Beyond the Reward Pathway: Coding Reward Magnitude and Error in the Rat Subthalamic Nucleus

Sylvie Lardeux, Remy Pernaud, Dany Paleressompuolle, and Christelle Baunez

Laboratoire de Neurobiologie de la Cognition, Centre National de la Recherche Scientifique Unité Mixte de Recherche 6155, Aix-Marseille Université, Marseille, France

Submitted 8 September 2008; accepted in final form 19 August 2009

Lardeux S, Pernaud R, Paleressompoule D, Baunex C. Beyond the reward pathway: coding reward magnitude and error in the rat subthalamic nucleus. J Neurophysiol 102: 2526–2537, 2009. First published August 26, 2009; doi:10.1152/jn.91009.2008. It was recently shown that subthalamic nucleus (STN) lesions affect motivation for food, cocaine, and alcohol, differentially, according to whether the nature of the reward or the preference for it. The STN may thus code a reward according to its value. Here, we investigated how the firing of subthalamic neurons is modulated during expectation of a predicted reward between two possibilities (4 or 32% sucrose solution). The firing pattern of neurons responding to predictive cues and to reward delivery indicates that STN neurons can be divided into subpopulations responding specifically to one reward and less or giving no response to the other. In addition, some neurons (“oops” neurons) specifically encode errors as they respond only during error trials. These results reveal that the STN plays a critical role in ascertaining the value of the reward and seems to encode that value differently depending on the magnitude of the reward. These data highlight the importance of the STN in the reward circuitry of the brain.

INTRODUCTION

A major challenge in the field of addiction is to understand if and how the brain differentiates natural rewards and drugs of abuse, since indeed, all rewards act on the reward circuit, involving the mesocorticolimbic pathway. To date, only lesions of the subthalamic nucleus (STN) have shown differential behavioral responses to different rewards. Such lesions increase motivation for food (Baunez et al. 2002) while decreasing motivation for cocaine assessed by conditioned place preference or by motivation to produce an effect to obtain the reward (Baunez et al. 2005). The initial level of preference for the reward also plays a role in STN effects on motivation because STN lesions increase motivation for alcohol in outbred rats showing a preference for alcohol (i.e., “high drinker”), and decrease it in rats showing a lower alcohol preference (i.e., “low drinker”) (Lardeux and Baunez 2008).

This focus of STN involvement in motivational functions is recent. Indeed for many years, the STN has been mainly considered as a motor structure, particularly in light of the obvious motor consequences of its lesion, such as ballism (Whittier 1947), or its reactivity in movement disorders such as Parkinson’s Disease (PD). The involvement of the STN in cognitive functions has also been demonstrated in clinical and experimental studies (Baunez and Robbins 1997; Eagle et al. 2008; Temel et al. 2006; Trillet et al. 1995; Voon et al. 2006; Winstanley et al. 2005; Witjas et al. 2005).

The STN is a major component and the sole excitatory nucleus of the basal ganglia. Much like the striatum, the STN is an input structure, receiving direct projections from the cortex and the thalamus (Afsharpour 1985; Sugimoto et al. 1983). The STN also receives projections from the striatum, the pallidum, and the midbrain dopaminergic nuclei. The STN is thus in a key position within the cortico-basal ganglia-thalamocortical loops to modulate basal ganglia outflow (Maurice et al. 1998a,b).

Interestingly, in various structures connected directly or indirectly with STN, such as orbitofrontal cortex or striatum, electrophysiological data have reported neuronal responses modulation to different rewards (Bowman et al. 1996; Carelli et al. 2000; Cromwell and Schultz 2003; Hassani et al. 2001; Roesch and Olson 2004; Roesch et al. 2006; Setlow et al. 2003; Tremblay and Schultz 1999). Other studies have shown that STN neurons respond to cues predicting reward and delivery of that reward (Darbaky et al. 2005; Matsumura et al. 1992; Teagarden and Rebec 2007). However, none of these results provide a clear picture of how the STN encodes reward value. Therefore demonstrating that STN neurons encode specifically different rewards might provide neurophysiological basis for our previous behavioral findings.

In the present work, we recorded STN neurons in rats performing a customized task derived from the discriminative stimuli reaction-time task (Hauber et al. 2000) using two rewards, differing only by their sweetness: 4 versus 32% sucrose solutions. In this task, discriminative cue lights predict the upcoming reward. This procedure allows the recording of neuronal responses during reward expectation and reward delivery in the same conditions for two rewards differing only by their magnitude.

METHODS

Animals

Male Long-Evans rats (n = 7; Janvier, Le Genest St Isle, France), weighing 350–400 g at the time of their surgery, were maintained on a 12-h light-dark cycle at an ambient temperature of 21°C. Before surgery, animals were housed in pairs in clear Perspex cages (42 × 26.5 × 18.5 cm). After electrode implantation, they were housed individually. During the entire experiment, rats were kept at 85% of their free feeding weight by restricting their food to 15–20 g/day per rat. Water was provided ad libitum, except during testing sessions. All procedures were conducted in accordance with the French Agriculture and Forestry Ministry decree 87–849.
STANDARD CONDITIONS. At the beginning of the session, the house light was turned on and the lever extended. Rats were trained to press the lever for 1 s. During this period, one of the two cue lights was randomly illuminated for 100 ms, 400 ms after the start of the lever press. A trigger tone was delivered 500 ms after the extinction of the light, indicating that the rat could release the lever and the cued reward was delivered (Fig. 1). Each cue light (either right or left of the lever) was associated with a specific reward if the response was correct (i.e., release of the lever after the tone). Half of the rats were trained with the following rule: left light indicated that 4% sucrose was the reward and right light indicated that the reward was 32% sucrose. The other rats were trained with the opposite rule (i.e., left light: sucrose 32%, right light: sucrose 4%). Immediately after the rat released the lever, this latter was retracted and the pump was activated. Detection of the animal’s head entry in the magazine after a correct lever release began a 5-s intertrial interval. Anticipatory lever releases (release before the trigger tone) were not rewarded and led to the retraction of the lever and to the extinction of the house light for 2 s. Anticipatory lever releases were recorded independently. Each session ended after 120 trials (60 trials with each reward randomly distributed) or if 30 min had elapsed. The timestamp clocks for behavioral events were sent to the electrophysiological recording system to enable subsequent evaluation of neuronal activity related to each behavioral event.

The animals were subjected to this task for 20–30 standard recording sessions (except for 2 rats, which lost their electrodes after 12 and 13 sessions).

CHALLENGES: CHANGES IN REWARD MAGNITUDE. For two sessions, 32% sucrose was replaced by water, leading to a possibility for the animals to get either 4% sucrose or water (4% becoming then the most salient reward; challenge 1, n = 4 rats). Then 32% sucrose was reintroduced, but 4% sucrose was replaced by water for another two sessions (challenge 2, n = 3 rats). Each challenge session was followed by a session in standard conditions.

Electrophysiology

Each multiwire electrode bundle comprised eight formvar-insulated 25-μm nichrome electrodes threaded through a 26-gauge stainless steel cannula that served as the ground. Rats were placed in the operant chamber and connected to a multichannel commutator (Crist Instruments) via a length of flexible cable that allowed the rat free movements. Electrophysiological signals were transmitted to a preamplifier (Neuralynx) that was located on the headstage, then to an amplifier (Neuralynx) and to data-acquisition hardware (Datawave Technologies). The recording of neuronal activity began every day before the start of the behavioral session, so that the threshold on the various channels could be determined. All waveforms exceeding an amplitude threshold (2 × above the noise level) were recorded. Unit discriminations were performed off-line using Sciworks clustering software (Datawave Technologies). In many cases, more than one waveform shape could be isolated on a single wire. When these waveforms could not be easily separated, they were discarded from the analysis. Autocorrelograms were then constructed for each unit using Neuroexplorer (Nex technologies), and units without 1.2-ms refractory periods were either rejected or re-sorted. Although a sample of several hundred units was recorded, it is likely that some signals were recorded more than once over the course of the experiment for different sessions. To avoid analyzing twice the same neuron in consecutive sessions, waveform of neurons recorded on the same electrode were compared according to the Grossman et al. analysis (Grossman et al. 2008). Briefly, multivariate ANOVAs allowed the comparison of the waveform recorded in the same electrode between two consecutive sessions. If the waveforms had a probability <0.001 to be different, the two neurons were considered as different, otherwise, they were considered as the same neurons and the second recording of this neuron was discarded from the analysis. Furthermore, if two neurons recorded on the same wire during two consecutive sessions, and considered as different, responded to the same event in these consecutive sessions, the second neuron was also discarded from the analysis.

Data analysis

BEHAVIORAL ANALYSES. The behavioral performance with regard to the two different rewards [anticipatory responses, reaction time (i.e., latency between the tone onset and the lever release) and movement time (i.e., latency between the lever release and the magazine entry)] were recorded and compared with a t-test using Statview (SAS Institute, Cary, NC).
ELECTROPHYSIOLOGICAL ANALYSES. Analyses were based on binned perievent firing rates (50-ms bins) obtained for each session. For each event, a perievent time histogram (PETH), centered on that event, was made with Neuroexplorer. The neuronal response to the different events were analyzed separately for the two different rewards: lever press, cue light, trigger tone, lever release, and magazine entry. The responses to the cue light was analyzed separately for correct and error trials. The responses at anticipatory lever releases were also analyzed separately from all correct lever releases.

As there are many events occurring within a short period in this task, and as STN neurons may respond to several events, it was necessary to compare the neuronal activity following an event with the basal neuronal activity just before it to demonstrate the neuronal responses to this specific event. Indeed we were interested in the specific neural response to each event individually, whether or not the neuron had responded to the previous event. Indeed, if a neuron exhibited a strong excitation after the lever press (which could have been expected with STN neurons regarding STN role in motor functions), followed by an inhibition or no response after the cue light, with a baseline taken during the intertrial interval, the analysis would have indicated that this neuron was excited by the cue light instead of revealing an inhibition or no response. Each response to an event was analyzed for 500 ms after the event (except for the lever press that occurred 400 ms before the cue light) and compared with the activity, 400 ms before this event. The baseline lasted 400 ms because it was the shortest period between two consecutive events (the lever press and the illumination of the cue light).

As there was often an anticipatory response before the lever press and before the magazine entry, the basal activity taken was between –600 and –200 ms before the event.

Analyses were performed according to Teagarden’s analysis (2007). Briefly, each perievent bin was expressed as a z score based on the mean ± SD of the firing rate during the respective basal period of this event. Three or more consecutive bins (>150 ms) with z scores > 1.64 SD (95% confidence interval) away from the baseline mean firing rate were considered as an activation or an inhibition. To investigate the neuronal response during the consummatory phase, long-lasting responses after magazine entry were analyzed with 500-ms bins, for a period of 8 s after magazine entry.

The firing rate of neurons that responded to one event for both rewards were compared with a t-test on the normalized data using Statview (SAS Institute). The average responses of neuronal populations to one event were analyzed with an ANOVA with the different neuronal subpopulations responding to each reward as between factors and the event as within factor when appropriate. In addition, the average neuronal responses of the populations exhibiting either inhibition or activation were analyzed separately. When a significant effect was found, post hoc comparison was performed using the Student-Newman-Keuls (SNK) test to correct the type I error.

The proportions of neuronal populations were compared with a χ² test.

Histology

At the end of the experiment, all rats were decapitated. The brains were removed, frozen and cut in coronal section with a cryostat. Frontal 40-μm-thick sections of the STN were stained with cresyl violet for assessment of the electrode placement.

RESULTS

Histology

Of the 14 electrode bundles implanted in the seven rats, 12 had their tips within the STN. Therefore the data for these 12 electrode bundles were kept for the analysis. In most cases, the electrodes were in the medial half of the STN (Fig. 2, A and B).
and the number of anticipatory lever releases did not differ between rewards. The shorter movement time for 32% sucrose, when compared with 4% sucrose, indicates that the rats discriminated the two cue lights as well as the two rewards and suggests a preference for 32% sucrose. This behavioral observation supports the electrophysiological responses in relation to the reward.

Neurons

A total of 380 isolated neurons (Fig. 2D see typical waveform) were recorded, of which 78.68% (299/380) responded to at least one event of the task. Of these, only 21.84% (83/389) responded to only one event. These neurons had a mean firing rate of 6.32 ± 0.51 Hz for the entire session (Fig. 2E). This relatively low firing rate is in line with rates reported in recent studies carried out in freely moving rats (Shi et al. 2004; Teagarden and Rebec 2007).

For each event, neurons were classified in different categories (Table 1). Neurons were “specific” if they responded to one event for one reward only (“exclusive neurons”) or for both rewards with significantly higher responsiveness to one reward than the other (“selective neurons”; t-test; P < 0.05). For example, one neuron responding only after the cue light announcing 32% sucrose (exclusively 32%) and one neuron that responded more after the cue light announcing 32% sucrose than after that announcing 4% sucrose were classified as specific 32% for the cue light. Neurons were “similar” neurons if they responded in a similar manner for both rewards (t-test; P > 0.05).

Reward expectation activity

We first investigated if STN neurons encode differentially, the incentive stimulus (the cue light announcing the reward) and the expectancy phases for the two rewards (32 and 4% sucrose). The expectation phase began when one of the cue lights predicting the reward was illuminated and ended when the rat obtained the reward. Three events took place during this phase: the cue light, the trigger tone, and the lever release. The cue light was illuminated while the rat was holding its paw on the lever, so the motor behavior was held constant. The measurement of neuronal responses to incentive cue lights was therefore facilitated. The trigger tone was a nonlateralized diffuse stimulus indicating that the rat could release the lever and the neuronal response could reflect sensory activation or motor preparation for lever release. As neuronal activity in the orbitofrontal cortex, that sends projections to the STN, is modulated by the movement direction (Feierstein et al. 2006), we delivered both rewards in the same magazine to ensure that the movements required to reach the reward were nonlateralized and identical for both rewards. The differential modulation of neuronal activity after lever release and at reward delivery according to the predicted reward is thus unlikely to be due to a different movement or direction of the animal.

To study the responses during the expectation phase of both rewards, each neuronal response was analyzed after the cue light, after the trigger tone, and after lever release.

During the expectation phase, 29.10% of the neurons (87/299) responded after the cue light, 40.80% (122/299) after the trigger tone, and 35.12% (105/299) after the lever release. Activation and inhibition were observed after each event (Table 2). Interestingly, inhibition was mostly seen after the cue light, whereas activation was predominantly observed after the other events.

After the cue light, trigger tone and lever release, the response of most neurons was modulated by the expected reward (86.21, 84.43, and 81.90% of specific neurons, respectively; Table 1). Figure 3, A and B, shows examples of the different activities in response to the cue light for one specific 32% (A) and one specific 4% (B) sucrose neurons. The raster are centered on the occurrence of the cue light (t = 0) announcing 32% sucrose (left) and 4% sucrose (right). On the top, the raster plot of spike firing on each trial is represented with the top row dots corresponding to the first trial. At the bottom, the mean firing rate across all trials is represented, the bin size is 50 ms. The consecutive bins showing a significant different firing rate than the baseline are represented in gray. The first vertical black line indicates the occurrence of the cue light and the second vertical black line indicates the end of the 500-ms analyzed window (see also another example of differ-

<table>
<thead>
<tr>
<th>Event</th>
<th>Total</th>
<th>Exclusive 32%</th>
<th>Specific 32%</th>
<th>Total</th>
<th>Exclusive 4%</th>
<th>Similar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct trials:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cue light</td>
<td>43.7 (38/87)</td>
<td>39.1 (34/87)</td>
<td>42.5 (37/87)</td>
<td>40.2 (35/87)</td>
<td>13.8 (12/87)</td>
<td></td>
</tr>
<tr>
<td>Trigger tone</td>
<td>42.6 (52/122)</td>
<td>36.9 (45/122)</td>
<td>41.8 (51/122)</td>
<td>36.1 (44/122)</td>
<td>15.6 (19/122)</td>
<td></td>
</tr>
<tr>
<td>Lever release</td>
<td>41.0 (43/105)</td>
<td>32.4 (34/105)</td>
<td>41.0 (43/105)</td>
<td>36.2 (38/105)</td>
<td>18.1 (19/105)</td>
<td></td>
</tr>
<tr>
<td>Magazine entry</td>
<td>41.1 (79/192)</td>
<td>29.2 (56/192)</td>
<td>37.0 (71/192)</td>
<td>28.6 (55/192)</td>
<td>21.9 (42/192)</td>
<td></td>
</tr>
<tr>
<td>Long responses</td>
<td>55.7 (64/115)**</td>
<td>42.6 (49/115)</td>
<td>33.0 (38/115)</td>
<td>26.1 (30/115)</td>
<td>11.3 (13/115)</td>
<td></td>
</tr>
<tr>
<td>Incorrect trials:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cue light</td>
<td>47.3 (26/54)</td>
<td>45.5 (25/55)</td>
<td>49.1 (27/55)</td>
<td>45.5 (25/55)</td>
<td>3.6 (2/55)</td>
<td></td>
</tr>
<tr>
<td>Lever release</td>
<td>41.9 (18/43)</td>
<td>41.9 (18/43)</td>
<td>53.5 (23/43)</td>
<td>53.5 (23/43)</td>
<td>4.7 (2/43)</td>
<td></td>
</tr>
</tbody>
</table>

The specific population includes the population of neurons responding exclusively (exclusive neurons) to one reward and the population responding to both rewards but with higher magnitude for one. The specific 32% exhibited a response when the predicted reward was sucrose 32%. The specific 4% exhibited a response when the predicted reward was sucrose 4%. For each specific population, the number of exclusive neurons is indicated. For the neurons that responded to magazine entry, many of their responses could begin before magazine entry. These neurons are thus included in the population that respond to magazine entry and in the anticipatory magazine entry (anticip. magazine). The error trials correspond to the trials during which the rat released the lever before the trigger tone.
TABLE 2. Proportion of neurons activated and inhibited after each event

<table>
<thead>
<tr>
<th>Event</th>
<th>Responsive</th>
<th>Activation</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct trials:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cue light</td>
<td>29.1 (87/299)</td>
<td>35.6 (31/87)</td>
<td>64.4 (56/87)</td>
</tr>
<tr>
<td>Trigger tone</td>
<td>40.8 (122/299)</td>
<td>68.0 (83/122)</td>
<td>32.0 (39/122)</td>
</tr>
<tr>
<td>Lever release</td>
<td>35.1 (105/299)</td>
<td>76.2 (80/105)</td>
<td>23.8 (25/105)</td>
</tr>
<tr>
<td>Magazine entry</td>
<td>64.2 (192/299)</td>
<td>66.1 (127/192)</td>
<td>33.9 (65/192)</td>
</tr>
<tr>
<td>Long response</td>
<td>38.5 (115/299)</td>
<td>62.6 (72/115)</td>
<td>37.4 (43/115)</td>
</tr>
<tr>
<td>Incorrect trials:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cue light</td>
<td>18.4 (55/299)</td>
<td>69.1 (38/55)</td>
<td>30.9 (17/55)</td>
</tr>
<tr>
<td>Lever release</td>
<td>14.4 (43/299)</td>
<td>65.1 (28/43)</td>
<td>34.9 (15/43)</td>
</tr>
</tbody>
</table>

For the neurons that responded to magazine entry, responding often began before magazine entry. These neurons are thus included in the population responding to magazine entry and in the anticipatory magazine entry (anticipatory response in Fig. 4A illustrating the response after the trigger tone announcing the reward and B illustrating the response after the lever release).

The populations responding to each reward were significantly inhibited after the cue light [SNK after an event × bin interaction: $F(7,721) = 3.080; P < 0.05$; Fig. 3C] and activated after the trigger tone and the lever release [SNK after an event effect: trigger tone: $F(1,143) = 20.230$; lever release: $F(1,136) = 55.030; P < 0.001$; Fig. 4, C and D].

Reward-delivery-related activity

We also investigated if the STN neurons encode the reward delivery. After the expectation period, the magazine entry was the event indicating the access to the predicted reward. After magazine entry, 64.21% (192/299) of the neurons were responsive and most of them activated (Table 2). Most neuronal responses were modulated by the reward (78.13%; Table 1) again with the separation of STN neurons into subpopulations that responded to either 32 or 4% sucrose solution (see Fig. 5, A and B). The average firing rate of the population responding at magazine entry, regardless of the reward involved, was significantly increased after the magazine entry when compared with before [SNK after an event effect: $F(1,271) = 31.948; P < 0.001$; Fig. 5C].

The possibility that the observed neuronal response at magazine entry might be caused by different tongue or jaw movements cannot be entirely discarded; however, this interpretation is unlikely because visual observation indicated that consumption behavior did not start during the analyzed period (1st 500 ms after magazine entry). The neuronal responses observed at magazine entry are unlikely to be due to an interruption in the rat’s movement. Furthermore, neuronal responses began commonly before magazine entry (62.96% of the responses to magazine entry) while the animal was still in movement.

During the consummatory phases, 38.46% of the neurons (115/299) exhibited a long-lasting response and were mostly activated (Table 2). Most of them (88.70%) responded specifically to one or the other reward (Table 1). Interestingly, more neurons responded specifically to 32% sucrose than to 4% sucrose (55.65 vs. 33.04%; $\chi^2 = 10.36, P < 0.001$). Furthermore, neuronal population responding to 4% sucrose exhibited a significantly higher firing rate than neuronal population responding to 32% sucrose [SNK after a reward effect: 32% sucrose $P < 0.05$, 4% sucrose $P < 0.0001$; Fig. 5C].
F(1,149) = 4.811; P < 0.001; Fig. 5D]. Indeed, 1 s after magazine entry, the population responding to 32% sucrose was inhibited, whereas the mean firing rate for 4% sucrose was around 0. Furthermore, whereas the response for 32% sucrose remains an inhibition, the response for 4% sucrose becomes an activation ~4 s after magazine entry.

**Error-related activity—“oops neurons”**

We investigated the STN neuronal responses in error trials, when the rat released the lever prematurely, before the trigger tone. We analyzed the neuronal responses after the cue light in error trials (18.39% of neurons, 55/299; Fig. 6, A and C). Most of these 55 neurons were activated (69.09%, 48/73; Table 2). Thus, there was a significantly higher proportion of neurons that exhibited an activation in error trials than in correct trials (68.09 vs. 35.63%; χ² = 22.44, P < 0.001). Furthermore, the neuronal population responding to the cue light during error trials exhibited a higher firing rate than the population responding during correct trials [SNK after a trial effect: F(1,153) = 6.895; P < 0.001]. These responses may probably be related to difficulty in maintaining the lever press (i.e., impulse control) or related to motor preparation to release the lever.

After the premature lever release in error trials, 14.38% of all the neurons (43/299) exhibited a significant response (Table 2). The neurons analyzed here did not respond after lever release in correct trials nor after the cue lights in the error trials as illustrated in Fig. 6, B and D, showing thus a specific response during premature lever release in error trials (i.e., “oops neurons”). Interestingly, the neuronal population responding to the lever release in errors trials exhibited a lower firing rate than the population responding in correct trials [SNK after a trial effect: F(1,181) = 7.473; P < 0.001].

After cue light and lever release in the error trials, more neuronal responses were specific to one predicted reward than in correct trials (cue light: 96.30 vs. 86.21%; P < 0.05; lever release: 95.35 vs. 81.90%; χ² = 8.97, P < 0.001; Table 1). For example, some neurons exhibited a response at premature lever release only when the predicted reward was 4% sucrose (see Fig. 6, C and D). For each reward, the firing rate of the population was significantly higher after the event than before [SNK after a event effect: cue light: F(1,48) = 5.818; P < 0.05; after an event × reward × bin interaction: lever release: F(7/301) = 2.304; P < 0.05].

**Responses to a change in magnitude of one reward**

To assess if and how STN neurons responded to a change between the two rewards, one sucrose concentration was replaced by water in “challenge” sessions. To obtain a small contrast (small difference in magnitude) between the two rewards available, and a high magnitude change, 32% sucrose was replaced by water (challenge 1). The two rewards available then became 0 and 4% sucrose (i.e., small contrast). During these sessions, the movement time was increased for the two rewards, one sucrose concentration was replaced by water (mean: 2,005 ms; t-test, P < 0.05), suggestive of a diminished motivation to obtain water instead of 32% sucrose. In contrast, there was no statistical difference in the movement time for 4% sucrose in challenge 1 and standard sessions (mean: 5,503 ± 3,601 vs. 3,365 ± 1,426 ms; t-test P > 0.05; Fig. 7A), suggesting that, although 4% sucrose became the more salient reward that was
available, it was insufficient to shorten the time to reach the magazine.

To get a bigger contrast with a change of smaller magnitude, 4% sucrose was replaced by water (small magnitude change; challenge 2). The two rewards available were thus 0 and 32% sucrose (big contrast). There was no significant change in behavioral measures (Fig. 7D).

During challenge 1 and 2 sessions, 22 and 17 neurons were recorded. Of these neurons, 16/22 and 15/17, respectively, reacted to at least one event. Except for magazine entry, the limited number of neurons responding to each event did not allow us to carry out an additional statistical analysis. For challenges 1 and 2, 11 and 10 neurons, respectively, responded to the magazine entry. Most of these neuronal responses were specific to one predicted reward (see examples in Fig. 7, C and F).

Interestingly, in challenge 1 sessions, fewer neurons responded for water than for 4% sucrose (18.18 vs. 45.45%; $\chi^2 = 17.14, P < 0.01$; Fig. 7B). In addition, the proportion of neurons responding to 32% sucrose under standard conditions decreased when the sucrose was replaced by water (18.18 vs. 49.03%; $\chi^2 = 24.37, P < 0.001$; Fig. 7B). In contrast, in challenge 2 sessions, more neurons responded for water (replacing 4% sucrose) than for 32% sucrose (70 vs. 50%; $\chi^2 = 4.76, P < 0.05$; Fig. 7E). In addition, the proportion of neurons responding to 4% sucrose under standard conditions increased when sucrose was replaced by water (70 vs. 45.14%; $\chi^2 = 20.45, P < 0.001$; Fig. 7E).

**DISCUSSION**

The present results show for the first time that a majority of STN neurons respond to different events in a discriminative
They also show that STN neurons respond differentially for 32 and 4% sucrose during the expectation phase (from the cue light onset to the magazine entry) and at the delivery of the reward. These data indicate that the STN encodes reward salience and that predicted reward can affect the coding of errors in a differential manner.

**STN and the coding of the reward magnitude**

The present study is the first to show that STN neurons respond to two distinct cues that announce two different rewards. Only two studies have previously reported STN neuronal responses to a single incentive cue (Matsumura et al. 1992; Teagarden and Rebec 2007). In our study, most neurons that responded to incentive cue lights were modulated by the predicted reward (i.e., specific neurons that respond mostly or only after 1 of the 2 cue lights), indicating that separate STN neurons encode the expectation of rewards according to their magnitude. The neuronal response to the cue light could reflect a sensory response, a response to the spatial location of the cue light, or the expectation of the predicted reward. Analysis of the recorded side with regard to the selective response to the cue light indicated that there was no systematic, specific response toward the contralateral side, ruling out a pure sensory or spatial response of STN neurons to the light (Coizet et al. 2009). The high proportion of neurons inhibited after the cue light is consistent with the observation that an STN lesion increases responding to conditioned stimuli associated with food or sucrose solution (Baunez et al. 2002). This inhibition is more likely due to reward-related processes than to motor-related processes. The classical hypothesis of the role of STN...
in motor processes predicts that the STN suppresses undesired movements by stimulating the inhibitory influence of the output nuclei of the basal ganglia (Isoda and Hikosaka 2008; Mink 1996). Therefore activation, rather than inhibition, of STN neurons would have been expected in our task to maintain the lever press.

After the trigger tone and the lever release most of the responding neurons were specialized for one predicted reward, thus reflecting a difference in the expectation of the two rewards and illustrates the convergence of motor and limbic information in the STN. This interpretation is consistent with similar findings in the striatum and the orbito-frontal cortex (OFC) (Arkadir et al. 2004; Cromwell and Schultz 2003; Hassani et al. 2001; Pasquereau et al. 2007; van Duuren et al. 2007). The high proportion of neurons responding according to the predicted reward during the expectation phase is in accordance with the interpretation of a role of STN neurons in encoding reward expectation and reward magnitude.

The present study also shows that STN neurons respond differentially to the delivery of two rewards. STN neurons have been reported to react to one reward delivery in the monkey (Darbaky et al. 2005) and in the rat (Teagarden and Rebec 2007). In line with these previous studies, we found both excitatory and inhibitory responses at reward delivery. In contrast to Teagarden and Rebec (2007), however, a majority of neurons exhibited excitatory responses. This discrepancy

---

**FIG. 7.** Challenge sessions: behavioral results and neuronal responses. A: movement time between lever release and magazine entry in the 2 challenge 1 sessions (water replaced 32% sucrose) compared with the 2 standard sessions on each prior day. Left bars represent the means ± SE movement time per standard session for 4% (●) and 32% (■) sucrose and right bars represent the means ± SE movement time per challenge session for 4% sucrose (●) and water (■). B: proportion of neuronal population responding at magazine entry in standard (left) and challenge 1 (right) sessions. □, neuronal populations responding similarly for the 2 rewards; ● and ■, those responding specifically to each reward for 32 and 4% sucrose, respectively. C: example of 1 STN neuron firing pattern to water (left) and 4% sucrose (right) delivery in a challenge 1 session (specific water). Rasters are centered on the magazine entry (t = 0, 1st vertical black line). Top: raster plot of spike firing on each trial with the top row of dots corresponding to the 1st trial. Bottom: mean firing rate across all trials, bin size is 50 ms. The consecutive bins showing a significant different firing rate than the baseline are represented in gray. The 2nd vertical black line indicates the end of the 500-ms period analyzed after the event.

D: movement time between lever release and magazine entry in the 2 challenge 2 sessions (water replaced 4% sucrose) compared with the 2 standard sessions on each prior day. Left bars represent the means ± SE movement time per standard session for 4% (●) and 32% (■) sucrose, and right bars represent the means ± SE movement time per challenge session for water (□) and 32% sucrose (■). E: proportion of neuronal population responding at magazine entry in standard (left) and challenge 2 (right) sessions. □, neuronal populations responding similarly for the 2 rewards; ● and ■, those responding specifically to each reward for 32 and 4% sucrose, respectively. F: example of one STN neuron firing pattern to 32% sucrose (left) and water (right) delivery in a challenge 2 session (specific 32%). Rasters are centered on the magazine entry (t = 0, 1st vertical black line). Top: raster plot of spike firing on each trial with the top row of dots corresponding to the 1st trial. Bottom: mean firing rate across all trials, bin size is 50 ms. The consecutive bins showing a significant different firing rate than the baseline are represented in gray. The 2nd vertical black line indicates the end of the 500-ms period analyzed after the event.
could be due to a difference in encoding reward when there is only one reward available in the task (as in Teagarden study) and when there are various rewards (present study). The activity of the majority of STN neurons at reward delivery was modulated by the reward. During the reward consumption, STN neurons responded mostly to one or the other reward. Furthermore, more neurons responded during 32% sucrose consumption and with a lower firing rate than during 4% sucrose consumption. This difference may reflect a motivational difference between the consumption of the preferred reward and the consumption of the less preferred reward. Moreover, this result may also reflect that rats take more time to consume 32% sucrose than 4% sucrose or that there is a higher rewarding effect of 32% sucrose than 4% sucrose, this latter hypothesis being supported by the higher number of neurons responding to 32% sucrose.

The high degree of selectivity of STN responses to one specific reward suggest that there are different neuronal subpopulations encoding specifically the value of the upcoming reward during the expectation phase and encodes differentially the delivery of a reward depending on its value.

Reward magnitude change

In the challenge 1 sessions, the increase of the latency to reach the magazine when water replaced 32% sucrose clearly indicates that rats perceive the change in reward salience. The reduction in the number of neurons responding to water when water replaced the most reinforcing reward, suggests that STN neurons are less prone to react to a high magnitude change (from 32 to 0%) for a low contrast between the rewards available (0 and 4% sucrose). On the contrary, the increase in the proportion of neurons responding to water when water replaced the less reinforcing reward, suggests that when the change in magnitude is low (from 4 to 0%), but the contrast between the available rewards is high (0 and 32% sucrose), STN neurons predominantly encode the contrast between rewards (i.e., difference between the rewards available). Thus the subpopulations specifically encoding the reward adapt to the change in reward salience and in the contrast between the rewards available.

STN neurons and errors

We have found neurons responding after the cue light or after the lever release in error trials that do not respond in correct trials. The proportion of neurons inhibited or activated after the cue light differed completely between correct and error trials (more activation in error trial), and the firing rate of the neural population responding after cue light was higher in errors trials than in correct trials, indicating that the response in error trials was specific to errors. The neuronal responses observed after the cue light more likely reflect a motor difficulty in maintaining the lever press and are thus related to an error in behavioral performance. The responses observed after the anticipatory lever release could be related to a specific motor activity during the error trials or to the extinction of the house light or to “disappointment,” as reward expectation is negated. The firing rate of the neural population responding after lever release was lower in error trials than in correct trials, reinforcing the idea that these separate populations encode different processes. Furthermore, in error trials, both after the cue light and after the lever release, the activity of most neurons was under the influence of the predicted reward, thus reinforcing the hypothesis of a disappointment-related reactivity in the STN.

When a fully predicted reward is missing, there is an important focus on coding of error prediction, which points specifically to the role of the dopaminergic neurons in this coding (Schultz and Dickinson 2000). Failing the trial while having the indication regarding the upcoming reward may engage the error prediction system as the expected reward will not be delivered. It is thus possible that the dopaminergic system influences the STN error responses observed here. There are very few reports of neuronal responding during or after behavioral errors during task execution. Some studies in the prefrontal cortex (particularly the anterior cingulate cortex), globus pallidus (GP), nucleus accumbens (NAc), and motor cortex report that neurons respond specifically in error trials (Amiez et al. 2005; Arkadir et al. 2004; Laubach et al. 2000; Taha et al. 2007; Watanabe 1989). Because these brain regions and the midbrain dopaminergic system are connected directly or indirectly to the STN, they could be responsible for the neuronal responses observed in the STN on error trials.

STN in the reward circuit

The STN role in motivational and cognitive processes is now established in rats (Baunez and Robbins 1997; Baunez et al. 2002, 2005; Chudasama et al. 2003; Eagle et al. 2008; Lardeux and Baunez 2008; Teagarden and Rebec 2007; Uslaner and Robinson 2006; Uslaner et al. 2007; Winstanley et al. 2005), monkey (Darbaky et al. 2005), and human (Temel et al. 2006; Witjas et al. 2005). As previously said, STN lesions have opposite effects on motivation depending on the reward involved or the level of initial preference for it (Baunez et al. 2002, 2005; Lardeux and Baunez 2008).

The prefrontal cortex, particularly the OFC, NAc and dorsal striatum, GP and ventral pallidum (VP), as well as the midbrain dopaminergic neurons are involved in coding incentive cues predicting different rewards and access to different rewards in primates (Amiez et al. 2006; Cromwell and Schultz 2003; Hassani et al. 2001; Hosokawa et al. 2004; Roesch and Olson 2004; Schultz 2002; Schultz et al. 1992; Tremblay and Schultz 1999; Wallis and Miller 2003; Watanabe 1996) and in rats (Carelli et al. 2000; Miyazaki et al. 2004; Nicola et al. 2004; Roesch et al. 2006; Roitman et al. 2005; Setlow et al. 2003; Taha and Fields 2005; Tindell et al. 2004, 2006; van Duuren et al. 2007; Wilson and Bowman 2005, 2006). Most of these studies report a modulation of neuronal responses depending on the reward, either for the incentive cues and/or reward delivery. The importance of the connection between prefrontal cortex and STN has been shown to functionally mediate complex behavior in rats (Chudasama et al. 2003). The present results showing neuronal responses in the STN to cues predicting the reward and to reward delivery itself are thus likely to be due in part to this so-called “hyperdirect” pathway. It might well be possible that NAc/striatum and VP/GP also contribute to this specific responding in the STN, especially because microcircuits of specific neuronal subpopulations have been suggested at the level of NAc (Carelli et al. 2000). Considering that STN neurons code reward properties, as well as error, like
dopaminergic neurons (Schultz 2002), it is highly probable that STN responses are under the modulatory influence of dopaminergic inputs.

The position of the STN within the basal ganglia may explain the complex and integrative neuronal responses that we observed here. Indeed our results show a partial overlap in the response of neurons to different events of the task, regardless of whether they responded with activation or inhibition. Thus the STN seems to integrate, in a subtle way, motivational, cognitive, and motor information from the prefrontal cortex, the midbrain dopaminergic nucleus, the Nac, and the VP. This high level of integration may allow the STN to modulate the activity of the output structures of the basal ganglia.

In conclusion, this is the first study highlighting the role of the STN in encoding reward magnitude. The results reported here show that the STN codes differentially the expectation and delivery of a natural reward such as sucrose, depending on its concentration. Thus the STN encodes the salience of a reward by activating subpopulations that respond to a specific reward. These subpopulations are able to adapt to a change in reward magnitude. Our study also shows that the STN codes behavioral performance errors and/or reward-prediction errors. Taken together, these results reveal a critical role for the STN in reward information processing and more generally in motivated behavior. Given that the STN is the current target for the treatment of Parkinson’s disease and now for obsessive-compulsive disorders treatment (Mallet et al. 2008), it is important to better understand its functions and contributions to the various neural circuits to which it belongs.

ACKNOWLEDGMENTS

The authors are greatly indebted to Dr. G. E. Meredith for helpful comments and corrections of the manuscript, to Dr. B. Pouzet for technical advice and critical reading and corrections of the manuscript. We are also grateful to Drs. P. Apicella and S. Ravel for helpful comments on the manuscript.

GRANTS

This research was supported by the CNRS, Aix-Marseille University and by a funding from the French national agency for research ANR (contract JC05_48262) to C. Baunez. S. Lardeux was supported by a grant from the French national agency for research ANR (contract 2007 BORIS 014 01).

REFERENCES


Roitman MF, Wheeler RA, Carelli RM. Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron* 45: 587–597, 2005.


