits, in this case, govern the behavioral responses to reward and aversion. If activation of these circuits underlies the behavioral responses to intraoral infusions

to some degree, it is not surprising that there would be a segregation of neural responses. It is well established that sucrose elicits appetitive oromotor behavior (i.e., mouth movements, tongue protrusions), whereas quinine elicits a very different set of orofacial movements aimed at rejection (i.e., gapes) (Grill and Norgren, 1978). Here, far less EMG activity was generated by quinine than by sucrose. Undoubtedly, much of the EMG response to quinine infusions consisted of gapes, which involve activation of the anterior digastric muscle, just as licking does (Travers and Norgren, 1986). Thus, if NAc output contributes to the motor response to intraorally infused tastants (as the data here would suggest), it is not surprising that we observed a segregation of NAc responses into sucrose-selective and quinine-selective cells. The fact that relatively selective responses were observed on the very first day of exposure in naive rats strongly suggests that the functional ensembles underlying responses to reward and aversion are innate.

That sucrose infusions elicited inhibitory responses from NAc neurons is striking but consistent with reports that inhibition of the NAc can be associated with feeding (Kelley, 2004; Nicola et al., 2004b; Yun et al., 2004). Indeed, Nicola et al. found that the most common firing pattern of NAc neurons observed when rats were consuming a sucrose solution following a nose-poke response was a long-lasting inhibition similar to that observed here (see Figures 3 and 4). Nicola and colleagues suggest that inhibitory responses may facilitate consummatory behavior, but to support that notion “a direct relationship between NAc neuronal firing and specific consummatory muscle movements” needs to be demonstrated (Nicola et al., 2004b). In this study, we have clearly established that such a relationship exists, as change in neuronal firing rate tightly correlated with increased EMG activity during infusions. In addition, EMG activity was high for sucrose infusions, which elicited an overwhelming majority of inhibitory responses from sucrose-responsive NAc neurons. On the other hand, quinine infusions elicited little EMG activity (which consisted of mainly rejection responses) and evoked a majority of excitatory responses from quinine-responsive NAc neurons. Thus, we have found a direct relationship not only between NAc cell firing and consummatory muscle movements, but also between NAc cell firing and rejection responses. While previous experiments have argued for dissociation between NAc activity and motor responses during goal-directed behavior (Carelli, 2002), these data suggest that the NAc may, in part, direct the unconditioned behavioral responses to the taste stimuli. It is interesting to note that Travers and colleagues have suggested that the basal ganglia plays a role in oromotor behavior (Travers et al., 1997).

Both glutamate receptor antagonists (Kelley and Swanson, 1997) and GABA receptor agonists (Stratford and Kelley, 1997) infused into the medial shell region of the NAc elicit robust feeding behavior, even in sated rats. In addition, exogenous opioid injections into the NAc, which presumably inhibit medium spiny neurons, also elicit feeding (Zhang and Kelley, 1997). Endogenous opioids have been implicated in mediating the rewarding aspect of sucrose (Berridge, 1996; Pecina and



Berridge, 2000). Thus, their potential role in sucrose-evoked inhibitions is an especially intriguing possibility. The predominant excitatory response to quinine may result from glutamatergic signals emanating from cortical (i.e., gustatory cortex) or subcortical (i.e., amygdala) regions. The amygdala is an intriguing possibility, as it plays a strong role in aversion (Schafe and Bernstein, 1996; Schafe and LeDoux, 2000). Understanding the relative contributions of afferents to the NAc in the generation of taste-related NAc firing patterns will be the subject of future research.

Another goal of this study was to examine the firing rate of individual NAc neurons during exposure to novel sensory stimuli that become predictive of tastant infusions in this Pavlovian conditioning paradigm. The NAc clearly plays a role in Pavlovian associative learning (Di Ciano et al., 2001; Parkinson et al., 2002). However, to date, no studies have characterized the activity of individual NAc neurons in response to conditioned stimuli in a purely Pavlovian behavioral paradigm (i.e., when conditioned stimuli are paired with unconditioned stimuli regardless of the subject's behavior). We found that ~40% of NAc neurons responded to conditioned stimuli. The majority of cue-responsive cells responded with a short-latency brief excitation relative to cue onset, and these cells also demonstrated a second increase in the milliseconds after cue offset. While about half the cue-responsive cells were activated selectively for only one of the tastant-associated cues, a large proportion of cells had responses to both sucrose-associated and quinine-associated cues. However, the magnitude of the increase was greater for the sucrose cue than the quinine cue.

These results are consistent with a recent report by Nicola and colleagues showing that a significant proportion of cells responding to a discriminative stimulus for sucrose reward also respond to a stimulus signaling no reward, but to a lesser magnitude (Nicola et al., 2004a). In addition, Mirenowicz and Schultz found that dopamine (DA) neurons that respond to cues associated with juice reward also respond to cues that signal aversive consequences, but to a lesser extent (Mirenowicz and Schultz, 1996). The authors attribute this finding to response generalization, which could be playing a role here as well. Indeed, DA may in part be responsible for cue-evoked changes in NAc firing. DA is released within milliseconds of a cue that signals the opportunity to work for sucrose reward (Roitman et al., 2004). In addition, DA is required for NAc responses to presentation of a discriminative stimulus (Yun et al., 2004).

A subset of NAc neurons developed responses to conditioned stimuli over trials. Specifically, cells that exhibited excitations to the cue predicting sucrose infusion had a gradual increase in signal-to-baseline ratio over the first few trials. At the same time, EMG activity gradually began to invade the cue period. This correlation between unit activity and behavior suggests that NAc cells encode the association between the conditioned and unconditioned stimuli.

The findings presented here continue to support the idea that the NAc comprises part of the brain's reward circuit. However, the data clearly indicate that individual NAc neurons play a role in aversion. Neural re-

sponses are organized on a microcircuit level—that is, rewarding and aversive responses are segregated. This organization is innate in that responses were segregated on the first day of exposure. This is consistent with other findings from our lab that recently demonstrated that NAc neurons are selective for operant responding for either “natural” or drug reward on the very first day of drug exposure (Carelli and Wondolowski, 2003). Individual NAc neurons also play a role in Pavlovian learning without any operant component. The results presented here provide valuable insight into how the NAc is organized for “natural” hedonic stimuli and their predictors.

## Experimental Procedures

### Subjects

Eight male Sprague-Dawley rats (280–350g) were individually housed with ad libitum food and water with a 12:12 light:dark cycle (lights on at 7:00 a.m.). All experiments were conducted in the light phase between 10:00 a.m. and 3:00 p.m.

### Surgery and Histology

Rats were anesthetized with a ketamine (100 mg/kg)-xylazine (20 mg/kg) mixture. For EMG recordings, the uninsulated tips of two seven-strand stainless steel wires (A-M Systems, Carlsborg, WA) were implanted into the anterior digastric muscle, and the other ends were led subcutaneously out of an incision in the top of the head, where they mated with a connector (see Kaplan et al., 1995, for details). Another wire wrapped around a skull screw served as ground for EMG recordings. For electrophysiological recordings, eight-wire microelectrode arrays (NB Labs, Dennison, TX) were implanted bilaterally in the NAc. The coordinates used, in accordance with the atlas of Paxinos and Watson (1997), were as follows: AP, +1.7 mm; ML,  $\pm 0.8$  to  $\pm 1.3$  mm; and DV,  $-6.5$  mm from the surface. For each array, another wire was wrapped around a skull screw to serve as ground. Finally, rats were bilaterally implanted with intraoral cannulae. Each cannula consisted of an approximately 6 cm length of PE-100 tubing, which was flanged at one end with a Teflon washer. The cannula was inserted just lateral to the first maxillary molar, with the Teflon washer flush against the molar. The other end was exteriorized out of the incision at the top of the head and held in place along with the EMG connector and arrays with dental acrylic. Rats were permitted at least 1 week to recover from surgery.

Following experiments, rats were deeply anesthetized with ketamine/xylazine, and electrode tips were marked by passing current (10  $\mu$ A, 5 s) through the electrodes. Rats were then transcardially perfused with phosphate buffer and 40% paraformaldehyde; their brains were removed and, after post-fixing and freezing, 40  $\mu$ m sections were sectioned through the forebrain. Sections were then mounted on slides and stained with potassium ferrocyanide and counterstained with thionin to visualize electrode tips. We implanted a total of 128 wires in eight rats. Of those 128, 112 wires were located in the nucleus accumbens, with 60 wires in the shell and 52 wires in the core subregions. There appeared to be no difference in response types across core and shell subregions, although subsequent experiments will explore this further. Only data from electrode placements within the borders of the NAc, as depicted in the atlas of Paxinos and Watson (1997), are presented here.

### Electromyographic and Electrophysiological Recordings

Electrophysiological procedures have been described in detail previously (Carelli and Deadwyler, 1994; Carelli et al., 2000). Briefly, before the start of the behavioral session, the rat was placed into a plexiglas chamber within a sound-attenuating box. Rats were connected to a flexible recording cable (Plexon Inc., Dallas, TX) attached to a commutator (Med Associates Inc., St. Albans, VT), which permitted virtually unrestrained movement within the chamber. The headstage contained 16 miniature unity-gain field effect

transistors. NAc activity was recorded differentially between each active wire and an inactive wire chosen for an absence of neuronal activity. Online isolation and discrimination were accomplished using a commercially available neurophysiological system (multi-channel acquisition processor [MAP] system; Plexon Inc., Dallas, TX). Multiple window discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals on the basis of 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 the behavioral events of the experiment (Med Associates Inc.) and sent digital outputs corresponding to each event to the MAP 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 one or two neurons were recorded per microwire, as in previous reports (Carelli et al., 2000; 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., 2000; Chang et al., 1994; Nicolelis, 1999; Nicolelis et al., 1997). Briefly, discrimination of individual waveforms corresponding to a single cell was accomplished using template analysis procedures provided by the neurophysiological software system (MAP system). The template analysis procedure involves taking a "sample" of the waveform and building a template of that extracellular waveform. Subsequent neurons that "match" this waveform are included as the same cell. Cell sorting was further accomplished after the experiment, using principal components analysis in Offline Sorter (Plexon Inc., Dallas, TX). After sorting, firing rates of individual cells were aligned to both pump onset for the intraoral infusion and cue presentation, which preceded pump onset by 6 s. Perievent histograms were constructed using commercially available software (NeuroExplorer; Plexon Inc., Dallas, TX). Firing rates per second were calculated for each cell from -12 to +10 s relative to pump onset in 100 ms bins. Data were then imported into Excel.

For EMG recordings, rats were attached to a second flexible cable, and EMG potentials were recorded differentially. Briefly, wires were led to an amplifier (Grass Instruments, West Warwick, RI) and signals were amplified (100 $\times$ ) and filtered (0.3 Hz low-pass; 3 Hz high-pass). Processed analog signals were then led through a national instruments board to the same computer that collected electrophysiological data. The same program (Sort Client; Plexon Inc., Dallas, TX) that collected electrophysiological data also collected EMG data. To analyze EMG signals, a horizontal threshold was positioned higher than at least 3 $\sigma$  of the noise. Threshold crossings were time-stamped and examined relative to pump and cue onsets in NeuroExplorer. Statistical analyses of both electrophysiological and EMG signals were performed using commercially available software (Prism).

#### Experimental Design

On the test day, naive rats were placed in the recording chamber and connected to EMG and electrophysiological cables as well as infusion lines to intraoral cannulae. After cells were initially sorted online, the experimental session began. Initially, the chamber was illuminated by a light on the side of the chamber. After a 30–60 s variable delay, the light was extinguished and one of two possible cues was delivered. Cues consisted of either a tone stimulus (65 dB, 2900 Hz) paired with illumination of a house light at the top of the chamber or a set of clicks (10 clicks/s: 80 dB, 800 Hz) paired with darkness. The cue was delivered for 6 s, after which the light on the side of the chamber was illuminated again. In addition, an infusion pump (model PHM-100; Med Associates Inc.) turned on for 6 s to deliver 200  $\mu$ l of fluid. Following the infusion, there was another 30–60 s variable delay before the next trial. Each cue was paired with either 0.3 M sucrose or 0.001 M quinine, and pairings were counterbalanced across rats. After a block of 30 trials of one cue-tastant pairing was completed, a second block of 30 trials of the other cue-tastant pairing was delivered.

#### Data Analysis

Most analyses are suitably described in the [Results](#) section above. Here, a few analyses are discussed in detail.

##### *Latency to First Burst*

EMG activity on each trial was analyzed. The average activity during -12 to -6 s relative to pump onset was calculated on each trial and served as that trial's baseline. Next, each 100 ms bin from -6 to +6 s was examined. The first burst in EMG activity was determined to be the time relative to pump onset at which three consecutive bins exceeded 140% of baseline activity. If criterion was not met on a given trial, that trial's latency was scored as "6." This happened most frequently on quinine trials, when EMG activity was suppressed.

##### *Determination of Phasic Responses*

Consistent with other reports from our lab (Carelli et al., 2000; Carelli and Wondolowski, 2003; Roop et al., 2002), NAc cells were considered to have a phasic response if their activity during critical periods of the trial deviated from baseline (average firing rate, from -12 to -6 s relative to pump onset) by 40%. For phasic responses to intraoral infusions, the average firing rate in each second of the intraoral infusion was evaluated for a 40% deviation from baseline, and cells were classified as having either an inhibitory or excitatory response. The same analysis was performed for each second of the cue period. Thus, a single cell could potentially belong to four different categories of responses: response to sucrose infusion, response to quinine infusion, response to sucrose-cue, and response to quinine-cue.

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