Nucleus accumbens responses differentiate execution and restraint in reward-directed behavior

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Roitman JD, Loriaux AL. Nucleus accumbens responses differentiate execution and restraint in reward-directed behavior. J Neurophysiol 111: 350–360, 2014. First published October 30, 2013; doi:10.1152/jn.00350.2013.—Our behavior is powerfully driven by environmental cues that signal the availability of rewarding stimuli. We frequently encounter stimuli—a bowl of candy or an alert from our smartphone—that trigger actions to obtain those rewards, even though there may be positive outcomes associated with not acting. The inability to restrain one’s action in the presence of reward-associated cues is one type of impulsive behavior and a component of such maladaptive behaviors as overeating, gambling, and substance abuse. The nucleus accumbens (NAc) is ideally situated to integrate multiple cognitive and affective inputs to bias action via outputs through the basal ganglia. NAc neurons have been shown to respond to cues that predict reward availability, goal-directed behaviors aimed at obtaining them, and delivery of the reward itself. As these processes are typically associated, it is difficult to discern whether signals in the NAc are more closely related to processing reward-predictive aspects of goal-directed behavior or selection of behavioral response. To dissociate these possibilities, we recorded the activity of NAc neurons while rats performed a task in which two different cues both informed rats of reward availability but required them to either press a lever while rats performed a task in which two different cues both informed rats of reward availability but required them to either press a lever (Go) or withhold pressing (NoGo) to obtain the reward. Individual cue-responsive neurons showed either increases or decreases in activity at cue onset. Increases in activity were larger, and decreases smaller, when rats withheld lever pressing, whether correctly for NoGo trials or in error on Go trials. Thus NAc cue responses correlated with action, regardless of cue type or accuracy.

nucleus accumbens; rodent; executive control; reward

ANIMALS SHOULD adaptively navigate their environment by approaching beneficial, and avoiding harmful, stimuli. However, even when restraint may be a more beneficial course of action, we often have difficulty in suppressing our approach for immediately rewarding stimuli. This impulsivity is a multidimensional construct that likely has several underlying processes governed by distributed neural systems (de Wit 2009). One aspect of impulsivity is the incapacity to restrain a behavioral response to a potent cue, which is manifested in patients with attention deficit hyperactivity disorder, drug addiction, and obesity (Volkow et al. 2012) as well as in “healthy” populations (Hamidovic et al. 2009).

Fronto-striatal systems are thought to play multiple, critical roles in approach behavior, with prefrontal cortical regions computing the value of goals (Padoa-Schioppa and Cai 2011; Schoenbaum et al. 2011) and actions (Roitman and Roitman 2010) and the striatum biasing motor sequences (Jin and Costa 2010; Kravitz et al. 2010; Pavuluri et al. 2012). The nucleus accumbens (NAc) is a striatal subterritory long associated with goal-directed behavior and approach. Disruption of NAc function impairs both Pavlovian approach and operant behavior (Ambroggi et al. 2011; Blaiss and Janak 2009; Cardinal et al. 2002). Pharmacological studies point to NAc in the control of appropriate versus inappropriate responding, suggesting a likely role in impulsive behavior (Ambroggi et al. 2011; Basar et al. 2010; Wiskerke et al. 2011). Moreover, individual neurons within the NAc respond to the receipt of rewards, the cues that predict them, and operant approach behaviors to obtain them (Carelli 2004; Day et al. 2006; Nicola et al. 2004). NAc neurons respond to such salient events with phasic changes in activity, which appear to play different roles in reward-directed behaviors (Ambroggi et al. 2011; Krause et al. 2010; Roitman et al. 2005; Taha and Fields 2006).

The degree to which NAc neurons signal contingencies between predictive environmental stimuli and expectations about outcome, goal-directed actions, and the outcomes themselves remains unclear. Go/NoGo paradigms have been widely used to study the mechanisms of behavioral inhibition, but these tasks often confound the reward-predictive nature of cues with the appropriate behavioral response. In all Go/NoGo tasks, the Go cue instructs approach behavior for reward, so that neural responses to Go cues may be related to the reward-predictive nature of the cue, the planned approach behavior, or both. In contrast, NoGo cues typically promote an omission of approach behavior either to avoid an aversive outcome (Mirenowicz and Schultz 1996; Setlow et al. 2003) or because the behavior is unrewarded (Boure and Sara 2004; Brown et al. 2011; Nicola et al. 2004). In these cases, the reward outcomes associated with Go and NoGo behavioral responses differ. Alternatives to Go/NoGo tasks that require subjects to discriminate between two spatially distinct approach options represent a different potential confound whereby neural responses can depend on the direction of the behavior. This potential directional bias is more likely to be observed in more dorsal subregions of the striatum (Stalnaker et al. 2012).

Here we measured the responses of NAc neurons while rats performed a novel task designed to dissociate the confound between approach behavior to achieve reward and inhibition of behavior to avoid a neutral/aversive outcome. In this symmetrical Go/NoGo paradigm, both Go and NoGo cues predict reward but command different behavioral responses. Rats were trained to press a lever after a Go cue and withhold presses of the same lever after a different NoGo cue. All successful behavioral responses were rewarded, while all failures to
approach or withhold appropriately led to a time-out. We recorded the activity of individual NAc neurons during task performance and hypothesized that cue-related activity would not differ between Go and NoGo trials if they encoded reward prediction, as both cues predict reward availability, but would differ if they contributed to the determination of behavioral response. Comparisons of correct and error trials following both Go and NoGo cues lend particular insight as to whether NAc activity is more strongly associated with reward prediction or behavioral response selection.

MATERIALS AND METHODS

Subjects. Eleven male Sprague-Dawley rats (300–350 g) were individually housed with access to a minimum of 16 g of chow per day and ad libitum water with a 12:12-h light-dark cycle (lights on at 7:00 AM). All experiments were conducted in the light phase between 10:00 AM and 5:00 PM. Animals were treated in accordance with the guidelines put forth by the National Institutes of Health and under the approval of the Animal Care Committee of the University of Illinois at Chicago.

Go/NoGo task. All testing was conducted in operant chambers housed within a sound-attenuating cubicle, equipped with a house light, white noise generator, tone generator, pellet dispenser, and food receptacle cup, with two retractable operant levers situated to the left and right of the food receptacle and two white cue lights, each located above one lever (Med Associates, St. Albans, VT). Because of the difficulty of the task, training for the Go/NoGo task was conducted in several phases detailed below to shape task performance.

Magazine training. Rats underwent magazine training during which they received sucrose pellets (45 mg; Bioserv, Frenchtown, NJ) on an imposed variable-interval schedule between 60 and 120 s and were trained under a fixed ratio 1 schedule with one lever (alternating right or left) extended into the operant chamber on each day. When rats reached a criterion of 100 lever presses in 30 min on two consecutive days, they began training for the Go/NoGo task. For each rat, one lever (right or left) was assigned as the Go lever, which was maintained throughout testing and was counterbalanced across rats.

Phase 1: Go+/NoGo− two-lever task. The goal of this phase of training was to train rats to complete a test session comprised of trials with two behavioral options, to press or not to press. Two operant levers were used, on either side of the central sucrose pellet receptacle, with one designated as “Go” and the other as “NoGo.” Sessions were composed of 100 trials, with 75% Go and 25% NoGo trials selected randomly. Presses of the Go lever were reinforced (+) with the delivery of a sucrose pellet, and presses of the NoGo lever were not reinforced (−). At the start of each daily session, a house light was illuminated in the operant chamber, which was enclosed in a dark, sound-attenuating cubicle. After an intertrial interval (ITI) of 5–13 s, one lever was extended. On Go trials, the Go lever was extended with a cue light illuminated above it and a Go press (correct) was immediately followed by the simultaneous presentation of a brief (0.2 s) tone and the delivery of one 45-mg sucrose pellet. If the rat failed to press (Go-error) within the assigned interval (described below), the lever retracted, the cue light was extinguished, and no reward was delivered, followed by a 40-s time-out period in which the house light was off. On NoGo trials, the second lever in the chamber was extended with no other environmental cues presented. If the rat did not press the NoGo lever (correct) within the assigned interval (described below), the lever retracted and no reward was delivered. If pressed (NoGo-error), the lever retracted, followed by a 40-s time-out period in which the house light was off. At the end of each trial, a new ITI began. On correct trials, the house light remained illuminated through the ITI; on error trials, the beginning of the new trial ITI was signaled by illumination of the house light.

Over the course of 4–5 days, the durations of Go and NoGo lever availability, Dp and Dn, respectively, were adjusted with the goal of setting them at 4 s and 4.5 s, respectively. On the first day Dp was set at 15 s, and as the rat learned to press more quickly it was gradually reduced to 4 s without disrupting performance (as evaluated by Go-errors). Dn was initially set at 1 s and was gradually increased to 4.5 s as the rat learned to withhold pressing of the lever. Thus, at the conclusion of phase 1, rats had a 4-s time window to press the Go lever and a 4.5-s interval in which they had to withhold pressing the NoGo lever. This ensured that the rat could not adopt a strategy of delaying the Go press until the NoGo interval had expired. This phase of training was similar to that of a discriminative stimulus paradigm, in which the Go cue/response was rewarded (DS+) and the NoGo response was not (DS−). Although this paradigm accomplished the goal of training rats to press on Go+ trials but not NoGo− trials, the lack of lever pressing on NoGo− trials occurred because these presses were not associated with reward. Ultimately, our goal was to train rats to withhold pressing in order to obtain a reward.

Phase 2: Go+/NoGo+ two-lever task. The goal of this phase of training was to train rats to associate NoGo cues with reward (+). In this phase, the following modifications were made to Go and NoGo trials to transition to the final symmetrical Go/NoGo task: otherwise the task sessions were the same. First, a cue light was illuminated above the NoGo lever when it was extended. Second, a 0.5-s white noise cue was assigned as either a Go or a NoGo cue for each rat and was presented simultaneously with presentation of the lever and cue light to which it had been assigned. The auditory cue was added to make the difference between Go and NoGo cues more salient. Third, correct NoGo trials were rewarded with a sucrose pellet. Thus the addition of the white noise, visual NoGo cue, and NoGo reward were used to facilitate association between these cues and their outcomes. Fourth, the timing between cue onset and reward delivery was roughly equated for correct Go and NoGo trials. On correct Go trials reinforcement (tone + pellet) was delivered 4 s after lever press, and on correct NoGo trials reinforcement (tone + pellet) was delivered immediately after lever retraction at 4.5 s. In this manner, the Go cue did not signal an immediately available reward, and rewards were similarly delayed from cue onset for all correct trials.

Symmetrical Go/NoGo task: Go+/NoGo+ single lever. During testing with the symmetrical Go/NoGo task, all trials began with presentation of the lever that had been assigned as “Go” during training (Fig. 1). Rats were given an instructional cue to press the lever on 75% of trials (Go) by illumination of the cue light above the lever simultaneous with its presentation. The remaining 25% of trials were NoGo, in which the same lever was presented simultaneously with the illumination of the cue light on the opposite side of the central pellet receptacle. This spatially distinct cue light was associated with the NoGo reward in the previous phase of training. For each rat, the 0.5-s white noise stimulus was maintained as a Go or NoGo cue (according to how it was assigned in the previous phase of training) and was presented simultaneously at the onset of that cue.

To summarize, for each rat, one lever was presented on every trial, with a cue light illuminated above it for Go trials and on the opposite side of the pellet tray for NoGo trials, and white noise was presented on either Go or NoGo trials. On Go trials, lever presses within 4 s were followed by sucrose pellet reinforcement after a 4-s delay. Failure to press on Go trials was followed by lever retraction and a 40-s time-out. On NoGo trials, withholding a lever press for 4.5 s was reinforced immediately upon lever retraction, while presses were followed by a 40-s time-out. After a correct trial, the next trial began with cue onset/lever extension after a 5–13-s ITI. During the time-out, the house light was extinguished and no trials were initiated. At the conclusion of error trials, the house light was illuminated and a 5–13-s ITI preceded the onset of the next trial. A session consisted of 150 correct trials.

Surgery. Once rats were trained to successfully perform the Go/NoGo task with 80% accuracy on Go trials and >50% accuracy on...
organized into two columns of four microwires (50-µm diameter; tip
<4 s) and sent digital outputs corresponding to each event to the
MAP System to be time stamped along with the neural data. During
the recording session, waveforms that matched templates generated by
principal component analysis (PCA) were collected as the same
neuron. Cell recognition and sorting was finalized after the experiment
with Offline Sorter (Plexon), which assessed neuronal data based on
PCA of the waveforms, cell firing characteristics, and interspike
intervals. Data were exported to MATLAB (MathWorks, Natick, MA)
and STATISTICA (StatSoft, Tulsa, OK) for statistical analyses.

Analysis of neural data. For each neuron, the timing of action
potentials was calculated from a -4 s to 8 s relative to trial onset (cue
presentation). Baseline activity was measured as the average firing
rate (sp/s) in the 4 s preceding cue presentation. We first tested
whether activity in the 8 s following trial onset, calculated in 0.5-s
bins across the population of neurons recorded, differed from baseline
by two-sided paired t-test with Bonferroni correction for multiple
comparisons. Next, for correct and incorrect trials separately, we
compared whether activity following Go and NoGo cues differed by
comparing average firing rate in 0.5-s bins with two-sided paired
t-tests with Bonferroni correction.

To identify individual neurons with phasic responses to cue onset,
which coincided with lever presentation, we calculated average firing
rate during the 1-s epoch following cue onset for increasing and decreasing
neurons separately to detect differences in response due to cue type
(Go/NoGo), accuracy (correct/error), and action (press/withhold) and
to determine interactions between these factors. Main effects were
tested with unequal N honestly significant difference (HSD) post hoc
tests.

We used regression models to test for differences between the
time courses of neural activity for different sets of trials. First, we were
interested in whether the time course of the neural response on correct
Go trials depended on the latency to lever press. For each rat, we
divided Go correct trials into “short”- and “long”-response time (RT)
trials based on the median RT for that test session. We modeled NAc
activity for these groups of trials by fitting

\[ Y = b_1 + b_2 T + b_3 I_{RT} + b_4 (T^* I_{RT}) + \varepsilon \]  

where \( Y \) is the firing rate in 100-ms bins, \( T \) is the time from cue onset
from 0.1 to 0.5 s, \( I_{RT} \) is an index for short- or long-RT trial, and \( T^* I_{RT} \)
is an interaction term to indicate a change in the time course of the
response between RT groups. The null hypothesis, that the slope
of the response does not depend on RT, is tested by setting \( b_4 = 0 \).

Next, we tested whether the time course of the response immedi-
ately following cue onset differed between trials in which the animal
presssed the lever (Go correct/NoGo error) and those in which pressing
was withheld (Go error/NoGo correct). We estimated the time course
of the neural response using

\[ Y = b_1 + b_2 T + b_3 I_p + b_4 (T^* I_p) + \varepsilon \]  

where \( Y \) is the firing rate in 100-ms bins, \( T \) equals time from cue onset
from 0.1 to 0.5 s, \( I_p \) is an index for press or no press, and \( T^* I_p \)
is an interaction term to indicate a change in the time course of the response
between groups. The null hypothesis, that the slope of the response
does not depend on the animal’s behavioral response, is tested by
setting \( b_4 = 0 \).

Histology. At the conclusion of testing, rats were injected with a
sublethal dose of pentobarbital sodium (100 mg/kg). Current (100
µA) was passed for 4 s through each electrode with a lesion-making device (Ugo Basile, Comerio, Varese, Italy) to mark recording sites. Rats were then transcardially perfused with physiological saline followed by 10% paraformaldehyde mixed with 3% potassium ferrocyanide. Brains were extracted and stored in paraformaldehyde-potassium ferrocyanide for at least 24 h. Potassium ferrocyanide reacts with iron deposited after lesions and causes a Prussian blue reaction product, which was used to help visualize electrode placements. After perfusion and storage, brains were sectioned at 50 µm in a cryostat (−20°C). Tissue was mounted on gelatin-subbed slides and viewed under a light microscope to verify electrode or cannula placement according to visual landmarks (Paxinos and Watson 2007).

RESULTS

Rats performed the Go/NoGo task with a high level of accuracy. Each trial began with the presentation of either a Go or a NoGo cue (75% or 25% of trials, respectively) simultaneous with the extension of the test lever into the chamber (Fig. 1). All correct trials—lever presses for Go, inhibition of pressing for NoGo—led to delivery of a sucrose pellet reward, while all errors—withheld response for Go, lever press for NoGo—resulted in a time-out. Average performance was 89.6 ± 1.4% correct on Go trials and 76.5 ± 6.4% correct on NoGo trials (Fig. 2A). Accuracy on Go trials tended to be higher than accuracy on NoGo trials [2-sided paired t-test on accuracy, t(10) = 1.81, P = 0.10, confidence interval (CI) = −3% to 29.2%]. The average number of completed trials in each session was 154.5 ± 29.2%. The average number of completed trials in each session was 31.4 ± 1.7 Go errors, and 9.6 ± 2.7 NoGo errors. Thus, on most trials, a Go cue was followed by the correct response of a lever press. The majority of NoGo cues resulted in an inhibition of pressing, although there were approximately equal numbers of trials in which rats failed to press when they should have or pressed when instructed to withhold.

When rats did press the lever, they responded quickly after the onset of the cue. The distribution of RTs for lever presses following Go (correct) and NoGo (error) trials is shown in Fig. 2B. Average RT on correct Go trials was 955 ± 24 ms, while presses following the NoGo cue were faster (677 ± 77 ms) (Fig. 2B). The pattern of faster latencies to press the lever on NoGo errors, compared with correct Go trials, was evident in 10 of the 11 rats tested (Fig. 2B, inset). Rats also engaged in making nose pokes at the central port where reward would be delivered during trials. Figure 2C shows the distribution of latencies to the first nose pokes at the central port following cue onset. The average latency to nose poke following Go cues was 2.61 ± 0.05 s, and that following NoGo cues was 3.06 ± 0.12 s (Fig. 2C). Across all rats, there was greater variability in the pattern of latency to nose poke following Go and NoGo cues (Fig. 2C, inset). The pattern of central nose pokes during trials provides evidence that rats attended to the central port on all trials—Go and NoGo—during the interval between cue and reward. The behavioral performance provides evidence that rats performed the task accurately and quickly, pressing the lever without delay in response to the more likely Go cue. To correctly withhold a press following NoGo cue presentation required rats to inhibit this prepotent tendency to press.

While rats performed the symmetrical Go/NoGo task, we recorded the responses of 127 NAc neurons. Ninety-two (72.4%) of these neurons were histologically verified to be localized within the core region of the NAc, while the remaining 35 neurons (27.6%) were localized within the NAc shell. Figure 3 shows the locations of the electrode tips from which the neurons were recorded. The categorization of neurons according to their individual response properties is discussed below. Our main focus was on the pattern of responses to the cue onset that initiated each trial. Core and shell neurons did not show significantly different patterns of activity in response to Go or NoGo cue onset [region × cue type: F_1,119734 = 0.11, P = 0.75; main effect of region: F_1,119734 = 0.87, P = 0.35; main effect of cue type: F_1,119734 = 13.47, P < 0.001]. Therefore, analyses were based on all 127 neurons verified to be located within the NAc. At the onset of each trial, signaled by the simultaneous occurrence of cue presentation and lever

![Fig. 2. Rats performed the Go/NoGo task accurately and did not delay execution of responses. A: the proportion of correct trials was high for both Go (89.6 ± 1.4% of 1,248) and NoGo (76.5 ± 6.4% of 451) trials. B: response times (RTs) are shorter for lever presses in error on NoGo trials than correct presses on Go trials. Distributions of RT are shown for correct Go trials (black, n = 1,118) and error NoGo trials (gray, n = 106). Average RT for presses following the NoGo cue (677 ± 77 ms, gray arrowhead) was shorter than that for the Go cue (955 ± 24 ms, black arrowhead). Inset: mean RT for lever presses on NoGo trials plotted as a function of mean RT for Go presses for each rat. For 10/11 subjects, RT was faster for incorrect NoGo presses than correct Go presses, suggesting that rats' failure to inhibit presses occurred in the immediate response to the cue. C: latency to nose poke at the central port is shorter after Go cues (2.61 ± 0.05 s) than NoGo cues (3.06 ± 0.12 s). Inset: mean latency to nose poke after cue onset on NoGo trials plotted as a function of mean latency to nose poke after Go cue for each rat. Although the latency is shorter on Go trials, there is not a consistent pattern of behavior between subjects.

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extension, the average firing rate across 127 neurons showed a transient increase in activity (Fig. 4A). The green curve in Fig. 4A shows the average firing rate (+1 SE in sp/s) for all Go trials, both correct and error combined, and the red curve shows the average response for all NoGo trials. The horizontal green and red lines beneath the firing rate traces in Fig. 4A indicate epochs (in 0.5-s time bins relative to cue onset) in which firing rate differed from baseline level. During the first 0.5 s following cue onset, firing rate transiently increased from baseline activity on both Go and NoGo trials, although activity was higher after the NoGo cue (2.49 ± 0.06 sp/s) compared with the Go cue [2.28 ± 0.03 sp/s, t(19,736) = 3.40, P < 0.001]. The response on Go trials then dropped below baseline during the delay period when rats were waiting for either reward delivery (which occurred at 4.99 ± 0.02 s) or lever retraction (at 4 s). After trial termination by reward delivery or lever retraction, average activity on Go trials fell below baseline again briefly, during the time of reward consumption. On NoGo trials, activity returned to baseline within 1 s and did not differ from baseline until a transient increase in activity when the lever retracted and a reward was delivered on correct trials (4.5 s after cue), which was followed by a brief reduction in activity.

The bias in firing rate to higher levels on NoGo trials following cue onset reflects the trend in the activity of the population of individual neurons recorded. For each neuron, we computed the difference in firing rate between the baseline level of activity and the response during the first second of the Go and NoGo cues separately. Figure 4B shows a scatterplot for the 127 neurons studied, with the change in firing rate in response to the NoGo cue plotted as a function of the change in firing rate in response to the Go cue. The diagonal line of unity marks equal responding to both cues. As such, points that fall above the line had a larger-magnitude response to NoGo cues and points below the line showed a larger response to Go cues. Neurons that had a significant change in firing rate at cue onset are outlined in black in Fig. 4B, while those that did not respond with a significant difference are outlined in gray; points filled with red had a significantly higher response to the NoGo cue compared with Go, while those filled with green had a significantly higher response to the Go cue. The yellow arrow in Fig. 4B marks the average change in firing rate from baseline in response to Go (x-component) and NoGo (y-component) cues, such that the response to NoGo cues was 0.21 sp/s higher than the response to Go cues (CI = 0.09–0.33). This small magnitude of firing rate difference persists despite the averaging together of trials in which the cue differed (Go vs. NoGo), the behavioral responses differed (press vs. withhold), and neurons exhibited different response profiles (increase, decrease, or no change in firing rate). We examine the contribution of each of these factors to the modulation of NAc responses below.

Because rats’ performance included errors on both Go and NoGo trials, it is difficult to ascertain whether the differences between Go and NoGo responses in Fig. 4A are related to the cues themselves or the behavior that followed. Figure 4, C and D, show correct and error trials separately, so that only trials with the same cue and behavioral response contribute to each average. All correct Go and NoGo trials for all neurons recorded are shown in Fig. 4C. These responses bear strong resemblance to those in Fig. 4A, as the majority of trials were completed with the correct response. Here responses to cues were higher for NoGo trials in the first 1.5 s of the trial (Fig. 4C; horizontal red line indicates NoGo > Go). There was a brief increase in activity on correct NoGo trials when the lever retracted and reward was delivered at the end of the delay period. Unlike NoGo trials, a large, transient response at the time of reward delivery at ~5 s after Go cue onset was absent. Neural responses on error trials (Fig. 4D) showed a different pattern of activity. Responses to cue onset were higher for Go trials in the first 0.5 s of the trial (horizontal green line indicates Go > NoGo). Error trials concluded and the time-out period commenced upon lever retraction for Go trials and lever press for NoGo trials. For all errors, there is a brief increase in activity when the time-out begins, which is signaled by extinguishing the house light in the operant chambers. For both correct and error trials, greater levels of neural activity at the
time of cue onset were associated with withholding of the behavioral response; thus the overall difference across all trials (Fig. 4A) may be attributable to a greater proportion of withheld responses for NoGo than for Go trials. These results suggest that elevated neural responses were associated with withholding the behavioral act of lever pressing rather than the NoGo cue itself, but, again, the magnitude of the difference is small, potentially because of the averaging together of neurons with different response profiles.

The overall modulation of activity shown in Fig. 4 was driven by individual neurons that responded to cue onset with transient changes in firing rate. Because differences between Go and NoGo cue responses were evident in the first 1.5 s of correct trials and the first 0.5 s of errors, we identified which neurons exhibited a change in firing rate relative to their baseline activity during the first second of Go trials. We found that the activity of 43 neurons responded at the onset of the trial with a significant increase in firing rate (INC; 32 core, 11 shell), while 22 neurons responded with a significant decrease in firing rate (DEC; 18 core, 4 shell). The distribution of INC and DEC neurons along the anterior-posterior axis did show a significant interaction between the subregion (core, shell) × response type (INC, DEC, nonphasic); $F_{2,121} = 345, P < 0.05]$. While there was no systematic relationship between the location of neurons and response type in the core, DEC neurons were located at more posterior locations in the shell than INC neurons (Tukey HSD, $P < 0.01$). An example
of an increasing neuron is shown in Fig. 5A. Go and NoGo trials are shown separately, aligned to the time of cue onset. In this example, the neuron’s firing rate increased from a baseline of 2.05 ± 0.14 sp/s to 5.79 ± 0.43 sp/s in response to the Go cue and to 10.97 ± 1.20 sp/s in response to the NoGo cue. A second group of neurons exhibited decreased firing rate at the onset of the cue, as shown in Fig. 5B. This neuron had a baseline firing rate of 2.43 ± 0.09 sp/s, which decreased to 1.65 ± 0.17 sp/s at the onset of the Go cue but remained at 2.28 ± 0.30 sp/s on NoGo trials. Although the population (Fig. 4A) and individual neuron (Fig. 5) data suggest differences in the neural responses to cues, these differences may also be related to whether they executed a correct behavioral response, as suggested by Fig. 4, C and D. Therefore, we examined the subgroups of neurons that showed increase or decrease in firing rate separately to determine whether their cue responses were modulated by whether their subsequent behavior was correct or not.

For INC neurons, larger elevations in firing rate were associated with withheld lever presses following both Go (error) and NoGo (correct) trials. In Fig. 6A, onset of the Go cue evoked larger elevations in activity on trials in which the rats failed to press the Go lever than on correct trials in which they did press. NAc activity to the Go cue increased in the first second for both correct and error trials and then decreased below baseline level from 1.5 to 7 s after trial onset for correct trials. At the time of reward delivery (at ~5 s after cue), firing rate remained below baseline level until after reward consump-

tion, when activity returned to baseline. For error trials, activity returned to baseline after the transient cue response and then increased again at 4 s, when the lever retracted and reward was not delivered. After the onset of the Go cue, firing rate rose from a baseline of 1.75 ± 0.03 sp/s to a maximum level of 3.57 ± 0.11 sp/s for correct trials and 4.60 ± 0.43 sp/s for errors. The average magnitude of the neural response during the first second of the cue epoch was lower for Go cues followed by lever presses (2.61 ± 0.05 sp/s) compared with Go cues followed in error by withheld responses (3.23 ± 0.20 sp/s, P < 0.05; Fig. 6A, inset).

The population of INC neurons showed a similar pattern of activity in response to NoGo cues (Fig. 6B). On correct NoGo trials, neural activity increased above baseline both at the time of cue onset (0–1.5 s) and at lever retraction/reward delivery (4.5–6 s). On NoGo trials in which rats pressed the lever in error, there was also an increase in firing rate in the first second of the trial, followed by a return to baseline. Again, the response of increasing NAc neurons to the same NoGo cue differed depending on whether the animal selected the correct behavioral response. After the onset of the NoGo cue, firing rate rose to a maximum level of 4.51 ± 0.25 sp/s for correct trials and 2.73 ± 0.39 sp/s for errors. The activity during the first second of the cue period did not reach as high a level for error trials, in which the lever was pressed (2.25 ± 0.19 sp/s), compared with correct trials, in which rats withheld pressing the lever (3.36 ± 0.12 sp/s, P < 0.001; Fig. 6B, inset). On NoGo trials, this pattern of activity is due to both the lower baseline firing rate on error trials compared with correct trials (1.17 ± 0.09 vs. 1.91 ± 0.06 sp/s, P < 0.0001) and a smaller increase in firing rate over baseline during the first second of the trial on errors (1.08 ± 0.16 vs. 1.45 ± 0.10 sp/s, F = 6.31, P < 0.05). Overall, for increasing neurons, there was a significant interaction between cue type (Go/NoGo) and accuracy (correct/error) for the cue response (F(1,6666) = 36.94, P < 0.00001) but no main effect of cue type (F(1,6666) = 0.59, P = 0.44) or whether the response was correct (F(1,6666) = 2.88, P = 0.09). This interaction can be explained by the overall larger increase in activity on trials in which lever pressing was withheld. This pattern of activity did not depend on which NAc subregion neurons were recorded from (region × cue type × accuracy: F(1,6662) = 1.82, P = 0.18). Thus, regardless of cue type, higher elevations in activity were associated with suppression of the behavioral response, whether appropriate (NoGo cue) or in error (Go cue).

In the group of neurons that showed reductions in firing rate following cue onset, greater decreases in activity level were associated with the execution of lever pressing. Figure 6C shows the average response of 22 decreasing neurons on Go trials. The onset of the cue resulted in a transient decrease in activity from baseline (2.28 ± 0.05 sp/s) for correct trials to a minimum of 1.09 ± 0.08 sp/s on correct trials, remaining significantly below baseline for the first 3 s of trials on correct trials. During the first second after cue onset, the average firing rate was 1.40 ± 0.04 sp/s (Fig. 6C, inset). In contrast, on Go error trials, firing rate transiently decreased to a minimum of 1.41 ± 0.22 sp/s and averaged 2.17 ± 0.18 sp/s during the first second following cue onset (Fig. 6C, inset). This brief and smaller-magnitude decrease in activity over the first second differed from correct trials (P < 0.001) but not baseline. On NoGo trials (Fig. 6D), this same group of neurons showed a

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**Fig. 5.** Examples of single neurons with significant modulations of activity at the onset of the Go cue and lever presentation. A: NAc neuron with significantly higher activity during the 1-s epoch following Go trial onset. Top: raster plots in which each row show the time of action potentials relative to cue onset (time = 0 s) for 1 trial. All Go trials (left), both correct and error combined, are shown separately from all NoGo trials (right). Bottom: PEHs show the firing rate averaged in 100-ms bins. In this example, the NoGo cue elicited a significantly higher level of activity than the Go cue during the first second of the trial. B: NAc neuron with a significant reduction in activity in the 1-s epoch following Go trial onset. Same conventions as A. This neuron showed a significant decrease in activity in response to the cue on Go trials but was not modulated on NoGo trials.
small reduction in firing rate for correctly withheld lever presses (1.76 ± 0.09 sp/s) and larger decreases in activity when rats pressed the lever in error (1.18 ± 0.15 sp/s, P < 0.01; Fig. 6D, inset). For both NoGo correct and error trials, decreases in firing rate below baseline were transient and modulations from baseline were not observed for the remainder of the trial period. Overall, the cue response of NAc decreasing neurons was modulated by an interaction between cue type (Go/NoGo) and accuracy (correct/error) (F₁,₃₄₆₁ = 30.72, P < 0.00001). There was no main effect of accuracy on neural response (F₁,₃₄₆₁ = 0.64, P = 0.43), but there was a main effect of behavioral response (press/withhold: F₁,₃₄₆₁ = 6.84, P < 0.0001). This pattern of activity did not depend on which NAc subregion neurons were recorded from (region × cue type × accuracy: F₃,₃₄₅₇ = 1.00, P = 0.32).

The pattern of results reveals a higher level of activity in NAc neurons for trials in which the rats did not execute the behavioral response of pressing the available lever—for both correct NoGo and incorrect Go trials. This elevation in response is driven by larger increases and smaller reductions in activity at the time of cue presentation on trials in which the rats ultimately withhold lever presses. We considered the possibility that these higher levels of activity might persist longer on trials in which no press occurred because the act of pressing could have engaged circuitry that suppressed the NAc response, and thus the differences observed here may be a consequence of action commission rather than a differential response to cues that biased action selection. To address this question, we examined the NAc population response separately for Go trials with different response latencies. For each rat, we represented in both groups. The mean RTs for the short-RT and long-RT trials were 510 ms and 1,442 ms, respectively. Figure 7 shows the average firing rate across all neurons, grouped by RT for Go correct trials and plotted to the median RT for each group. Responses during this time frame are also shown for trials in which lever pressing was withheld (P < 0.01).

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Go correct trials (Eq. 1, $H_0: b_4 = 0, P = 0.10$). However, we did verify that the rate of decline in activity was more rapid for trials in which the rats pressed the lever compared with those in which they did not press (Eq. 2, $H_0: b_4 = 0, P < 0.001$; fits shown in black in Fig. 7). Thus it appears that the weaker response and rapid decline following cue onset precede the commission of the lever press and are similar regardless of press RT.

DISCUSSION

The NAc plays a role in establishing cue-outcome relationships as well as cue-evoked goal-directed behavior (Smith-Roe and Kelley 2000; van der Meer and Redish 2011; Yawata et al. 2012). NAc neurons transiently respond to reward-predictive cues as well as during goal-directed behaviors (Carelli 2002; Day et al. 2006), which are strongly correlated in most studies of operant responding. Here we measured the activity of individual NAc neurons while rats performed a Go/NoGo task with symmetrical outcomes. In this task, correct responses for all trials—Go and NoGo—were rewarded and all errors were followed by a time-out period from the task. Thus both cues predicted the availability of reward but signaled that different behavioral responses were required to obtain them. Rats performed this task with a high level of accuracy on both Go and NoGo trials, with short response latencies on both Go correct responses and NoGo errors, indicating that rats were biased to press the lever when it was presented. However, on the majority of NoGo trials they succeeded in inhibiting this behavioral response according to the instructional cue. Since both cues here are reward predictive, we would hypothesize that both cues would elicit equivalent responses if reward expectation drove the neural response. Although there was not a significant difference in accuracy between Go and NoGo trials, there was a trend for Go trials to be more accurate, and therefore more frequently rewarded (89% vs. 76%).

Previous work has shown that dopamine neurons show increasingly strong responses to cues as their certainty in predicting reward increases from 0 to 100% (Fiorillo et al. 2003). If the NAc exhibited similar responding, we would predict slightly stronger responses to the Go cue compared with the NoGo cue. Alternatively, it has been suggested that NAc biases motor systems toward appropriate behavioral responses, such as to approach or withdraw (Ambroggi et al. 2011; Carlezon and Thomas 2009; Krause et al. 2010), which predicts that NAc responses would differentiate motor plan, rather than reward expectation. The symmetrical Go/NoGo task allowed us to dissociate whether NAc responses are equivalent because of similar reward-association, stronger for Go cues because they may be slightly more reward predictive, or associated with the subsequent act—to engage in or withhold lever pressing.

We found that the response of NAc neurons across the entire population recorded to instructive reward-predictive cues was more closely associated with the subsequent behavioral response rather than reward expectation. That is, transient responses to the Go or NoGo cue elicited higher levels of activity on trials in which rats withheld the production of a lever press, even when, in the case of Go errors, such restraint led to reward omission (Fig. 4). In the population, the transient cue response was weaker on both correct Go and incorrect NoGo trials, in which rats subsequently pressed the lever. The pattern of activity, with stronger cue responses on correct NoGo trials relative to Go, was reversed for error trials, for which Go cues elicited a stronger response than NoGo cues. Thus the overall elevated NAc response correlated with the behavior produced rather than specific cue identity or whether the trial was rewarded or not. This pattern of population activity was driven by subpopulations of neurons that showed transient changes in activity at the time that the cue and lever were presented to trigger a behavioral response (Figs. 5 and 6). We found that both subpopulations responded to both Go and NoGo cues, but the differences in neural activity did not correlate specifically with whether the behavioral response was correct or an error but rather with whether the lever press would be performed or withheld. Two-thirds of the neurons that responded to cue onset/lever presentation showed a brief increase in activity. Regardless of whether the cue instructed a Go or NoGo response, these increases had a larger magnitude on trials in which lever pressing was withheld, whether correctly (NoGo) or in error (Go). The remaining third of neurons that responded to cue onset/lever presentation showed a reduction of activity. Larger decreases in activity preceded approach and lever pressing, whether correct (Go) or in error (NoGo). Thus the data suggest that shifts in the levels of excitation and inhibition in the NAc participate in a more subtle, moment-to-moment, control of behavior. The overall shift toward elevated activity preceding behavioral inhibition suggests that excitation in the NAc may support the encoding of a salient stimulus, holding behavior in check. This is beneficial when withholding a response is appropriate but perhaps disruptive of a prepotent, prepared response.

Previous findings offer a model for how this balance of decreases and increases in NAc activity may bias the animal toward action or restraint. In the dorsal striatum, optogenetic activation of dopamine D1 receptor-expressing medium spiny output neurons (MSNs) projecting to the substantia nigra...
tions of the NAc have been shown to bias animals toward action. This supports the intriguing possibility that cortico-striatal circuits may be crucial in providing an excitatory stop signal to exert inhibitory control over behavior.

We speculate that excitatory inputs to the NAc influence control over behavior in this task. Pharmacological manipulations of the NAc have been shown to bias animals toward approach or inhibition of action. μ-Opioid agonists, which exert an inhibitory effect on NAc neurons, elicit feeding in satiated rats (Smith and Berridge 2005). Pharmacological inhibition of the NAc shell in particular appears to disinhibit behavioral responding (Ambroggi et al. 2011). Our finding that reduced NAc activity was correlated with approach and behavioral responding is consistent with these pharmacological effects. Our results also suggested that elevations in activity were associated with behavioral restraint. One source of glutamatergic inputs into the NAc that may serve to inhibit goal-directed behavior is the prefrontal cortex (PFC). Regions of the PFC have been shown to be necessary for successful inhibition of behavior in stop-signal reaction time tasks (Bari et al. 2011). Inactivation of NAc-projecting subterritories of the PFC can lead to an increase in inappropriate responding (Ghazizadeh et al. 2012) and behavioral disinhibition (LaLumiere et al. 2012). Top-down control over striatal regions in general appears critical in tests of response inhibition in human subjects (Jahfari et al. 2011). Consistent with the idea that excitatory input is necessary for restraint, we observed that responding in error on NoGo trials was preceded by lower baseline firing rates in increasing neurons (Fig. 6B). This lower level of activity may have resulted in a bias for behavioral approach, in that a larger excitatory drive would be needed to sufficiently halt the initiation of the behavioral response.

Using a symmetrical Go/NoGo task, we have found that transient responses of NAc neurons to reward-predictive cues that instruct behavior show a stronger correlation with initiation of action than accuracy. The modulations of firing rate are evident in populations of neurons that increase and decrease activity concurrently in response to cue presentation for all trials, with the strength of response predicting whether rats will engage in behavior or restrain it. Behavioral restraint was associated with larger elevations from increasing neurons and smaller reductions from decreasing neurons. These findings suggest that active engagement of circuitry in the NAc contributes to the ability to restrain behavior, a critical component of impulsivity.

REFERENCES


