Subthalamic and striatal neurons concurrently process motor, limbic, and associative information in rats performing an operant task.

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RUNNING HEAD: STN and STR physiology during operant performance

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ABSTRACT

Although the subthalamic nucleus (STN) is commonly assumed to be a relay for striatal (STR) output, anatomical evidence suggests the two structures are connected in parallel, raising the possibility that parallel STN and STR firing patterns mediate behavioral processes. The STR is known to play a role in associative and limbic processes, and while behavioral studies suggest that the STN may do so as well, evaluation of this hypothesis is complicated by a lack of pertinent STN physiological data. We recorded concurrent STN and STR firing patterns in rats learning an operant nose-poke task. Both structures responded in similar proportions to task events including instructive cues, discriminative nose-pokes, and sucrose reinforcement. Neuronal responses to reinforcement comprised phasic excitations preceding reinforcement and inhibitions afterwards; the inhibition was attenuated when reinforcement was absent. Reinforcement responses occurred more frequently during later training sessions in which discriminative action was required, suggesting that responses were context-dependent. Nose-pokes were typically preceded by excitations; there also was a nonsignificant trend towards inhibition encoding correct nose-pokes. Sustained changes in firing rate coinciding with specific task events suggested that both nuclei were encoding behavioral sequences; this is the first report of such behavior in the STN. Our findings also reveal complex STN responses to reinforcement. Thus, both STN and STR neurons show concurrent involvement in motor, limbic, and associative processes.
Introduction

Interpretation of theoretical and empirical research within the basal ganglia is colored, if not constrained, by the notion of an architecture in which the striatum (STR) receives cortical and thalamic input and routes its output to the substantia nigra along two pathways, one of which comprises the globus pallidus and subthalamic nucleus (STN) (Albin et al. 1989; DeLong 1990). In this view, these nuclei had no extra-basal ganglia inputs of their own, and so they were considered relays for striatal firing patterns. However, there is considerable evidence that the STN receives direct cortical (Hartmann-von Monakow et al. 1978; Nambu et al. 1996), thalamic (Sugimoto et al. 1983), and midbrain (Hassani et al. 1997) input. Because the STN and STR receive direct input from the same cortical and midbrain regions, the STN is in a position to act as a second, independent input area of the basal ganglia. Both of these nuclei project directly to the output areas of the basal ganglia (the substantia nigra pars reticulata and internal globus pallidus) and both are reciprocally connected with the external globus pallidus. Thus, an alternative interpretation of basal ganglia connectivity is that the STN and STR provide parallel cortico-nigrothalamic pathways (Levy et al. 1997; Nambu et al. 2002; Kita et al. 2004). If such parallelism exists, we may well ask whether it extends to function as well.

An extensive literature highlights parallel STR (Rolls et al. 1983; Crutcher and DeLong 1984; Anderson et al. 1979; Haracz et al. 1993) and STN (Carpenter et al. 1950; Georgopoulos 1983; Matsumura et al. 1992; Bergman et al. 1994; Wichmann et al. 1994; Cheruel et al. 1996; Shi et al. 2004; Chang et al. 2005) involvement in normal and pathological movement. However, while striatal involvement in associative and limbic processes has been thoroughly documented (Schultz and Romo 1992; Apicella et al.
1991, 1992, 1997; Tremblay et al. 1998; Jog et al. 1999; Packard and Knowlton 2002), it is unclear if the STN plays a parallel role. Lesions of the STN in rats performing a multi-choice attentional task induced deficits that suggest decreased impulse control (Baunez and Robbins 1997, but see Winstanley et al. 2005), increased behavioral measures of motivation (Baunez et al. 2002), and differentially impaired craving for rewards (Baunez et al. 2005). Single-unit recordings in primate STN (Matsumura et al. 1992; Darbaky et al. 2005) and cat (Cheruel et al. 1996) show that these neurons increase firing rate during reinforcement.

Lesions (Obeso et al. 1997) or high-frequency stimulation of the STN (Limousin et al. 1995) are effective treatments for the dyskinesia characteristic of Parkinson’s disease. If the STN is indeed involved in non-motor aspects of behavior that relate to impulse control, surgical manipulation of this nucleus would be expected to induce corresponding behavioral deficits; such deficits have already been reported. Parkinson’s patients undergoing high-frequency stimulation of the STN exhibit overeating (Moro et al. 1999), uncontrollable laughter (Krack et al. 2001), and increased hypersexuality (Absher et al. 2000; Romito et al. 2002).

Although behavioral and physiological studies collectively suggest that STN neurons are involved in operant behavior, there are no studies in the rat that examine concurrent behavior and single-unit electrophysiology during operant learning. We therefore recorded STN and STR neuronal firing patterns in rats learning an operant task comprising motor, limbic, and associative processes in order to 1) obtain information about STN firing patterns during operant behavior; 2) examine any involvement of STN neurons in non-motor aspects of behavior; and 3) compare STN activity with concurrent
recordings of STR neuronal activity with an eye towards assessing functional parallelism within the basal ganglia.

Materials & Methods

Animal Care

Male Sprague-Dawley rats (250-400 g, Harlan Industries, Indianapolis, IN) were used in all experiments. For the duration of the study, rats were food-restricted to 85% of free-feeding weight, but had *ad libitum* access to water except for two instances of overnight water deprivation. Rats were housed in the Department of Psychology animal colony on a 12:12 h light:dark cycle. All experimental protocols were approved by the local Institutional Animal Care and Use Committee (IACUC), and followed guidelines established by the National Institutes of Health (Institute of Laboratory Animal Resources, 1996).

Stereotaxic surgery

Rats were under general anesthesia during all surgical procedures. Atropine sulfate (0.05 mg / kg, s.c.) was given preoperatively to facilitate breathing. Anesthesia was induced with ketamine / xylazine (90/10 mg/kg, i.m.) and maintained with 0.2 mL ketamine i.p. as needed. We blocked all incision areas and pressure points with lidocaine, and applied a moisturizing lubricant (Moisture Eyes PM, Bausch & Lomb) to prevent corneal drying. We used blunt ear bars coated with antibiotic jelly to reduce the risk of infection due to accidental rupture of the eardrum. All implanted materials were sterilized in 1:4 diluted Cidex/dH₂O. During recovery, rats were given 10 mL lactated Ringer’s solution s.c. to counteract dehydration and closely monitored until they awoke.

Following incision and reflection of the scalp and periosteum, we drilled two
holes in the skull over the STN and ipsilateral STR, and removed the exposed dura. Multiwire bundle electrodes were lowered into the target structures at a rate of 50-100 μm / 30 s. STR coordinates (AP and ML) were +1.0 and +2.5 mm relative to bregma, and -5.5 mm ventral to skull surface (Paxinos and Watson 1998). Due to the small volume and deep location of the STN, we estimated its location by averaging the results from two sets of calculations, one measured from bregma (-3.8 mm AP, +2.5 mm ML) and skull surface (-8.35 mm DV), the other from the interaural line (+5.2 mm AP, +2.5 mm ML, +1.65 mm DV). Four to six additional holes were drilled for stainless steel anchor screws. The electrode assemblies were fixed in place with super glue and dental acrylic.

**Electrophysiology**

Each multiwire electrode bundle comprised seven 25 μm-diameter, Formvar-insulated stainless steel wires (California Fine Wire) threaded through a 27-gauge stainless steel needle that served as the ground. The cannula was soldered to an 8-pin male strip connector (Omnetics PS2). The wire bundle was drawn out of a hole at the base of the needle and each wire soldered to a connector pin. Wires were trimmed so that they protruded 1-2 mm past the end of the needle. On recording days, an 8-pin plug interfaced with the head-affixed PS2 assemblies. Electrode impedances were typically on the order of 1 MΩ, although this was not quantified systematically.

Rats were placed in the operant chamber and connected to a multichannel electrical commutator (Plastics One) via a length of flexible shielded cable, allowing them complete freedom of movement. Electrophysiological signals were transmitted via the commutator to a preamplifier, and then to data acquisition hardware (MNAP, Plexon...
Inc, Dallas TX) that allowed PC-based user control of unit discrimination and recording. Signals were also fed to an oscilloscope and audio monitor to facilitate unit discrimination.

Using the Plexon system software (SortClient), we were able to discriminate and simultaneously record up to four signals per channel, for a maximum of 56 possible units per recording session. Unit discrimination was accomplished using a combination of template-matching, principal components analysis, and k-means clustering algorithms. All units had at least a 3:1 signal: noise ratio. Prior to recording, the power spectra and either an autocorrelogram or an interspike interval histogram were examined for each putative unit to maximize the probability that units consisted of only one signal (reflected in a trough in the interspike interval (ISI) histogram around the absolute refractory period), and were free of 60-cycle interference. The typical yield was 5-7 units per recording session. Although we report here a total sample size of several hundred units, it is likely that many signals were recorded more than once over the course of several days. However, as we had no criteria for determining a common signal source over repeated recordings, we were obliged to treat them as independent entities. Our reported values are presented as the average number of recorded or responsive neurons per training/recording session.

**Operant training**

Recording sessions took place in a custom-built Plexiglas operant chamber. Two nose-poke detectors recesses were located along one wall, ~ five cm above the floor. Each recess contained a green LED and an infrared beam-break detector. A recess on the opposite wall, ~ five cm above the floor, contained a sucrose delivery spout, infrared
beam-break detector, and yellow LED. Tone generators (1.9 kHz and 4.2 kHz) provided auditory stimuli. Figure 1 depicts the physical layout of the operant chamber as well as schematic diagrams of the training protocols.

During the first three operant training sessions, rats learned to lick a spout to obtain sucrose (LICK sessions). Coincident auditory (1.9 kHz, 70 dB, 3 x 300 ms pulses, 100 ms interpulse interval, “feed tone”) and visual (illumination of the spout by the yellow LED) cues signaled the start of a trial and the onset of a “reward-ready” period that persisted until the rat licked the spout (the feed tone occurred only at the start of the reward-ready period, but illumination was maintained until the rat licked the spout). If, during this period, the rat licked the spout thereby breaking the IR beam, a valve opened making 10% w/v sucrose solution available at the spout for 1 s. Initially, the intertrial interval (ITI) was set to 5 s. After every fifth valve opening, the ITI increased by 5 s, up to a maximum of 30 s. All trials were reinforced during these LICK sessions. Rats were water-deprived the night before the first LICK session.

After three days of LICK training, rats underwent nine daily sessions in which they learned to perform a discriminatory nose-poke to obtain reward (POKE sessions). Rats were water-deprived the night before the first POKE session. A trial began with concurrent auditory and visual cues (auditory: 4.5 kHz, 70 dB, 2 x 500 ms pulses, 100 ms interpulse interval, “nose-poke tone”; visual: pseudo-random illumination of one of the nose-poke recesses by a green LED). A nose-poke into the lit recess elicited the previously learned feed tone / yellow LED cues signaling the reward-ready period, which in this protocol lasted only 5 s. A trial ended when either the reward-ready period elapsed without any spout licks, or 1 s after valve opening. The ITI varied
pseudorandomly from 5-8 s. The tone/light combinations were not counterbalanced, nor was the association between the green LEDs and “correct” nose-pokes.

To determine the effects of reward prediction, the final two operant sessions used a modified POKE protocol in which reward occurred in 75% of the trials (POKE75 session). In the remaining 25% of trials, all cues were presented normally but spout licking during the reward-ready period did not open the valve. Non-rewarded trials occurred on a pseudorandom basis.

All rats learned the task within the two-week training period. One rat underwent LICK training alone; those data were pooled with the other LICK data. A second group of rats underwent the two-week training regimen before being implanted with bundle electrodes; they were then recorded for a further two weeks performing the POKE and POKE75 protocols. We shall refer to this group as the “pretrained” group.

Histology

Upon completion of all recordings, rats were deeply anesthetized with a mixture of chloral hydrate and sodium pentobarbital (chloropent). A 30 µA, 5s current pulse was passed through each electrode where a unit was recorded. Rats were then transcardially perfused via the ascending aorta with 10% formosaline / 19% w/v potassium ferrocyanide (K₄Fe(CN)₆), producing small blue deposits at the site of the recording electrode (“Prussian blue” reaction). Brains were removed, fixed in 10% formosaline, cryoprotected in 30% phosphate-buffered sucrose, sliced into 80 µm coronal sections, and stained with Cresyl violet. We included for analysis all units from animals with large visible lesions in the STN or along the border with the cerebral peduncle (Figure 2A).

Analysis
All of our statistical analyses were based on binned perievent firing rates obtained during each recording/training session. Our first experimental aim was to describe the qualitative features of STN and STR perievent responses to operant events. Towards this end, we determined the latency, duration, and magnitude of significant perievent responses. We obtained measures that described the response profile for each neuronal sample i.e. the probabilities of significant excitations and inhibitions, as well as whether either of these response types was predominant at a given time relative to the event. Our second experimental question was whether there were differences in the proportion of neurons within each sample that responded to operant events, either within or across nuclei, training session, or reinforcement condition. The raw data in this case were the numbers of responsive neurons within a sample, responsiveness having been determined during the qualitative phase of analysis. We looked at the number of responses to each event out of a) the entire neuronal sample for that nucleus, or b) a subsample comprising only neurons that showed significant perievent responses to the chosen event. We compared these response rates using Fisher’s Exact Test with a conservative alpha value, to compensate for the large number of comparisons.

Our qualitative analysis began by expressing each binned perievent record as z-scores based on the mean and standard deviation of the firing rate during the period from -2 to -1 s preceding the event. Bins with z-scores more than 1.64 standard deviations (95% confidence interval) away from the baseline mean firing rate were considered significant. A “response” to an event was defined as three or more (>=150 ms) consecutive, significant bins. In certain cases, indicated in the text, we analyzed prolonged neuronal responses. These we defined as 10 or more consecutive significant
100 ms bins, yielding a minimum response duration of 1 s. We included for analysis only those responses that fell within certain time windows. For phasic responses, that window was ± 1 s, and for long responses the window extended from -1 to +5 s, as we wished to examine some responses that corresponded to the end of the reinforcement period.

We determined, for each response, the onset latency (defined as the time of occurrence of the first of the consecutive bins), duration (defined as the total number of consecutive bins with significant z-scores), and magnitude (defined as the mean z-score across all the consecutive bins constituting an individual response). Plotting the response magnitude vs. the onset latency gave us the response distribution.

Because neuronal responses might correspond to either the beginning or the end of a particular behavior, we obtained the aggregate response probability for both excitations and inhibitions. Our thinking was that such an analysis would illustrate any overlap between the endings and beginnings of perievent responses, thereby highlighting time points within a given behavioral sequence that were particularly emphasized by changes in firing rate, and providing us with a slightly different perspective on the temporal structure of perievent responses than that afforded by traditional perievent analysis. We obtained these aggregate response probabilities by first grouping together all of the neurons that showed significant responses to a particular behavioral event. Each neuron’s perievent response was described by a string of values, one for each bin. A value of +1 meant that that bin occurred during a significant excitation; a value of -1 meant a significant inhibition was ongoing, and a value of zero meant that firing during that bin was not different from baseline. We then summed the +1 responses across the entire sample and divided by the sample size to obtain the aggregate, binwise probability
of excitation. We performed a similar summation on the -1 values to obtain the probability of inhibition. To obtain a measure of the strength and polarity of neuronal responses vs. time, we also took the total sum of positive and negative responses, and divided by the sample size. The sign of this aggregate probability corresponded to whether the predominant response in a given bin was excitatory or inhibitory, and its magnitude gave a measure of the degree of predominance of that response. See Figure 3 for an example of this technique.

In some instances, it was useful to compare differences in neuronal responses to a pair of related behavioral events e.g. correct vs. incorrect nose-pokes. In such a case, we developed a technique called a differential perievent histogram (dPEH) that allowed us to assign a measure of statistical significance to differences in perievent firing within a sample. For every pair of events, we included for analysis any neuron that showed a significant response (as defined above) to either of the events in the pair. We obtained, for each included neuron, the binned perievent firing rates for those events. Having normalized these data as previously detailed, we obtained the binwise difference score. The result of this operation was a list of numbers of the same length as a PEH, centered at $t = 0$; this point no longer had an exact behavioral counterpart, but rather represented time of onset of either of the paired events. In the hypothetical case of Event A - Event B, dPEH bins with positive values suggest that firing was faster in relation to Event A than at the same time-point relative to Event B; they make no claim, however, about the mechanism underlying the difference – the result would be the same whether neuronal firing sped up during Event A or slowed down during Event B.

To determine the significance of these differences, we took the mean dPEH across
all neurons included for analysis. We also generated a control event by taking the dPEH, for each neuron, of pairs of randomly picked time points occurring during the ITI; we repeated this 100 times for each neuron, and then obtained the mean dPEH for this “null” event across all the neurons previously selected for analysis. We used this null dPEH to generate a binwise mean and standard deviation; our confidence interval was 3 standard deviations from the bin mean, corresponding to a p value of 0.0027 (confidence interval = ±99.865). We then applied this confidence interval to the testable event dPEHs. Responses were considered significant if any of the bins exceeded the confidence interval.

After determining the qualitative parameters of each neuron’s perievent responses as described above, we obtained three sets of sums for each event during each training session type: 1) the numbers of responsive and non-responsive STN and STR neurons, 2) the sums of neurons that did and did not show responses preceding event onset (t <= 0), and 3) the numbers of neurons that did and did not respond following event onset (t > 0 s). By specifying and summing over different combinations of training sessions, we were able to examine specific conditions and factors that might have influenced response rates. For example, by comparing the response data from POKE sessions to that from LICK sessions, we could examine whether the behavioral context affected response rates. Other conditions included pretrained vs. non-pretrained, all POKE and POKE75 sessions vs. LICK sessions, and POKE vs. POKE75 sessions. Such comparisons allowed us to determine not only whether STR or STN neurons were more likely to respond to a given stimulus, but also whether the responses observed in each nucleus were context- or training-dependent.
To calculate the significance of differences in within-session/across-nucleus response proportions (disregarding whether the responses preceded or followed the specified behavioral event) we used Fisher’s Exact Test, with a conservative value of alpha = 0.005 to compensate for the large number of comparisons. For each session or group of sessions, the numbers of responsive and non-responsive neurons from one nucleus constituted the expected values, while the observed values were obtained from the other nucleus. For example, to compare the proportions of STN and STR responses to reinforcement during POKE sessions, we would set the expected values to the numbers of responsive and non-responsive STN neurons across all POKE sessions; the observed values would be taken from the STR group. It made no difference which nucleus was designated as observed or expected. Within-nucleus/across-session significances were calculated in a similar fashion, with the expected values taken for one session or group of sessions, and the observed values taken from a second session or group of sessions e.g. the expected values were taken from the numbers of recorded and responsive STN neurons during all POKE sessions, and the observed values were taken from the numbers of recorded and responsive STN neurons during all LICK sessions. Although we performed our statistics on the raw totals of recorded neurons, our tables present these values as the average numbers of neurons per training session; the statistical results were unaffected.

Results

We recorded 743 STN and 405 STR signals from 31 animals. STN signals had biphasic or triphasic waveforms ≤ 1 ms wide, and had mean firing rates of 2.40 ± 2.7 spikes·s⁻¹ (range = 0.0005 to 24.34 spikes·s⁻¹). STR waveforms were also biphasic or
triphasic, with a long afterhyperpolarization; a typical STR waveform was ~ 1.5 ms wide. Mean STR firing rate was $2.69 \pm 2.9$ spikes·s$^{-1}$ (range = 0.0043 to 19.15 spikes·s$^{-1}$).

Typical waveforms are illustrated in Figure 2B. Our reported STN firing rates are lower than those previously reported for awake, unrestrained rats (Olds et al. 1999), a discrepancy that may reflect methodological differences. A recent study using an electrode technique similar to ours also reported relatively slow firing rates (5-10 spikes·s$^{-1}$) (Shi et al. 2004). To address the possibility that we recorded from distinct subpopulations with different firing rates, we split the neuronal population into two groups based on each neuron’s mean firing rate ($\leq 1$ spikes·s$^{-1}$), and re-ran all our analyses. We found no significant differences in the percent of neurons responding to each event or the response parameters (latency, duration, magnitude), although slower firing neurons did show a floor effect with regard to event-related inhibitions. We present here the pooled neuronal populations.

**Operant behavior**

We used two behavioral metrics to monitor learning: discriminative accuracy, measured as the ratio of the number of incorrect nose-pokes to the total number of nose-pokes, and latency to lick the spout following the feed tone. These results are shown in Figure 4. Dots indicate the mean values for each training session; vertical lines indicate the standard error. Solid lines show the best fit to the raw data. The data from non-pretrained rats were fit with an exponential function, while the data from pretrained rats were fit with a line.

Non-pretrained rats approached an asymptotic level of discriminative accuracy, or one mistake per ten trials, around the fifth POKE session (Figure 4A). The empirically
determined asymptotic value (mean of the last ten training sessions) for naïve rats was 0.12 ± 0.01, while the exponential fit gave an asymptote of 0.11. Discriminative accuracy was unaffected by the switch to POKE75 sessions in which reinforcement was less predictable. The relatively high score observed at the outset of training may be related to the training protocol, in which a few drops of sucrose solution were placed inside the lit nose-poke recess to guide the rat to poke his nose into the hole. Pretrained rats performed at a stable level throughout the two-week recording period. The empirically determined asymptote, determined over the same range as the non-pretrained rats, was 0.05 ± 0.009, while the linear fit gave a y-intercept of 0.08. Discriminative accuracy in pre-trained rats continued to increase over the two-week recording period, even during the switch to POKE75 sessions. The empirically determined asymptotic values of discriminative accuracy were significantly lower in pretrained rats than in non-pretrained rats (ANOVA, F(1,18) = 18.67, p<0.0005), which probably reflect an overtraining effect.

Latency to lick the spout (Figure 4B) was initially high in non-pretrained rats but decreased exponentially until it reached an asymptotic value of 2.92 ± 0.22 s (mean of the last ten training sessions); exponential curve-fitting gave an asymptotic value of 2.78 s. Pretrained rats had an empirically determined asymptotic value of 2.48 ± 0.13 s, while linear curve fitting yielded a y-intercept of 3.04 s. As with discriminative accuracy, pretrained rats showed decreasing latencies during the two-week recording period. Switching from POKE to POKE75 training sessions had no effect on lick latency in either set of rats. The asymptotic latencies of pretrained and non-pretrained rats were not significantly different (ANOVA, F(1,18) = 3.02, p = 0.1). Both of these measures
demonstrated that rats successfully learned the discriminative task within the two week recording / training period.

**STN and STR firing decreases during reinforcement**

STN and STR firing patterns during reinforcement were complex, often multiphasic; they comprised both excitations and inhibitions of various durations (Figure 5A). Responses were characterized relative to the time of the first spout lick during the “reward-ready” period; a “response” was any significant deviation from baseline firing rate beginning within ± 1 s of reinforcement. For a qualitative description of reinforcement-related firing patterns, we plotted the onset latency vs. the mean amplitude of every response (Figure 5B, top row) for both the STN (left) and STR (right). Additionally, we plotted the probability of an ongoing response vs. time, as determined by summing across all neurons and dividing by the sample size (Figure 5B, bottom row). This plot highlighted the times at which different responses intersected e.g. if, at a particular time, one neuronal response terminated as another one in a different neuron began, or two responses with different onset latencies terminated at the same time. Peaks indicate an overlap between two responses that might not have been obvious from the scatter plot. In Figure 5B and in subsequent plots of this nature, the black line represents the probability of excitation vs. time, the light gray line represents the probability of inhibition vs. time, and the dark gray line reflects the aggregate probability i.e. the predominant response vs. time for the entire neuronal sample, taken as the sum of the black and light gray lines. This graph shows that the prototype neuronal response of both STN and STR neurons included a period of excitation preceding reinforcement (t < 0 s, when the rat was approaching the spout) and a pronounced, persistent decrease in firing
rate during and following reinforcement (t >= 0 s).

Inspection of perievent rasters based on reinforcing events (valve opening) revealed many trials in which sucrose delivery was accompanied in STN and STR neurons by long responses lasting 1 s or more. These responses were primarily inhibitory, although some excitations were observed. In order to examine these long responses without contamination from the short responses that typically preceded reinforcement, we re-analyzed the data but increased the minimum response length criterion from 150 ms (3 x 50 ms bins) to 1 s (10 x 100 ms bins). Responses had to occur between t = -1 and +5 s in order to be counted. We extended the time window in order to examine events that corresponded to the end of the reinforcement period at t = +1 s. (The analysis of long responses was only performed on reinforcement-related events, as long responses to nose-pokes and tones were rarely observed.) Although it is true that the 5 s post-event window overlaps with the intertrial interval, it was necessary in order to analyze responses that corresponded to the end of the reinforcement period. The behaviors exhibited during the intertrial interval were minimal; rats typically persisted in (non-reinforced) spout licking, or sat quietly until the start of the next trial.

The aggregate probability profiles for short (>150 ms; Fig. 5B, left) and long (i.e. > 1s; Fig. 5B, right) responses were similar in shape; both indicated a pronounced decrease in firing rate during and following reinforcement, although the profile for long responses also shows a high probability of excitation following reinforcement. In both nuclei, there was a transition point at which a steadily increasing probability of excitation dropped sharply and an initially low probability of inhibition increased. This transition point occurred around t=0 in the STN and t=-500 ms in the STR; these differences may
provide a clue as to the meaning of the increase in firing rate preceding reinforcement.

Although Figure 5B clearly indicates that reinforcement was typically preceded by excitation and accompanied by inhibition, there were some neurons whose firing rates instead switched from low to high (Figure 5A, STN, upper right). These results show that STN and STR firing patterns during reinforcement were not uniform, and raise the possibility that changes in firing rate during reinforcement may reflect transitions between behavioral states or "chunks" (Graybiel 1995, 1998) of behavioral sequences.

We noted in particular one STR neuron (Figure 5A, lower right) in which examination of the raster plot revealed a response that developed over the course of this training session. Early in the recording session (upper rows of the raster), the short excitation that follows the end of the 1 s reinforcement period was brief, limited to one or two spikes, and its occurrence was intermittent. Three quarters of the way through the session, the response became more pronounced, and indeed began to widen. Such responses were rarely observed, but provided an exciting in vivo example of real-time neuronal plasticity that accompanied learning.

Having qualitatively described STN and STR responses to reinforcement, we wanted to know whether there were significant differences in the proportions of responsive neurons, either across nuclei or within nuclei but across conditions i.e. pretrained vs. non-pretrained, LICK vs. POKE, etc. A neuron was considered responsive if it demonstrated any significant deviation from baseline firing rate as determined during the qualitative analysis (see above, Methods). The proportions of responsive neurons were compared using Fisher’s exact test, with the expected and observed values obtained from the two neuronal samples (in the case of across-nuclei comparison) or two groups of
sessions (for within-nucleus comparisons). In some cases, which will be noted as appropriate, we confined the samples to only those neurons whose responses occurring before or after reinforcement i.e. valve-opening.

The numbers of reinforcement-responsive STN and STR neurons per session are shown in Table 1. Short responses (>150 ms) were more likely to occur in the STN than in the STR (p<0.005) when the data were pooled across all training sessions. Within each nucleus, the proportion of responsive neurons was context-dependent, occurring more frequently during POKE sessions than during LICK sessions (STN: p<0.0001, STR: p<0.0001). There was no effect of pretraining on the proportion of responsive neurons. STN and STR responses were equally likely to precede or follow reinforcement, regardless of whether we expressed responsive / non-responsive totals based on a) the entire sample of recorded neurons (before: p<0.01, after: p<0.18) or b) only those neurons showing reinforcement responses (before: p<0.72, after: p<0.09). Within-nucleus, across-session comparisons revealed a context-dependence identical to what we observed when we considered all responses together: STN and STR neurons were more likely to respond to reinforcement during POKE sessions than LICK sessions. This was true for responses preceding and following reinforcement (p<0.0001 in each case), making it unlikely that this context-dependence arose solely from the distinct motor behaviors immediately preceding reinforcement in the two sessions (approach vs. stationary spout-licking). Our analysis revealed a context-dependent bias towards responses preceding reinforcement in the STN (p<0.005), but not the STR (p=0.197). During POKE sessions, early STN responses were more likely than late responses (p<0.002), while during LICK sessions the proportions were equal. This difference may
reflect the differing behavioral patterns immediately preceding spout licking.

Our quantitative analysis of long responses revealed that overall, STN and STR neurons were equally responsive to reinforcement (p=0.67). STR neurons were more likely to respond during POKE sessions (p<0.005 vs. LICK sessions), but STN neurons showed no such context-dependence (p=0.086). STN and STR responses were equally likely to precede (p=1.0) or follow (p=0.52) reinforcement. Although we did not observe an overall bias within either nucleus towards early or late responses, we did observe that in the STR (p<0.0005), early responses were less common during LICK sessions.

**STN, STR inhibition was attenuated when reinforcement was withheld**

We used a 75% reinforcement schedule (see Methods) to control for the motor aspects of spout licking and to assess the influence of prediction error on neuronal firing patterns. Comparisons were made between the reinforcement condition, where t = 0 corresponded to the first lick that opened the spout, and the unexpectedly non-reinforced condition in which the first lick at the spout did not elicit sucrose. Rats did not show any overt changes in behavior during this protocol, although they did lick the spout significantly less during the 1 s following the first non-reinforced lick (5.71 ± 2.54 licks) than during normal reinforcement (8.33 ± 1.58 licks, p<0.001, t-test).

Figure 6A illustrates paired firing rate histograms centered on valve opening (Reward) or on the analogous, non-reinforced first spout lick in the POKE75 trials (No Reward). In both examples, the firing pattern observed during the 1 s reinforcement period (indicated by vertical lines) was markedly altered when a predicted reinforcement was withheld. In the STN neuron, the large decrease in firing rate was greatly attenuated when reinforcement was withheld. The STR neuron responded to reinforcement with a
strong short excitation coupled with a sustained decrease in firing that persisted throughout the reinforcement period and the remained of the ITI. When reinforcement was withheld, the initial excitation persisted for 1-2 s and the long inhibition disappeared. Furthermore, the large excitation seen at the start of the subsequent trial (Reward, t = +5) was absent following the withholding of reinforcement. Thus, in both of our examples, firing rate was higher following an incorrect prediction of reinforcement than following normal reinforcement.

Because long responses following an incorrect prediction were rare (Table 2), we focused on short responses in relation to non-reinforced spout licking. Equal proportions of the STN and STR samples showed short responses in the window surrounding non-reinforced spout licking (p=0.23); similarly, equal percentages of STN and STR neurons changed their firing rate preceding (p=0.5) and following the non-reinforced spout lick (p=0.35).

Figure 6B shows the probability vs. time for short (left graphs) and long (right graphs) responses associated with the withholding of reinforcement. In both cases, the probabilities of excitation and inhibition preceding the first non-reinforced lick were similar to those observed preceding reinforced valve opening: increases in the probability of excitation that peak at t=0 in the STN and t = -500 ms in the STR. In the STR, the decrease in the probability of excitation was accompanied by a sharp increase in the probability of inhibition, but this was not observed in the STN. In both nuclei the probability of inhibition was basically zero after t = 0, indicating an absence of inhibitory responses. Thus, the aggregate response was dominated by excitations, the probability of which increased in both the STN and STR as t approached 0. These graphs also indicate
the rarity of long changes in firing rate during withholding of reinforcement, most
notably with regard to inhibitions. This is the single most noticeable difference between
the probability profiles of reinforced and non-reinforced spout licking.

To confirm the significance of the lack of inhibition following withholding of
reinforcement, we used a differential perievent histogram (see Methods) to visualize the
relative firing rates of neurons during the two reinforcement conditions. Confidence
intervals generated using a control dPEH based on random points within the intertrial
interval allowed us to determine the times at which firing rate during one event was
significantly different from the firing rate during the paired event. The results are shown
in Figure 7. Differences scores for short responses to reinforcement were not significant.
Long responses, however, were significantly different beginning near the zero point of the
dPEH, were we observed a large negative region, indicating a higher firing rate following
the withholding of reinforcement. This negative region was shorter in the STN than in the
STR, and began later (STN: t = +500 ms, STR: t = 0). Our dPEH analysis confirmed
that individual neurons’ firing rates were higher following the unexpected absence of
reinforcement.

One possible explanation for the difference in responses could arise from different
behavioral patterns. Rats’ behavior was identical during the approach period preceding
spout licking, as there was no indication that reinforcement would be withheld. We have,
however, observed that when predicted reward was withheld, rats licked the spout
significantly less during the 1 s period in which they would have received it. Lick-related
motor behavior was clearly reflected in STN and STR firing rates in the form of periodic
excitations that were time-locked to individual licks. Because these periodic excitations
were identical in reinforced and non-reinforced trials, we can rule out the possibility that inhibition of firing rate during reinforcement was due to lick-related motor activity. Examples of this periodic type of firing are illustrated for three different reinforcement conditions in Figure 6C. These perievent histograms were built on individual licks, some of which arrived in quick temporal succession. This had the effect of distorting the perievent record, as samples taken at increasingly later times were aligned and averaged together. In all cases, periodic firing was evident, with a frequency near eight Hz, consistent with the frequency of licking measured with the infrared beam detector; this periodic signal was less clear during Non-Reinforced licks (right graph), consistent with our observation of decreased licking when reinforcement was withheld. During reinforcement, this periodic signal is superimposed on a background of low, decreasing firing rate, reflected in the early trough. These features were absent during ITI licking (middle graph) and non-reinforced licking observed in POKE75 sessions (right graph). Thus, differences in firing pattern could not be attributed to different behavioral profiles.

**STN, STR responses to nose-pokes do not reflect discriminative accuracy**

STR and STN neurons both responded to nose-pokes. Figure 8A shows typical examples of responses to correct and incorrect nose-pokes. In these instances, firing rate increased before or coincident with nose-pokes, regardless of their accuracy. Firing rate decreased following a correct nose-poke, such decrease being absent following the incorrect nose-poke.

For our quantitative analysis of response rates, responses were characterized relative to the time of the poke; as with reinforcement, a response had to occur within ± 1 s of the poke to be counted. Approximately 30% of recorded STN and STR neurons
responded to correct nose-pokes (p = 0.34); approximately 15% responded to incorrect nose-pokes (p=0.63) (Table 3). There were no significant differences in the relative rates of early (t < 0) or late (t >= 0 s) responses, either within or between nuclei. Nor did we observe context-dependent response rates, or effects of pre-training. Similar proportions (~14%) of STN and STR neurons responded to nose-pokes occurring during the ITI; these response rates were independent of context or pre-training. We ignore ITI poke responses for the remainder of our analysis.

Figure 8B shows the response probability profiles for correct (left graphs) and incorrect (right graphs) nose-pokes. In the STN, the probabilities vs. time of excitation and inhibition for correct nose-poke responses both peak coincident with poke onset, declining thereafter to a modest nonzero level. The aggregate response probability vs. time was positive before the nose-poke but went negative afterward; these effects were modest. In the STR, the probabilities vs. time of excitation and inhibition began increasing early in the period before the nose-poke. The probability of inhibition increased at t = 0 s, with the result that the aggregate response probability switched from positive to negative, similar to the pattern shown in Figure 8A. The major difference in the response probability profiles for incorrect nose-pokes was the low probability of inhibition, especially after t = 0 s; the probability of excitation was similar to that seen for correct nose-pokes. These changes were seen in both the STN and STR.

Although the examples and response probability profiles in Figure 8 suggest that a decrease in firing rate following a nose-poke accompanies a correct discrimination, analysis of dPEHs for correct and incorrect nose-pokes showed that although there was a trend in this direction, the differences in relative firing rates were not significant. While
it is possible that a significant effect might be seen with a larger sample size, we conclude that STN and STR firing rates do not encode the “correctness” or “success” of a discriminative nose-poke.

*STN, STR neurons differentially respond to tone stimuli*

Figure 9 and Table 4 summarize the STN and STR responses to tone stimuli. Approximately 30% of the recorded neurons in each nucleus responded to the feed tone (p = 0.49), and approximately 18% of the neurons in each nucleus responded to the nose-poke tone presentation (p=0.06). Responses were characterized in relation to the onset of the first pulse of each of the tones, and were only counted if they occurred within ± 1 s of the tone onset.

The proportion of neurons showing feed tone responses was context-dependent, with an increased likelihood of response during the pooled POKE sessions vs. the LICK sessions. This context-dependence was evident in the STN with regard to all responses, regardless of whether they preceded or followed tone onset (p<0.0001) and to responses following feed tone presentation (p=0.0005); the corresponding STR responses were not context-dependent (p<0.01). In contrast, responses preceding feed tone presentation were context-dependent in both the STN (p<0.0001) and STR (p=0.0001), occurring more frequently during POKE sessions. The response probability profiles for feed tone responses (Figure 9B, left) show that in STN neurons, the probabilities vs. time of excitation and inhibition peaked early in the pre-tone period; the aggregate probability vs. time shows only one distinct peak, a positive deflection peaking around -500 ms. The response profile for STR neurons shows peaks in the probability of excitation around +200 ms; this peak is also present in the aggregate probability. These results suggest that
the context-dependence observed in STN response rates arose because of the increase in firing preceding the tone presentation; if this increase was due to the preceding nose-poke, then the absence of nose-pokes during LI CK sessions would explain the low response rates during those sessions. The same explanation holds for STR responses, whose response probability profile showed some modest positive deflections in the period preceding tone presentation.

Equal proportions of STN and STR neuron responses preceded (p=0.18) or followed (p = 0.18) nose-poke tone presentation. We did not observe any effects of pre-training, context-dependence, or reinforcement condition on the rates of nose-poke tone response in either nucleus. In both nuclei, response probability profiles revealed peaks in the probability of excitation (with a corresponding positive peak in the aggregate probability) ~ 200 ms after tone onset. The probability of inhibition remained flat except for a brief increase ~500 ms after tone onset; the aggregate probability showed no negative peaks, confirming the neurons were typically excited during nose-poke presentation.

Discussion

General comments

We report here, for the first time in rats, that the STN and STR are concurrently involved in the processing of events related to operant performance. We found that neurons in both structures responded to task events including reinforcement and instructive cues, and showed differential responses to the presence and absence of reinforcement. Although reinforcement-related firing patterns have been reported in primates (Matsumura et al. 1992; Darbaky et al. 2005), this is the first study in behaving
rats to examine STN neuronal responses to reinforcement in detail.

Reinforcement-related activity

Our results show that STN firing patterns can represent operant reinforcement. In fact, STN and STR neurons respond concurrently, but with subtle differences, to reinforcement. The predominant motif of reinforcement-related neuronal firing, both in the STN and the STR, was a phasic excitation preceding reinforcement coupled with a pronounced inhibition during consumption of sucrose reward (Fig. 5A). Response latency histograms revealed differences in the latencies of phasic STN and STR excitations preceding valve opening, suggesting that these neurons' firing rates related to ongoing behavioral sequences in different ways.

In the STN, the aggregate response probability increased steadily preceding valve opening but dropped off sharply at the time of valve opening. Increased firing could have reflected either an increasing expectation of reward or motor activity as the rats approached and positioned themselves at the spout. The proportion of STN neurons showing responses preceding valve opening was higher during POKE sessions, in which rats had to cross the chamber in order to lick the spout, than during LICK sessions in which rats remained in front of the spout. Because the change in the aggregate probability decreased so abruptly at t = 0 s (valve opening), and because the proportion of STN excitations preceding valve opening depended on the training protocol, which differed in the nature of behavior expressed immediately before valve opening, it is likely that excitatory STN responses preceding valve opening were related to approach/positioning behavior. In the STR, the analogous sudden decrease in the aggregate response probability occurred about 500 ms before valve opening.
Furthermore, the percentage of responsive STR neurons was independent of training protocol and thus of behavior immediately preceding spout licking. STR responses preceding valve opening may therefore have related to cue presentation; perievent histograms based on consecutive events confirmed that some STR neurons’ firing rates increased after the feed tone but before valve opening.

During sucrose reinforcement, neurons were predominantly inhibited. This inhibition was greatly attenuated, or even reversed (Figure 6A, right) when spout licking was not reinforced, whether it occurred during the ITI or during non-reinforced trials in the POKE75 sessions. Several reports have shown a “pause” response in tonically active STR neurons during reinforcement (Apicella et al. 1991, 1992, 1997; Aosaki et al. 1995). In primates and cats, STN neurons were typically excited during reinforcement (Cheruel et al. 1996; Matsumura et al. 1992; Darbaky et al. 2005). Although we did observe reinforcement-related excitations in the STN, the majority of our responses were inhibitory, as illustrated by the aggregate response probability graph in Figure 5B.

Cue-related activity

Both STN and STR neurons increased their firing rates during the period surrounding presentation of auditory cues. Several reports have documented STN and STR responses to auditory stimuli as well as innervation of these areas by auditory cortex afferents (Gardiner & Kitai 1992; Jog et al. 1999; Cromwell and Schultz 2003; Kolomiets et al. 2003; Cheruel et al. 1996; Shi et al. 2005). STN and STR response profiles differed both within and across nuclei. Although we report differences in the rates of response to the feed tone stimulus as a function of training protocol, these differences were confined to the pre-tone period, and may arise from the nature of the behavioral tasks: during
LICK sessions, rats remained stationed in front of the reinforcement spout, whereas during POKE sessions they had to approach it from the other side of the recording chamber. Thus, it is likely that pre-feed-tone responses were related to approach behavior.

Neurons in both the STN and STR responded to the nose-poke tone with phasic excitations occurring within 200 ms after the tone presentation, reflected in the positive aggregate response probability in Figure 9B. STR neurons showed a similar response profile following the feed tone. STN neurons, however, showed equal proportions of inhibitions and excitations following feed tone presentation; there was no peak in the aggregate probability. STR firing patterns may reflect either purely auditory responses, or the instructive value of the tone. Conversely, while STN neurons clearly responded to the nose-poke tone, responses occurred throughout the feed tone perievent window, and the only evident peak in the aggregate probability occurred early in the perievent period. This peak was likely due to a residual response to the preceding correct nose-poke. The response profiles suggest that STN neurons were not merely responding to auditory stimuli, but rather to the differential salience of each cue within the operant paradigm.

Nose-pokes

Both STN and STR neurons responded to correct and incorrect nose-pokes, although responses to incorrect nose-pokes were much less common. In both nuclei, the aggregate response probability indicated that firing rate increased before all nose-pokes, regardless of whether they were correct or not. This consistency is not surprising, and the most parsimonious explanation would be that increases in firing rate relate to the motor aspects of approach and poke behavior. However, our recordings from the STR were
located in the medial portion of the caudate, a region not noted for its innervation by motor cortical afferents (Parent and Hazrati 1995a). We think that approach behavior was the most likely explanation for the increase in STN firing because we saw a similar pattern of responses preceding reinforcement (valve opening). The STR response may instead reflect that the nose-poke is the first motor action in a behavioral sequence, which could account for the lack of a difference between correct and incorrect nose-pokes. We observed several STR (and STN) neurons that exhibited sustained periods of increased or decreased firing whose onsets corresponded with nose-poke performance; one such neuron is shown in Figure 5A, where the pronounced “hump” occurring late in the perievent histogram corresponds with the nose-poke at the start of the subsequent trial.

The response probability graphs further revealed that inhibitions often followed the performance of a correct nose-poke, inhibitions that were attenuated or even reversed following an incorrect nose-poke. This was an exciting finding because it suggested that STN and STR neurons could encode discriminative accuracy or successful fulfillment of part of a sequence. However, the dPEH scores for these events were not significant, and so we must conclude that these neurons were incapable of differentially coding discriminative accuracy.

Functional implications

One interesting hypothesis concerning the role of the basal ganglia in behavior is that they mediate the acquisition of habits or sequences of behavior by coding for the start and stop times of the “chunks” constituting those sequences (Graybiel 1995, 1998, Jog et al. 1998). When we examined the responses to different task events, we frequently observed neurons in both the STN and STR that would exhibit sustained changes in firing
rate coincident with a particular behavioral event. During reinforcement we often observed transitions from one firing regime to another e.g. a slow-firing neuron transitioned to fast firing during reinforcement, and this fast firing persisted until the start of the next trial. Other neurons showed just the opposite transition (cf. Figure 5A, STN: upper right and lower left, STR: upper and lower left; Figure 6A). Such transitions suggest that STN and STR firing patterns could reflect more than simply a motor or reinforcement roles, including 1) the start of a new trial or behavioral sequence, 2) the discriminative action phase of the trial, and 3) the reinforcement phase of the trial as well as the completion of a behavioral sequence and entry into a behaviorally neutral period. Such “behavioral chunking” could explain the similarity in STR firing patterns preceding correct and incorrect nose-poke; since we are not recording from motor areas of striatum, the similarity could reflect that a nose-poke was the first event in a behavioral sequence, regardless of its accuracy. Similarly, it could explain why STN responses were locked to the nose-poke tone but not the feed tone. Although there is already evidence in rats for STR neurons encoding of the beginning and end of operant behavioral sequences (Jog et al. 1999), we are unaware of any reports documenting such coding by STN neurons.

Although STN firing patterns during operant performance have not been widely documented (but see Darbaky et al. 2005), lesion and pharmacological studies have suggested that the STN is involved in motivated behavior. STN-lesioned rats exhibited perseverative and premature responses in an attentional task, and demonstrated higher scores on behavioral measures of motivation including latency to consume food and locomotion in anticipation of reward (Baunez and Robbins 1997; Baunez et al. 2002, 2005). Lesions of the prefrontal cortex-STN pathway induced similar behavioral deficits
(Chudasama et al. 2003). Clinical reports have described inappropriate, uncontrollable laughter (Krack et al 2001), increased appetite and overeating (Moro et al. 1999), and increased sex drive (Absher et al. 2000; Romito et al. 2002) in Parkinson's patients having undergone STN deep-brain stimulation. These findings suggest that disruption of STN function may interfere with the suppression of impulsive behaviors. More recent behavioral work, however, suggests that rather than gating impulsive behaviors, the STN may instead mediate the association of conditioned and unconditioned stimuli (CS/US) (Winstanley et al. 2005). Not only did our STN neurons respond to sucrose reinforcement (a US), but they also respond to tone cues that might serve as conditioned stimuli, and thus are in an excellent position to mediate the Pavlovian association of stimuli. Indeed, STN and STR neurons both responded to these events with similar, but subtly differing, firing patterns. It is intriguing to consider the interplay, at the output nuclei, of these similar firing patterns given the different learning deficits induced by STN and STR lesions (Baunez and Robbins 1997; Winstanley et al. 2005; Baunez et al. 2005; Packard and Knowlton 2002). Although we can draw no conclusions about impulse control or CS-US pairing, we have demonstrated that STN neurons are responsive during an operant task engaging limbic, associative, and motor faculties, and that the firing patterns of these neurons are capable of encoding information related to each of these processes. If STN neurons were responsible for CS-US pairing or the gating of behavior based on motivational (limbic) state, such coding would be a prerequisite.

*Basal ganglia function*

One of our goals in performing these recordings was to gain some insight into the
functional anatomy of the basal ganglia. Specifically, we hoped to determine whether the STN, as part of the “indirect” striato-nigral pathway that received no external input, acted as a passive relay for the firing patterns of striatal output neurons (Albin et al. 1989; DeLong et al. 1990), or whether the STN and STR, each of which receives direct, monosynaptic cortical, thalamic, and midbrain inputs from the same regions, acted as parallel cortico-nigral pathways to process afferent input and shape the firing patterns of the substantia nigra (Levy et al. 1997; Nambu et al. 2002; Kita et al. 2004). The issue is complicated in this case by the fact that the STN contains no interneuron population, while many of our recorded STR neurons bore the physiological hallmarks of cholinergic interneurons (wide action potential, spontaneous activity during quiet rest, a pause in firing during reinforcement; Aosaki et al. 1995, Wilson et al. 1990). We found that similar proportions of the STN and STR samples modulated their firing rates in association with operant events. We also observed qualitative similarities in many STN and STR responses e.g. reinforcement was associated with sustained inhibition that was attenuated when reinforcement was withheld, correct nose-pokes were preceded by excitation and followed by an inhibition.

These findings are consistent with both the relay and parallel input hypotheses, although it is of interest to note that we sampled from all areas of the STN whereas our STR sample was relegated to the head of the caudate, a primarily associative area. As an aside, if our STR sample indeed comprised tonically active, cholinergic interneurons, the similarities in STN and STR responses are interesting in light of the fact that the tonically active neurons show periodic burst firing in MPTP-primates, similar to that observed in the STN (Raz et al. 1996; Wichmann et al. 1994). We did observe some quantitative and
qualitative differences in STN and STR event responses, e.g., the differential responses of
STN and STR neurons to the feed tone, the difference in the probability of excitation
preceding reinforcement, and the differences in context-dependence for some events,
suggesting that firing patterns in the STN and STR samples encoded different
information. In light of the known projections from motor, associative, and limbic
cortex, thalamus, and midbrain to the STN, we believe that the STN and STR do act
independently, but in parallel, to process similar sets of afferent firing patterns, and that
while similarities in event responses reflect common afferent input, any differences in
event responses reflect differential local processing of these afferent patterns.
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Table 1. Responses during reinforcement. Reinforcement was defined as the first spout lick during the reward-ready period, after which sucrose solution was available at the spout for 1 s. The numbers in each cell represent the mean number of neurons per session. N = neurons recorded during each type of experimental session, regardless of whether they responded to the event. All = all neurons showing responses during the perievent window: ±1 s for short responses and -1 to +5 s for long responses. A response was defined as a certain number of consecutive bins whose z-transformed firing rates were more than 1.64 standard deviations away from the mean firing rate, determined by the period from t = -2 to -1 s before the event. The criterion length for short responses was 150 ms (3x50 ms bins) and for long responses was 1 s (10x100 ms bins). Pre = neurons with at least one response onset latency before the event (t < 0). Post = neurons with at least one response onset latency after event onset (t > 0). Bold numbers, where present, indicate percentages based on the N values for that nucleus and session. Pre and Post percentages are based on the values in the All column, while the All percentages are based on the values in the N column. LICK sessions were those in which rats were trained to lick at the sucrose spout; these sessions had no discriminative/nose-poke component. POKE and POKE75 sessions required a correct discriminative nose-poke before reward was made available; POKE75 sessions differed from POKE sessions in that 25% of POKE75 trials were not reinforced. Pretrained animals underwent the two-week training protocol before being implanted with electrodes, and then underwent two weeks of recording during POKE and POKE 75 sessions. Non-pretrained rats were implanted with electrodes prior to the beginning of operant training, and thus were
recorded during task learning. We recorded 743 STN neurons during 33 LICK sessions, 68 POKE sessions and 19 POKE75 sessions (non-pretrained rats), and 14 POKE and 11 POKE75 sessions (pretrained rats). We recorded 405 STR neurons during 64 LICK sessions, 109 POKE sessions, and 33 POKE75 sessions (non-pretrained rats), and 40 POKE and 30 POKE 75 sessions (pretrained rats). The numbers of sessions are the same for each subsequent table.

Table 2. Responses during the absence of reinforcement. Responses were analyzed relative to the first spout lick during the reward-ready period. In contrast to reinforced trials, this spout lick did not make sucrose available. Non-reinforced trials only occurred on 25% of the trials during POKE75 sessions; these responses are thus taken only from those trials. All abbreviations are as described for Table 1.

Table 3. Nose-poke responses. Responses were analyzed relative to the detection of the nose-poke by an infrared beam detector. Correct nose-pokes were those into the hole illuminated by the green LED; incorrect nose-pokes were those into the unlit hole. All responses occurred within ±1 s of nose-poke detection. All abbreviations are as described for Table 1.

Table 4. Tone responses. Responses were analyzed relative to the onset of the tone stimuli. The feed tone comprised three 300 ms, 1.9 kHz tone pulses (70 dB). The nose-poke comprised two 500 ms, 4.2 kHz tone pulses (70 dB). All responses occurred within ±1 s of tone onset. All abbreviations are as described for Table 1.
Figure 1. Schematic representation of the operant tasks. The first three days of operant training comprised one daily session in which rats learned to pair a 1.9 kHz "feed tone" and yellow LED with a "reward-ready" period of indefinite length, during which spout licking yielded sucrose for 1 s ("LICK"). The subsequent nine days involved single daily sessions ("POKE") in which rats learned to poke their noses into one of two wall-mounted holes, indicated by a green LED and a 4.2 kHz "nose-poke" tone. A correct nose-poke was followed by the previously learned yellow LED/ feed tone and a 5 s reward-ready period. An incorrect nose-poke caused a 30 s "timeout" when all lights were extinguished, and a new trial began. A trial ended when either the reward-ready period elapsed without a spout lick, or when the rat had received sucrose for 1 s. The intertrial interval varied between 5-8 s. POKE and POKE75 sessions differed only in that 25% of trials were non-reinforced during POKE75 sessions.
Figure 2. Histology and representative STN and STR waveforms. A. Coronal section illustrating a K$_4$Fe(CN)$_6$ lesion (dark spot) marking electrode placement in the medial portion of the STN (bounded ventrally by the cerebral peduncle (CP) and dorsally by the ventral zona incerta (ZI)). B. Representative extracellular waveforms for STN and STR neurons.
Figure 3. Schematic representation of perievent analysis techniques. A. The determination of significant perievent responses. The upper two graphs show the raw data from a single neuron during reinforcement (t = 0). Raster plots and perievent histograms (upper two graphs) represent the trial-by-trial and average perievent activity, respectively. In the lower graph, we have normalized the perievent firing data by converting it to z-scores based on the mean and standard deviation of the firing rate during the first 1 s of the record (t from -2 to -1 s). We set confidence intervals (dashed lines) equal to 2 standard deviations. A specified number of consecutive suprathreshold bins was considered to be a response. Horizontal bars underneath the graph indicate significant perievent responses, with black bars indicating excitations and gray bars indicating inhibitions. B. The determination of aggregate, excitatory, and inhibitory response probabilities. For each event, we summed across vectors containing the...
excitatory and inhibitory responses of every neuron that responded to that event. These vectors were simply numerical versions of the horizontal bars seen at the bottom of A, depicted in the upper graph. We summed the numbers of excitatory (black) and inhibitory (light gray) responses within each bin, and divided by the number of neurons in the sample, to yield the black and light gray lines seen in the lower graph. The aggregate probability was taken by summing all responses within each bin and dividing by the sample size, yielding the dark gray line.
Figure 4. Behavioral measures of operant task learning vs. training session. Black lines: naive rats recorded during task acquisition (non-pretrained). Gray lines: rats having undergone two weeks of training prior to electrode implant surgery and recording (pretrained). Breaks in the two lines indicate a switch from POKE to POKE75 sessions. A. For the purposes of graphing, discriminative accuracy was depicted as $100 \times \frac{\text{incorrect}}{\text{correct} + \text{incorrect}}$. The asymptotic degree of discriminative accuracy of the non-pretrained group was significantly lower (ANOVA, $F(1,18) = 18.664, p<0.0005$) than that of the pretrained animals, which may reflect an overtraining effect. B. Latency to lick was calculated as the time between completion of a correct nose-poke and the first subsequent spout lick. Latency scores in the non-pretrained group converged relatively quickly to those in the pretrained group; the differences were not significant (ANOVA, $F(1,18) = 3.02, p = 0.09$).
Figure 5. STN, STR responses to reinforcement. A. Examples of perievent firing patterns in four STN neurons (two left columns) and four STR neurons (two right columns) preceding, during, and following reinforcement, which began at t = 0 and persisted for 1 s. Reinforcement, which occurred at t = 0 s, was defined as the first spout lick during the reward-ready period. This lick would open a valve that allowed sucrose solution to drip out of the valve for 1 s. The upper graph for each neuron is a raster plot of spike firing on each trial, with the top row of dots corresponding to the first trial. Although the box heights are identical, differing numbers of trials (events) yielded rows of different heights. The lower graph is the mean firing rate across all trials; the reinforcement period is bounded by vertical black lines. Bin sizes were 10 ms for the raster plots, and 50 ms for the perievent histograms. B. Response magnitudes and probabilities vs. time. The two left columns show the STN (left) and STR (right) response parameters for short changes in firing rate (> 150 ms); the right columns show the parameters for long responses. The
scatter plot indicates the response magnitude vs. onset latency for all recorded responses to valve opening. Black dots indicate excitations, gray dots indicate inhibitions. The lower graph indicates the response probability vs. time. The black and light gray lines represent the probability that either an excitation (black) or inhibition (gray) was ongoing at a particular time. The probability of inhibition was multiplied by -1, for the purposes of illustration; a probability of -1 means that an inhibition always occurred at the specified time. The dark gray line is a binwise sum of all ongoing responses (1=excitation, -1 = inhibition) divided by the number of ongoing responses, yielding a value between -1 and 1 that indicates the polarity of the most frequently occurring response during that bin; this quantity is referred to as the aggregate probability of response.
Figure 6. Characteristics of non-reinforced spout licking. Graphs are as described in Figure 5. There were approximately 75 reinforced trials and 25 non-reinforced trials per recording session. Both reinforcement and non-reinforcement trials were aligned on the same event, namely the first spout lick during the reward-ready period. Sucrose reinforcement was withheld on non-reinforced trials. A. Examples of single unit responses to reinforced and non-reinforced spout licking. Left graphs: this STN neuron (left) showed a decrease in firing rate during reinforcement that was attenuated when reinforcement was withheld (right). Right graphs: The sustained decrease in firing rate accompanying reinforcement in this STR neuron (left) was reversed when reinforcement was withheld, with a prolonged increase in firing rate following non-reinforced spout licking (right). These results suggest that firing rate decreases following valve opening may indicate ongoing reinforcement. C. Response parameters for non-reinforced spout licking. As during
reinforced trials, STN and STR firing rates increased preceding the first spout lick, although STR neurons did show a brief pause preceding reinforcement. In both cases, the aggregate response probability following the first spout lick (t = 0) was non-negative, in contrast to the negative values observed during reinforced licking. Long responses were rare, and were excitatory when they occurred. C. Periodic firing pattern observed during different types of licking behavior. This STN neuron showed periodic firing with a characteristic frequency of about 7 Hz; this periodic firing was most pronounced during ITI licking, and was attenuated during reinforced and non-reinforced licking. Note the increase in firing rate throughout the reinforced lick PEH as subsequent licks occurred closer to the ITI and the attendant reduction of reinforcement-related inhibition.
Figure 7. Differential perievent histograms (dPEHs) representing the relative firing rates of neurons during two behavioral events. Only neurons that showed a significant response to one of the two events were used to generate the dPEH. The Z value represents the mean difference in the perievent histograms for the two events. We generated confidence intervals by obtaining, from each neuron, the difference scores of 100 randomly paired, single-trial spike trains taken during the ITI, and then taking the binwise mean. We set the confidence interval 3.0 standard deviations away from the bin mean. Upper row: STN responses. Lower row: STR responses. Left column: difference score for short responses during reinforced and non-reinforced trials. STN neurons showed two late, brief periods with significant negative difference scores, consistent with our observation of a decrease in firing rate following reinforcement. STR neurons showed brief negative scores late in the perievent period. Center column: difference score of long
responses during reinforced and non-reinforced trials. STN neurons showed significant difference in firing rate - firing rate was faster preceding, and slower during, reinforced spout licking than during the corresponding periods for non-reinforced spout licking. In the STR, there was a pronounced negative region indicating that firing rate was lower during and after reinforcement than during non-reinforced licking; firing rate was significantly faster during the ITI following reinforcement than following the analogous non-reinforced event. Right column: the difference score for correct and incorrect nose-pokes showed no significant differences in perievent activity for these two events in either nucleus.
Figure 8. Responses to nose-pokes. Trials were aligned on the detection of the nose-poke event. A. Examples of STN and STR neurons to correct and incorrect nose-pokes. In both cases there was a decrease in firing rate coincident with or following correct nose-poke that was attenuated or reversed following incorrect nose-pokes. In the STN neuron, this decrease was difficult to make out following incorrect nose-pokes. In the STR neuron there was an increase in firing following the incorrect nose-poke. B. Response parameters for correct (left columns) and incorrect (right columns) nose-pokes. STN neurons showed increases in the probabilities vs. time of excitation and inhibition following correct nose-pokes, with the aggregate response probability being slightly positive; the probability of inhibition was attenuated following incorrect nose-pokes, yielding a positive aggregate probability. In the STR, the increase in the probability of inhibition following correct nose-pokes was large enough to push the aggregate probability negative; this inhibition was absent following incorrect nose-pokes.
Figure 9. Responses to tone events. Trials were aligned on tone onset. A. Left column, STN response to nose-poke tone. Right column, striatal response to feed tone. B. Parameters of tone responses. Most tone responses were characterized by a brief excitation following tone presentation, reflected in a positive aggregate response probability shortly after $t = 0$. The exception to this trend was STN responses to the feed tone; the aggregate probability was basically flat throughout the peri-event period except for a brief peak early on, which may correspond to a residual response to correct nose-poke performance.
Table 1. Responses during reinforcement

### Phasic (>150 ms) responses

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<tr>
<td>Session:</td>
<td>N</td>
<td>N (%)</td>
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<tr>
<td>LICK</td>
<td>4.93</td>
<td>1.09 (22)</td>
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### Non-pretrained rats

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</tr>
<tr>
<td>POKE</td>
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### Pretrained rats

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### Average

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### Sustained (>1s) responses

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### Non-pretrained rats

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### Pretrained rats

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### Average

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Table 2. Responses during the absence of reinforcement

### Phasic (>150 ms) responses

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### Sustained (>1s) responses

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Table 3. Responses during nose pokes

Correct nose poke responses

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Incorrect nose poke responses

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Table 4. Responses to tone cues

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<td>Session:</td>
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<td>N (%)</td>
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<tr>
<td>LICK</td>
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<td>5.88</td>
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