

Neural Encoding in Ventral Striatum during Olfactory Discrimination Learning

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Summary

A growing body of evidence implicates the ventral striatum in using information acquired through associative learning. The present study examined the activity of ventral striatal neurons in awake, behaving rats during go/no-go odor discrimination learning and reversal. Many neurons fired selectively to odor cues predictive of either appetitive (sucrose) or aversive (quinine) outcomes. Few neurons were selective when first exposed to the odors, but many acquired this differential activity as rats learned the significance of the cues. A substantial proportion of these neurons encoded the cues' learned motivational significance, and these neurons tended to reverse their firing selectivity after reversal of odor-outcome contingencies. Other neurons that became selectively activated during learning did not reverse, but instead appeared to encode specific combinations of cues and associated motor responses. The results support a role for ventral striatum in using the learned significance, both appetitive and aversive, of predictive cues to guide behavior.

Introduction

There is current interest in the role of ventral striatum (VS) in associative learning (Cardinal et al., 2002; Kelley, 1999; Schultz et al., 2000). In particular, VS is important for the use of learned cues (stimuli that predict the occurrence of biologically significant events) to guide or modulate behavior, as experimental manipulations of VS affect performance when those functions are taxed (Cardinal et al., 2002; de Borchgrave et al., 2002; Hauber et al., 2000; Setlow et al., 2002b; Tzschentke, 1998; Wyvell and Berridge, 2000). Electrophysiological recordings have also shown that neurons in VS respond to learned cues that predict biologically significant outcomes, as well as to the outcomes themselves (Carelli, 2002; Schultz et al., 2000; Shidara et al., 1998; Williams et al., 1993; Woodward et al., 1999). Given the historical emphasis placed on the role of VS in reward mechanisms, most studies have used cues predictive of rewarding events. However, emerging evidence implicates VS in processing of aversive information and aversively motivated learning as well (Breiter et al., 2001; Parkinson et al., 1999; Ravel et al., 1999; Salamone et

al., 1997; White et al., 1994; Williams et al., 1993). This evidence has provided an increasing awareness that VS function is not restricted to learning motivated by reward and indicates that VS may be more generally involved in the use of learned cues predictive of biologically significant outcomes, either rewarding or aversive. Such a role is consistent with Mogenson's (1980) conception of VS as a "limbic-motor interface" through which motivationally significant information guides behavior. However, relatively little is known about how such information becomes encoded in VS during the learning process, especially in settings that involve aversive events.

In the present experiment, we recorded neural activity from VS in rats performing a task previously used in our laboratory to examine the properties of neurons in the basolateral amygdala (ABL) and orbitofrontal cortex (OFC) during learning (Schoenbaum et al., 1998, 1999). In this symmetrically reinforced go/no-go odor discrimination task (see Figure 2), thirsty rats learn to discriminate between two odor cues, one which signals delivery of a rewarding sucrose solution, and the other which signals delivery of an aversive quinine solution. The current study of VS neurons had three main objectives. The first was to compare the role of VS in processing information about cues associated with either appetitive or aversive outcomes. The second was to investigate the role of VS in the learning process by determining how neural correlates of learning develop in relation to the acquired significance of the odor cues and/or behavioral responses. The third was to relate the encoding properties of VS neurons to those observed in two VS afferent structures (ABL and OFC) within the same behavioral paradigm. Neurons in both ABL and OFC encode information about the learned motivational significance of cues, but appear to do so in different ways (Breiter et al., 2001; Horvitz, 2002; Ono et al., 1995; Quirk et al., 1995; Rolls, 2000; Schoenbaum et al., 1999; Schultz et al., 2000). We looked for similarities between encoding properties previously observed in ABL and OFC and those observed in VS in order to better understand how these component structures of a forebrain system interact to guide goal-directed behavior.

Results

Neural activity in VS was recorded using a drivable bundle of ten microwires. This bundle was advanced between recording sessions so that data from new neurons could be acquired in each session. We recorded from VS in eight rats during 40 sessions of odor discrimination learning. In each session, thirsty rats were presented with a problem consisting of two novel odors so that data could be acquired from neurons during new learning. As shown in Figure 1A, electrode tracks were located in the core subregion of the nucleus accumbens and the ventral caudate-putamen just dorsal to accumbens. These sites yielded 256 neurons recorded in the behavioral task with baseline firing rates >0.1 Hz (mean

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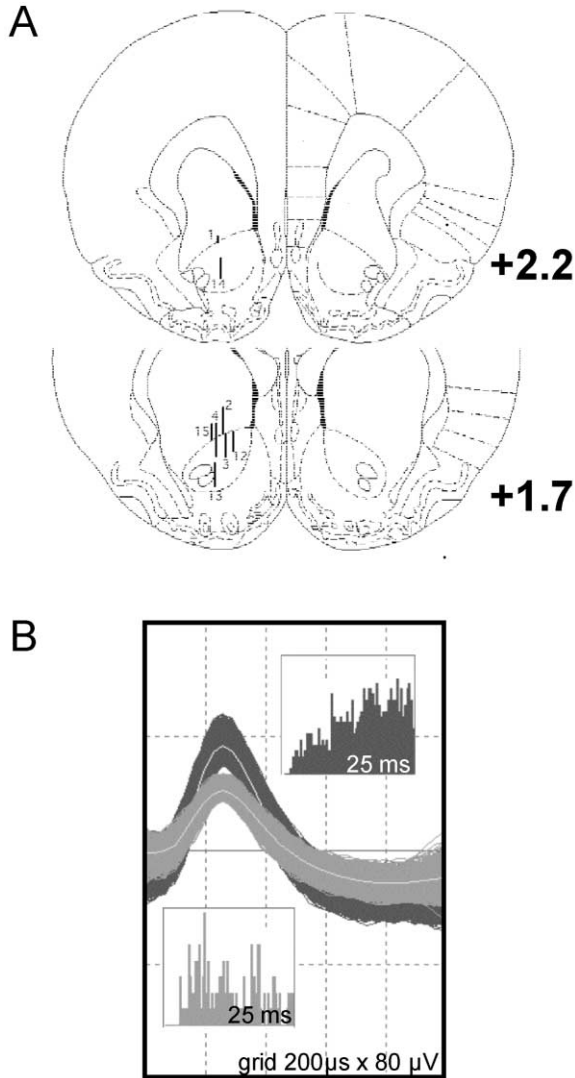


Figure 1. Locations of Recording Electrode Tracks in Ventral Striatum and Examples of Sorted Units

(A) Electrode tracks (vertical lines) for all rats are illustrated on the left hemisphere. Numbers next to electrode tracks refer to individual rats. Plates were adapted from the atlas of Paxinos and Watson (1997).

(B) Example of two units sorted on one channel. The waveforms sorted for each unit are shown along with their interspike interval histograms. Note the refractory period in the histograms for both units. The image was adapted from Offline Sorter (Plexon Inc.).

baseline firing rate = 2.12 Hz, range = 0.1–23.5 Hz). A substantial proportion (52/256, 20%) of these neurons had baseline firing rates (>3 Hz) in the range of fast-spiking and tonically active interneurons observed in striatum (Apicella, 2002; Kawaguchi et al., 1995). Given that the proportion of these neurons is estimated to be only 4%–7% of the striatal population, it is likely that they were somewhat overrepresented in our sample due to their larger size and high firing rate (Kawaguchi et al., 1995; Wilson et al., 1990). The remaining neurons (80%) with baseline firing rates <3 Hz were likely medium spiny neurons, which comprise the majority of the striatal population (Kawaguchi et al., 1995).

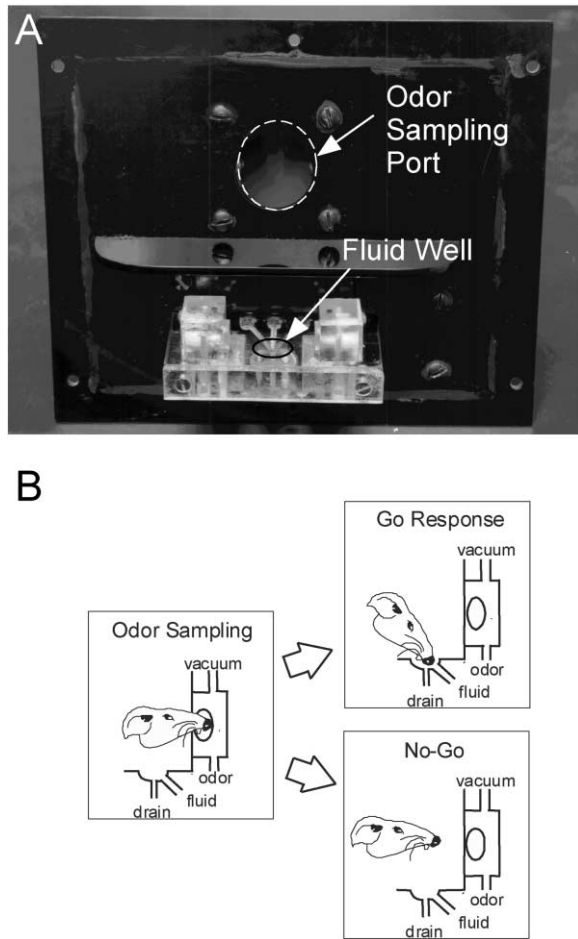


Figure 2. Training Apparatus and Behaviors in the Task

(A) Photograph of the training apparatus removed from the training chamber to show the odor sampling port (white circle) and the fluid delivery well (black circle).

(B) Schematic illustration of behaviors in each trial type.

Behavior

On each trial of a session, a nosepoke into an odor port (Figure 2) resulted in delivery of one of two odors. One of the odors (positive) signaled availability of a sucrose solution at a fluid well located below the odor port. The other odor (negative) signaled availability of an aversive quinine solution at the fluid well. Within each session, rats initially sampled the fluid following either odor (“go” responses), but then learned to withhold responding after sampling the negative odor cue (“no-go” responses). Rats reached criterion performance (18/20 correct responses in a moving block of 20 trials) in all sessions in a mean of 96 trials. According to previous methods (Schoenbaum et al., 1999), the block of trials before criterion performance was achieved was divided into an early (mean 14 trials) and late (mean 82 trials) precriterion phase for further analysis. Rats performed a mean of 84 trials after reaching criterion (postcriterion phase). Choice (go/no-go) accuracy during early, late, and postcriterion trials is illustrated in Figure 3A. As expected, performance improved significantly over the three phases of training [repeated measures ANOVA, $F(2, 38) = 130.3$, $p < 0.01$].

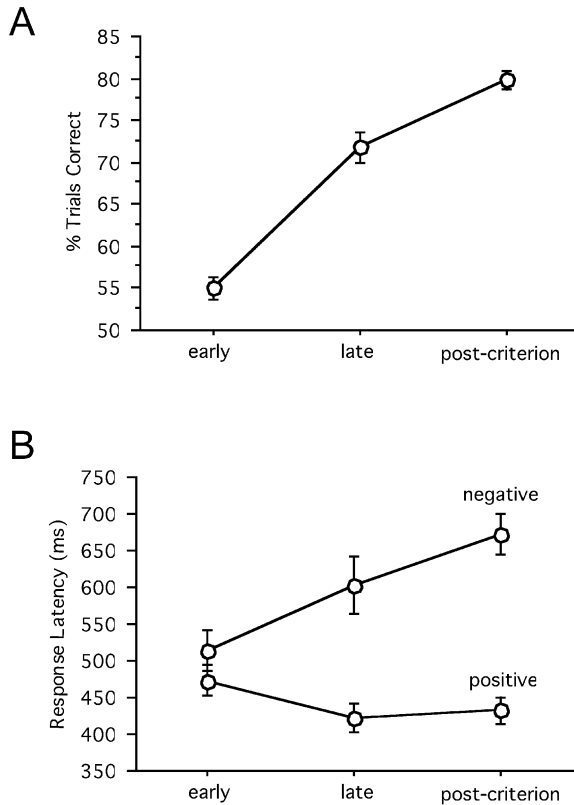


Figure 3. Behavioral Measures of Performance during the Early and Late Precriterion and the Postcriterion Phases of the Odor Discrimination Sessions

(A) Percent trials correct.

(B) Latencies to respond at the fluid well following sampling of positive and negative odors (no-go trials were not included in this analysis).

In addition to accuracy of go and no-go responses, we also measured latency to respond at the fluid well following odor sampling, excluding trials on which rats made no-go responses (Schoenbaum et al., 2000). As shown in Figure 3B, response latencies changed across session phases (early and late precriterion, and postcriterion). Latencies following sampling of positive odors decreased, whereas latencies following sampling of negative odors increased, yielding significant effects of session phase for latencies on both positive [$F(2, 38) = 7.87, p < 0.01$] and negative [$F(2, 38) = 7.75, p < 0.01$] trials. Latencies to respond for the two outcomes did not differ in the early phase of training ($t = 1.95, n.s.$), but did differ significantly in the late precriterion and postcriterion phases ($t_s = 4.54, 8.36$, respectively; $p < 0.01$). Interestingly, there were no significant correlations between response latency differences (negative latencies minus positive latencies) and performance accuracy (or trials-to-criterion) in any session phase (for all measures, $r < 0.30, n.s.$), suggesting that these two measures may assess different associative processes (Schoenbaum et al., 2003).

Selective Firing during Sampling of the Odor Cues

We examined neural activity during odor sampling to determine whether this firing differed between cues as-

sociated with positive and negative outcomes. Such selective activity (greater firing during sampling of one odor cue than the other) has been observed in this task in structures (ABL and OFC) that project to VS (Schoenbaum et al., 1999). As in those investigations, we first looked for selective activity during postcriterion trials, when rats had learned the odor-outcome associations as evidenced by having reached criterion performance. We found that a large proportion of VS neurons (103/256, 40%) fired differentially to the two odor cues during postcriterion trials. Of this population, 26% (27/103) fired more strongly to the odor cue predicting sucrose, whereas 74% (76/103) fired more strongly to the odor cue predicting quinine.

Few ($n = 3$) of the 103 neurons observed to have selective activity in postcriterion trials had selective firing to the odors during early precriterion trials. This suggests that selective firing during odor cue sampling (cue-selective activity) was related to learning rather than encoding of sensory features of the odor cues. In order to examine whether cue-selective activity encoded the anticipated instrumental responses associated with the odors (go versus no-go), we examined neurons that fired more to the negative odor cue and compared firing on trials when the rat subsequently made a go response versus a no-go response after sampling the negative cue. This analysis was restricted to postcriterion trials. Only 21/76 (28%) neurons showed differential firing dependent on the subsequent instrumental choice response, suggesting that cue-selective activity could not be generally attributed to anticipation of the instrumental responses. Further evidence in support of this conclusion is indicated by the analyses in the sections that follow.

In these analyses, unless otherwise indicated, data from neurons selective for the positive or the negative odor cues are presented together, as there were no differences in any of their firing properties. For example, the baseline firing rates of the two groups of neurons did not differ (unpaired t test, $t = 1.43, n.s.$), and similar proportions of each group developed selective firing during precriterion trials and reversed selectivity after reversal of odor-outcome contingencies (see below and Table 1; chi-squares $< 0.18, n.s.$).

Development of Odor Cue-Selective Firing during Learning

A subpopulation of the 103 cue-selective neurons (46/103, 45%) developed that selectivity prior to the point at which rats reached criterion go/no-go performance (that is, during precriterion trials). The remaining 57 neurons (55%) developed cue-selective activity only during postcriterion trials. For subsequent analyses, we examined neurons in these two subpopulations (which will be abbreviated as *rapidly selective* and *slowly selective*, respectively) separately. Their selective activity showed systematic differences in relation to other neural properties and behavior, suggesting that activity in these subpopulations encoded different types of information. Notably, rapidly selective neurons tended to have higher baseline firing rates than slowly selective neurons (means = 4.70 versus 2.08 Hz, unpaired t test, $t = 3.43, p < 0.01$), implying that the former subpopulation con-

Table 1. Odor Cue-Selective Activity of Neurons Selective for the Positive or Negative Cue during Postcriterion Trials

Population	Neurons Selective during Postcriterion Trials	Neurons also Selective during Precriterion Trials	Neurons Reversing Selectivity ^a
Positive cue	27/103 (26%)	12/27 (44%)	5/19 (26%)
Negative cue	76/103 (74%)	34/76 (45%)	13/41 (32%)

^aIn the reversal phase of training.

tained a greater number of neurons with firing rates in the range of tonically active and fast-spiking neurons (Kawaguchi et al., 1995). Figure 4 shows the firing rate distributions for these two subpopulations. There were no obvious differences between these two subpopulations in bursting or waveform characteristics, or in location within VS.

Firing in rapidly selective neurons appeared largely to reflect the acquired motivational significance of the odor cues rather than their sensory properties or anticipated go/no-go motor responses. By definition, neurons in this subpopulation developed cue-selective firing prior to the most accurate phase of choice performance (Figure 5), suggesting that this activity was not highly correlated with discriminative instrumental behavior. Further support for this conclusion comes from a comparison of cue-selective activity in recording sessions divided (median split) based on behavioral choice accuracy during late precriterion trials. Neurons that developed selective firing to the odor cues in this phase were actually more

often found in recording sessions with poor behavioral choice performance (64% correct, comparable to what we have previously observed; Schoenbaum et al., 1999) relative to sessions with more accurate (81% correct) performance (chi-square = 5.24, $p < 0.05$). This demonstration that development of cue-selective firing during precriterion trials was not associated with greater choice accuracy further dissociates differential firing to the odor cues from instrumental choice performance in this subpopulation.

In order to examine in greater detail the relationships between development of cue-selective firing and behavior in these two subpopulations, we performed correlations between the trial on which a neuron developed cue-selective firing within a session and either the trial on which the rat developed a significant difference in response latencies on positive and negative trials or the trial on which the rat reached criterion behavioral performance. By this analysis, the rapidly selective subpopulation showed a near-significant correlation ($r = 0.27$, $p = 0.07$) between the trials on which cue selectivity and latency differences emerged, but not between the trials on which cue selectivity and criterion performance emerged ($r = 0.11$, n.s.). In contrast, the slowly selective subpopulation showed the opposite pattern of results, in that there was no significant correlation ($r = -0.02$, n.s.) between the trials on which cue selectivity and latency differences emerged, but a significant correlation was present between the trials on which cue selectivity and criterion performance emerged ($r = 0.30$, $p < 0.05$). These data suggest that activity in the rapidly selective subpopulation was related most strongly to development of response latency differences, whereas activity in the slowly selective subpopulation was related most strongly to development of discriminative instrumental responding.

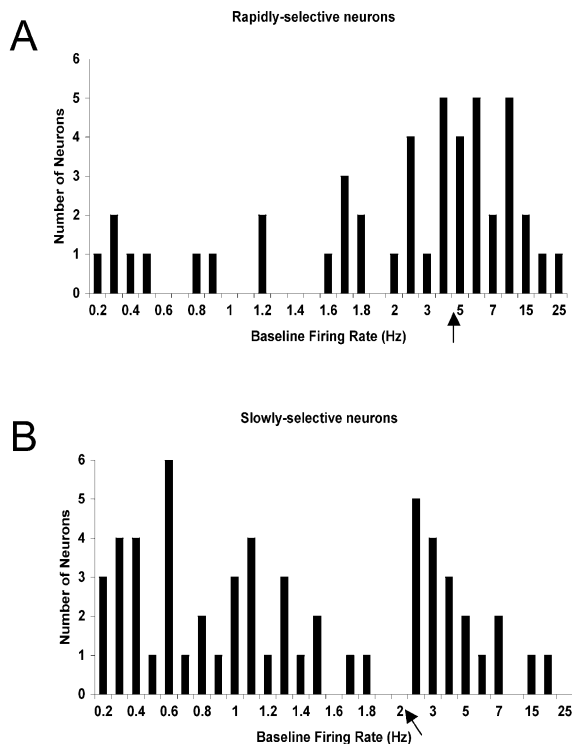


Figure 4. Distribution of Baseline Firing Rates in the Two Subpopulations of Odor Cue-Selective Neurons

(A) Firing rate frequency histogram for rapidly selective neurons.
(B) Firing rate frequency histogram for slowly selective neurons.
Arrows indicate mean baseline firing rates for each group.

Odor Cue-Selective Activity during Reversal of Odor-Outcome Contingencies

In 22 of the sessions, the odor-outcome contingencies were reversed, such that the previously positive odor cue signaled quinine delivery and the previously negative odor cue signaled sucrose delivery. These reversals were conducted within the same sessions as acquisition, immediately after data were acquired from a set of 60–100 postcriterion trials. A mean of 109 trials occurred after reversal, during which performance averaged 69% correct. Data from 148 of the 256 VS neurons were acquired during reversals in these sessions, including 60 neurons that showed selective firing to the odor cues during postcriterion trials prior to reversal.

Few of these neurons (3/60, 5%) maintained firing selectivity for the same odor after reversal, consistent with other evidence of little activity attributable to en-

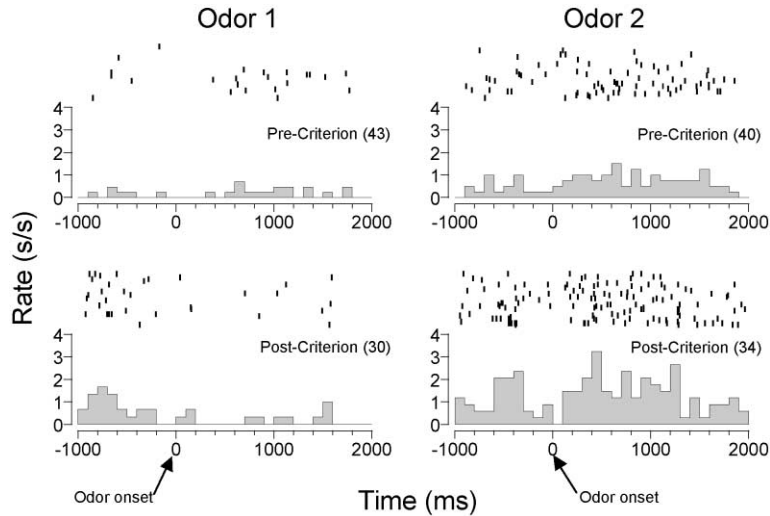


Figure 5. Odor Cue-Selective Neural Activity during Learning

A neuron that developed selectivity for the negative cue (odor 2) during precriterion trials and maintained this selectivity during postcriterion trials. Raster displays show spikes on individual trials. Histograms show neural activity in spikes/second in 100 ms bins synchronized to onset of odor 1 (positive, left panels) and odor 2 (negative, right panels) trials. Each panel shows activity on trials in a particular phase of the session as indicated by the labels in the upper right corner. The number of trials in each phase for each valence is given in parentheses.

coding of sensory properties of the odors (see above) or maintenance of information about the original odor-outcome contingencies. Instead, changes in firing to the odor cues occurred in most neurons when odor-outcome contingencies were altered in the reversal phase (Figure 6). Notable differences were observed in the predominant pattern of change observed for the two subpopulations described previously. Rapidly selective neurons were more likely to reverse their selectivity after reversal of odor-outcome contingencies (chi square = 7.71, $p < 0.05$). In contrast, slowly selective neurons were more likely to become nonselective after reversal (chi square = 10.28, $p < 0.05$). These data are summarized in Table 2. In addition, very few neurons that showed no cue-selective firing prior to reversal developed cue selectivity after reversal (5/88, 6%).

Selective Activity during Other Trial Intervals and Its Relation to Odor Cue-Selective Activity

If neural activity during sampling of the odor cues encodes information about the cues' motivational significance based on association with rewarding or aversive

outcomes, it might be expected that neurons selective for a particular cue would also exhibit selective activity either in anticipation or during sampling of the associated outcome. To look for such a relationship, we determined whether cue-selective neurons also showed corresponding selective activity in anticipation of the outcomes (during a delay period after a go response had been made but prior to fluid delivery) and during sampling of the sucrose and quinine outcomes themselves. Analysis of delay- and outcome selectivity was done only on go trials (when outcomes were delivered) during the precriterion phase, when there were sufficient negative go trials (errors) to perform such analyses.

The results of these analyses are shown in Table 3. Significant proportions of the cue-selective neurons also fired selectively in anticipation or during sampling of the associated outcome, representing a substantial proportion of the delay- and outcome-selective populations as a whole. Rapidly selective neurons showed the greatest degree of this overlap with delay- and outcome selectivity, whereas overlap in the slowly selective subpopulation was no greater than expected by chance. As was

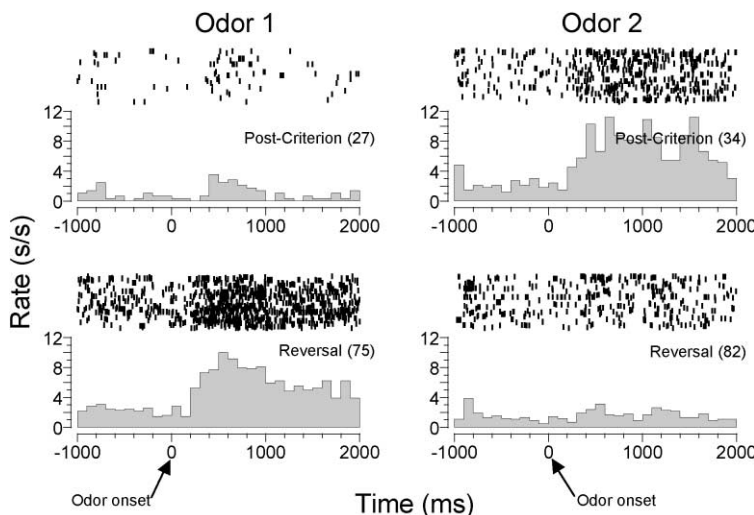


Figure 6. Odor Cue-Selective Neural Activity before and after Reversal of Odor-Outcome Contingencies

A neuron that was selective for the negative cue (odor 2) during postcriterion trials and reversed its selectivity after reversal of odor-outcome contingencies. Conventions are as in Figure 5.

Table 2. Activity after Reversal in the Two Subpopulations of Odor Cue-Selective Neurons

Subpopulation of Neurons	Reversed Selectivity	Maintained Same Selectivity	Lost Selectivity
Rapidly selective (n = 21)	11 (52%)	2 (10%)	8 (38%)
Slowly selective (n = 39)	7 (18%)	1 (3%)	31 (79%)

the case with the population of cue-selective neurons as a whole, neurons with overlapping selectivity were more frequently selective in anticipation or during sampling of quinine. Similar preferential encoding of the negative outcome was observed in the delay- and outcome-selective populations as a whole (Table 3). Figure 7 shows examples of neurons displaying selective firing during the delay period and during sampling of the sucrose and quinine outcomes.

Discussion

The results of this experiment show that neural activity in VS is related to the learning of associations between predictive cues and outcomes during acquisition of novel problems in a go/no-go odor discrimination task. Neurons developed differential firing to odor cues that signaled either positive or negative outcomes as rats learned to discriminate. One subpopulation of these neurons (rapidly selective) seemed to best encode the learned motivational significance of the odor cues, whereas another subpopulation of these neurons (slowly selective) seemed to best encode conjunctions of the odor cues and anticipated instrumental responses. The associative encoding properties of these neurons support a role for VS in using predictive cues to guide behavior.

Neural Activity in Ventral Striatum Encodes Both Rewarding and Aversive Task Events

A striking feature of the results was a preponderance of neurons that fired selectively to odor cues that signaled the aversive quinine outcome. A large proportion of neurons also had greater firing during the delay that preceded quinine delivery and during sampling of quinine itself. Several studies report striatal (including VS) activity selective for primary and conditioned aversive stimuli (Blazquez et al., 2002; Ravel et al., 1999; Williams et al., 1993), as well as enhanced VS dopamine release in response to similar events (Horvitz, 2002). Various manipulations of VS can also affect behavioral performance in aversively motivated tasks (Lorenzini et al., 1995; Parkinson et al., 1999; Salamone et al., 1997). However, while acknowledging that VS encoding and

its role in learning may not be limited to circumstances involving reward, several investigations have indicated a predominance of reward-related encoding when comparing responses of VS neurons to either aversive versus rewarding or nonpreferred versus preferred outcomes (Breiter et al., 2001; Hassani et al., 2001; Tremblay et al., 1998; Williams et al., 1993). Such findings have suggested a preferential sensitivity of VS neurons for encoding events with rewarding properties and positive hedonic value.

One potential account for why the current findings differ from those in prior reports involves the demands for learning in the go/no-go task used in this investigation. After familiarization with this task, rats begin performance in a session when a novel odor problem is presented by making go responses following both positive and negative odors. As training progresses, rats learn to withhold responding (i.e., no-go) to the negative odor, whereas responding to the positive odor remains relatively unchanged. Thus, the negative odor (and associated outcome) is of particular importance for guiding task acquisition, and, as a consequence, these task demands might bias VS toward encoding the aversive outcome and cues predictive of that outcome. At the same time, the current results may reflect the relative values of the rewarding and aversive events (that is, the highly aversive quinine outcome we used might have greater motivational power or intensity than the relatively dilute sucrose). This interpretation is consistent with the results of Schultz and colleagues (Cromwell and Schultz, 2003; Hassani et al., 2001), which showed that striatal neurons tend to fire more strongly to cues associated with more-preferred outcomes (which presumably have greater motivational value). By this interpretation, manipulations of the relative motivational properties of the two outcomes (e.g., by increasing the sucrose concentration or decreasing the quinine concentration) might increase the proportion of neurons selective for appetitive task events. The relatively large proportion of neurons responsive to direct exposure to quinine relative to sucrose in our recording population could reflect either of the above accounts; that is, either a tendency to process aversive events as a basis for learning or a relative difference in the motivational properties of the reinforcers used in the task. Note that the high proportion of neurons selective for, and in anticipa-

Table 3. Overlap between Subpopulations of Odor Cue-Selective Neurons and Populations of Neurons Showing Selective Firing during the Delay and During Fluid Outcome Sampling

Time Window of Analysis	Overlap with Rapidly Selective Neurons (n = 46)	Overlap with Slowly Selective Neurons (n = 57)
Delay (n = 70; positive = 13, negative = 57)	25/46 (54%) ^a	11/57 (19%)
Outcome (n = 58; positive = 10, negative = 48)	21/46 (46%) ^a	9/57 (16%)

^aOverlap is significantly greater than expected by chance ($p < 0.05$, Pearson chi-square).

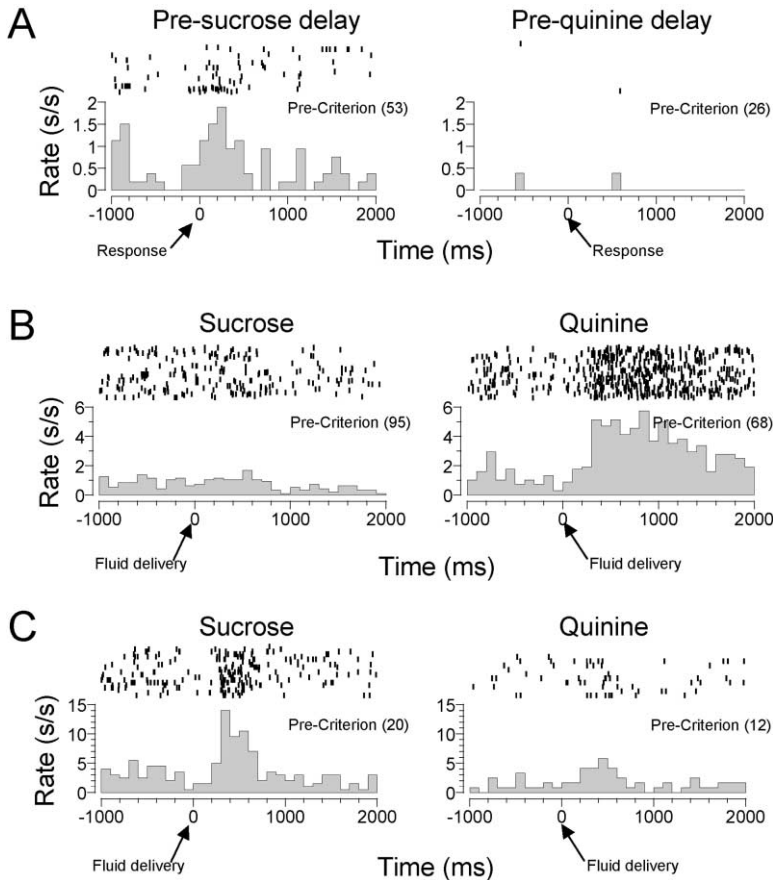


Figure 7. Selective Neural Activity during the Delay and Fluid Outcome Sampling

(A) A neuron that fired selectively during the delay prior to sucrose delivery. Raster displays show neural activity on individual trials. Histograms show neural activity in spikes/second in 100 ms bins synchronized to response at the fluid well on trials following sampling of the positive cue (left panel) and the negative cue (right panel). Each panel shows activity during pre-criterion trials. The number of trials is given in parentheses.

(B) A neuron that fired selectively during quinine sampling. Neural activity is synchronized to delivery of sucrose (left panel) or quinine (right panel). Other conventions as in (A).

(C) A neuron that fired selectively both during the delay prior to sucrose delivery and during sucrose sampling. Conventions are as in (B).

tion of, the quinine outcome suggests that the similarly high proportion of negative odor cue-selective neurons was not simply due to encoding of anticipated no-go instrumental responses, but rather includes other factors inherent in the task design.

Whatever the basis for the greater proportion of neurons selective for the negative stimuli, it is important to note that in all cases examined, parallel findings were observed across the sets of neurons encoding appetitive and aversive contingencies. Neurons selective for the positive and negative odor cues had a similar distribution of baseline firing rates and also developed and reversed their cue selectivity similarly (Table 1). Such observations suggest that these neurons were drawn from populations with very similar properties for encoding oppositely valenced information.

Odor Cue-Selective Activity Encodes the Learned Significance of the Cues

A large proportion of VS neurons showed cue-selective activity during post-criterion trials in the task. Such patterns of differential firing activity to cues that predict different outcomes have been observed in primate VS. Schultz and colleagues found selective firing in striatum (including VS) to visual cues predictive of differently preferred rewards (Cromwell and Schultz, 2003; Hassani et al., 2001). Similarly, Williams et al. (1993) described neurons in VS that fired differentially to visual cues predictive of an appetitive or aversive outcome (see also

Blazquez et al. [2002] for related findings in dorsal striatum). The present report expands upon the findings in these studies by examining how differential firing developed during learning and was affected by reversal of the odor-outcome contingencies. This approach allowed us to dissociate firing related to the learned significance of the odor cues from firing that might be related to sensory features of the odors or to the anticipated motor responses. Our analyses suggested the existence of two subpopulations of cue-selective neurons, which encoded different types of task-related information.

One subpopulation of cue-selective neurons (rapidly selective) seemed to encode the acquired motivational significance of the cues. Although this subpopulation developed cue selectivity during pre-criterion trials, very few of these neurons displayed activity consistent with sensory encoding, as there was little cue-selective activity during early training trials and little maintenance of selectivity for the same odor cue after reversal. Activity in these neurons also did not seem to encode anticipated go/no-go instrumental responses; these neurons were actually more prevalent in recording sessions with poor pre-criterion behavioral choice performance than in sessions with relatively more accurate performance. In addition, over half of the neurons in this subpopulation reversed their selectivity upon reversal of odor-outcome contingencies, and many also showed selective activity in anticipation or during sampling of the outcomes associated with the odor cues, providing further support for

encoding of the motivational significance of the cues. Encoding of cue significance, distinct from associated motor responses, has also been observed in striatum (including VS) in monkeys (Kawagoe et al., 1998; Schultz et al., 2000; Shidara et al., 1998), as well as in human VS (Breiter et al., 2001; O'Doherty et al., 2002). Interestingly, as in the present study, Tremblay et al. (1998) also reported that reward-selective firing to predictive visual cues in primate striatum tended to develop prior to accurate choice performance.

In contrast, the other subpopulation of neurons (slowly selective) seemed to encode a conjunction of information about the odor cues and the go/no-go instrumental responses. These neurons developed cue-selective firing only during the most accurate phase of behavioral choice performance, suggesting that selective firing was linked to discriminative instrumental responding. Importantly, however, these neurons largely failed to reverse their selectivity after reversal of odor-outcome contingencies (and the concomitant behavioral responses), but instead lost their selective firing altogether. This implies that their cue-selective activity encoded particular conjunctions of odor cues and instrumental responses rather than anticipated instrumental responses alone.

Subpopulations of Odor Cue-Selective Neurons: Relation to Neural Systems and Behavior

Our findings suggest that cue-selective firing in the rapidly selective subpopulation encoded the learned motivational significance of the odor cues. Activity in these neurons may play a role in biasing response times to reflect the motivational significance of the outcomes. Development of cue-selective activity in this subpopulation paralleled the development of differences in latency to respond at the fluid well on go trials following sampling of the positive versus negative cues. Latency to respond to obtain an outcome following a predictive cue has been shown in other settings to reflect associative information about the motivational significance of the outcome (Holland and Straub, 1979; Sage and Knowlton, 2000; Watanabe et al., 2001). Thus, it is likely that the development of response latency changes seen here reflected learning of associations between the odor cues and motivational properties of the outcomes. Further evidence for a role of VS in using the motivational significance of cues to bias response latency comes from an experiment in which pharmacological disruption of VS function impaired expression of differences in latency to respond for different quantities of reward (Hauber et al., 2000). Interestingly, this idea is conceptually similar to that of a "response invigorating" function of VS, in which VS is involved in modulating the vigor of instrumental behavior (Cardinal et al., 2002).

In contrast to the rapidly selective subpopulation, encoding in the slowly selective subpopulation did not appear to be tightly coupled to any one task event. Development of cue-selective firing in this subpopulation was correlated with attainment of criterion choice performance. However, these neurons largely failed to reverse their selectivity, suggesting that their cue-selective firing reflected particular combinations or conjunctions of cue significance and motor responses (go or

no-go), or perhaps even specific stimulus-response associations (Schoenbaum and Setlow, 2001). Consistent with such speculation, neurons in this subpopulation were seldom active in anticipation or during sampling of the associated outcome. Activity in these neurons might play a role in the use of cue significance to guide discriminative behavior (Corbit et al., 2001).

The encoding properties of these two subpopulations of cue-selective neurons appear in many ways similar to those of two VS afferent structures, ABL and OFC, from which we have recorded previously in rats performing this task (Schoenbaum et al., 1998, 1999). Both ABL and OFC (including dorsal and ventral agranular insular cortex) send strong, direct, and overlapping projections to VS (Berendse et al., 1992; Kelley et al., 1982; Wright et al., 1996). Although properties of OFC inputs to VS have been not been investigated, ABL and medial prefrontal cortical inputs can project to the same VS neurons and may interact to influence VS function (Goto and O'Donnell, 2002; Jackson and Moghaddam, 2001; O'Donnell and Grace, 1995). There are no direct VS projections back to ABL or OFC; however, firing in VS may influence activity in OFC indirectly through striato-pallido-thalamo-cortical feedback "loops" (Alexander et al., 1990; O'Donnell, 1999).

Selective firing to the odor cues in the rapidly selective subpopulation resembled that observed previously in ABL, where neurons tended to develop cue selectivity during precriterion trials and where the majority of cue-selective neurons reversed selectivity after reversal of odor-outcome contingencies. Indeed, there were further overall similarities between encoding properties of VS neurons and those of ABL, including the large proportion of negative cue-selective neurons and the small proportion of previously nonselective neurons that developed cue selectivity after reversal (Schoenbaum et al., 1999). These data are consistent with behavioral evidence that ABL and VS function together in encoding the learned motivational significance of cues to guide behavior (Cardinal et al., 2002; Setlow et al., 2002b). Interestingly, the role of ABL in such functions suggests that cue-selective activity in the present experiment may reflect plastic change in VS rather than (or in addition to) simple transmission of ABL input (Hernandez et al., 2002). Lesion studies have shown that although ABL is necessary for a cue to acquire motivational significance, it may not be necessary for its expression, implying that VS may be a locus of long-term plasticity related to maintenance of this information (Fuchs et al., 2002; Setlow et al., 2002a).

Firing in the slowly selective subpopulation of neurons may reflect input from OFC. Similar to neurons in this subpopulation, OFC neurons also developed selective firing to the odor cues predominantly during postcriterion trials and largely failed to reverse this selectivity, instead becoming nonselective after odor-outcome contingencies were reversed (Schoenbaum et al., 1999). One crucial difference between neurons in this subpopulation and neurons in OFC, however, is that in OFC, a substantial proportion of cue-selective neurons also exhibit outcome-related activity during precriterion trials (G.S., B.S., M.P. Saddoris, and M.G., unpublished data). Thus, neurons in this subpopulation in VS may lack an associatively activated representation of the outcome

which is present in OFC. At the same time, the delay-selective activity observed in the present experiment may be related to OFC influences on VS (Schoenbaum et al., 1998). Recent work from our laboratory has shown that delay-selective activity in OFC of rats performing the odor-discrimination task is unaffected by ABL lesions, indicating relative independence of those inputs (G.S., B.S., M.P. Saddoris, and M.G., unpublished data). Discriminative activity that anticipates outcome delivery during delays has been observed in both rat and primate VS and OFC (Chang et al., 2002; Lavoie and Mizumori, 1994; Miyazaki et al., 1998; Rolls, 2000; Schultz et al., 2000), and such activity may support a prospective form of working memory that encodes expected outcomes (Schoenbaum and Setlow, 2001; Schultz et al., 2000). These data are consistent with behavioral evidence that both OFC and VS (particularly the core subregion of the nucleus accumbens) may be important for maintaining outcome representations across delays (Cardinal et al., 2001; DeCoteau et al., 1997; Mobini et al., 2002; Otto and Eichenbaum, 1992).

The presence of these two subpopulations of neurons is compatible with the idea that VS contains different neuronal ensembles that are activated by distinct afferents or sets of afferents (O'Donnell, 1999; Pennartz et al., 1994). In the context of our dataset, the rapidly selective ensemble (possibly related to ABL input) strongly encodes the learned significance of cues based on the associated motivational properties of the outcomes in the task, whereas the slowly selective ensemble (possibly related to OFC input) encodes particular conjunctions of events (cue significance and anticipated motor response) involved in each discrimination problem. There is also likely to be considerable overlap between ABL and OFC inputs in VS (O'Donnell and Grace, 1995). Further investigation of how activity in these three structures interacts will enable a better understanding of how they function as a system in using predictive cues to guide behavior.

Experimental Procedures

Subjects

Eight male Long-Evans rats, obtained from Charles River Laboratories (Raleigh, NC) at approximately 3 months of age, served as subjects. Rats were housed individually on a 24 hr light/dark cycle with ad libitum access to food and water except during testing, when water access was restricted for the preceding 24 hr.

Electrodes, Surgery, and Histology

Rats weighed 300–400 g at the time of surgery. Procedures were identical to those used previously for implantation of recording electrodes (Schoenbaum et al., 1999). Rats were anesthetized with isoflurane and placed in a stereotaxic frame. The skull was exposed and holes drilled in the skull over the recording site and for anchoring screws. A driveable electrode bundle was implanted dorsal to VS in the left or right hemisphere at +1.6 mm anterior and ± 1.5 mm lateral from bregma, –5.5 mm ventral from the skull surface. The electrode bundle was composed of ten 25 μm diameter FeNiCr wires insulated except at the tips (Stablohm 675, California Fine Wire, Grover Beach, CA) and threaded through a 27 gauge thin-wall cannula (Small Parts, Miami Lakes, FL). Immediately prior to implantation, these wires were freshly cut at a 45° angle with fine surgical scissors to extend ~ 1 mm beyond the cannula and electroplated with platinum (H_2PtCl_6 , Aldrich, Milwaukee, WI) to an impedance of ~ 300 kOhms. Inspection of histological material showed

that the wires occupied an area 0.25–0.33 mm across in the medial-lateral plane and 0.5 mm in the dorsal-ventral plane.

During recording, the electrode bundle was advanced in 40 μm increments to acquire activity from new neurons for the following day. The final electrode position was marked by passage of a 15 μA current through each microwire for 10 s to create a small iron deposit. The rats were then perfused with formaldehyde and potassium ferrocyanide solution to visualize the iron deposit. The brains were removed from the skulls and processed for histology as described elsewhere (Setlow et al., 2002b).

Behavioral Apparatus and Training

Electrophysiological recording was conducted in an aluminum chamber measuring approximately 45 cm on each side but with inward-sloping walls narrowing to an area of 30 \times 30 cm at the floor. The front wall was hinged to open outward and provide access to the interior of the chamber. Two panel lights were located on the right wall of the chamber above the odor delivery port and fluid well (see below). A commutator (Crist Instrument Co, Damascus, MD) was mounted on the ceiling of the chamber and was mated to equipment from Datawave Technologies (Longmont, CO) for gathering neurophysiological data. A flexible cable connected the electrode assembly on the rat's head to the commutator. Task events and behavioral data were controlled by custom subroutines written in C++ and running within the Datawave program. A speaker broadcast output from a white noise generator to mask extraneous sound in the room.

Odor discrimination problems were composed of odor pairs chosen from compounds obtained from International Flavors and Fragrances (New York, NY). Discrimination problems were constructed from dissimilar odors, and the odor discrimination sequence was arranged such that similar compounds were counterbalanced by valence of the associated outcome and did not repeat across days. Odors were isolated on removable cartridges that could be connected to a system of solenoids and flowmeters to allow each odor to be individually delivered to the training chamber. All tubing and valves associated with an odor were dedicated to that odor to prevent any cross-contamination between cues (see Schoenbaum [2002] for further details).

Trials were signaled by illumination of the panel lights. When these lights were on, a nosepoke into the odor port (Figure 2) resulted in delivery of the preselected odor cue to a small hemicylinder located behind this opening. The rat was required to remain in the odor sampling port for 250 or 500 ms after odor onset. The rat terminated odor sampling by leaving the odor port. The rat then had 3 s to make a go response at the fluid well located below the port. If a response was made after sampling a positive odor, then a 0.05 ml bolus of a 5% sucrose solution was delivered to the well after a variable delay of 500–1500 ms. If a response was made after sampling a negative odor, then a 0.05 ml bolus of a 0.02 M quinine solution was delivered after a similar delay. If the rat did not respond within 3 s, the trial was counted as a no-go (Figure 2). A go response after a negative odor was considered an error and was followed by a prolonged intertrial interval (9 s versus 4 s after a correct response). The panel lights were extinguished at the end of a trial (either after a correct no-go or after the rat left the fluid well).

After recovery from surgery (2 weeks), rats were first shaped to nosepoke at the odor port to receive a water reward and then to solve an odor discrimination problem in a single session. Recording began once rats showed stable acquisition and reversal of odor discrimination problems in a single session while attached to the cable and commutator. Each odor discrimination problem consisted of two odors selected from the set described above. Rats were trained on a discrimination until they met a criterion of 18 correct responses in a moving block of 20 trials. In some sessions, odor-outcome contingencies were reversed after 60–100 trials of postcriterion performance.

Data Acquisition and Analysis

For each recording session, the rat was placed in the training chamber, and the electrode wires were screened for neural activity while the rat explored the open chamber. If no activity was detected, the rat was removed, and the electrode was advanced 40 or 80 μm .

Otherwise, active wires were selected for recording, and a training session was begun. Neural activity was recorded using a Datawave Enhanced Discovery system, capable of recording neural waveforms on up to eight channels. Signals from active wires were passed through a unity-gain JFET headstage, bandpass filtered at 300–3000 Hz, and amplified differentially (relative to a silent reference electrode) at 5000× (Neuralynx). Waveforms (>2.5:1 signal-to-noise) were digitized at 25 kHz and recorded to disk by the data acquisition software along with timestamps indicating when task events occurred (odor onset, nosepoke, fluid delivery, etc). These files were analyzed offline using software from Plexon Inc. (Dallas, TX). For this analysis, files were first imported into Offline Sorter where waveforms on each channel were sorted using principal components and a template-matching algorithm. These waveforms were compared to notes regarding the waveforms made during the session, and the interspike interval histograms were inspected to ensure that spike events were separated by >1 ms. Typically, one to three waveforms could be isolated on an active channel. An example of two units sorted on a single channel is shown in Figure 1B.

Sorted files were then processed in Neuroexplorer to extract unit timestamps and relevant event markers. These data were subsequently analyzed using statistical routines in Matlab (Natick, MA) to examine firing activity during odor sampling (from 50 ms after odor onset to 50 ms after odor offset), during the variable delay after a response at the fluid well (from 50 ms before the response until fluid delivery), and after fluid delivery (first 500 ms). Firing activity (spikes/s) in these time windows was compared on positive and negative trials during pre- and postcriterion phases and after reversal using ANOVA ($p < 0.05$). Neurons with a significant difference in activity were categorized as “selective” in that time window and phase. Baseline firing rates were calculated using the intertrial interval (from panel light offset to panel light onset on the subsequent trial).

To further explore the relationships between development of cue selectivity and changes in behavior, we examined correlations between development of firing rate differences during odor sampling and development of response latency differences and accurate choice performance. For this analysis, the trial within each session on which cue selectivity or a latency difference emerged was determined by comparing the firing rate or response latency on each negative trial (only negative go trials in the case of latency) with the values from all positive trials in the preceding block of 20 trials. A Z test was applied to determine whether the sample value from the negative trial fell outside of the normal distribution of values from the positive trials ($p < 0.05$). Correlation coefficients were then calculated between the first trial on which a significant difference in firing rate was detected and the first trial on which the rat showed a significant increase in response latency. Correlation coefficients were also calculated between the first trial on which a firing rate difference was detected and the trial on which the rat met criterion choice performance on the discrimination problem. The significance of the results was assessed using Fisher's r to z transformation.

For analysis of overlap between selective activity in different time windows, a Pearson chi-square test was used to compare the proportions of neurons with different firing properties and to ask whether the degree of overlap between two populations was greater than that expected by chance. For these comparisons, chance was calculated based on the actual proportion of neurons in the population that exhibited each type of response. For example, if 50 of 100 neurons fired selectively during sampling of the positive odor cue in a given phase, and 50 of 100 neurons fired selectively while the rat was waiting for sucrose delivery in that same phase, then the number of cells likely to be in both populations by chance would be $(0.5)(0.5)(100)$, or 25 neurons. This proportion was calculated for both valences and added to obtain the results in Table 3.

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findings are of great interest from a scientific perspective, but they are also clearly important from a clinical and societal perspective. Even though the meningoencephalitis side effect remains a problem, this article shows that the concept of vaccination is alive.

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Learning Is Bitter and Sweet in Ventral Striatum

The ventral striatum (VS) plays a key role in motivationally guided learning. Setlow et al. show that VS neurons encode the significance of cues associated with both aversive and rewarding outcomes and that this neural linkage develops over time in a fashion roughly paralleling the expression of learned behavior. Subpopulations of VS neurons may contribute distinct signals to the learning process, reflecting either cue significance or learned sensory-motor associations.

Back in the early 1990s, Michael Stipe of the band R.E.M. wryly crooned “your head is there to move you around.” Without a means for modifying movement in response to changing external conditions and internal milieu, however, Stipe’s “head” would be useless. Not surprisingly, animals with even the simplest of nervous systems have evolved mechanisms for assessing the outcome, either good or bad, of behavior and linking these outcomes to salient cues in the environment. By potentiating responses to cues associated with good outcomes and inhibiting responses to cues associated with bad outcomes, the nervous system adapts behavior to the current environment and thereby achieves a positive internal milieu (Thorndike, 1898). In mammals, this type of motivationally guided learning involves neural circuits within the basal ganglia, including the dorsal and ventral striatum (VS), as well as inputs to these nuclei from dopaminergic neurons in the midbrain ventral tegmental area (VTA) and substantia nigra pars compacta (SNc; reviewed in Schultz, 2002). In turn, these circuits participate in larger signaling loops involving the amygdala

(AMG), orbitofrontal cortex (OFC), and prefrontal cortex (PFC) during the learning and performance of contextually appropriate behavior (Alexander et al., 1990).

Current models posit that learning occurs following a mismatch in the response of midbrain dopamine neurons to cues that predict rewards and actual reward outcomes, thereby potentiating neural responses in target structures such as VS (reviewed in Schultz, 2002). Both VS and VTA/SNc responses have recently been observed in association with aversive events as well, suggesting that these circuits may serve a broader function in linking cues with biologically significant outcomes, either rewarding or aversive (Blazquez et al., 2002; Becerra et al., 2001; Horvitz, 2002). Until recently, relatively little was known about how such links are formed during learning and how they change when predictions fail, particularly in contexts involving both aversive and rewarding outcomes. In this issue of *Neuron*, Setlow and colleagues extend prior investigations of neural encoding in VS by examining neural responses and behavior during cue learning involving both rewarding and aversive outcomes. Their data suggest that VS processes cues associated with both aversive and rewarding outcomes and that selective neuronal responses in this area evolve over time in a fashion roughly paralleling learned behavior (Setlow et al., 2003). These data powerfully document the role of VS in linking environmental stimuli with biologically significant outcomes during learning.

In this study, Setlow and colleagues used an olfactory go/no-go task that required rats to learn to discriminate two odors, one of which predicted a palatable sucrose solution in the drinking well (positive odor) and another which predicted a bitter quinine solution (negative odor). The rat’s job was to learn the significance of the odor cues and drink following presentation of the positive odor and avoid drinking following presentation of the negative odor. Behavioral performance, as well as neuronal activity in VS, including both the core region of the nucleus accumbens and the ventral caudate-putamen, was recorded during learning. In a further set of experimental sessions, behavior and neuronal activity were recorded following reversal of odor-outcome pairings.

Rats rapidly learned the significance of the odor cues, performing better than 90% correct within 100 trials of odor-outcome pairing. Moreover, rats were faster to begin drinking from the fluid well on positive odor trials than when they erroneously drank on negative odor trials, and these differences in response latency became more pronounced with experience. Changes in discrimination accuracy were not closely related to changes in response latency, suggesting that these two behavioral measures might reflect different components of the learning process.

The authors also found that, once rats had mastered the odor discrimination, about 40% of neurons in the VS responded differentially to the two odor cues. Intriguingly, about 1/4 responded more strongly to odors predicting sucrose, and about 3/4 responded more strongly to odors predicting quinine. These neuronal responses did not merely reflect impending behavior, i.e., withholding or initiating drinking, suggesting that VS neurons encode the motivational significance of behavioral cues rather than a planned or anticipated movement.

The temporal dynamics of responses by VS neurons roughly paralleled behavioral learning and suggest the possibility that different subpopulations of these neurons might contribute different signals to the learning process. Specifically, one subpopulation of VS neurons rapidly developed selectivity for odor-outcome pairings, prior to accurate discrimination performance by rats, while a second subpopulation of VS neurons developed odor cue selectivity only after rats had mastered the odor discrimination. These two subpopulations may reflect physiologically identified neuronal subtypes with different neurochemical properties. Rapidly selective neurons tended to have higher baseline firing rates in the range characteristic of cholinergic fast-spiking and tonically active interneurons, whereas slowly selective neurons tended to have lower baseline firing rates typical of GABAergic medium spiny projection neurons.

These two VS subpopulations may contribute different signals to the learning process. The development of odor selectivity in rapidly selective neurons was correlated with the emergence of differences in the time it took rats to respond differentially to the odor cues. Conversely, the development of odor selectivity in slowly selective neurons was correlated with the emergence of accurate performance but not the development of differential latencies in behavior. Taken together, these data invite the hypothesis that rapidly selective, presumably fast-spiking and tonically active, VS neurons serve to highlight biologically significant cues and thereby potentiate the initiation of adaptive behavioral responses, whereas slowly selective, presumably medium spiny, VS neurons serve to link particular cues with particular behavioral responses.

In an important and compelling addition to the literature on VS function, Setlow and colleagues also studied behavioral responding as well as VS neuronal activity following the reversal of odor-outcome pairings. They found that neuronal selectivities reversed following reversal of odor-outcome contingencies. Intriguingly, rapidly selective neurons were more likely to reverse cue selectivity following reversal in odor-outcome pairings, whereas slowly selective neurons were more likely to become nonselective. The responses of rapidly selective VS neurons following odor-outcome reversal echo the finding by Kawagoe et al. (1998) that the positional tuning of neurons in the primate dorsal striatum systematically shifts to match changes in position-reward mapping in an eye movement task.

Finally, the authors demonstrated that many VS neurons responded selectively in anticipation of or during sampling of liquid in the drinking well. Most of these neurons belonged to the rapidly selective subpopulation and maintained selectivity for the same odor-outcome pairing during odor sampling, preceding liquid delivery as well as during drinking. In contrast, slowly selective VS neurons generally did not respond selectively prior to or during drinking.

The authors point out that these responses, segregated in separate subpopulations of VS neurons, resembled the responses of neurons in AMG and OFC during the same types of tasks. In fact, the authors have studied neurons in all three areas using the same task, permitting them to relate the results and proffer a model for inputs to VS from AMG and OFC that could produce the ob-

served results in VS (Schoenbaum et al., 1998, 1999). Specifically, the authors propose that the responses of rapidly selective VS neurons are heavily influenced by neurons in the basolateral nucleus of the AMG, while the responses of slowly selective VS neurons are derived, at least in part, from OFC inputs.

This model accounts for a number of observations. Both rapidly selective VS neurons and basolateral amygdala neurons develop selectivity to odor cues prior to accurate behavioral discrimination, reverse selectivity following reversal in odor-outcome pairings, and appear to be biased toward signaling cues predicting aversive outcomes. In contrast, both slowly selective VS neurons and OFC neurons do not develop cue selectivity until well after rats accurately classify odor cues and largely fail to reverse selectivity following reversal of odor meaning. OFC neurons, however, do respond during and after reward delivery, suggesting that the responses of slowly selective VS neurons are not a passive reflection of activity in OFC.

The rich behavioral and electrophysiological data communicated in this paper strongly support the idea that VS contributes to learning the biological significance of external cues, both rewarding and aversive, as well as organizing appropriate behavioral responses to them. Moreover, separate subpopulations within VS may participate in distinct neural circuits that either highlight biologically relevant cues or link these cues with specific behaviors. The intriguing model derived from these data should provide compelling food for thought for researchers studying learning and motivation and the representation of these processes in the brain.

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