View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Neuron, Vol. 33, 973–982, March 14, 2002, Copyright ©2002 by Cell Press

A Network Representation of Response Probability in the Striatum

Pablo M. Blazquez,[1] Naotaka Fujii,[2] Jun Kojima,[3] and Ann M. Graybiel[2,4]
[1]Washington University School of Medicine
4566 Scott Avenue
St. Louis, Missouri 63110
[2]Department of Brain and Cognitive Sciences
and the McGovern Institute for Brain Research
Massachusetts Institute of Technology
45 Carleton Street, E25-618
Cambridge, Massachusetts 02139
[3]Sayama Neurology Hospital
246 Higashi-mitsugi
Sayama-shi, Saitama 350-1302
Japan

Summary

The striatum of the basal ganglia is considered a key structure in the learning circuitry of the brain. To analyze neural signals that underlie striatal plasticity, we recorded from an identifiable class of striatal interneurons as macaque monkeys underwent training in a range of conditioning and non-associative learning paradigms, and recorded eyeblink electromyographs as the measure of behavioral response. We found that the responses of these striatal interneurons were modifiable under all training conditions and that their population responses were tightly correlated with the probability that a given stimulus would evoke a behavioral response. Such a network signal, proportional to current response probability, could be crucial to the learning and decision functions of the basal ganglia.

Introduction

A leading hypothesis about the neural basis of learning and memory is that different neural circuits are specialized for different types of learning. The basal ganglia have been proposed as structures critical for reward-based learning. The main input side of the basal ganglia, the striatum, is thought to receive teaching signals from the reward-sensitive dopamine-containing neurons of the substantia nigra (Schultz, 1998). Many neurons in the striatum also respond to reward or are influenced by reward-based conditioning (Aosaki et al., 1994a, 1994b, 1995; Apicella et al., 1997; Hollerman et al., 1998; Jog et al., 1999; Kawagoe et al., 1998; Rolls et al., 1983; Shidara et al., 1998). Yet the striatum, as the origin of the major output pathways of the basal ganglia, is centrally implicated in the control of movement and cognitive functions, many of which are not obviously related to reward. Likewise, many striatal neurons have response properties that do not seem dependent on reward: they may respond to neutral stimuli, to aversive stimuli, or in relation to movements or movement sequences (Aosaki et al., 1994a; Crutcher and DeLong, 1984; Hikosaka et al., 1989; Kermadi and Joseph, 1995; Mink, 1996; Ravel et al., 1999; Schneider, 1987; White et al., 1994).

To address this paradox, we designed a set of experiments in macaque monkeys to test the relation between the responses of striatal neurons and behavioral responses under different learning conditions including aversive as well as reward-based conditioning, extinction, and habituation. We focused on delay eyeblink conditioning and extinction (Gruart et al., 1995; Richardson and Thompson, 1985; Steinmetz and Thompson, 1991; Thompson and Kim, 1996; White et al., 1994) and blink responses to neutral stimuli (Blumenthal and Goode, 1991), because the basal ganglia are known to be involved in the control of eyeblink (Basso and Evinger, 1996; Basso et al., 1996, 1993; Blaxton et al., 1996; Evinger et al., 1993; Logan and Grafton, 1995), and we could accurately monitor the behavioral responses of the monkeys by recording the EMG activity of a single muscle, the orbicularis oculi.

Throughout the experiments, we recorded the responses of the striatal interneurons known as tonically active neurons (TANs) because their response properties in reward-based conditioning have been extensively studied (Aosaki et al., 1995, 1994b; Apicella et al., 1996, 1997; Ravel et al., 1999; Raz et al., 1996; Sardo et al., 2000; Shimo and Hikosaka, 2001) and are known to be influenced by dopamine-containing inputs from the substantia nigra (Aosaki et al., 1994a; Raz et al., 1996). Moreover, because the TANs have distinctive firing characteristics, they can be readily recognized during recording experiments. This allowed us to monitor the activity of a single type of local circuit neuron in the awake behaving primate in relation to the activity of a single muscle.

We found that the population responses of striatal TANs to any given stimulus were tightly linked to the probability that the stimulus would evoke a behavioral response. The probabilistic signal carried by these striatal network neurons could be used in computations underlying motor and cognitive functions of the basal ganglia.

Results

TANs Acquire Responses to Aversive Conditioning Stimuli

We recorded the neuronal activity of striatal TANs and the EMG activity of the left orbicularis oculi muscle in three monkeys as they underwent training and then extinction in a delay eyeblink conditioning protocol in which an auditory tone stimulus (CS) was associated with an aversive airpuff directed toward the left eye (US). Before conditioning, two of these animals and a fourth monkey received repeated exposure to tone stimuli in order to induce behavioral habituation, during which recordings were also made. Throughout the conditioning and extinction experiments, the monkeys also performed a conventional reward conditioning task so that TANs could be recorded during blocks of both aversive

[4]Correspondence: graybiel@mit.edu

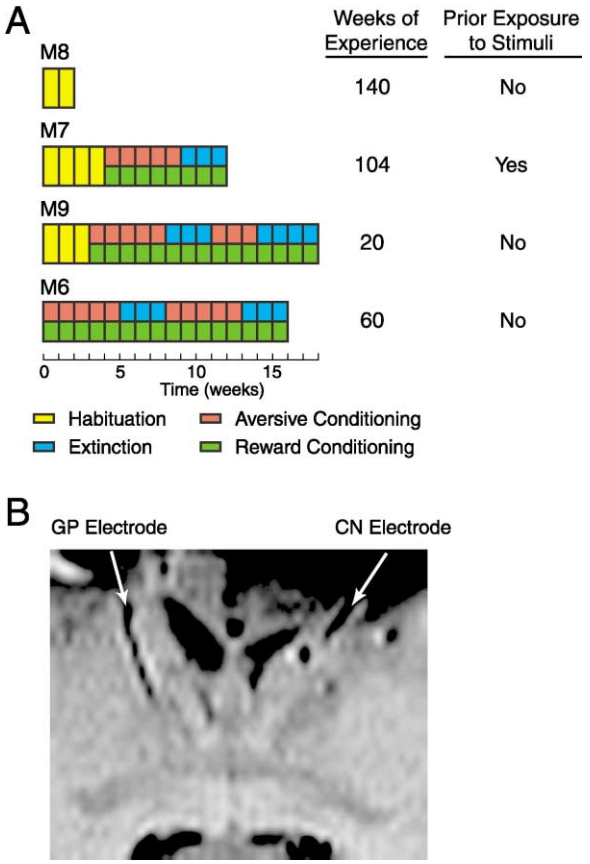

Figure 1. Experimental Schedules and Sites of Neuronal Recording

(A) All monkeys except M6 (M7, M8, and M9) were given habituation training in which they were exposed to repeated presentations of tone stimuli. Following habituation training (or initially for M6), all but M8 were trained in aversive delay eyeblink conditioning and extinction protocols, and during the same periods were trained in reward conditioning. Table to right indicates the range of experience of the four monkeys.

(B) shows MRI image taken in M6, in which two electrodes (see arrows) were left in place after recording from the globus pallidus (GP, left) during mapping session, and after recording the activity of a TAN in the caudate nucleus (CN, right).

and reward conditioning (Figure 1A). We identified TANs by their irregular tonic 2–10 spikes/s activity and by their spike waveforms, characterized by a wide (2–5 ms) action potential and prolonged after-hyperpolarization (Aosaki et al., 1994b). In all, we recorded from 986 TANs in the caudate nucleus and putamen (Figure 1B) from seven hemispheres in the four monkeys.

We first recorded TAN and EMG activity during conditioning in the two monkeys thoroughly habituated to the conditioning stimuli (M7 and M9; Figure 1A). Figure 2 illustrates the results for monkey M7. At the beginning of conditioning, the monkey exhibited a strong EMG response to the airpuff (average latency 37 $\pm$ 16 ms) but little response to the tone that served as a CS. With training, however, EMG responses to the CS increased rapidly, and by behavioral asymptote, they occurred in response to about 97% of the CS presentations. The mean latency of the conditional response was 118 $\pm$ 50 ms. During the same training period, there was a sharp increase in the number of TANs responding to the CS, from a low of 11% of those recorded during the last week of habituation to a high of 92% of those recorded during subsequent conditioning.

Like TAN responses previously reported for reward-associated conditioning stimuli (Aosaki et al., 1995, 1994b; Apicella et al., 1996, 1997; Sardo et al., 2000), the responses to the CS during aversive conditioning typically included a ca. 120 ms long decrease in firing rate ("pause" response) about 110 ms after the CS, followed by an increase in firing (Figure 2C). Some TANs also had an initial excitatory response, and the rebound excitation following the pause response often was followed by a further pause-rebound cycle (cf. Aosaki et al., 1994b; Ravel et al., 1999; Raz et al., 1996). Early in training, a majority of the TANs also responded to the airpuff that served as the US, but responses to the US decreased during conditioning (from 100% to 40% of the TANs recorded in M7).

Extinction training, in which the aversive US was omitted, led to a rapid decline in the behavioral responses to the CS and also in the responsiveness of the striatal TANs. In M7 (Figure 2A), EMG responses fell to 24% and TAN responses fell to 20%. Thus, during the eyeblink conditioning and subsequent extinction training, both the behavioral responses and the responses of striatal TANs showed marked plasticity.

Responses to Aversive and Rewarding Stimuli Can Co-Occur in a Single TAN

The fact that we trained the monkeys in both reward and aversive conditioning paradigms meant that we could determine whether individual TANs could respond both to the CS indicating the rewarding US (water) and to the CS indicating the aversive US (airpuff). We held 305 TANs during both training procedures, and found that about a third (30%) responded both to the aversive conditioning CS and to the reward conditioning CS in M6, and the corresponding values were about 20% in M9 and about 18% in M7, in which the reward-associated CS was presented 750 ms before reward (Figure 3). Calculated as a percent of the less represented stimulus category (rewarding for M6 and M7, and aversive for M9), over half of all TANs responded to both types of stimuli (61% for M6, 78% for M7, and 65% for M9). These data, together with those of Ravel et al. (1999), demonstrate that many TANs respond to conditioning stimuli whether they are associated with rewarding or aversive unconditioned stimuli.

TAN Responses to Sensory Stimuli Vary with Behavioral Habituation

To test whether familiarity with the stimuli used as CSs would also systematically influence TAN responsiveness, we examined the effect of repeated exposure to neutral tone stimuli on TAN and EMG responses in three of the monkeys (M8, M7, and M9; Figure 1). As shown for monkey M8 in Figure 4, both the behavioral blink responses as measured by orbicularis oculi EMG recordings (Figure 4A) and the neuronal responses as measured by the percentage of responsive TANs (Figures 4B and 4C) declined sharply during such habituation training. We took the opportunity to look for any

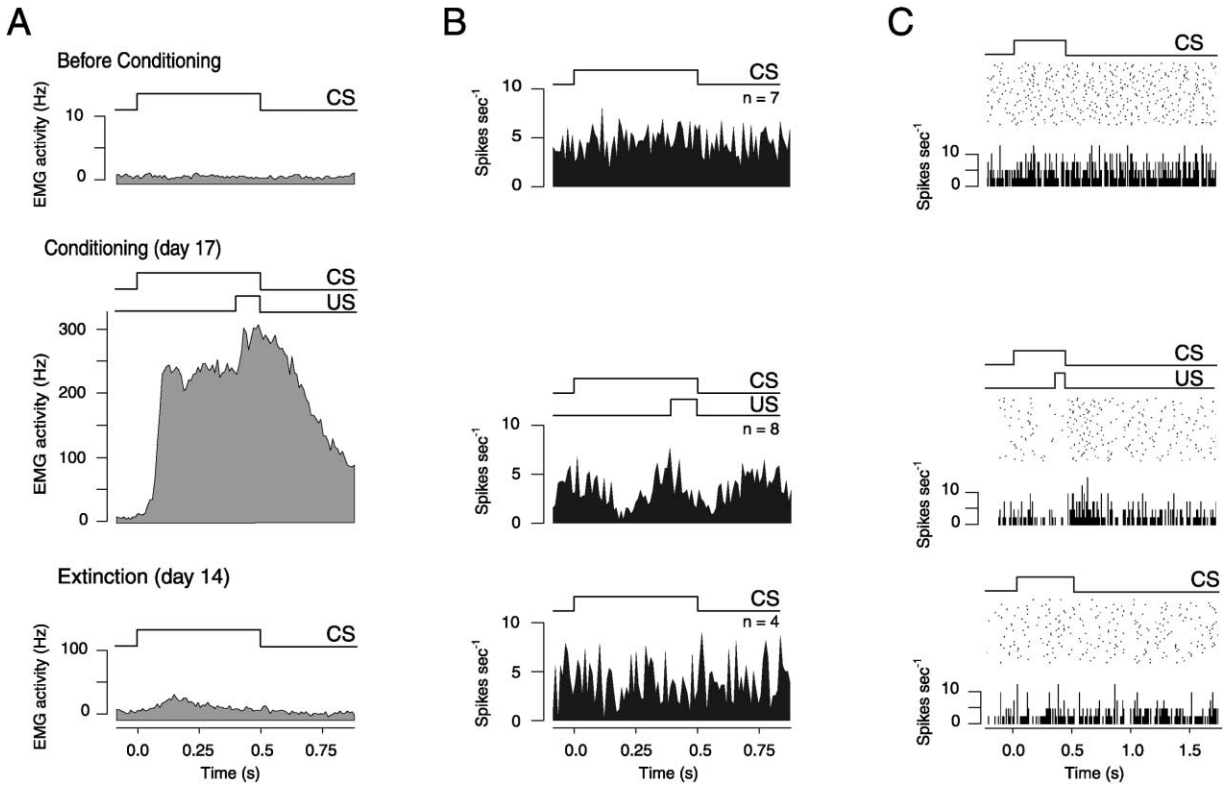

Figure 2. Tonically Active Neurons (TANs) in the Striatum Exhibit Response Plasticity during Aversive Conditioning and Subsequent Extinction

(A) shows pooled EMG responses of left orbicularis oculi muscle recorded before and during eyeblink conditioning and after extinction in monkey M7. (B and C) Population response histograms (B) and raster plots of individual TANs (C) recorded on corresponding days are shown. The onset and offset times of the conditioned stimulus (CS, a 333 Hz, 63.4 dB tone) and unconditioned stimulus (US, airpuff) are plotted above by step-up and step-down deflections in (A)–(C). The n values shown in (B) indicated the number of TANs analyzed to make the histograms.

obvious differences in behavioral and neural responsivity to the tones that might be related to general laboratory experience. Monkey M7 had more than two years of lab experience, during part of which she had been exposed to the tone sounds, whereas monkey M9 had participated in other experiments for only 20 weeks and had no experience with the tone stimuli used in the present experiments (Figure 1). The initial responses to the tones were lower in M7, which had previous exposure to these tone stimuli (24% responsive TANs), than in M9, which did not have such previous exposure (35% responsive TANs) (Figure 5). Correspondingly, the effects of habituation were smaller in the more experienced monkey (M7) and were larger in the less experienced monkey (M9), which remained reactive (across all stimulus conditions, responses fell from 35% to 17% in M9 and from 24% to 11% in M7). In both monkeys, for any given level of exposure, the louder the tone stimulus, the more responsive were the TANs (Figures 4D–4G). These results raise the possibility that not only associative learning brought about by the formal conditioning and extinction training, but also simple stimulus intensity and incidental learning that occurred as a result of repeated exposure to the same stimuli, systematically altered responsiveness of striatal TANs as well as the behavioral responses to the same stimuli (cf. Grunewald et al., 1999).

The Population Responses of Striatal TANs to Conditioned Stimuli Parallel the Probability of Conditioned Responses to These Stimuli

The fact that many TANs responded to conditioning tone stimuli in both reward and aversive conditioning, and also in proportion to the novelty and intensity of the tone stimuli, suggested that the TAN responses might reflect general stimulus salience (cf. Aosaki et al., 1994b; Ravel et al., 1999). An alternative interpretation, however, was that the responses of these striatal neurons were more closely related to the behavioral response itself, indicating, for example, whether a response was occurring. To approach this issue, we first asked whether TAN responses were more tightly linked to the CS or to the conditioned eyeblink (CR) that followed it during aversive conditioning.

We carried out a trial by trial analysis of the TAN pause-rebound responses recorded in M6, M7, and M9 in relation to the CS and CR events in the same trials. We then calculated the coefficients of variation (CV = SD/mean) for the occurrence and the latencies of these events. We found that TAN responses to the CS were not invariably present in every trial in which an EMG response occurred, so that for any given blink response, the responses of the individual TANs being recorded at that time were not accurate predictors of the blink. The results of the CV analysis suggested that the occurrence

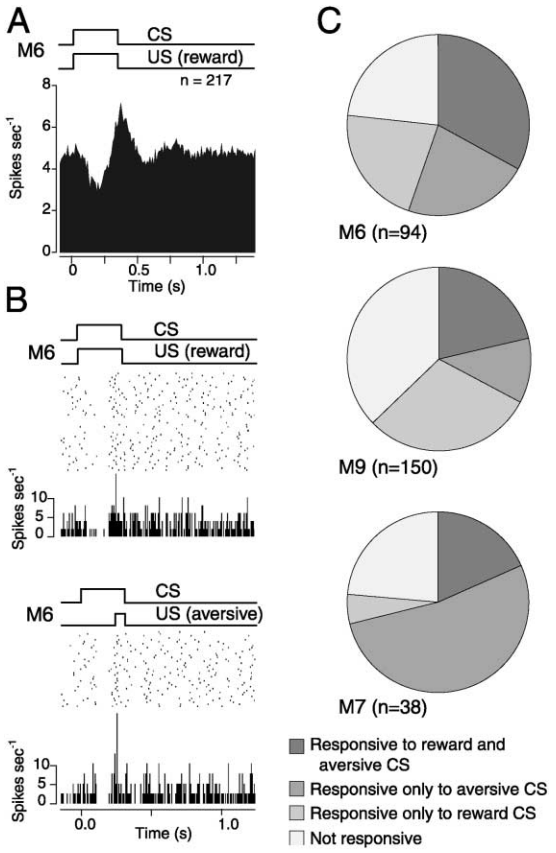

Figure 3. Single TANs Can Respond Both to a CS Associated with an Aversive US and to a CS Associated with Reward

(A) Population histogram of TANs recorded in response to CS reward presentation in M6 is shown; n indicates number of neurons used for the histogram. (B) shows raster plots of a representative TAN in M6 responding both to CS associated with reward (above) and to the CS associated with an aversive airpuff (below). (C) shows plots of percentages of TANs recorded during both aversive and reward conditioning that responded to both reward-conditioning CS and aversive-conditioning CS, to reward-conditioning CS only, to aversive-conditioning CS only, or to neither CS in monkeys M6, M9, and M7. Numbers of TANs for each monkey are indicated for each plot.

of TAN responses was no better linked to the occurrence of the CSs than to the occurrence of the CRs. For the neural and behavioral response occurence, the TAN-CR CVs were 1.1 times (M6), 1.5 times (M7), and 1.4 times (M9) larger than the TAN-CS CVs. Temporally, however, the TAN responses in any given trial were clearly much better linked to CS onset than to EMG onset. The CVs for TAN responses in relation to CR onset were 2.15 times (M6), 1.47 times (M7), and 2.38 times (M9) larger than the CVs for the same TAN responses in relation to CS onset.

Despite the lack of one to one correlation of TAN and EMG responses for any given TAN in any given trial, the overall percentage of responsive TANs paralleled remarkably closely the percentage of behavioral responses. The graphs in Figure 5 plot these percentages of neural and behavioral responses week by week for the 2 week habituation training of M8 (Figure 5A), the 12 and 11 week habituation and initial conditioning and extinction training of M7 and M9 (Figures 5B and 5C), and the entire two-conditioning and extinction training cycles given over 16 weeks to M6 (Figure 5D). Throughout training, whether habituation, conditioning, or extinction, the neural and behavioral responsivities changed concordantly. Both fell sharply during habituation in M8. Both started low in the highly experienced (already habituated) M7 (Figure 5B), and both started very high in the non-habituated M6 (Figure 5D). M6's responses actually fell during the first conditioning; but after this initial period, the TAN and EMG responses of M6 were similar in pattern to those of M7, falling during extinction and then systematically rising and then falling again during the second conditioning and extinction periods. The behavioral and TAN responses both showed the smallest changes and the greatest variability during training in monkey M9, the least experienced monkey (Figure 5C).

To obtain a quantitative estimate of the degree of correlation between the TAN and EMG responses, we performed regression analyses on the values for the neural and behavioral responses for each monkey averaged for each week of each behavioral schedule and for each schedule collapsed across weeks. The resulting correlation coefficients for the by-week analysis (Figure 5E) yielded a Pearson R^2 value of 0.79. The individual R^2 values for the three conditioned monkeys were 0.68 for M6, 0.86 for M7, and 0.66 for M9. The Pearson value for the by-task analysis was $R^2 = 0.9$. These data indicate that, under the assumption of bivariate normal distribution, as much as 79%–90% of the variance of the EMG responses to the CS could be predicted by the responses of the recorded TANs to the same CS.

The fact that the week by week and condition by condition TAN responses were so tightly correlated with the occurrence of behavioral responses, even though the responses of individual TANs in any given trial were not, suggested that it was the population responses of TANs that were such remarkably accurate predictors of the behavioral blink responses. If TANs were to respond as a population, then different TANs recorded simultaneously should have similar response profiles. For technical reasons, we could not record simultaneously from large numbers of TANs, but we were able to record simultaneously from 133 pairs or triplets of TANs during the experiments. We compared their response profiles (response or no response) across conditions during aversive conditioning and extinction. Of the TANs simultaneously recorded within the same hemisphere, 67% had similar response patterns. For the sample of TANs recorded simultaneously in opposite hemispheres, 58% of the TANs had similar responses.

To estimate how the size of the responding TAN population would influence the accuracy with which the TAN responses could predict behavioral responses, we used a modified version of the neuron-dropping analysis of Wessberg et al. (2000). On the basis of our data (Figure 5), we calculated the correlation coefficients and R^2 values that would occur for the correlation between TAN responses and EMG responses upon increasing the numbers of TANs included in the sample. The samples of TANs were chosen randomly from the entire data set condition by condition, up to a total of 40 TANs, the maximum number of TANs that were recorded for any

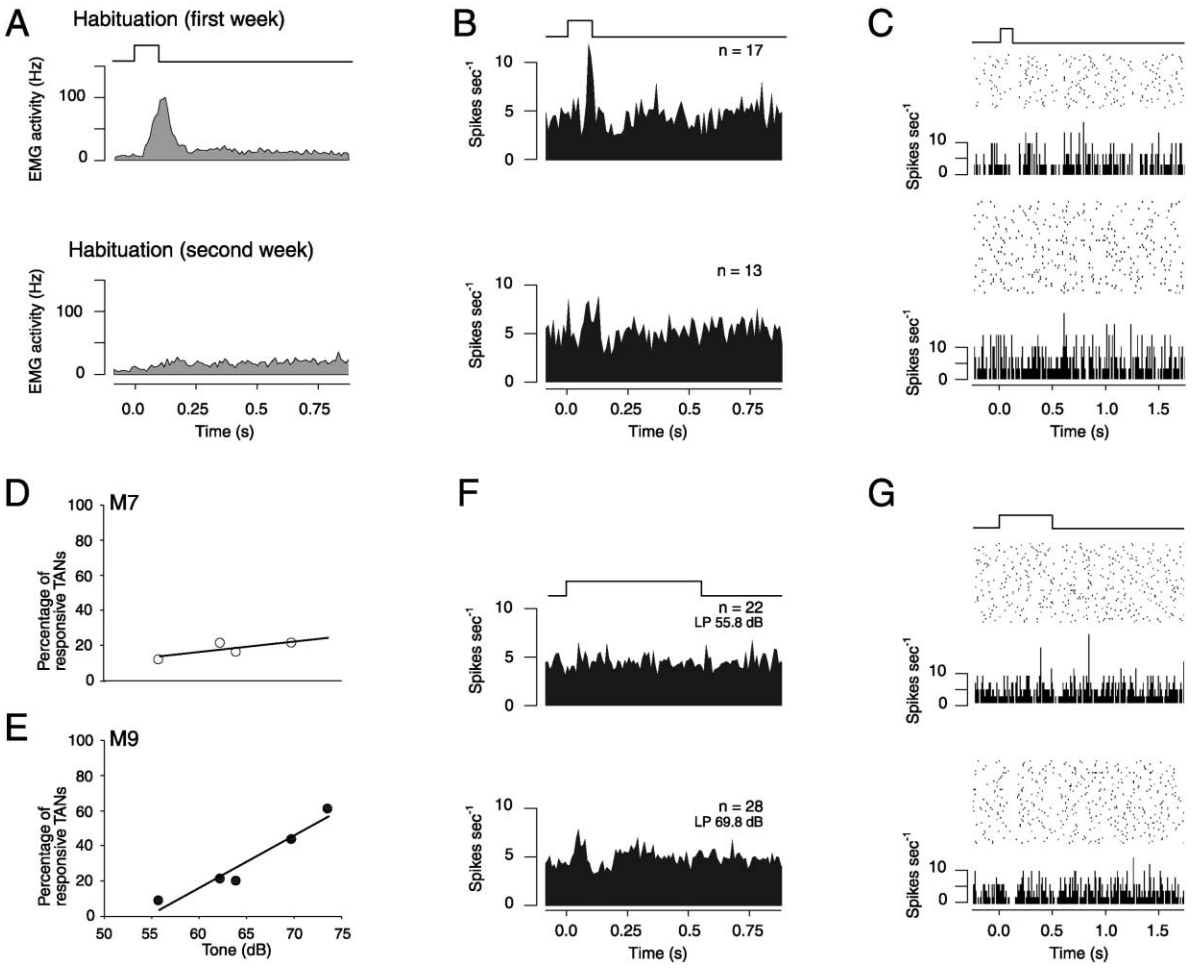

Figure 4. TAN Responses Vary as a Function of Unconditional as Well as Conditional Attributes of Sensory Stimuli

(A) Averaged EMG activity; (B) population histograms of TAN responses; and (C) raster plots of single TAN responses during the first and second weeks of habituation training in M8 to the sound of the airpuff (directed away from the monkey). (D–G) Increases in stimulus intensity produce increases in TAN responsiveness are shown. (D) and (E) show plots of TAN responsiveness as a function of stimulus intensity for monkeys M7 (D) and M9 (E), collapsed across four weeks (M7) and three weeks (M9) of exposure that the monkeys were given. Monkey M7 (D) was exposed to all sounds except the airpuff, and M9 (E) was exposed to all of the sounds. (F) Population histograms of the responses of all TANs recorded with 333 Hz tone presented at 55.8 dB (above) and 69.8 dB (below) are shown. (G) shows raster plots illustrating the response of a single TAN to 333 Hz tone presented at 55.8 dB (above) and 69.8 dB (below), recorded during the second week of habituation in M9.

one condition across all conditions (Figures 5A–5C). The data were fitted to the function $y = \sqrt{(cx/1 + cx)}$ (Djurfeldt et al., 1999; Wessberg et al., 2000). The results (Figure 6) indicate that to reach a Pearson value of $R^2 = 0.95$ would require about 300 responsive TANs ($n = 310$), and to reach an $R^2 = 0.90$ would require on the order of 150 responsive TANs ($n = 140$).

Discussion

Our experiments demonstrate that the striatum contains a population of neurons whose response to sensory stimuli reflects the likelihood that the stimuli elicit a behavioral response. The close correlation between the neural and behavioral responses was apparent across a range of behavioral conditions including classical conditioning, extinction, and non-associative learning. Striatal TANs thus stand in a position to influence activity in cortico-basal ganglia loops based on estimates of the action-based salience of stimuli acquired through experience. Such network signals could contribute to computations used in on-going learning functions of the basal ganglia (Graybiel, 1998; Hikosaka et al., 1998) as well as in their action-oriented decision functions (Gold and Shadlen, 2001; Graybiel and Kimura, 1995; Platt and Glimcher, 1999; Schall, 2001).

TANs Have a Broad Range of Potential Sensory Responsiveness

The hypothesis that TANs participate in the positive reinforcement circuitry of the basal ganglia is based on evidence that their reward-associated responses are quite similar to those of midbrain dopamine-containing neurons and that their responses are influenced by ni-

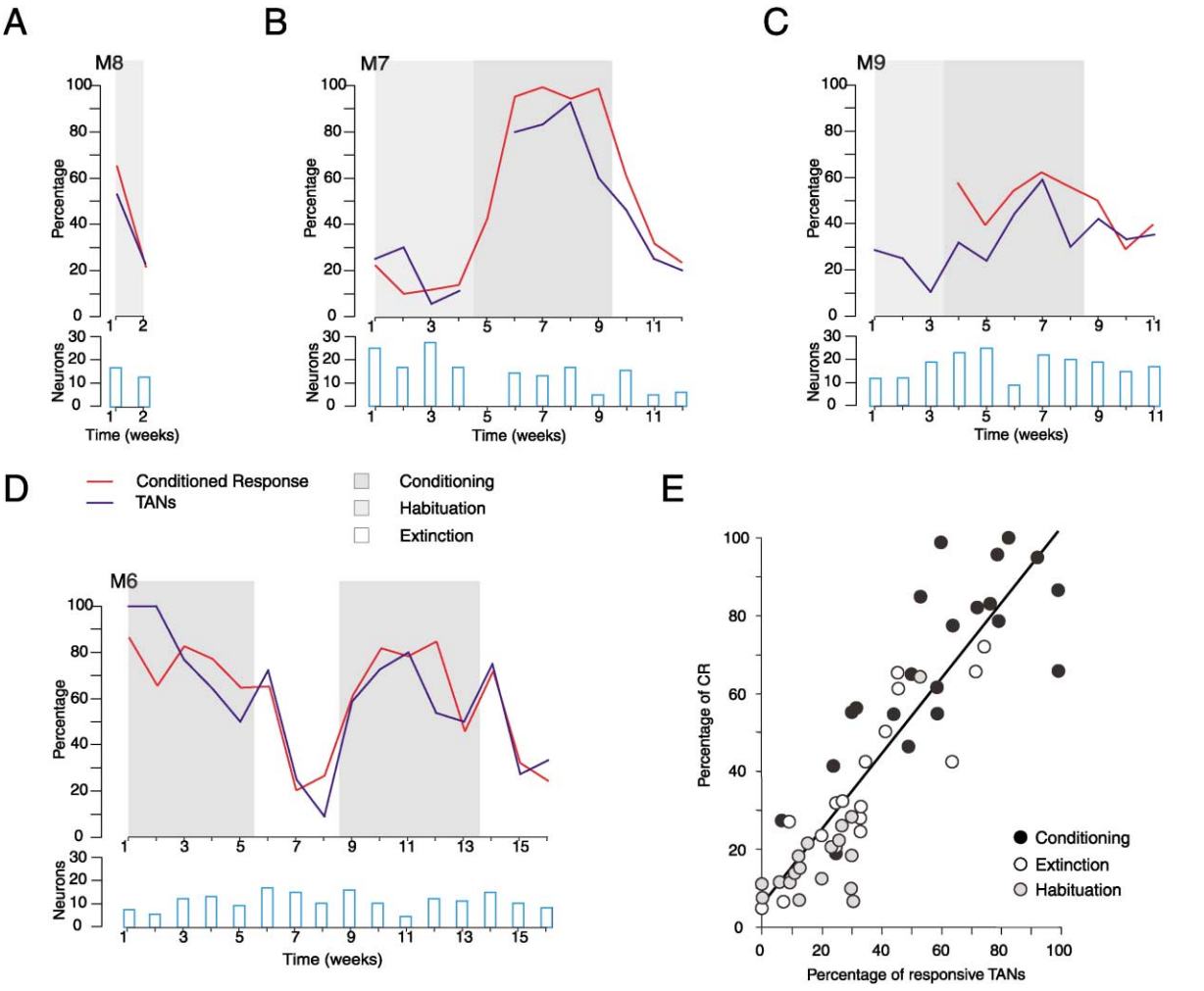

Figure 5. The Responses of Striatal TANs Parallel the Behavioral Responses of Monkeys to Sensory Stimuli

(A)–(D) show plots of the percentages of TAN responses (blue) and EMG responses (red) for consecutive weeks of training in monkeys M8 (A), M7 (B), M9 (C), and M6 (D). Bar graphs below show numbers of TANs recorded each week. Light stipple indicates habituation training, dark stipple indicates delay eyeblink conditioning, and no stipple indicates extinction condition. (A) Habituation of M8 to sound of airpuff (directed away from the monkey); (B) habituation (333 Hz, 63.9 dB tone), conditioning (400 ms CS-US interval), and extinction (CS without US) in M7; (C) habituation (333 Hz, 63.9 dB tone), conditioning (500 ms CS-US interval), and extinction in M9; and (D) conditioning (250 ms CS-US interval), extinction, second conditioning (500 ms CS-US interval), and extinction periods in M6. (E) Regression analysis of neural responses of TANs in each behavioral condition relative to the blink EMG responses recorded. Data points represent pooled results for the four monkeys obtained during week by week habituation, conditioning, and extinction, as shown in (A)–(D).

grostriatal dopamine (Aosaki et al., 1994a, 1994b; Apicella et al., 1996, 1997; Raz et al., 1996; Sardo et al., 2000; Shimo and Hikosaka, 2001), even though the TANs appear to respond more readily to neutral and aversive stimuli than do the dopamine-containing neurons, at least in primates (Aosaki et al., 1994b; Mirenowicz and Schultz, 1996; Ravel et al., 1999; Schultz and Romo, 1987).

Our findings suggest an alternate interpretation of the responses of TANs to rewards and reward-associated stimuli, in which these are instances of a more general category of potential responses influenced by negative as well as positive reinforcers, and by stimulus familiarity and intensity as well as motivational valence. Our results argue that a principal unifying characteristic of stimuli eliciting the population responses of TANs lies not in a single sensory or affective quality, but in the probability that the stimuli will evoke a behavioral response.

Population Response of Striatal TANs as a Predictor of Behavioral Outcome

A key advantage of monitoring eyeblink in our experiments is that we could measure the onset latencies of EMG activity in a single muscle (orbicularis oculi) and compare these to the onset latencies of TAN responses. These measures demonstrated that the TAN responses occurred at about the same time as the EMG responses. The responses of any one TAN to the CS thus do not represent premotor responses used to direct the blink response to that CS. Moreover, on a trial by trial basis,

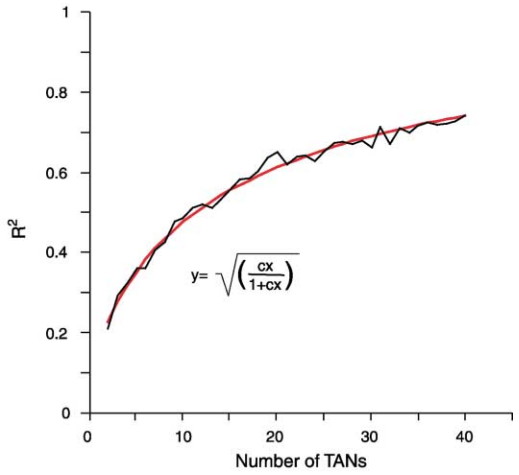

Figure 6. The Activity of TANs in the Striatum Predicts Behavioral Outcome

Pearson R^2 values for correlation between TAN responses and EMG responses, calculated from data pooled for the four monkeys condition by condition for habituation, delay eyeblink conditioning, and extinction. Calculations were made by taking samples of increasing numbers of TANs up to 40, the maximum available for all of the behavioral protocols. Data were fitted to the function $y = \sqrt{(cx/1 + cx)}$ by means of a nonlinear Fletcher-Reeves conjugate gradient method available in the See neuron simulation package (Djurfeldt et al., 1999), according to a modified neuron-dropping analysis (Wessberg et al., 2000); $c = 0.03$.

the responses of any one TAN were poor indicators of whether or not a blink occurred. The responses of single TANs thus do not signal each occurrence of a movement. What the TANs do appear to signal are values that, when summed over a population of TANs, indicate the probability that a behavioral response to a given stimulus will occur.

Our observations strongly suggest that this probabilistic signal is built up by past experience. The responsiveness of the TANs was adjusted from day to day and week to week to signal the likelihood of a response under the current training conditions. This plasticity suggests that the TANs of the striatum are exquisitely sensitive to the statistical environment in which the CSs and CRs occur. Striatal TANs thus, as a population, carry a signal that predicts context-dependent behavioral outcome.

TANs as Integrators of Inputs Related to the Relevance of Stimuli for Behavior

Our experiments did not address the issue of whether the probabilistic signal carried by the TANs is conveyed to them by striatal afferents or is generated within the striatum by the TANs themselves by integration of different inputs related to affective valence, salience, and behavioral outcome. In addition to inputs from the reward-sensitive dopamine-containing neurons of the substantia nigra, TANs receive input from centre median and parafascicular nuclei of the thalamus (Lapper and Bolam, 1992; Wilson et al., 1990), in which neurons exhibit strong responses to sensory stimuli that do not appear to be reward dependent, but do show rapid habituation (Matsumoto et al., 2001). Inactivation of these thalamic nuclei reduces the responsiveness of TANs to reward-associated CSs, just as depletion of nigrostriatal dopamine does (Matsumoto et al., 2001). If the TANs correspond to the cholinergic interneurons of the striatum, as evidence suggests (Aosaki et al., 1995; Bennett and Wilson, 1998; Bolam et al., 1986; Kawaguchi et al., 1995), then they also receive inputs from the neocortex (Thomas et al., 2000), in which stimulus salience is widely represented (Colby and Goldberg, 1999; Gold and Shadlen, 2001; Schall and Bichot, 1998). Moreover, the cholinergic interneurons of the striatum receive local input from the class of striatal projection neurons giving rise to the "direct," movement-releasing pathway of the basal ganglia (Aosaki and Kawaguchi, 1996; Bolam et al., 1986; Kawaguchi et al., 1995). This intrastriatal input could be one source of information used by TANs to estimate likely behavioral outcome.

TANs as Local Network Interneurons Biasing Cortico-Basal Ganglia Circuits

From the results reported here, we estimate that on the order of 300 TANs should be sufficient to predict the probability of a behavioral response with 95% accuracy. TANs are sparsely, but widely, distributed within the striatum (Aosaki et al., 1994b). Given the estimated density distribution of TANs, a population of 300 TANs could, at closest spacing, span several striatal modules (striosomes and matrisomes) (Flaherty and Graybiel, 1994). The TANs thus could locally tune or bias striatal network function.

Because widespread TANs can acquire similarly timed responses, it has been suggested that they could have a reset or latch-on function in the striatum and that they could thus contribute to motor and cognitive binding (Graybiel, 1997; Graybiel et al., 1994). Our present results suggest that such a binding function could involve a population response related to outcome probability. Our experiments were limited to measuring one response to the tone stimuli, so that how general such a population signal might be in predicting response outcome is a matter of speculation. Nor have we addressed possible attentional effects. As a working hypothesis, however, we suggest that the TANs could signal the probability of response outcome across a wide range of potential responses. Such a generalized function would fit their broad distribution and similarity of response characteristics in a given context.

How much biasing of striatal network function could occur as a result of TAN activity is an open question, because the output targets of physiologically identified TANs have not been identified. Experimental evidence is available, however, about the output targets of striatal cholinergic interneurons, to which TANs are thought to correspond, and about the potential effects of striatal acetylcholine, which largely originates from these interneurons (Akins et al., 1990; Bennett and Wilson, 1998, 1999; Calabresi et al., 2000a, 2000b; DiChiara et al., 1994; Kaneko et al., 2000; Kawaguchi et al., 1995). The cholinergic interneurons—and thus putatively TANs—can influence both projection neurons and interneurons in the striatum by interacting with postsynaptic receptors and also striatal afferent fibers by way of presynaptic receptors (Akins et al., 1990; Alcantara et al., 2001; Howe and Surmeier, 1995). Thus, depending on the cho-

linergic receptor subtypes affected, the cholinergic interneurons could shift glutamatergic and dopaminergic functions within the striatum as well as the response properties of recipient striatal neurons.

Particularly interesting from the point of view of our results is the recent demonstration that acetylcholine strongly affects the activity of the fast-spiking interneurons of the striatum (Chang and Kita, 1992; Kita et al., 1990; Koós and Tepper, 2002). These fast-spiking neurons are electrotonically coupled interneurons that, once activated, are potentially capable of inhibiting a large number of striatal projection neurons synchronously (Koos and Tepper, 1999). It has been suggested that these fast-spiking interneurons focus the effects of striatal inputs from the neocortex (Parthasarathy and Graybiel, 1997). In slice preparations, acetylcholine can either activate or inhibit fast-spiking neurons, depending on the acetylcholine receptors involved (Koos and Tepper, 2002). Potentially, then, the cholinergic interneurons—putatively the TANs of the striatum—could phasically increase or decrease levels of inhibition of striatal output neurons (via pause-rebounds) and could initiate longer term tonic changes in striatal network function (via intracellular signaling).

There is a striking similarity between the organization of cortico-basal ganglia circuits and the architecture of computational mixture-of-experts models of learning networks (Graybiel et al., 1994). TANs could modulate such networks by signaling the probability of actions or the sensory consequences of such actions. They thus could be part of a forward model network used in cortico-basal ganglia learning circuits. One possibility raised by our findings is that the population response of TANs could serve a corollary discharge function, signaling to the striatal network the on-going status of sensorimotor responses. If so, striatal TANs could carry a signal related to "readiness-to-respond" in a given context. Such a signal has been predicted on the basis of clinical and experimental work implicating the striatum in movement initiation and release (Denny-Brown and Yanagisawa, 1976; Jog et al., 1999; Robbins and Everitt, 1992). In this view, populations of TANs in the striatum could provide an experience-based scale of outcome probability to modulate cortico-basal ganglia release circuits. This conclusion fits well with the hypothesis that the basal ganglia are part of the forebrain circuitry implementing the decision to act.

Experimental Procedures

Four female macaque monkeys (M6, M7, M8, and M9; 4.5–5.2 kg) were trained 5 days per week in a monkey chamber while seated in a primate chair with head fixed and fitted with a recording chamber. Auditory stimuli were presented in blocks of 30–50 trials either alone (habituation and extinction protocols) or paired with water reward (reward conditioning) or with an airpuff (20 psi) directed to the left eye (aversive conditioning). Training schedules were varied across monkeys (Figure 1A), and the monkeys were chosen to span a range of laboratory experience. Neuronal and EMG recordings were made, with rare exceptions, throughout training. Experimental protocols were approved by the MIT Committee on Animal Care and performed in accordance with NIH guidelines.

Behavioral Training and Recording

During reward conditioning, a 4 kHz, 62.3 dB tone was presented as a CS either simultaneously with a drop of water (M6 and M9) or 750 ms before water delivery (M7). For the aversive delay eyeblink conditioning, a 333 Hz, 63.9 dB tone was presented as a CS either 250 ms (M6 and M9), 400 ms (M7), or 500 ms (M6 and M7) before the airpuff, which occurred during the last 100 ms of CS presentation. During extinction, the unconditioned stimuli (airpuffs) were omitted. During habituation (M7, M8, and M9), monkeys were repeatedly exposed in a given block to the same tone stimulus (333 Hz at 55.8 dB, 63.9 dB, or 69.8 dB; 4 kHz at 62.3 dB; or to the airpuff, at 73.6 dB, directed away from the monkey) in randomly ordered blocks, for 2 to 4 weeks. Intertrial intervals varied from 6–14 s for all training schedules.

Before training, monkeys received one or more MRI scans to facilitate accurate electrode placement, and underwent sterile surgery for implantation of a subconjunctival eyecoil to monitor eye movements (Fuchs and Robinson, 1966), a headpost for head fixation, and a recording chamber (Crist et al., 1988). EMG electrodes to record orbicularis oculi activity were implanted chronically, or, initially for M9 and M6, acutely. Two to three tungsten microelectrodes (1–2 Mohm, FHC, Inc., Bowdoinham, Maine) were inserted into the caudate nucleus and/or the putamen of one or both hemispheres with independently movable custom-made microdrives. During training, neuronal signals were recorded conventionally (Discovery, Datawave Technologies, Longmont, CO), synchronized with EMG (captured at 1 kHz) and eye position data (captured at 250 Hz), and sorted and analyzed offline.

Data Analysis

Neuronal signals were sorted offline on the basis of spike shape (DataWave Autocut used in operator-controlled mode). Each identified unit was then analyzed with respect to each task event. The spike activity of TANs was analyzed by block or by trial, depending on the time scale required for the analysis. Wilcoxon tests were applied to evaluate the significance of neural responses to the stimuli in the block-based analysis. For each neuron, a peri-stimulus time histogram (PSTH) with a 10 ms bin-width was made for the activity in a block of trials. Based on this histogram, we compared the average firing rates of each TAN after the CS presentation with the average firing rate during two different control periods, one 0–500 ms before CS onset and a second 3000–3500 ms after CS offset (i.e., during the 6000–14,000 ms intertrial interval). To make the comparisons, a 100 ms-wide window was moved in 10 ms steps from CS onset to US onset (or, for extinction and habituation, stimulus offset). Values for each window-step were compared to control values for the first control window by a Wilcoxon test. If at least four consecutive windows showed a significant increase or decrease in firing frequency, then the TAN firing was reanalyzed with respect to the second control period by the same method. The p values used to consider a TAN as responsive were normalized for each cell to take account of possible variability in firing frequency during the control period and thus to control for the occurrence of false positives. To do this, a 1 s time period, from 3.75–4.75 s after US offset, was analyzed as though it were the response period (response emulation period, REP). If, during the 1 s REP, four consecutive windows were found to differ from either of the control windows used for the response analysis at $p < 0.05$ by Wilcoxon tests, then the p value used to accept as significant a response during the actual response period for that particular cell was $p < p(REP)$. Otherwise, the p value used was $p < 0.05$.

For the trial by trial analyses, we selected TANs that showed a statistically significant excitation after the pause response detected in the PSTH generated for the block analyses. We designed this method to eliminate the problem in accurately detecting the onset of a pause response on a trial by trial basis due to the low tonic firing rates (2–10 spikes/s) of the TANs. This method allowed us to measure reliably the latency of the TAN responses as the first spike evoked in the post-pause excitatory rebound response. For each TAN with a PSTH showing a pause (for example, 100–160 ms after stimulus onset) followed by an excitation (for example, 200–250 ms after stimulus onset), we constructed a template of the pause-peak response. If, in a given trial, the neuron did not fire during the pause period of the template, and fired at least one spike during the excitatory period of the template, the trial was defined as a positive response trial in which the TAN showed a response.

EMG data were rectified and integrated over 10 ms bins. A 500 ms window immediately before stimulus onset was used as the control period. To detect an EMG response, a threshold was placed one standard deviation above the mean of the rectified-integrated EMG values during the control period. If the rectified and integrated EMG exceeded the threshold at any time in the interval between CS onset and US onset (or stimulus offset for extinction and habituation), we considered there to be a positive behavioral response. This method allowed us to detect a CR in single-trial analyses and the percentage of CRs in analyses across blocks of trials. To obtain the latencies of positive responses, we again rectified the raw EMG data and integrated with 1 ms bins instead of 10 ms bins. The latency of the behavioral responses detected corresponded to the first time that the rectified and integrated EMG crossed the threshold. To illustrate the raw EMG data (Figures 2 and 4), the EMG data were digitized into event data by using a threshold that was placed one standard deviation above the average EMG activity during the control period for each trial. Each time the EMG activity exceeded the threshold, the activity was counted as a single event. This method allowed data presentation as frequency (Hz).

Regression analyses were carried out conventionally (MS Excel platform). For the modified neuron-dropping analysis (Wessberg et al., 2000), we obtained Pearson R^2 values by randomly choosing "n" number of responsive TANs from those recorded during a given condition for each monkey and the corresponding EMG response values (% behavioral response). Then we averaged the TAN responses (i.e., for n = 1, the possibilities are 0 = no response, or 1 = response; for n = 2, the possibilities are 0 = both TANs unresponsive, 0.5 = 50% of TANs responsive, and 1 = both TANs responsive) and used the EMG response average. The results obtained from all samples were then adjusted by a simple linear regression (% responsive TANs versus % EMG response) and an R^2 value was obtained. This procedure was repeated 20 times for different random samples of "n" cells, and the final R^2 value was obtained by averaging the results of these 20 repetitions.

Acknowledgments

This work was supported by a Javits award (NIH/NINDS R01 NS25529), the National Parkinson Foundation, the Stanley Foundation, and the A.A. Scottish Rite Schizophrenia Research Program. We thank Dr. Mikael Djurfeldt and Dr. Paul DiZio for their valuable help with the data analysis, Dr. Richard Courtemanche for participating in some experiments, and Dr. Bruce Jenkins and Dr. Robert Marini for help with MRI acquisition.

Received: September 10, 2001
Revised: February 15, 2002

References

Akins, P.T., Surmeier, D.J., and Kitai, S.T. (1990). Muscarinic modulation of a transient K^+ conductance in rat neostriatal neurons. Nature *344*, 240–242.

Alcantara, A.A., Mrzljak, L., Jakab, R.L., Levey, A.I., Hersch, S.M., and Goldman-Rakic, P.S. (2001). Muscarinic m1 and m2 receptor proteins in local circuit and projection neurons of the primate striatum: anatomical evidence for cholinergic modulation of glutamatergic prefronto-striatal pathways. J. Comp. Neurol. *434*, 445–460.

Aosaki, T., and Kawaguchi, Y. (1996). Actions of substance P on rat neostriatal neurons in vitro. J. Neurosci. *16*, 5141–5153.

Aosaki, T., Graybiel, A.M., and Kimura, M. (1994a). Effects of the nigrostriatal dopamine system on acquired neural responses in the striatum of behaving monkeys. Science *265*, 412–415.

Aosaki, T., Tsubokawa, H., Ishida, A., Watanabe, K., Graybiel, A.M., and Kimura, M. (1994b). Responses of tonically active neurons in the primate's striatum undergo systematic changes during behavioral sensorimotor conditioning. J. Neurosci. *14*, 3969–3984.

Aosaki, T., Kimura, M., and Graybiel, A.M. (1995). Temporal and spatial characteristics of tonically active neurons of the primate's striatum. J. Neurophysiol. *73*, 1234–1252.

Apicella, P., Legallet, E., and Trouche, E. (1996). Responses of tonically discharging neurons in monkey striatum to visual stimuli presented under passive conditions and during task performance. Neurosci. Lett. *203*, 147–150.

Apicella, P., Legallet, E., and Trouche, E. (1997). Responses of tonically discharging neurons in the monkey striatum to primary rewards delivered during different behavioral states. Exp. Brain Res. *116*, 456–466.

Basso, M.A., and Evinger, C. (1996). An explanation for reflex blink hyperexcitability in Parkinson's disease. II. Nucleus raphe magnus. J. Neurosci. *16*, 7318–7330.

Basso, M.A., Powers, A.S., and Evinger, C. (1996). An explanation for reflex blink hyperexcitability in Parkinson's disease. I. Superior colliculus. J. Neurosci. *16*, 7308–7317.

Basso, M.A., Strecker, R.E., and Evinger, C. (1993). Midbrain 6-hydroxydopamine lesions modulate blink reflex excitability. Exp. Brain Res. *94*, 88–96.

Bennett, B.D., and Wilson, C.J. (1998). Synaptic regulation of action potential timing in neostriatal cholinergic interneurons. J. Neurosci. *18*, 8539–8549.

Bennett, B.D., and Wilson, C.J. (1999). Spontaneous activity of neostriatal cholinergic interneurons in vitro. J. Neurosci. *19*, 5586–5596.

Blaxton, T.A., Zeffiro, T.A., Gabrieli, J.D.E., Bookheimer, S.Y., Carrillo, M.C., Theodore, W.H., and Disterhoft, J.F. (1996). Functional mapping of human learning: a positron emission tomography activation study of eyeblink conditioning. J. Neurosci. *16*, 4032–4040.

Blumenthal, T.D., and Goode, C.T. (1991). The startle eyeblink response to low intensity acoustic stimuli. Psychophysiology *28*, 296–306.

Bolam, J.P., Ingham, C.A., Izzo, P.N., Levey, A.I., Rye, D.B., Smith, A.D., and Wainer, B.H. (1986). Substance P-containing terminals in synaptic contact with cholinergic neurons in the neostriatum and basal forebrain: a double immunocytochemical study in the rat. Brain Res. *397*, 279–289.

Calabresi, P., Centonze, D., Gubellini, P., Marfia, G., Pisani, A., Sancesario, G., and Bernardi, G. (2000a). Synaptic transmission in the striatum: from plasticity to neurodegeneration. Prog. Neurobiol. *61*, 231–265.

Calabresi, P., Centonze, D., Gubellini, P., Pisani, A., and Berenardi, G. (2000b). Acetylcholine-mediated modulation of striatal function. Trends Neurosci. *23*, 120–126.

Chang, H.T., and Kita, H. (1992). Interneurons in the rat striatum: relationships between parvalbumin neurons and cholinergic neurons. Brain Res. *574*, 307–311.

Colby, C.L., and Goldberg, M.E. (1999). Space attention in parietal cortex. Annu. Rev. Neurosci. *22*, 319–349.

Crist, C.F., Yamasaki, D.S., Komatsu, H., and Wurtz, R.H. (1988). A grid system and a microsyringe for single cell recording. J. Neurosci. Methods *26*, 117–122.

Crutcher, M.D., and DeLong, M.R. (1984). Single cell studies of the primate putamen. I. Functional organization. Exp. Brain Res. *53*, 233–243.

Denny-Brown, D., and Yanagisawa, N. (1976). The role of the basal ganglia in the initiation of movement. Res. Publ. Assoc. Res. Nerv. Ment. Dis. *55*, 115–149.

DiChiara, G., Morelli, M., and Consolo, S. (1994). Modulatory functions of neurotransmitters in the striatum: ACh/dopamine/NMDA interactions. Trends Neurosci. *17*, 228–233.

Djurfeldt, M., Sandberg, A., Ekeberg, B., and Lansner, A. (1999). See---a framework for simulation of biologically detailed and artificial neural networks and systems. Neurocomputing *26–27*, 999–1003.

Evinger, C., Basso, M.A., Manning, K.A., Sibony, P.A., Pellegrini, J.J., and Horn, A.K.E. (1993). A role for the basal ganglia in nicotinic modulation of the blink reflex. Exp. Brain Res. *92*, 507–515.

Flaherty, A.W., and Graybiel, A.M. (1994). Input-output organization of the sensorimotor striatum in the squirrel monkey. J. Neurosci. *14*, 599–610.

Fuchs, A.F., and Robinson, D.A. (1966). A method for measuring

horizontal and vertical eye movement chronically in the monkey. J. Appl. Physiol. *21*, 1068–1070.

Gold, J.I., and Shadlen, M.N. (2001). Neural computations that underlie decisions about sensory stimuli. Trends Cogn. Sci. *5*, 10–16.

Graybiel, A.M. (1997). The basal ganglia and cognitive pattern generators. Schizophr. Bull. *23*, 459–469.

Graybiel, A.M. (1998). The basal ganglia and chunking of action repertoires. Neurobiol. Learn. Mem. *70*, 119–136.

Graybiel, A.M., and Kimura, M. (1995). Adaptive neural networks in the basal ganglia. In Models of Information Processing in the Basal Ganglia, J.C. Houk, J.L. Davis, and D.G. Beiser, eds. (Cambridge, MA: M.I.T. Press), pp. 103–116.

Graybiel, A.M., Aosaki, T., Flaherty, A.W., and Kimura, M. (1994). The basal ganglia and adaptive motor control. Science *265*, 1826–1831.

Gruart, A., Blazquez, P., and Delgado-Garcia, J.M. (1995). Kinematics of spontaneous, reflex, and conditioned eyelid movements in the alert cat. J. Neurophysiol. *74*, 226–248.

Grunewald, A., Linden, J.F., and Andersen, R.A. (1999). Responses to auditory stimuli in macaque lateral intraparietal area. I. Effects of training. J. Neurophysiol. *82*, 330–342.

Hikosaka, O., Sakamoto, M., and Usui, S. (1989). Functional properties of monkey caudate neurons. III. Activities related to expectation of target and reward. J. Neurophysiol. *61*, 814–832.

Hikosaka, O., Miyashita, K., Miyachi, S., Sakai, K., and Lu, X. (1998). Differential roles of the frontal cortex, basal ganglia, and cerebellum in visuomotor sequence learning. Neurobiol. Learn. Mem. *70*, 137–149.

Hollerman, J.R., Tremblay, L., and Schultz, W. (1998). Influence of reward expectation on behavior-related neuronal activity in primate striatum. J. Neurophysiol. *80*, 947–963.

Howe, A.R., and Surmeier, D.J. (1995). Muscarinic receptors modulate N-, P-, and L-type $Ca2^{+}$ currents in rat striatal neurons through parallel pathways. J. Neurosci. *15*, 458–469.

Jog, M., Kubota, Y., Connolly, C.I., Hillegaart, V., and Graybiel, A.M. (1999). Building neural representations of habits. Science *286*, 1745–1749.

Kaneko, S., Hikida, T., Watanabe, D., Ichinose, H., Nagatsu, T., Kreitman, R.J., Pastan, I., and Nakanishi, S. (2000). Synaptic integration mediated by striatal cholinergic interneurons in basal ganglia function. Science *289*, 633–637.

Kawagoe, R., Takikawa, Y., and Hikosaka, O. (1998). Expectation of reward modulates cognitive signals in the basal ganglia. Nat. Neurosci. *1*, 411–416.

Kawaguchi, Y., Wilson, C.J., Augood, S.J., and Emson, P.C. (1995). Striatal interneurons: chemical, physiological and morphological characterization. Trends Neurosci. *18*, 527–535.

Kermadi, I., and Joseph, J.P. (1995). Activity in the caudate nucleus of monkey during spatial sequencing. J. Neurophysiol. *74*, 911–933.

Kita, H., Kosaka, T., and Heizmann, C.W. (1990). Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study. Brain Res. *536*, 1–15.

Koos, T., and Tepper, J.M. (1999). Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nat. Neurosci. *2*, 467–472.

Koos, T., and Tepper, J.M. (2002). Dual cholinergic control of fast-spiking interneurons in the neostriatum. J. Neurosci. *22*, 529–535.

Lapper, S.R., and Bolam, J.P. (1992). Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. Neuroscience *51*, 533–545.

Logan, C.G., and Grafton, S.T. (1995). Functional anatomy of human eyeblink conditioning determined with regional cerebral glucose metabolism and positron-emission tomography. Proc. Natl. Acad. Sci. USA *92*, 7500–7504.

Matsumoto, N., Minamimoto, T., Graybiel, A.M., and Kimura, M. (2001). Neurons in the thalamic CM-Pf complex supply neurons in the striatum with information about behaviorally significant sensory events. J. Neurophysiol. *85*, 960–976.

Mink, J.W. (1996). The basal ganglia: focused selection and inhibition of competing motor programs. Prog. Neurobiol. *50*, 381–425.

Mirenowicz, J., and Schultz, W. (1996). Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature *379*, 449–451.

Parthasarathy, H.B., and Graybiel, A.M. (1997). Cortically driven immediate-early gene expression reflects modular influence of sensorimotor cortex on identified striatal neurons in the squirrel monkey. J. Neurosci. *17*, 2477–2491.

Platt, M.L., and Glimcher, P.W. (1999). Neural correlates of decision variables in parietal cortex. Nature *400*, 233–238.

Ravel, S., Legallet, E., and Apicella, P. (1999). Tonically active neurons in the monkey striatum do not preferentially respond to appetitive stimuli. Exp. Brain Res. *128*, 531–534.

Raz, A., Feingold, A., Zelanskaya, V., Vaadia, E., and Bergman, H. (1996). Neuronal synchronization of tonically active neurons in the striatum of normal and parkinsonian primates. J. Neurophysiol. *76*, 2083–2088.

Richardson, R.T., and Thompson, R.F. (1985). Unit activity recorded from the globus pallidus during classical conditioning of the rabbit nictitating membrane response. Brain Res. *332*, 219–229.

Robbins, T.W., and Everitt, B.J. (1992). Functions of dopamine in the dorsal and ventral striatum. Semin. Neurosci. *4*, 119–127.

Rolls, E.T., Thorpe, S.J., and Maddison, S.P. (1983). Responses of striatal neurons in the behaving monkey. 1. Head of the caudate nucleus. Behav. Brain Res. *7*, 179–210.

Sardo, P., Ravel, S., Legallet, E., and Apicella, P. (2000). Influence of the predicted time of stimuli eliciting movements on responses of tonically active neurons in the monkey striatum. Eur. J. Neurosci. *12*, 1801–1816.

Schall, J.D. (2001). Neural basis of deciding, choosing and acting. Nat. Rev. Neurosci. *2*, 33–42.

Schall, J.D., and Bichot, N.P. (1998). Neural correlates of visual and motor decision processes. Curr. Opin. Neurobiol. *8*, 211–227.

Schultz, W. (1998). Predictive reward signal of dopamine neurons. J. Neurophysiol. *80*, 1–27.

Schultz, W., and Romo, R. (1987). Responses of nigrostriatal dopamine neurons to high intensity somatosensory stimulation in the anesthetized monkey. J. Neurophysiol. *57*, 201–217.

Schneider, J.S. (1987). Basal ganglia-motor influences: role of sensory gating. In Basal Ganglia and Behavior: Sensory Aspects of Motor Functioning, J.S. Schneider, and T. Lidsky, eds. (Toronto: Hans Huber), pp. 103–121.

Shidara, M., Aigner, T.G., and Richmond, B.J. (1998). Neuronal signals in the monkey ventral striatum related to progress through a predictable series of trials. J. Neurosci. *18*, 2613–2625.

Shimo, Y., and Hikosaka, O. (2001). Role of tonically active neurons in primate caudate in reward-oriented saccadic eye movement. J. Neurosci. *21*, 7804–7814.

Steinmetz, J.E., and Thompson, R.F. (1991). Brain substrates of aversive classical conditioning. Neurobiology of learning. In Emotion and Affection, J. Madden IV, ed. (New York: Raven Press), pp. 97–120.

Thomas, T.M., Smith, Y., Levey, A.I., and Hersch, S.M. (2000). Cortical inputs to m2-immunoreactive striatal interneurons in rat and monkey. Synapse *37*, 252–261.

Thompson, R.F., and Kim, J.J. (1996). Memory systems in the brain and localization of a memory. Proc. Natl. Acad. Sci. USA *93*, 13438–13444.

Wessberg, J., Stambaugh, C.R., Kralik, J.D., Beck, P.D., Laubach, M., Chapin, J.K., Kim, J., Biggs, S.J., Srinivasan, M.A., and Nicolelis, M.A. (2000). Real-time prediction of hand trajectory by ensembles of cortical neurons in primates. Nature *408*, 361–365.

White, I.M., Miller, D.P., White, W., Dike, G.L., Rebec, G.V., and Steinmetz, J.E. (1994). Neuronal activity in rabbit neostriatum during classical eyelid conditioning. Exp. Brain Res. *99*, 179–190.

Wilson, C.J., Chang, H.T., and Kitai, S.T. (1990). Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. J. Neurosci. *10*, 508–519.