Rat Nucleus Accumbens Neurons Persistently Encode Locations Associated With Morphine Reward

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German PW, Fields HL. Rat nucleus accumbens neurons persistently encode locations associated with morphine reward. J Neurophysiol 97: 2094–2106, 2007. First published November 8, 2006; doi:10.1152/jn.00304.2006. When rats and mice are free to explore a familiar environment they spend more time in a previously rewarded location. This conditioned place preference (CPP) results from an increased probability of initiating transitions from an unrewarded location to one previously paired with reward. We recorded nucleus accumbens (NAc) neurons while rats explored a three-room in-line apparatus. Before place conditioning, approximately equal proportions of NAc neurons show excitations or inhibitions when the rat is in each of the rooms (morphine paired, center or saline paired). Conditioning increased the proportion of neurons inhibited while the rat was in the morphine room and neurons excited in the saline room. Many of the neurons in these two groups responded during room transitions. Furthermore, the postconditioning increase in the population of neurons with room-selective responding persisted for several weeks after the last morphine treatment. This long-lasting change in population responses of NAc neurons to initially neutral locations is a neural correlate of the change in location preference manifest as CPP.

INTRODUCTION

Remembering reward-associated cues and the actions successfully used to obtain reward is essential for survival and reproductive success. Reward memory is also a major challenge to the treatment of drug addiction because it contributes to relapse after long periods of abstinence. Approach to reward-associated locations involves memory of the reward quality and of the spatial cues required for return navigation. One manifestation of this process is the increased time an animal spends in a previously rewarded location, even when the reward is no longer present. Such conditioned place preference (CPP) offers a measure of reward location memory, motivation for reward, and the ability to apportion limited time resources to maximize the benefit of global exploration with local preferences.

Behavioral analysis of CPP to opioid (morphine) reward reveals that the increased time spent in the previously morphine-paired room is explained by an increased probability of initiating location-directed movements that begin in the saline-paired room and end in the morphine-paired room (German and Fields 2007). This observation suggests that the neural circuits contributing to this behavior encode activity leading to the initiation of room transitions as well as the sensory cues that distinguish locations (rooms) within the test chamber.

Previous studies of the neural mechanisms that underlie CPP implicated multiple brain regions (Bardo 1998; Tzschentke 1998) including the nucleus accumbens (NAc), γ-Aminobutyric acid (GABA) agonists microinjected into the rostral NAc induce place preference (Reynolds and Berridge 2002). Methamphetamine injection into the NAc also induces CPP (McBride et al. 1999). Significantly, local injections into the NAc of 6,7-dinitroquinolinicacidamine-2,3-dione (DNQX), an α-aminono-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA)/kainate receptor antagonist, blocks the expression of CPP previously conditioned by systemic amphetamine, cocaine, or morphine (Kaddis et al. 1995; Layer et al. 1993; Popik and Kolasiewicz 1999). Furthermore, nonselective dopamine antagonists injected into the NAc block expression of both amphetamine and cocaine induced CPP (Hiroi and White 1990, 1991) as well as morphine CPP in opiate-dependent rodents (Bechara and van der Kooy 1989; Laviolette et al. 2004). The NAc is also involved in CPP for natural reward: the expression of a CPP for a sucrose-paired room was blocked by excitotoxic lesions of the NAc after training (Everitt et al. 1991). These studies imply that NAc neuronal activity both encodes the location of previously learned reward associated cues and increases the probability of movements toward that location. NAc neuron firing patterns correlate with a variety of goal-directed behaviors. The NAc modulates consumption of palatable foods (Kelley et al. 2002). NAc neuron firing encodes palatability and correlates with motivation to consume palatable food (Roitman et al. 2005; Setlow et al. 2003; Taha and Fields 2005). In rats, NAc neurons respond to reward-associated or reward-predictive sensory stimuli (Wilson and Bowman 2004, 2005).

In primates, ventral striatum neurons respond to juice and cocaine reward (Bowman et al. 1996), reward magnitude, and relative reward value (Cromwell and Schultz 2003; Cromwell et al. 2005). However, studies of responses to discrete reward-associated cues while animals are engaged in reward acquisition are difficult to relate to CPP. For example, we would expect the transient discrete sensory cues to be processed differently from the contextual spatial cues in CPP because their time course is different.

Another difference between these behavioral paradigms is highlighted by the finding that NAc neurons encode progress through a series of motor actions that lead to reward (Shidara et al. 1998). In this behavior progress was in a behavioral sequence that was one way and toward a known and expected
ACCUMBENS PERSISTENTLY ENCODES REWARDED LOCATIONS

METHODS

Apparatus

The conditioned place preference (CPP) chamber was a rectangular Plexiglas box 84 × 46 cm with 45-cm-high walls. The long axis of the chamber was divided into three equal-size rooms (28 × 46 cm) using removable partitions. These partitions could be used to completely separate each room or they could be left partly open. All the walls were painted in semigloss medium gray. Each room could be distinguished by the color and texture of the floor. The central room was left with a smooth acrylic floor; one side room (left) was covered by a white plastic 1-cm-square grid. The opposite side room (right) was covered by a black plastic 2-cm-square grid. To remove odors, the floors were wiped with 70% ethanol. The rats had no initial bias toward either side room. The CPP chamber was enclosed in a cabinet that was lit from above by a 100-W incandescent light. The entire floor was of semitransparent acrylic, to allow tracking of the rat’s shadow by a CCD camera mounted below the chamber. The animals’ positions were digitized and tracked continuously using Ethovision software (Noldus Information Technology, Leesburg, VA) at a sampling rate of either 15 Hz (52 sessions) or 30 Hz (91 sessions).

Experimental subjects

We used a total of 23 male Long-Evans rats (nine were used for pretest only). Rats weighed 250–300 g on arrival from vendor (Harlan, Indianapolis, IN). Surgery was conducted under isoflurane anesthesia according to the Institutional Animal Care and Use Committee Protocol. Microwire arrays were implanted bilaterally in the NAc. The recording arrays had eight electrodes per side of 50-micron-diameter stainless steel wire with Teflon coating (Microwire Technologies, East Windsor, NJ). The wires were cut flat at the end with an impedance of 200–250 kOhms. Electrodes where set individually in each array at a distance of about 250 microns between adjacent electrodes in either two rows of four electrodes or three rows with three, three, and two electrodes each. The target stereotaxic coordinates were (in mm): AP +1.7, ML ±1.0, DV –7.1. After surgery, animals were handled and habituated to the lab environment for 1 wk before beginning experiments. The animals were housed individually and received a daily ration of rat chow that maintained a stable weight of 400 g.

Behavioral paradigm

For the 3 days before the first behavioral session, the recording arrays were attached to the connecting cable in each animal’s home cage to habituate it to the recording situation. Each subject was randomly assigned to one of the two side rooms for morphine pairing. On day 1, each animal was placed in the center room of the chamber at the start of a 30-min pretest. During the pretest, all doors were open and the rats were free to explore all three rooms. On 4 of the next 8 days, the animals received 5 mg/kg subcutaneous injections of morphine paired with confinement to one of the side rooms for 30 min. On alternate days, they received pairings of saline vehicle injections with the opposite side room for 30 min. After conditioning the animals were again tested in the open chamber for 30 min. We minimized the number of animals used by repeatedly testing animals at approximately 1-wk intervals until their preference behavior diminished.

Extracellular recording

The recording apparatus consisted of a head stage with field effect transistors, cable, and commutator to allow the animal free movement within the chamber. We used Plexon (Dallas, TX) recording hardware and software. At the beginning of every session, the animals were connected to the recording apparatus and then returned to their home cage, with a special lid to allow free movement of the cable. At this
time the signals were assigned thresholds and units were isolated using the Plexon signal-acquisition software. The system used preamplifier filtering 150–9 kHz, one pole; digital sampling 12 bit, 40 kHz, with filters 400 Hz, two-pole low cut, and 5 kHz, two-pole high cut. Waveforms that crossed a threshold were stored for subsequent spike sorting. One electrode in each array was designated as a reference electrode. The signal from the reference electrode was subtracted from the signal on the other electrodes to reduce noise and movement artifacts.

**Histology**

The electrode placements were verified by passing 20 μA of current through the electrodes to deposit iron at the tips of all electrodes. This was accomplished using a 9-V battery in series with a 500-kOhm resistor, with the ground inserted into the anesthetized rodent’s rectum. Animals were lethally anesthetized with pentobarbital and perfused transcardially with saline, followed by 10% formalin and 3% potassium ferrocyanide solution to develop the Prussian blue deposit. The brain was cut on a microtome into 40-μm sections, mounted, and counterstained with neutral red.

**Data analysis**

Off-line analysis of the behavior was conducted in Matlab (The MathWorks, Natick, MA), Neural Explorer (Plexon), and SigmaStat 3.0 (Systat Software, Richmond, CA). Much of our analysis focused on behavioral epochs we termed visits, defined as the time between an entry and the following exit of a room. Visit analysis began after the on behavioral epochs we termed.

For the present study, center room visits were divided into simple or complex visits. Simple visits were defined as any visit to the center room in which the rat did not deviate from the 10-cm-wide path between the two doorways. Only the complex center visits were counted for adjacent room transitions. Simple visits were defined as visits in which the animal remained in the corridor between the doorways. Occasionally, these included the animal poking its head into the center room and then returning. The simple visits that resulted in a return were not included in the analysis. Most of the simple visits (exiting one side room to the opposite side room) were calculated separately for each direction of movement to create the two constants for the location-directed transitions between the morphine and saline rooms. Changes to transition constants were compared with two-tailed, paired t-tests.

Pretest and postconditioning firing rate was compared with a t-test of the average firing rate of visits to the saline or morphine rooms. We included in the analysis all neuron sessions with four alternating visits to each room with a minimum visit time of 5 s. Postconditioning firing rate was compared with an ANOVA of the average firing rate of visits to all three rooms. For inclusion in this analysis, we set a minimum of four visits to each room. All neurons that were significantly different

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**FIG. 1.** Video tracking of conditioned place preference (CPP). Time spent in 3 rooms during 30-min pretest (A) and test (B) (n = 14). Path of one rat during a 30-min pretest (C) and test (D) after 4 pairings of morphine and 4 pairings of saline in left and right rooms, respectively. Errors bars represent SE for all figures.
and may reflect the restraining effect of the cable on the animals’ head that transitions into the center room. This increased probability of Complex visits (German and Fields 2007). The tendency for rats with con-
center room. In tests without a connecting cable, rats spent the extra time in the center room to the position of the recording saline-paired room (Fig. 1, A).

After morphine conditioning the animals increased the time on the time when the center of the rat’s body crossed the door threshold. The transitions were categorized by door, direction through the door, and as simple or complex.

RESULTS

Conditioned place preference behavior

During pretest sessions, the animals explored all areas of the CPP apparatus and spent similar cumulative amounts of time in both rooms used for subsequent training (Fig. 1, A and C). After morphine conditioning the animals increased the time they spent in the morphine-paired room compared with the saline-paired room (Fig. 1, B and D). The total time in the center room also increased during testing. We attribute the extra time in the center room to the position of the recording cable connecting the rat’s head to a commutator above the center room. In tests without a connecting cable, rats spent the same amount of time in the center room during test and pretest (German and Fields 2007). The tendency for rats with con-
nected electrode arrays to spend more time in the center room postconditioning could be explained by the slight restraining effect of the recording cable on the rats’ heads, which would tend to encourage movement to the center room.

The rats tended to move throughout the session and to explore all areas of the apparatus multiple times during the pretest, when the apparatus was completely novel (Fig. 1C). After conditioning with morphine and saline in the two side rooms, rats continued to explore all rooms, including the saline room, even though their explorations were not encouraged with rewards or the novelty of changing stimuli (Fig. 1D). This spontaneous repetition of room transitions made it possible to analyze neuronal responses during CPP testing by segmenting the behavior into room visits and room transitions. Neverthe-

Altered room transition probabilities

Dividing all room transitions into simple (Fig. 2A) and complex (Fig. 2B; see METHODS) creates a model of CPP in which there are six location-directed transition types. Each of these transitions types can be described by a single transition constant that represents the instantaneous probability of the animal making that transition. Constants for the transition probability model were calculated using the session’s mean visit frequency and duration for each visit type. We calculated the percentage change to these constants from pretest and the first test session after conditioning (Fig. 2C, Table 1).

The largest change to a transition coefficient was a 117% increase in the probability of initiating a simple transition from the saline room directly into the morphine-paired room (P = 0.008, n = 14, paired t-test, two-tailed). There was also a smaller, 47% decrease in the probability of initiating transitions from the center room into the saline room (P = 0.007). These changes are very similar to a previous behavioral study that identified these transition probabilities as the critical post-conditioning alteration in behavior that explains the increased cumulative time rats spend in the morphine-paired room.

Unlike the previous study on animals without recording electrodes and connecting cable, here we found an additional increase in complex movements into the center room from both the morphine-paired room (+86%; P = 0.006) and the saline-paired room (+98%, P = 0.003). Unlike the saline to mor-

TABLE 1. CPP model transition probabilities

<table>
<thead>
<tr>
<th>Transition Constant, s⁻¹</th>
<th>Pretest Mean</th>
<th>Pretest SD</th>
<th>Test Mean</th>
<th>Test SD</th>
<th>Percentage Change</th>
<th>p Value</th>
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<tr>
<td>K_Ms</td>
<td>0.0138</td>
<td>0.0070</td>
<td>0.0117</td>
<td>0.0093</td>
<td>−15.0</td>
<td>0.5</td>
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<td>K_Mc</td>
<td>0.0109</td>
<td>0.0045</td>
<td>0.0203</td>
<td>0.0112</td>
<td>85.8</td>
<td>0.006</td>
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<tr>
<td>K_sm</td>
<td>0.0224</td>
<td>0.0053</td>
<td>0.0232</td>
<td>0.0071</td>
<td>−3.4</td>
<td>0.7</td>
</tr>
<tr>
<td>K_sc</td>
<td>0.0206</td>
<td>0.0106</td>
<td>0.0110</td>
<td>0.0068</td>
<td>−46.7</td>
<td>0.007</td>
</tr>
<tr>
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<td>0.0051</td>
<td>0.0269</td>
<td>0.0127</td>
<td>98.0</td>
<td>0.003</td>
</tr>
<tr>
<td>K_Ms</td>
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<td>0.0104</td>
<td>0.0336</td>
<td>0.0244</td>
<td>117.3</td>
<td>0.008</td>
</tr>
</tbody>
</table>
electrode implants, it likely reflects the restraining effect of the recording cable pulling the animals toward the center of the cable line. Taken together with our earlier findings in completely unrestrained rats, these results indicate that the primary behavioral effect of morphine conditioning in this apparatus is an increased instantaneous probability of initiating location-directed “simple” transitions from the saline into the morphine room.

**Room conditioning alters NAc neuron firing**

To test whether NAc neurons firing rate correlated with room location after morphine conditioning, we analyzed the within-room firing rate of neurons during pretest and test sessions. For inclusion in the analysis, each neuron was required to be from a session with a minimum of four alternating visits to the morphine and saline rooms (891 of 951 test neurons, 187 of 190 pretest neurons). For each neuron, we compared the baseline firing rate during alternating visits to the morphine and saline rooms. During pretest, 9% (16/187) of neurons showed a significant selective alteration in firing when the rat was in one of the rooms. These neurons were fairly evenly distributed between higher firing rates in the room subsequently paired with morphine (44%, 7/16) and that to be paired with saline (56%, 9/16). After conditioning, the number of room-selective neurons increased to 12% (103/891). However, 75% (77/103) of these neurons had a higher firing rate in the saline-paired room (henceforth referred to as “saline-excited/morphine-inhibited”) compared with 25% (26/103) that had a higher firing rate in the morphine room (henceforth referred to as “morphine-excited/saline-inhibited”). This proportion of neurons with a higher firing rate in the saline room was significantly greater than chance (Fig. 3A) \( (P < 0.05) \) and represented roughly 9% of all recorded postconditioning neurons.

There was no apparent effect of the unconditioned room cues of the apparatus on these altered firing rates. The animals were conditioned with morphine in randomly assigned rooms. By re-sorting the neurons based on their selectivity for either the left or right room, without regard to which room was paired with morphine, we found that there was no difference between neurons with increased firing in the left room (50/103) compared with neurons with increased firing in the right room (53/103) (Fig. 3B). This indicates that the change in population selectivity for the morphine and saline rooms was a result of the conditioning treatment and not of a bias introduced by cues unrelated to the conditioning procedure.

Some individual electrodes recorded room-selective neurons on more than one test day. Because we cannot exclude the possibility that these recordings represented a single neuron encountered on multiple days, we recounted the room-selective neurons, accepting only the first room-selective neuron recorded on each channel. Using this conservative approach, the total number of room-selective neurons during the test session was reduced from 103 to 47; however, the proportion of morphine-excited/saline-inhibited neurons remained at 32% (15/47), whereas more than twice as many neurons (32/47) were saline-excited/morphine-inhibited. This proportion is significantly different from the null hypothesis of equal numbers in both groups \( (P < 0.05) \). Furthermore, when these neurons are divided by individual rats, only one of nine rats had more than 50% of their room-selective neurons composed of the morphine-excited/saline-inhibited type (Table 2).

To measure the persistence of learning, we tested animals approximately once a week for as long as they expressed a room preference. Some continued to show a preference >6 mo after the last conditioning session with morphine. This is shown for the sessions in which room-selective neurons were recorded. A t-test comparing the difference of the time spent in the morphine-paired room and the saline-paired room revealed a significant preference during the first 45 days after the initial test \( (P = 0.000003) \) (Fig. 4A). After 45 days, there was still a trend toward CPP \( (P = 0.056) \) (Fig. 4B). We use a balanced design, where the room in which the animals receive the drug was randomly assigned. Comparing the two rooms for non-morphine-related bias revealed no bias for the cues in either room before or after conditioning (not shown).

**FIG. 3.** CPP increased the number of nucleus accumbens (NAc) neurons excited in saline room and inhibited in morphine room. A: proportion of all recorded NAc neurons with significantly increased firing rate (neurons classified based on 2-room t-test, \( P < 0.05 \)) during morphine or saline room visits. Population of neurons excited by the saline-paired room during postconditioning tests was increased compared with neurons excited by morphine during postconditioning \( (P < 0.02, \text{Holm–Sidak comparisons, represented by horizontal bar}) \). B: same neurons as in A divided into left and right room visits (independent of morphine or saline conditioning) do not show an effect of room cues alone. C: proportion of all NAc neurons recorded with significantly different firing rate between rooms (neurons classified based on 3-room ANOVA, \( P < 0.05 \)). Data plotted separately for inhibitions and excitations in each of the 3 rooms. Neurons were assigned to the room where they displayed the most deviant firing rate from the other two. There was a significantly larger population of morphine room-inhibited neurons than either center-inhibited, saline-inhibited \( (P < 0.005, \text{Holm–Sidak comparisons, represented by black horizontal bars}) \), or morphine-excited \( (P < 0.05, \text{represented by gray horizontal bar}) \) neurons. Population of saline room excited neurons was larger than all other groups \( (P < 0.005, \text{for all Holm–Sidak comparisons}) \). Error bars are SE.
The proportion of saline-excited morphine-inhibited neurons was not affected by time from the last conditioning session. Looking at the 47 neurons from unique recording channels revealed that during the first 45 days after conditioning, 63% (17/27) were saline-excited/morphine-inhibited, compared with 37% (10/27) morphine-excited/saline-inhibited (not significant). Similarly, of neurons recorded >45 days after conditioning, 75% (15/20) were saline-excited/morphine-inhibited, compared with 25% (5/20) morphine-excited/saline-inhibited (not significant). Therefore the neuronal populations coding for the morphine and saline rooms were differentially altered by place conditioning. Saline-room–excited neurons increased, but saline-room–inhibited neurons did not change. In contrast, morphine-room–inhibited neurons increased, whereas morphine-room–excited neurons were unchanged. Finally, the populations of center-room–excited and –inhibited neurons were not altered by conditioning.

### Room transition response

To better understand the class of room-selective neurons, we plotted firing rate histograms triggered by transitions between rooms. The activity of a single saline-excited/morphine-inhibited neuron during transitions between rooms is shown in Fig. 5. This neuron shows an increase in activity when the rat is in the saline room. This excitation is seen immediately after the rat leaves the morphine room for the saline room in a simple (direct) transition (Fig. 5A) (Bonferroni-corrected Wilcoxon signed-rank test, \( n = 9, P < 0.05 \)). This neuron is also excited just before and after leaving the morphine room for center room complex visits (Fig. 5B) (Bonferroni-corrected Wilcoxon, \( n = 10, P < 0.05 \)). The same neuron does not show an alteration in firing rate during transitions in the opposite direction, i.e., from the saline into the center room. However, when the rat made simple transitions directly from the saline room into the morphine room, the neuron was inhibited (Fig. 5D) (Bonferroni-corrected Wilcoxon, \( n = 5, P < 0.05 \)). This neuron with a room-selective firing rate is altering its firing rate in a statistically significant way during the first seconds of the transition while the animal is still moving into the destination room.

We tested each room-selective neuron with a unique channel \( (n = 47) \) for room transition responses during three time periods: pretransition (−2 to 0 s before crossing doorway), early transition (0 to 2 s after crossing doorway), and late transition (4 to 6 s after crossing a doorway) (Fig. 6, timeline). Of the saline-excited/morphine-inhibited neurons, only a few responded for movements in any direction during the pretransition period (Fig. 6A). However, as many as a third of the neurons showed a significant change in firing rate during the early transition period (Fig. 6B). These early transition re-
responses were excitatory when the animal moved from the morphine toward the saline room and inhibitory when the animal moved in the other direction. This bidirectional pattern continued into the late transition phase (Fig. 6C).

Morphine-excited/saline-inhibited neurons displayed the opposite pattern of transition responses. Although almost no neurons responded in the pretransition period (Fig. 6D), as many as a third of morphine-excited/saline-inhibited neurons were excited during the early transition period (Fig. 6E) and late transition period (Fig. 6F). Of these neurons, most were inhibited during transitions from the morphine room into or toward the saline room. The same set of neurons was excited during transitions from the saline room into or toward the morphine room.

Pooled histograms show population response

Many neurons that altered firing rate in either the morphine or saline room responded during movements between rooms. To explore this pattern, we pooled all the morphine-excited/saline-inhibited neurons and plotted the population response during room transitions as perievent time histograms (PETHs).
triggered on the time the rat crossed a doorway. In this analysis, both simple and complex room transitions were pooled. As a population, morphine-excited/saline-inhibited neurons were inhibited beginning just as the animals exit the morphine-paired room (Fig. 7A) (Friedman’s test with Dunn’s pairwise comparisons to baseline against pre, early, and late periods, \( P < 0.05 \)). The firing rate remains depressed as the animals enter the saline room from the center room (Fig. 7B) (\( P < 0.05 \)). These same neurons are excited when the animal moves from the saline room back to the morphine room. The pooled morphine-excited/saline-inhibited neurons increase their firing immediately on exiting the saline room (Fig. 7C) (\( P < 0.05 \)). The firing remains elevated as they enter the morphine room from the center room (Fig. 7D) (\( P < 0.05 \)).

As a group, the saline-excited/morphine-inhibited neurons displayed essentially the same pattern of firing for movements but in the opposite direction. The population firing rate increased as the animals exited from the morphine room (Fig. 7E) (\( P < 0.05 \)) and remained elevated as they entered the saline room (Fig. 7F) (\( P < 0.05 \)). For movements in the other direction, the firing rate of neurons was inhibited during the late transition as the rats left the saline room (Fig. 7G) (\( P < 0.05 \)) and the firing rate also decreased as they moved from the center room into the morphine room (Fig. 7H) (\( P < 0.05 \)).

Histology

Reconstruction of electrode paths and tips from Prussian blue deposits revealed that our electrode tips were scattered throughout the NAc core and shell. Most electrodes terminated in the rostral portion of the NAc (Fig. 8). Many of the electrode arrays straddled the border between the NAc shell and core. It was not always possible to identify which electrode tip deposit corresponded to a specific channel. Therefore we classified electrode placement only if all the electrodes of an array were within the boundaries of either the shell or core. Using this criterion to identify the anatomy of the 47 units on unique electrodes revealed 78% of shell neurons were excited by the...
saline room (14/18, \( P < 0.05 \)), but only 50% of neurons in the core were excited by the saline room (6/12). The remaining 17 neurons could not be reliably classified as core or shell but were near the border. Of these, 71% were excited by the saline room (12/17). A Fisher exact test comparing shell and core did not find a significant difference between regions (\( P = 0.14 \)).

**DISCUSSION**

CPP is an important behavioral assay that is widely used to assess the reward value of drugs and natural reinforcers. This behavior has been used to guide an extensive body of research into the anatomic, pharmacologic, genetic, and molecular substrates of reward (Tzschentke 1998). Despite this, CPP behavior itself has not been subjected to detailed analysis. This failure has so far been an obstacle to using the analytical power of behavioral electrophysiology to understand CPP in the way that it has for studying self-administration behavior (Carelli 2004; Schultz 1998). Perhaps this is because self-administration paradigms of reward are easily understood as a series of brief motor actions often in response to discrete sensory cues. As a result, the contribution of changes in single-neuron firing rates to each cue, action, or outcome has shaped thinking on the role of the NAc in operant responding to reward-predictive cues (Schultz et al. 1992; Setlow et al. 2003; Yun et al. 2004b).

We set out to examine single-unit activity during CPP to more directly study the role of NAc neurons in reward directed behavior without the complication of an intervening learned instrumental task. Because inactivation of the NAc blocks the expression of CPP, we assumed that there would be a population of NAc neurons whose activity encodes both the previously rewarded location and the actions that increase the probability of being in that location. In fact, we found significant and long-lasting conditioning-related changes in NAc neuronal populations encoding location. Further analysis of these populations revealed rapid changes in firing associated with transitions between locations.

**NAc neurons encode rewarded locations**

Previous studies showed that NAc neurons encode spatial and movement parameters. For example, NAc neurons respond to particular locations in an eight-arm radial maze (Lavoie and Mizumori 1994). Although the location specificity of NAc neurons is not as precise as that of hippocampal neurons (Martin and Ono 2000; Shibata et al. 2001), the location responses in the NAc may depend on hippocampal input (Pennartz et al. 1994, 2004). NAc neurons also encode locomotion (Callaway and Henriksen 1992; Chang et al. 1994; Kiyatkin and Rebec 1996; Peoples et al. 1998) and directionally selective locomotor responses (Lavoie and Mizumori 1994; Peoples et al. 1998). These patterns of activity are consistent with a role for NAc neurons in spatial navigation tasks (Albertin et al. 2000; Floresco et al. 1997). Although reward tasks are often used in these studies, they have not shown an effect of reward learning on NAc location responses.

This is the first study to demonstrate that reward learning conditions spatial responses in NAc neurons. The number of neurons that selectively alter firing rate in rewarded locations was significantly increased. Specifically, during CPP expression, NAc neurons responded selectively in one of the three rooms and during transitions between rooms. Conditioning increased the number of neurons inhibited when rats were in the morphine-paired room or excited when they were in the saline-paired room. In contrast, the proportion of center-room neurons was not increased. This demonstrates that NAc neurons differentially encode cues for the unconditioned center room and the explicitly nonrewarded saline room. Furthermore, room-selective neurons tended to have statistically significant changes in their response during the transitions between rooms. The firing rate of these neurons increased as the animal transitioned in one direction and decreased for transitions in the opposite direction. These firing rate changes may reflect the cue salience of the current room and/or they may bias the motor system to initiate transitions into a different room.

We did not record from the same neuron during both pretest and test, and thus our data cannot determine whether the response of any individual neuron is changed by conditioning. However, the change in population response supports the idea that some NAc neurons have altered room responses as a result of conditioning.

We trained the animals in a counterbalanced design, so that half the animals received morphine in the right room and half in the left room of the CPP apparatus. Some neurons were selective for a single room during pretest, in agreement with earlier reports that location selectivity arises spontaneously (Shibata et al. 2001). However, the increased number of room-selective neurons during tests could not have arisen from experience with spatial cues alone because the asymmetric encoding of the conditioning rooms was observed only when comparing morphine to saline rooms and was unaffected by whether the drug or saline was given in the right or left room. Therefore the changes we observed in neuronal population responses in the saline and morphine rooms can be entirely attributed to the conditioning treatments.

**Relationship of NAc neuron firing to preference behavior**

We propose that during CPP expression, NAc neurons encode preference for a location in their population response. An increased proportion of NAc neurons with elevated firing rate in a location increases the probability of the animal leaving that location. Furthermore, the exit transition, once initiated, is most likely to take the animal toward the destination in which the NAc population will be maximally inhibited. In the case of our CPP chamber, this means that the rat is most likely to leave the saline room because of the relatively large number of excited NAc neurons. When the animal leaves the saline room, it is most likely to end the transition in the morphine room where the most NAc neurons are inhibited. In the center room, the animal is slightly more likely to exit than during the pretest, although it exits more often toward the morphine room, where the greatest proportion of neurons will be inhibited. The likelihood of leaving the morphine room is not increased because the NAc neuron population is not excited in this room.

The observed change in firing rate occurs primarily after the animal has left the room. Therefore if NAc activity is influencing room exits, it is probably the elevated steady-state firing rate that is carrying the signal to exit. This is consistent with the behavioral change, which affects the exit probability for the entire duration of a room visit. The change in firing rate during...
the transition would reflect the updating of this tonic signal to reflect the relative preference for (tendency to remain in) the animal’s new context. Therefore we hypothesize that the signal is a context (room) dependent preference signal, rather than a direct motor command or motor intention signal. Yet, this tonic signal does carry value and intention indirectly. The tonic activation reflects the constant level of transition probability measured behaviorally. When the rat enters the saline room, a greater number of neurons are excited and this population response may signal that there is less utility in being there, which increases the probability of initiating an exit for the duration of the visit.

Although the tonically excited NAc population in the saline room may act directly to influence behavior, it is not as clear how the subsequent inhibition of the population is influencing the behavior. One possibility is that the NAc neuron inhibition begins as the animal moves and the animal continues to move as long as the NAc population response continues to decline. Another possibility is that a separate population of neurons, perhaps in a separate brain region, encodes the optimal destination for maximizing future NAc neuron inhibition. This latter explanation is more consistent with the behavioral data, which suggests that some signal is mediating transitions from a starting room to a particular destination room.

The tonic excitation of some NAc neurons in the saline room may be related to the phasic excitation of neurons after cues that signal the availability of reward in operant reinforcement tasks (Nicola et al. 2004a). However, during CPP expression, there are no temporally discrete, salient cues of reward availability; therefore the excitation is tonic and results in a general increase in the probability to move to the rewarded location.

**The saline-paired room**

A surprising result is the prominence of the saline room in both our behavioral and NAc neural studies. One interpretation is that the saline room is associated with the “absence of reward,” so there is little value in staying and exploring further. In contrast, the center room is relatively novel and the availability of reward unknown.

**Conditioning affects a relatively small number of neurons**

Twenty-six percent of all neurons displayed a significantly different response when the animal was in one of the three rooms, yet the number of neurons that altered their response after conditioning appears to be only a few percent. This proportion is small, but within a range we might expect given the characteristics of the nucleus and the nature of the behavioral paradigm. Most electrophysiological studies report that relatively small percentages of NAc neurons show firing correlated with different types of stimuli and actions (Nicola et al. 2004a; Pennartz et al. 1994). Furthermore, most of these studies use repeated presentation of salient, transient stimuli and rewards in tasks involving operant response behaviors that are repeated frequently within a recording session. In contrast, CPP behavior tests use ongoing contextual stimuli and the actual number of behavioral responses relevant to CPP can be small. In light of the low firing rate in the NAc, the power of our statistical analysis was necessarily lower than that for behaviors that generate dozens of stereotyped responses. The rats in our study were free to wander or not and often a particular door was crossed only a half dozen times. Our visual inspections of PETHs often revealed neurons that appeared to have extremely robust transition responses, but because the rat made the transition only three or four times, it was not statistically significant. For this reason, the proportion of neurons we found showing CPP-related changes is likely to be conservative. A modified form of CPP that induced the rats to make more transitions would be valuable for future studies.

On the other hand, our data were sufficient to observe significant posttraining differences in the NAc populations based on room preference. Furthermore, the population of NAc neurons responding to conditioning is consistent with our behavioral analysis of CPP. Our behavioral data show that these room transitions are not timed actions in the way an animal presses a lever in response to a stimulus onset. Rather, they are tendencies to self-initiate locomotion. We propose that these CPP-conditioned NAc neurons provide a constant bias rather than an abrupt command to exit a room. We envision the saline-room–excited neurons as lowering the threshold for the initiation of an exit from the saline room, but it is likely that there are other factors and circuits that contribute to determining precisely when that threshold is crossed and the actual movement occurs. Similarly, we envision that the morphine-room–inhibited neurons lower the threshold for deciding to terminate a movement in the morphine room, but other factors ultimately play into the rat’s decision to stop in the center or the morphine room. We are proposing that NAc neurons have a measurable influence on the animal’s behavior over long intervals (i.e., 15 min). At any given time there are many different signals from many different brain regions. These signals are integrated and a single action is selected. The signals in the NAc that promote CPP behavior must compete with ongoing signals for other actions, such as grooming and exploration. This competition model is consistent with the behavioral observation that the initiation of saline room exits is temporally unpredictable but becomes biased with conditioning toward an increased probability of direct transitions to the morphine room.

**CPP as a model for behavioral electrophysiology of reward**

The activity of NAc neurons has been extensively studied in drug self-administration paradigms. CPP differs from the self-administration model in that reward learning is passive and animals are typically tested in a drug-free state. Self-administration models typically administer a reward to maintain behavior during recording. This can complicate interpretation of the relation of neuronal activity to reward or initiation of drug seeking because the drug may have direct pharmacological effects on NAc neurons that are unrelated to reward or motivational mechanisms. For example, cocaine reward inhibits the baseline firing rate of NAc neurons during self-administration (Peoples and Cavanaugh 2003). CPP removes this confound because CPP testing occurs in the absence of drug; therefore neuronal changes observed during CPP expression are independent of drug effects.

A close analog to the reward-free testing of CPP is operant responding in extinction, when the animal is no longer rewarded for responses. Extinction studies have provided some important insights about the relation of NAc neuronal firing to...
reward. For example, NAc-neuron responding for sucrose or cocaine is attenuated when reward is withheld (Hollander et al. 2002; Janak et al. 2004). When cocaine was withheld during self-administration, NAc responses were primarily altered during the postresponse period when the drug would have been administered. These results emphasize that the NAc firing changes observed in these studies are tied to the immediate reinforcement of the motor response, in contrast to CPP where entries to the rewarded location are not directly reinforced and therefore the location-dependent changes in neural firing reflect long-term learning.

Ghitza and colleagues conducted a cocaine self-administration study in animals in which the delivery of the cocaine was associated with an auditory cue. Animals were later tested with presentation of the cocaine-associated cue while in a drug-free state (Ghitza et al. 2003). They found a population of NAc neurons excited by the cocaine-associated cue. That study demonstrates that cues associated with reward in the setting of an operant task are persistently encoded in the NAc. The current study extends that finding by showing that NAc neurons can encode spatial information related to the location of and approach to a previous reward, even when an animal does not engage in the approach behavior during reward conditioning.

Persistent encoding of classically conditioned reward

Expression of a preference persists for several weeks after the last exposure to a reinforcer (Herzig and Schmidt 2005; Kim et al. 2004; Mueller and Stewart 2000; Mueller et al. 2002). We have seen an alteration in neuronal firing rate many weeks after the animal last experienced morphine. This persistent alteration in NAc neuron responses reflects plasticity in the reward circuit. Evidence points to the NAc as a potential site of this long-term plasticity to reward cues. For example, suppression of c-fos induction in the NAc prevents acquisition of morphine CPP (Tolliver et al. 2000) and antagonizing transcription factors in the NAc blocks both the acquisition of CPP to amphetamine (Aujla and Beninger 2003; Beninger et al. 2003; Gerdjikov et al. 2004) and the expression and reconsolidation of cocaine CPP (Miller and Marshall 2005). Morphine CPP is disrupted by protein synthesis inhibitors injected into the hippocampus, basolateral amygdala, or NAc (Mileck et al. 2006). Another possibility is that the observed alteration in NAc responses after CPP are secondary to long-term changes in regions projecting to the NAc such as the amygdala, medial prefrontal cortex, and hippocampus.

These studies may have relevance for understanding drug addiction, especially cue-induced craving and relapse. A major challenge for drug addiction treatment is craving that persists after long periods of abstinence. Exposure to drug-related cues increases craving in addicts (Childress et al. 1999). A goal of addiction research is to understand what types of cues induce craving and how craving is persistently encoded in neural circuits. To this end, we took advantage of the unusual passive conditioning component of CPP to ask how NAc neurons respond when reward learning was never contingent on a response by the animal. Our data indicate that NAc encoding of long-term reward learning does not require conditioning of specific reward-seeking instrumental behaviors, simply reward-associated sensory cues.

In conclusion, we propose a neural model that is consistent with the behavioral changes observed in CPP expression. This neural model consists of two processes. The first process (mediated by the relative excitation of the NAc neuron population in the saline room) is a constant bias on the animal’s behavior to exit the saline room. The second process (mediated by the relative inhibition of the NAc neuron population) then biases already initiated transitions to end in the morphine room.

These observations extend understanding of the contribution of NAc neurons to the expression of reward learning. Specifically, we have found that morphine conditioning persistently alters location responses in the NAc. The same neuronal population encodes specific directional room-transition responses and is a candidate to initiate or guide approach behavior to contexts associated with drug reward. A similar long-term encoding of passive stimulus–reward associations could be a mechanism by which cues induce craving in abstinent addicts.

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