COCAINE ABSTINENCE ALTERS NUCLEUS ACCUMBENS FIRING DYNAMICS DURING GOAL-DIRECTED BEHAVIORS FOR COCAINE AND SUCROSE

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ABSTRACT

COURTNEY CAMERON: Cocaine abstinence alters nucleus accumbens firing dynamics during goal-directed behaviors for cocaine and sucrose
(Under the direction of Regina M. Carelli)

Distinct subsets of nucleus accumbens (NAc) neurons differentially encode goal-directed behaviors for natural versus drug rewards, and the encoding of cocaine-seeking is altered following cocaine abstinence. Here, electrophysiological recordings were made to determine if NAc selective encoding of natural versus cocaine reward is: (1) maintained when the natural reinforcer is highly palatable and (2) altered by cocaine abstinence. Rats (n=14) were trained on a sucrose/intravenous cocaine multiple schedule and NAc activity was recorded before and after 30 days cocaine abstinence. Before abstinence, the majority of NA neurons displayed nonoverlapping patterns of activity during the task. After abstinence, this pattern was largely maintained; however, more neurons became selectively activated during cocaine- versus- sucrose-seeking. The results indicate that although the selective encoding of cocaine and natural rewards is maintained even with a highly palatable substance, 30 days of cocaine abstinence dynamically alters overall population encoding of natural and drug rewards.

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CHAPTER 1

INTRODUCTION

The ability to seek and acquire natural rewards such as food and water is essential for survival. As such, the brain evolved a highly dynamic system to process information about natural reinforcers. It is often hypothesized that drugs of abuse exert their effects by 'tapping into' this system, causing aberrant reward processing and, ultimately, addiction (Wise, 1997). The nucleus accumbens (NAc) is a critical component of this system and has been implicated in processing information about both natural and drug rewards (Robinson & Berridge, 2000; Kelley, 2004). This structure also plays a key role in addiction, as the dopaminergic projection from the ventral tegmental area to the NAc is a crucial substrate for the reinforcing properties of abused drugs (DiChiara, 1995; Koob & Nestler, 1997; Kalivas & McFarland, 2003; Carlezon & Thomas, 2009).

Electrophysiological recordings show that NAc neurons display patterned discharges (increases or decreases in firing rate) relative to operant responding for both natural and drug reinforcers (Carelli & Deadwyler, 1994; Peoples & West, 1996; Carelli *et al.*, 2000; Carelli, 2002; Nicola *et al.*, 2004). However, different populations of NAc neurons selectively encode information about goal-directed behaviors for natural rewards (food/water) versus intravenous cocaine (Carelli *et al.*, 2000; Carelli & Ijames, 2001). Conversely, natural reinforcers activate largely the same population of neurons in the NAc (Carelli *et al.*, 2000), even when one is highly palatable (Roop *et al.*, 2002). These

findings suggest that drugs and natural rewards activate a separate neural circuit in the NAc (Carelli *et al*., 2000).

However, the precise manner in which NAc neurons encode goal-directed behaviors for drug and natural rewards can be influenced by many factors, including the type of reinforcer and also the pattern of drug exposure (Hollander & Carelli, 2005; Hollander & Carelli, 2007). In human cocaine addicts, drug-taking behavior is often characterized by binges followed by periods of drug abstinence, increased craving, and relapse (Gawin, 1991). Further, animal studies revealed that cocaine abstinence leads to neuroadaptations in brain regions important for reward processing, including the NAc (Robinson *et al.*, 2001; Lu *et al.*, 2003; Conrad *et al.*, 2008; Pickens *et al.*, 2011). Importantly, the percentage of NAc neurons that encode goal-directed behaviors for cocaine, and cocaine-associated cues, is dramatically increased following 30 days of cocaine abstinence (Hollander & Carelli, 2005; Hollander & Carelli, 2007). It is therefore possible that drug abstinence may alter the differential processing of natural versus drug rewards by NAc neurons.

The present study was completed with two primary objectives. First, we determined if the selective encoding by NAc neurons of natural versus cocaine reward is maintained when the former is a highly palatable sweet tastant (i.e., sucrose), as opposed to less palatable food/water used in prior studies (Carelli *et al.*, 2000; Carelli & Ijames, 2001). Second, we examined if the selective encoding by NAc neurons of cocaine- and natural reward-seeking is altered by 30 days cocaine abstinence. To this end, NAc neurons were recorded during a sucrose/cocaine multiple schedule before and after 30 days of cocaine abstinence.

CHAPTER 2

METHODS

Animals

Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN, USA; *n*=14) aged 90-120 days and weighing 260-350g were used as subjects and individually housed with a 12/12-h light-dark cycle. Body weights were maintained at no less than 85% of pre-experimental levels by food restriction (10-15g of Purina laboratory chow each day). Water was available *ad libitum*. This regimen was in place for the duration of the experiment, except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the UNC Institutional Animal Care and Use Committee.

Surgery and behavioral training

All training was conducted in custom-made experimental chambers that consisted of a 43 x 43 x 53cm Plexiglass chamber housed within a commercial sound-attenuated cubicle (Med Associates Inc., St. Albans, VT, USA). One side of the chamber was equipped with two retractable levers, a corresponding cue light positioned above each lever, and a reward receptacle positioned between the two levers.

Rats were first trained to press one lever for sucrose (45mg pellet) on a fixed ratio 1 (FR1) schedule of reinforcement. The start of the sucrose training session was signaled by the onset of the cue light positioned above the active lever and extension of the lever

into the chamber. Lever depression resulted in delivery of a sucrose pellet to the reward receptacle, onset of a tone (65db, 2900Hz, 20s), and retraction of the lever (20s). Rats underwent daily 30 min training sessions until they reached criterion (at least 50 presses per session).

Rats were then prepared for extracellular recording in the NAc via implantation of microwire electrode arrays during the same surgery as catheter implantation using established procedures (Carelli *et al.*, 2000; Hollander & Carelli, 2005). Each array was custom-designed (consisting of eight microwires each with 50µm diameter and arranged in a 2 x 4 configuration), purchased from a commercial source (NB Labs, Denison, TX). Arrays were permanently implanted bilaterally into the NAc core or shell (AP: +1.7mm, ML: ±1.3mm for core and ±0.8mm for shell, DV:-6.2mm from brain, relative to bregma, level skull; Paxinos & Watson, 2007).

Following recovery from surgery, rats were trained to self-administer cocaine on an FR1 schedule of reinforcement during daily 2h sessions. The start of the self-administration session was signaled by the onset of the cue light positioned above the active lever and extension of the lever into the chamber. The cocaine-associated lever was spatially distinct from the lever previously used during sucrose training. Lever depression resulted in intravenous cocaine delivery (0.33 mg/infusion, 6s) via a computer controlled syringe pump, onset of a different tone (65db, 800Hz, 20s), and retraction of the lever (20s). The tones associated with cocaine versus sucrose were counterbalanced across animals.

Following acquisition of cocaine self-administration (2-3 weeks), rats underwent electrophysiological recording (described below) during a multiple schedule of

reinforcement for sucrose and cocaine. Specifically, rats had access to the sucrose-reinforced lever (FR1; 15min) followed by a 20s time out period (no lever extended; dark chamber) and extension of the second cocaine-reinforced lever (FR1; 2h). Illumination of a cue light above each lever signaled the phase (sucrose or cocaine) of the multiple schedule. The order of reinforcer availability (sucrose or cocaine) was varied across animals and recording days such that the same reinforcer was not always given first. In addition, the lever and tone associated with each reinforcer was counterbalanced across animals. Rats then underwent a 30 day abstinence period during which drug access was interrupted (rats remained in their home cages). After 30 days of abstinence, rats underwent a second recording session while performing an identical multiple schedule session for sucrose and cocaine. A timeline of the experimental design is shown in Fig. 1a. Fig. 1b shows an example of a multiple schedule session where phase 1 involved sucrose reward and phase 2 incorporated cocaine self-administration.

Electrophysiological recordings

Electrophysiological procedures have been described in detail previously (Carelli & Deadwyler, 1994; Carelli *et al.*, 2000; Hollander & Carelli, 2005; Roitman *et al.*, 2005). Briefly, before the start of the session rats were connected to a flexible recording cable attached to a commutator (Med Associates Inc., St. Alban, VT, USA) which allowed virtually unrestrained movement within the chamber. NAc activity was recorded differentially between each active and the inactive (reference) electrode from the permanently implanted microwires. The inactive electrode was examined before the start of the session to verify the absence of neuronal spike activity and served as the differential electrode for other electrodes with cell activity. Online isolation and

discrimination of neuronal activity was accomplished using a neurophysiological system commercially available (multi-channel acquisition processor, MAP System, Plexon, Dallas, TX). Multiple window-discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals based on waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal spike events to a Pentium computer. Another computer controlled behavioral events of the experiment (Med. Associates) and sent digital outputs corresponding to each event to the MAP box to be time-stamped along with the neural data. Principal component analysis (PCA) of recorded waveforms was performed prior to each session and aided in the separation of multiple neuronal signals from the same electrode. A projection of waveform clusters was presented in a three-dimensional space, enabling manual selection of individual waveforms. Before each session, an individual template made up of many sampled waveforms was created for each cell isolated using PCA. During the behavioral session, waveforms that matched this template were collected as the same neuron. Cell recognition and sorting was finalized after the experiment using the Offline Sorter program (Plexon), when neuronal data were further assessed based on PCA of the waveforms, cell firing characteristics, and interspike intervals.

Data analysis

Changes in neuronal firing patterns relative to sucrose- or cocaine-reinforced lever press responses were analyzed by constructing raster displays and peri-event histograms (bin width, 250 ms) surrounding each lever press response using

commercially available software (Neuroexplorer, Plexon). Cell firing was classified into one of three well-defined types of phasic neuronal firing patterns that occurred within seconds of the reinforced lever press response (Carelli & Deadwyler, 1994; Carelli *et al.*, 2000; Hollander & Carelli, 2005; Jones *et al.*, 2008). Specifically, cells were classified as type preresponse (PR) if they displayed an anticipatory increase in activity preceding the lever press. Cells were classified as type reinforcement-excitation (RFe) if they displayed an increase in firing rate immediately following a reinforced response. Cells were classified as type reinforcement-inhibition (RFi) if they displayed a decrease in firing rate surrounding a reinforced response. Cells that showed no significant change in firing rate (increase and/or decrease) relative to a reinforced response were classified as nonphasic (NP).

Individual units were classified as either type PR, RFe, or RFi if the firing rate was greater than or less than the 99.9% confidence interval projected from the baseline period (10s before lever response) for at least two 250 ms time bins. This confidence interval was selected such that only robust responses were classified as phasic. Some cells in this analysis exhibited low baseline firing rates, and the 99.9% confidence interval included zero. When this was the case, inhibitions were assigned only if the number of consecutive 0 spike/s time bins surrounding the reinforced lever response was more than double the number of consecutive 0 spike/s time bins in the baseline period. Cells with extremely low firing rates (<0.1 spikes/s) or relatively high firing rates (>15 spikes/s) were likely not medium spiny neurons and were excluded from further analysis.

Cells were then classified based on their phasic activity across both reinforcers (sucrose and cocaine) during performance of the multiple schedule. Cells that displayed

one of the three types of well-defined patterned discharges (type PR, RFe, or RFi) relative to sucrose-reinforced responding, but nonphasic activity relative to the cocaine-reinforced response were classified as 'Sucrose-Selective'. Cells that displayed one of the three types of patterned discharges relative to cocaine-reinforced responding, but nonphasic activity relative to the sucrose-reinforced response were classified as 'Cocaine-Selective'. Cells that displayed the same type of phasic activity to both reinforcers (for example, type PR to both cocaine- and sucrose-reinforced responses) were classified as 'Overlapping'. Finally, cells showing different phasic patterns of activity during responding for cocaine versus sucrose were classified as 'Differentially Phasic'. Comparisons of the number of cells in each category were made across recording days using Fisher's exact test. Comparisons of behavioral responding across recording days were accomplished with paired t-tests.

Histology

Histological reconstruction of electrode positions was accomplished using established procedures (Carelli *et al.*, 2000; Hollander & Carelli, 2005). After the experiment, rats were deeply anesthetized with a ketamine and xylazine mixture (100 and 20mg/kg, respectively) and a 13.5µA current was passed for 5s through all recording wires. Rats were perfused with 10% formalin and 3% potassium ferracyanide and brains were removed, blocked, and sectioned (40µm) throughout the rostral-caudal extent of the NAc. Sections were stained with thionin to aid with identification of structures and location of the blue dot reaction product corresponding to the location of the marked electrode tip. To reconstruct electrode positions, serial sections were examined under a light microscope and the locations of all marked electrode tips were plotted for all

subjects on coronal sections taken from the stereotaxic atlas of Paxinos and Watson (Paxinos & Watson, 2007). Only neurons recorded from wires positioned in the NAc were used in the present study.

CHAPTER 3

RESULTS

Behavior

An example of behavioral responding during the multiple schedule for one representative animal is shown in Fig. 1c. Before abstinence (Day 1) the rat completed 36 sucrose-reinforced responses with an average inter-response interval (INT) of 23.60 ± 1.11s and 24 cocaine-reinforced responses with an average INT of 4.99 ± 0.41 min. On Day 30 the same rat made 42 sucrose-reinforced responses with an average INT of 21.55 ± 0.32s and 30 cocaine-reinforced responses with an average INT of 4.07 ± 0.32min. Similar response patterns were observed across all animals (n=14). Specifically, during sucrose self-administration on Day 1, rats exhibited 37.14 ± 1.99 lever presses with an average INT of 30.50 ± 4.89s. On Day 30, the same animals exhibited 32.29 ± 2.73 lever presses with an average INT of 45.40 ± 13.69s. Cocaine self-administration responding on Day 1 was characterized by 22.43 ± 1.82 lever presses with an average INT of 6.00 ± 0.40 min. On Day 30, animals completed 22.54 ± 2.22 lever presses with an average INT of 5.68 ± 0.43 min. Importantly, behavioral response patterns during the multiple schedule were not altered by cocaine abstinence. Specifically, rats displayed a similar number of lever press responses during the first day of the multiple schedule (Day 1) and following 30 days of abstinence (Day 30) for both sucrose ($t_{(13)}$=1.861; p>0.05) and cocaine ($t_{(12)}$=0.075; p>0.05; Fig. 1d). Further, there was no significant difference in the

average INT from Day 1 to Day 30 for either sucrose ($t_{(13)}$=1.471; p>0.05) or cocaine ($t_{(12)}$=0.9195; p>0.05).

NAc neurons exhibit 3 types of neuronal firing patterns relative to reinforced responding for sucrose reinforcement or intravenous cocaine

Independent of abstinence conditions, three types of neuronal firing patterns (types PR, RFe & RFi) were recorded in the NAc during the multiple schedule relative to reinforced responding for either cocaine or sucrose. The rasters and peri-event histograms (PEHs) in Fig. 2 show examples of the activity of representative phasically active neurons recorded during the cocaine self-administration phase of the multiple schedule. The raster displays and PEHs encompass a 20s time interval surrounding the cocaine-reinforced response (represented by dashed line at time R). One neuron (left) exhibited an anticipatory increase in firing rate immediately before the response, classified as a type PR cell. Another neuron (middle) displayed an increase in firing rate immediately after the reinforced response, termed type RFe activity. The third neuron exhibited type RFi activity (right) showing a decrease in firing surrounding the reinforced response for intravenous cocaine.

Other neurons showed similar types of neuronal firing patterns relative to sucrose reinforced responding. The rasters and PEHs in Fig. 3 show examples of representative phasically active neurons recorded during the sucrose self-administration phase of the multiple schedule. In this case, a neuron classified as a type PR cell (left) exhibited an anticipatory increase in firing rate immediately before the sucrose-reinforced lever press, while another cell exhibited type RFe activity (middle) characterized by an increase in firing rate after the response. A third neuron displayed type RFi cell activity (right) with a decrease in firing rate relative to the reinforced response.

Of all neurons recorded on Day 1, 63% (82 of 130 cells) displayed one of the three types of phasic activity described above, regardless of reinforcer condition. On Day 30, 62% of recorded neurons (70 of 113 cells) exhibited one of the three types of patterned discharges noted above. Thus, there was no difference in the overall percentage of phasic cells from Day 1 to Day 30 independent of reinforcer type.

Populations of NAc neurons exhibit differential firing properties relative to goal-directed behaviors for sucrose versus cocaine prior to abstinence

In prior studies we showed that distinct subsets of NAc neurons differentially encode information about goal-directed behavior for a natural (water/food) reward versus cocaine self-administration using our multiple schedule design (Carelli *et al.*, 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003). One goal of the present study was to extend those findings and examine if the majority of NAc neurons continued to selectively encode goal-directed behaviors for cocaine versus a natural reward prior to abstinence when the later consisted of a highly palatable tastant, sucrose. Consistent with previous findings, distinct populations of NAc neurons differentially encoded information about lever press responding for cocaine or the palatable natural reward, sucrose, prior to abstinence. Specifically, as noted above, a total of 130 neurons were recorded during the multiple schedule for sucrose reinforcement and intravenous self-administration of cocaine on Day 1. Of 130 cells, 82 (63%) exhibited patterned discharges relative to the sucrose- or cocaine-reinforced response. Of 82 responsive neurons, 61 cells (74%) exhibited one of three types of patterned discharges (type PR, RFe, or RFi) relative to the sucrose- or cocaine-response during the multiple schedule, but not both. Only 17 cells (21%) showed similar patterned discharges relative to reinforced responding for sucrose

and cocaine. Finally, 5% were classified as 'Differentially Phasic', exhibiting different types of phasic activity relative to sucrose- and cocaine-reinforced responding.

Fig. 4 summarizes these findings and shows PEHs of normalized firing of all Cocaine-Selective (left), Sucrose-Selective (middle) and Overlapping neurons (right) during the multiple schedule prior to abstinence (Day 1). Only 4 cells displayed one of the three types of phasic activity relative only to cocaine-reinforced responses (nonphasic activity relative to sucrose-reinforced responding), termed Cocaine-Selective (Fig. 4, left; blue lines). Of the 4 neurons, 1 cell was classified as type PR, 2 cells as type RFe and 1 cell as type RFi. In all cases, the same neurons exhibited nonphasic activity relative to responding for sucrose (Fig. 4, left, gray PEHs). Interestingly, a much larger number of neurons (*n*=57) displayed one of the three types of phasic firing patterns relative to responding for sucrose, but not cocaine, and were classified as Sucrose-Selective (Fig. 4, middle; red lines). Of 57 cells, 8 neurons were classified as type PR, 34 as type RFe and 15 as type RFi cells. The same neurons exhibited nonphasic activity relative to lever press responding for cocaine (Fig. 4, middle, gray PEHs). Another population of neurons (*n*=17) exhibited similar patterns of phasic activity during responding for both cocaine and sucrose and were classified as Overlapping (Fig. 4, right). Finally, a small subset of cells (*n*=4) showed different patterns of phasic activity during responding for cocaine versus sucrose, classified as Differentially Phasic (not shown).

Cocaine abstinence shifts how NAc neurons encode goal-directed actions for cocaine versus sucrose reinforcement

A major goal of the present study was to examine if 30 days abstinence from cocaine altered how NAc neurons encode goal-directed actions for sucrose versus cocaine reinforcement during the multiple schedule. To address this issue, the

distribution of NAc patterned discharges was compared before and after 30 days of cocaine abstinence. Before abstinence a high percentage of Sucrose-Selective cells (57 of 82 cells, 70%) was observed while a relatively small percentage of Cocaine-Selective cells (4 of 82 cells, 5%) was recorded (Fig. 4, 5a). However, after abstinence (Fig. 5b) there was a significant increase in the percentage of Cocaine-Selective cells (to 12 of 70 cells, 17%; p=0.017, Fisher's exact test) and a significant decrease in the percentage of Sucrose-Selective cells (to 32 of 70 cells, 46%; p=0.005, Fisher's exact test). Further, while a slight increase was observed in the percentage of Overlapping cells following abstinence (from 17 of 82 cells, 21%, to 23 of 70 cells, 33%), this increase was not significant (p=0.099, Fisher's exact test). There was no change in the percentage of Differentially Phasic cells following abstinence (from 4 of 82 cells, 5%, to 3 of 70 cells, 4%; p=1.000, Fisher's exact test). Finally, the percentage of nonphasic cells was not altered as a function of abstinence (from 48 of 130 cells, 37%, to 43 of 113 cells, 38%; p=0.9027, Fisher's exact test).

The abstinence-induced changes in neuronal firing noted above were differentially distributed across the core and shell subregions of the NAc (Fig. 5c). In the core, there was a significant increase in the percentage of Cocaine-Selective cells (from 1 of 36 cells, 3%, to 6 of 27 cells, 22%; p=0.036, Fisher's exact test). There was also a non-significant decrease for the percentage of Sucrose-Selective cells from Day 1 to Day 30 (from 24 of 36 cells, 67%, to 11 of 27 cells, 41%; p=0.2883, Fisher's exact test). In the shell, there was a significant decrease in the percentage of Sucrose-Selective cells (from 33 of 46 cells, 72%, to 21 of 43 cells, 49%; p=0.032, Fisher's exact test). Further, there was a non-significant increase in the percentage of Overlapping cells from Day 1 to Day

30 (from 9 of 46 cells, 20%, to 16 of 43 cells, 37%; p=0.1821, Fisher's exact test). The increase in Cocaine-Selective cells from Day 1 (3 of 46 cells, 6%) to Day 30 (6 of 43 cells, 14%) in the shell was not significant (p=0.5246). As above, the percentage of nonphasic cells was not altered by abstinence in either the core (from 23 of 59 cells, 39%, to 22 of 49 cells, 45%; p=0.7245, Fisher's exact test) or the shell (from 25 of 71 cells, 35%, to 21 of 64 cells, 33%; p=0.8657, Fisher's exact test).

Histology

Histological reconstruction of electrode placement confirmed the location of recording wires in the NAc core or shell (Figure 6). A total of 130 NAc neurons were recorded on Day 1 (core, n=59; shell, n=71), while 113 neurons were recorded after 30 days of abstinence (core, n=49; shell, n=64). Only data from electrode placements within the borders of the NAc, as depicted in the atlas of Paxinos and Watson (2007) were included in the analysis.

CHAPTER 4

DISCUSSION

The present study was completed with two primary objectives. First, we examined if the selective encoding of goal-directed behaviors for cocaine versus natural rewards would be maintained when the later is a highly palatable tastant. Prior to abstinence, NAc neurons that encoded goal-directed behaviors for intravenous cocaine were largely separate from neurons activated during sucrose self-administration. These findings are consistent with earlier work (Carelli *et al.*, 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003), but extend those findings by showing that this differential encoding is maintained even when the natural reinforcer is highly palatable. Second, we examined if 1-month cocaine abstinence alters this predominantly selective encoding of cocaine- and sucrose-related information by NAc neurons. Results revealed that after abstinence the majority of NAc cells (67%) displayed differential, nonoverlapping patterns of phasic activity relative to cocaine- versus sucrose-reinforced responding. Further, there was a significant *increase* in the overall percentage of cells that were selective for cocaine-related information and a significant *decrease* in the percentage of cells that were selective for sucrose-related information. These findings may be relevant to the decrease in reinforcing properties of natural rewards often reported by human drug addicts (Gawin & Kleber, 1986; Gawin, 1991). Each of the primary findings of the present study is discussed in detail below.

NAc neurons differentially encode information about cocaine versus the highly palatable natural reward sucrose prior to abstinence

As noted above, the first objective of this study was to examine if the selective encoding of goal-directed behaviors for cocaine versus natural rewards (Carelli *et al.*, 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003) is maintained when the later is a highly palatable sweet tastant, as opposed to a more neutral food (Purina Lab chow) or water reinforcer. As noted above, this differential encoding by NAc neurons was largely maintained when sucrose was the natural reinforcer in the multiple schedule. Interestingly, in the present study the percentage of overlapping neurons (21% before abstinence) was higher than that observed during a multiple schedule for cocaine and water (8% overlapping) or cocaine and food (7% overlapping) in our prior work (Carelli *et al.*, 2000). Further, there was a greater percentage of neurons that were selective for sucrose-related information (70% in the present study) compared to neurons that were selective for water-related information (48% in the previous study). These differences may be related to the greater hedonic value of sucrose, compared to water.

However, our previous work also suggests that the percentage of NAc neurons that encode reinforcer-related information is not attributed solely to hedonics. For example, when animals performed a multiple schedule for water and sucrose, the majority of NAc neurons (65%) were similarly activated even though one was of greater hedonic value (Roop *et al.*, 2002). . Others have also shown that goal-directed behaviors for different types of abused drugs (cocaine and heroin) predominantly activate distinct subsets of NAc neurons (Chang *et al.*, 1998), suggesting that the functional segregation of NAc neurons is sensitive to not only natural versus drug rewards but also different classes of abused drugs. Together, these findings suggests that the degree of overlap in

populations of NAc neurons activated during goal-directed behaviors prior to abstinence is functionally complex and could be influenced by several interdependent factors including hedonics, reinforcer type (e.g., sweet tastant versus food/water), and the inclusion of a drug reinforcer in the multiple schedule task.

The selective encoding of cocaine versus the natural reward sucrose is largely maintained following 1 month of cocaine abstinence

A second objective of this study was to determine if 1-month cocaine abstinence alters the predominantly selective encoding by NAc neurons of cocaine- and sucrose-related information noted above. As previously reported, the percentage of NAc neurons phasically active during operant responding for cocaine, or during presentation of cocaine-related cues, is dramatically increased following 30 days of cocaine abstinence (Hollander & Carelli, 2005; Hollander & Carelli, 2007). Therefore, the current study was performed to determine if a specific population of NAc neurons account for the increase in cocaine-related information following abstinence. One possibility is that more NAc neurons exhibit overlapping phasic activity (i.e., similar types of phasic firing during cocaine and sucrose seeking) after abstinence. This finding would indicate that cells that normally process information about highly palatable natural reinforcers are also recruited to process information about goal-directed actions for cocaine following abstinence. However, this was not the case; differential encoding of goal-directed behaviors for the two reinforcers was largely maintained following 1 month abstinence. These results provide evidence that functionally segregated microcircuits exist in the NAc that selectively process specific types of reward-related information, and these microcircuits remain stable following 1-month abstinence.

Further, the anatomical organization of the NAc lends support to this model of functional segregation. The classical view of the NAc as a limbic-motor integrator (Mogenson *et al.*, 1980) is supported by anatomical studies which show that the NAc receives synaptic inputs from limbic areas including the ventral tegmental area, hippocampus, basolateral amygdala, and prefrontal cortex (Zahm & Brog, 1992; Brog *et al.*, 1993). In turn, the NAc can guide motor output through connections with the ventral pallidum and lateral hypothalamus (Zahm, 1999). However, it is unlikely that the NAc as a whole sends a single integrated output to its target structures in order to initiate behavior. Theories of basal ganglia function suggest that the NAc is embedded in a larger system that is organized into several structurally and functionally discrete circuits that are essentially parallel in nature (Alexander *et al.*, 1986; Alexander & Crutcher, 1990). Further, Pennartz *et al*. (1994) proposed that the NAc is composed of a collection of functionally heterogeneous 'neuronal ensembles' that are characterized by distinct afferent-efferent projections. Within this framework, unique sets of limbic inputs converge on specific ensembles of NAc neurons which then generate output to a particular set of target structures, inducing behavioral effects that are specifically linked with each ensemble. The present findings showing differential activation of discrete subsets of NAc neurons during goal-directed behavior for sucrose versus cocaine even after abstinence supports this view of NAc organization.

Cocaine abstinence increases population encoding of cocaine-related information

In the present study, before abstinence there was a relatively high percentage of sucrose-selective cells (70%) and a low percentage of cocaine-selective cells (5%). The exact reasons for this predominance of sucrose-selective encoding are not clear at the

present time. Regardless, it is clear that 1 month abstinence from cocaine alters this pattern of encoding of sucrose- and cocaine-related information by distinct populations of NAc neurons. That is, after 1 month of abstinence, there was an increase in the selective encoding of cocaine-related behaviors and a concomitant decrease in the selective encoding of sucrose-related behaviors. This switch was reflected in an increase in the percentage of Cocaine-Selective cells as well as a decrease in the percentage of Sucrose-Selective cells recorded in the NAc. These results could provide significant insight into the development of an addicted state.

That is, cocaine addicts going through withdrawal experience anhedonia, dysphoria, and an inability to perceive of anything other than cocaine as potentially pleasurable (Gawin & Kleber, 1986). Furthermore, the intensity of these symptoms is associated with the patient's degree of cocaine craving. Human imaging studies using positron emission tomography in cocaine addicts going through withdrawal have consistently demonstrated a reduction in striatal dopamine D_2 receptor availability as well as a reduction in dopamine release in the striatum (Volkow *et al.*, 1993; Volkow *et al.*, 1997; Volkow *et al.*, 1999). It is hypothesized that this hypodopaminergic activity in the striatum (including the NAc) could result in decreased activation of reward circuits by natural reinforcers, causing natural rewards to pale in comparison to drug rewards, thus leading to continued cocaine use as a means to compensate for this decreased reward sensitivity (Volkow *et al.*, 1999; Volkow *et al.*, 2010). The shift from primarily sucrose-related firing to more cocaine-associated discharges in the present study may therefore represent a neurophysiological correlate of this reduction in sensitivity to natural rewards relative to drug rewards following repeated cocaine administration and abstinence.

Implications for the accumbal hypoactivity model

Evidence of accumbal hypoactivity caused by repeated cocaine exposure comes from electrophysiological studies that have demonstrated depression of excitatory synaptic transmission onto NAc medium spiny neurons following repeated cocaine injections (Thomas & Beurrier *et al.*, 2001), sensitization to the inhibitory effects of dopamine on glutamate evoked firing of NAc neurons following cocaine self-administration (White & Harris *et al.*, 1995), and reduced whole-cell sodium (Zhang & Hu *et al.*, 1998) and calcium (Zhang & Cooper *et al.*, 2002) currents in NAc medium spiny neurons after repeated cocaine injections. However, given the selective potentiation of cocaine-related behaviors relative to other non-drug behaviors in addicts, it is difficult to explain how a general hypoactivity of all NAc neurons might lead to addiction. Therefore, Peoples *et al*. (2007) proposed that NAc neurons that encode drug-related behaviors may be spared from the hypoactivity induced in neurons that encode other types of motivated behavior, leading to a relative increase in the transmission of drug-directed behaviors through accumbal circuits and the differential changes in drug- and non-drug-directed behaviors that characterize addiction (termed the differential inhibition hypothesis). An important assumption of this hypothesis is that there exist populations of NAc neurons that are selectively activated during performance of cocaine-related behaviors, but not during the performance of other types of motivated behavior. The present study as well as our previous studies showing differential, nonoverlapping encoding of natural versus drug reinforcers by NAc neurons (Carelli & Ijames *et al.*, 2000; Carelli & Ijames, 2001; Carelli & Wondolowski, 2003) support this supposition.

Further, electrophysiological recordings of NAc neurons from behaving rats during cocaine self-administration show that neurons that encode events related to drug-taking behavior ('Task-Activated' neurons) exhibit no significant change in average firing rates across early (2 or 3 days) and late (30 days) sessions (Peoples & Kravitz *et al.*, 2007). In contrast, other NAc neurons that do not encode aspects of the cocaine self-administration task ('Task-Non-Activated' neurons) exhibit a significant decrease in average firing rates between early and late self-administration sessions, suggesting that these neurons undergo cocaine-induced hypoactivity. The results of our study are consistent with those findings and also include a direct comparison of NAc cell firing during responding for cocaine versus a natural reinforcer. Following abstinence, there was a significant increase in the percentage of cocaine-selective cells (comparable to 'Task-Activated' neurons mentioned above) and a significant decrease in sucrose-selective cells (comparable to 'Task-Non-Activated' neurons). Further, there was a slight, though non-significant increase in the percentage of overlapping cells, which could be considered comparable to 'Task-Activated' neurons as they displayed phasic activity during cocaine self-administration. It is possible that the cocaine-induced hypoactivity that occurred in 'Task-Non-Activated' neurons in the above study also occurred in sucrose-selective cells in our study, thus mediating the reduction in the percentage of sucrose-selective cells and associated increase in the percentage of cocaine-selective and overlapping cells reported here.

Importantly, in our study we examined changes in the percentage of phasic neurons (those that displayed short-duration changes in firing rate time-locked to specific behavioral events). While the Peoples *et al*. (2007) study reported changes in the average

basal firing rates of 'Task-Activated' versus 'Task-Non-Activated' neurons, they did not observe any changes in the percentage of neurons that exhibited phasic activity during drug-directed behaviors, even though the differential inhibition hypothesis might predict this. However, their study did not include an abstinence period, while the present study did. This suggests that a period of drug abstinence may be critical for the development of neuroadaptations mediating the increase in phasic activity observed in our studies.

In support, prolonged periods of cocaine abstinence have been shown to lead to a variety of neuroadaptations in the NAc. These include molecular neuroadaptations such as increased levels of GluR1, NMDAR1, GluR2, and PKA (Lu & Grimm *et al.*, 2003) as well as changes in neurotransmitter levels including increased extracellular GABA concentrations (Xi & Ramamoorthy *et al.*, 2003) and reduced extracellular glutamate concentrations mediated by a reduction in the activity of the cystine/glutamate exchanger (Baker & McFarland *et al.*, 2003). A variety of changes in gene expression have also been observed in the NAc following cocaine abstinence (Toda & McGinty *et al.*, 2002). Of particular interest is the finding that prolonged abstinence from cocaine self-administration leads to the formation of GluA2-lacking AMPARs in the NAc (Wolf & Ferrario, 2010). Relative to GluA2-containing AMPARs, GluA2-lacking AMPARs are more sensitive to excitatory stimulation as a result of their higher channel conductance, permeability to calcium ions, and inward rectification. Incorporation of GluA2-lacking AMPARs can therefore lead to enhanced responsiveness of NAc neurons to glutamatergic inputs (Wolf & Ferrario, 2010). One possibility is that this enhanced responsiveness of NAc neurons to excitatory inputs underlies the increase in cocaine-related phasic activity previously reported (Hollander & Carelli, 2005; Hollander &

Carelli, 2007). Importantly, formation of GluA2-lacking AMPAs in NAc neurons following prolonged cocaine abstinence has been shown to mediate the increase in cocaine-seeking behavior observed during this period (Conrad & Tseng *et al.*, 2008). Further studies will be necessary to determine whether the increases in phasic activity of NAc neurons that we have observed are a direct result of any of the neuroadaptations described above. Nevertheless, it is apparent that cocaine abstinence causes significant alterations in the NAc and larger mesocorticolimbic system that are correlated with changes in cell-firing and cocaine-seeking behavior.

Examination of NAc activity in the core versus shell

Here, abstinence-induced changes in cell-firing were different within the core and shell subregions. While the overall pattern of reduced sucrose encoding and increased cocaine encoding was seen in both the NAc core and shell, the increase in the percentage of Cocaine-Selective cells following abstinence was significant in the core but not the shell. Conversely, the decrease in the percentage of Sucrose-Selective cells following abstinence was significant in the shell but not the core. Considering the differences in afferent/efferent projections (Zahm & Brog, 1992; Zahm, 1999) and electrophysiological characteristics (Pennartz *et al.*, 1992) between the core and shell, it is not surprising that there were differential changes in these subregions following abstinence. The larger increase in the percentage of Cocaine-Selective cells after abstinence in the core may be related to the selective increase in the percentage of phasic neurons in the core following cocaine abstinence we reported in our earlier studies (Hollander & Carelli, 2005; Hollander & Carelli, 2007). However, it is important to note that in the current study the increase in Cocaine-Selective cells was only significantly enhanced in the core although

there was a tendency toward an increase in the shell. The earlier cocaine abstinence studies did not include a multiple schedule, therefore an interesting finding of the current study is that the performance of a sucrose/cocaine multiple schedule appears to recruit NAc shell neurons so that there is a change in both subregions of the NAc following a month of cocaine abstinence.

Effects of cocaine abstinence on behavioral responding during the multiple schedule

Animals displayed consistent cocaine and sucrose self-administration behavioral response profiles across recording days. There was no effect of abstinence on either number of lever press responses or inter-response interval for either reinforcer. These results are consistent with previous studies in which rats were allowed to resume self-administration of sucrose (Jones *et al.*, 2008) or cocaine (Hollander & Carelli, 2005; Hollander & Carelli, 2007) following abstinence. While cocaine abstinence has been shown to enhance motivation to obtain the drug as measured by an increase in operant responding after abstinence (termed 'incubation of drug craving'; Grimm *et al*. 2001), it is important to note that these experiments were performed under extinction conditions in which operant responding did not result in drug infusion. In the present study, rats were allowed to resume self-administration of cocaine after the abstinence period. Therefore, increases in operant responding were not predicted, consistent with similar experimental manipulations previously used in our lab (Hollander & Carelli, 2005; Hollander & Carelli, 2007). However it is important to note that animals also underwent a period of 'sucrose abstinence' as well as cocaine abstinence in the present study. Although others have documented an incubation of sucrose craving (Lu *et al.*, 2004; Grimm *et al.*, 2005),

we have not previously observed this effect in our prior studies (Jones *et al.*, 2008), or in the current study that incorporated the multiple schedule.

As discussed above, the present findings indicate that cocaine self-administration followed by a prolonged period of abstinence can lead to a disruption in reward processing such that encoding of cocaine is enhanced relative to the natural reward sucrose. This neural encoding mirrors the reduced sensitivity to natural rewards seen in cocaine addicts. In the present study, animals did not display a reduction in sucrose self-administration or an increase in cocaine self-administration following abstinence. However, given that the behavioral paradigm used here did not test operant responding under extinction conditions nor involve a direct choice between cocaine and sucrose, it is not unexpected that animals would respond similarly before and after abstinence. Many studies that used behavioral paradigms that more directly examined hedonic processing or reward choice provide evidence that both animals (Aigner & Balster, 1978; Aston-Jones & Harris, 2004; Harris *et al.*, 2007; Negus & Rice, 2008) and humans (Lubman *et al.*, 2009) experience a decrease in sensitivity to natural reinforcers with repeated drug exposure. Furthermore, work from our laboratory has shown that cocaine experience can alter the hedonic value of a natural reinforcer that predicts access to drug self-administration (Wheeler *et al.*, 2008; Wheeler *et al.*, 2011). Therefore, although no overt changes in behavior were observed here during the sucrose/cocaine multiple schedule following abstinence, our findings are consistent with prior work showing that natural reinforcers become devalued as a consequence of repeated drug experience and abstinence.

Conclusions

Overall, the results of this study indicate that drugs of abuse such as cocaine do not simply 'turn on' the same brain reward circuits that have evolved to process information about natural reinforcers. Rather, cocaine activates a neural circuit in the NAc that is largely separate from the one engaged during goal-directed behaviors for natural rewards. Further, it appears that following prolonged abstinence normal reward processing is dysregulated and the encoding of drug-related information is potentiated at the cost of natural physiological rewards. It is possible that with more drug exposure and extended or repeated periods of abstinence these effects will become even greater, leading to the loss of control over drug-directed behaviors that is characteristic of the addicted state in humans.

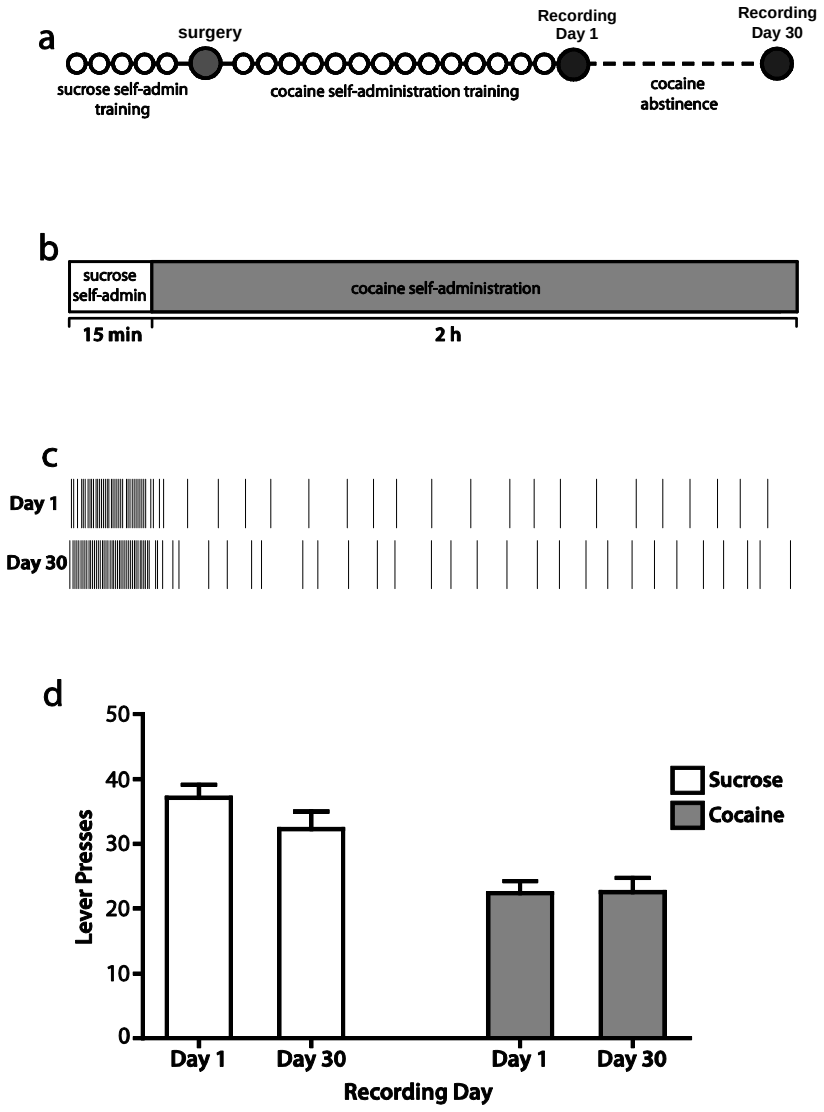

Figure 1. Experimental design and behavior. *a*, Diagram of the experimental timeline. Each circle represents 1 day. During sucrose self-administration training (~5 days), rats had access only to sucrose during daily 30 min sessions. During cocaine self-administration training (~14 days), rats had access only to cocaine during daily 2h sessions. On Recording Day 1 and Recording Day 30, rats performed a sucrose/cocaine multiple schedule. Cocaine abstinence lasted for 30 days, during which time rats

remained in their home cages without drug access. See Methods for details. *b*, Schematic diagram of the multiple schedule. On Recording Day 1 and Recording Day 30, rats had access to the sucrose-reinforced lever for 15 min followed by access to the cocaine-reinforced lever for 2h. See Methods for details. *c*, Example of a representative behavioral response pattern for one rat. Each vertical line indicates one lever press response. On Day 1 (top), the rat completed 36 responses on the sucrose lever and 24 responses on the cocaine lever. On Day 30 (bottom), the rat completed 42 responses on the sucrose lever and 30 responses on the cocaine lever. *d*, Rats *(n*=14) displayed consistent numbers of lever press responses across recording sessions (Day 1 and Day 30) for both sucrose and cocaine.

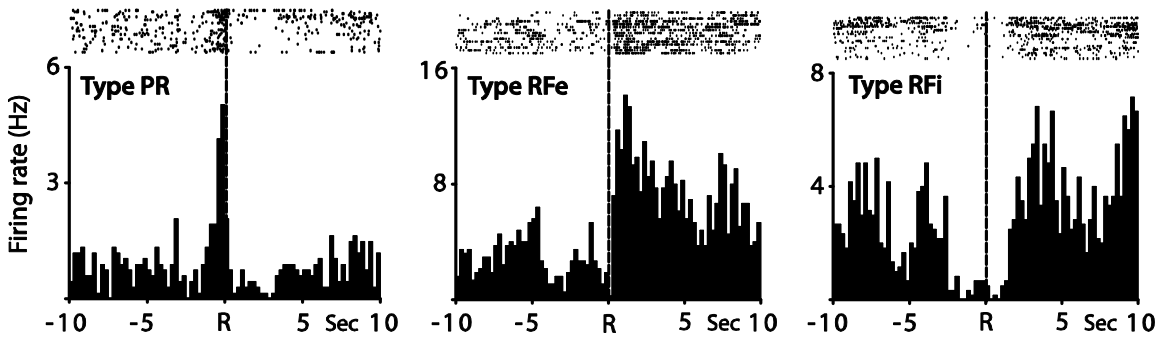

Figure 2. Examples of individual NAc neurons showing one of the three types of patterned discharges (PR, RFe, RFi) during cocaine self-administration. Raster displays and PEHs show the activity of each neuron recorded during a 20 s time period surrounding the cocaine-reinforced response (indicated by dashed line at time R). Individual cells exhibited type PR activity (left), type RFe activity (middle), or type RFi activity (right).

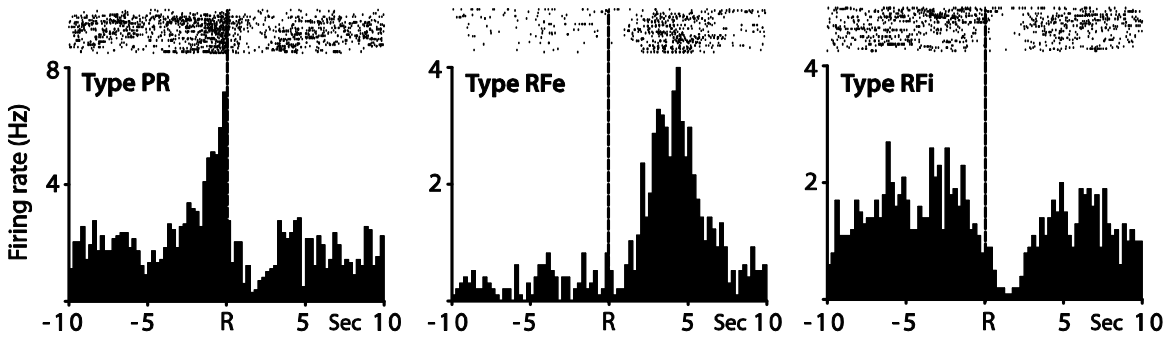

Figure 3. Examples of individual NAc neurons showing one of the three types of patterned discharges (PR, RFe, RFi) during sucrose self-administration. Raster displays and PEHs show the activity of each neuron recorded during a 20 s time period surrounding the sucrose-reinforced response (indicated by dashed line at time R). Individual cells exhibited type PR activity (left), type RFe activity (middle), or type RFi activity (right).

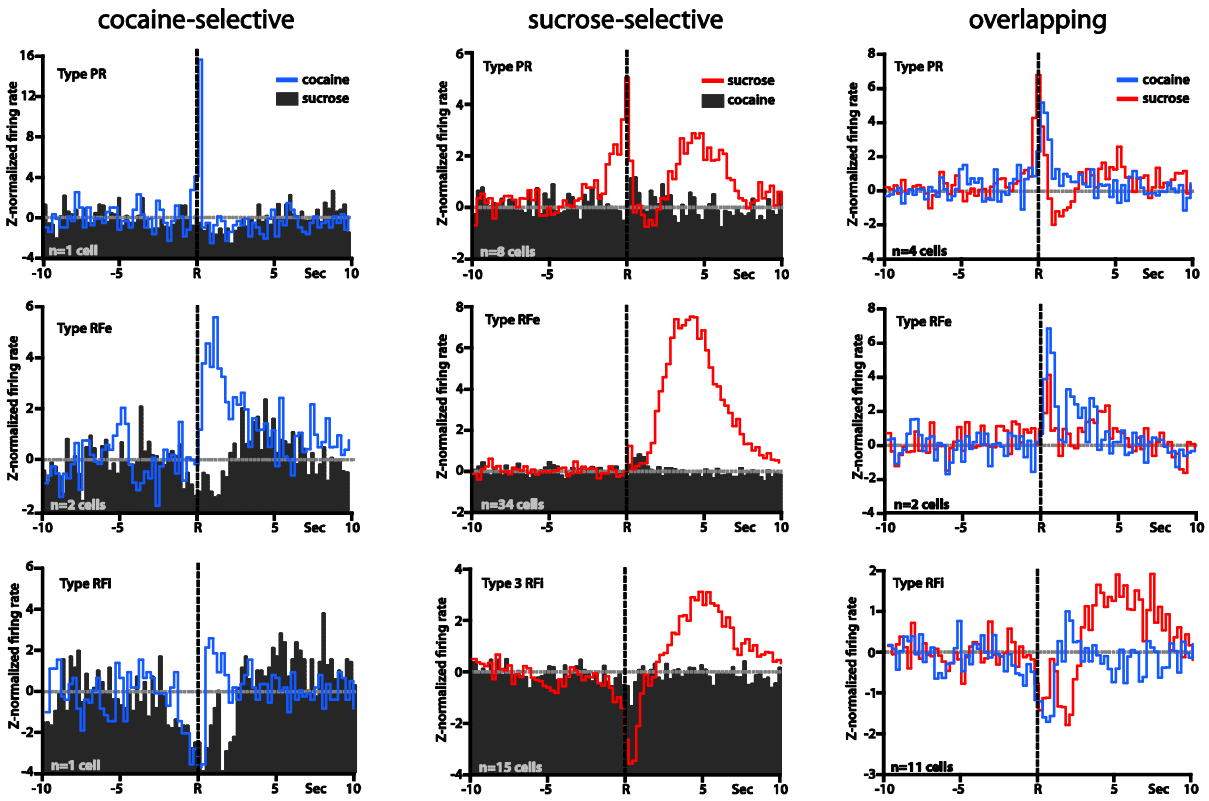

Figure 4. Population histograms of the three cell classifications (Cocaine-Selective, Sucrose-Selective, Overlapping) recorded prior to abstinence (Day 1), separated by type of phasic activity (PR, RFe, or RFi). Lever press responses are indicated by dashed black line at time R in all PEHs. Averages were z-normalized, and baseline is indicated by the dashed gray line at 0. *Left column*, Population averages of Cocaine-Selective cells. The activity of the same neurons relative to cocaine responding (blue lines) versus sucrose responding (gray filled histograms) plotted on the same graph. Normalized firing displayed one of the three well-defined patterns of phasic activity (PR, top; RFe, middle; RFi, bottom) relative to cocaine-reinforced responses but the same cells showed no change from baseline relative to sucrose-reinforced responses. *Middle column*, Population averages of Sucrose-Selective cells. Normalized firing displayed one of the three patterns of phasic activity (PR, top; RFe, middle; RFi, bottom) relative to sucrose-

reinforced responses (red lines), but no change from baseline relative to cocaine-reinforced responses (gray filled histograms). *Right column*, Population averages of Overlapping cells. Normalized firing displayed the same types of phasic activity (PR, top; RFe, middle; RFi, bottom) relative to both cocaine- (blue lines) and sucrose- (red lines) reinforced responses.

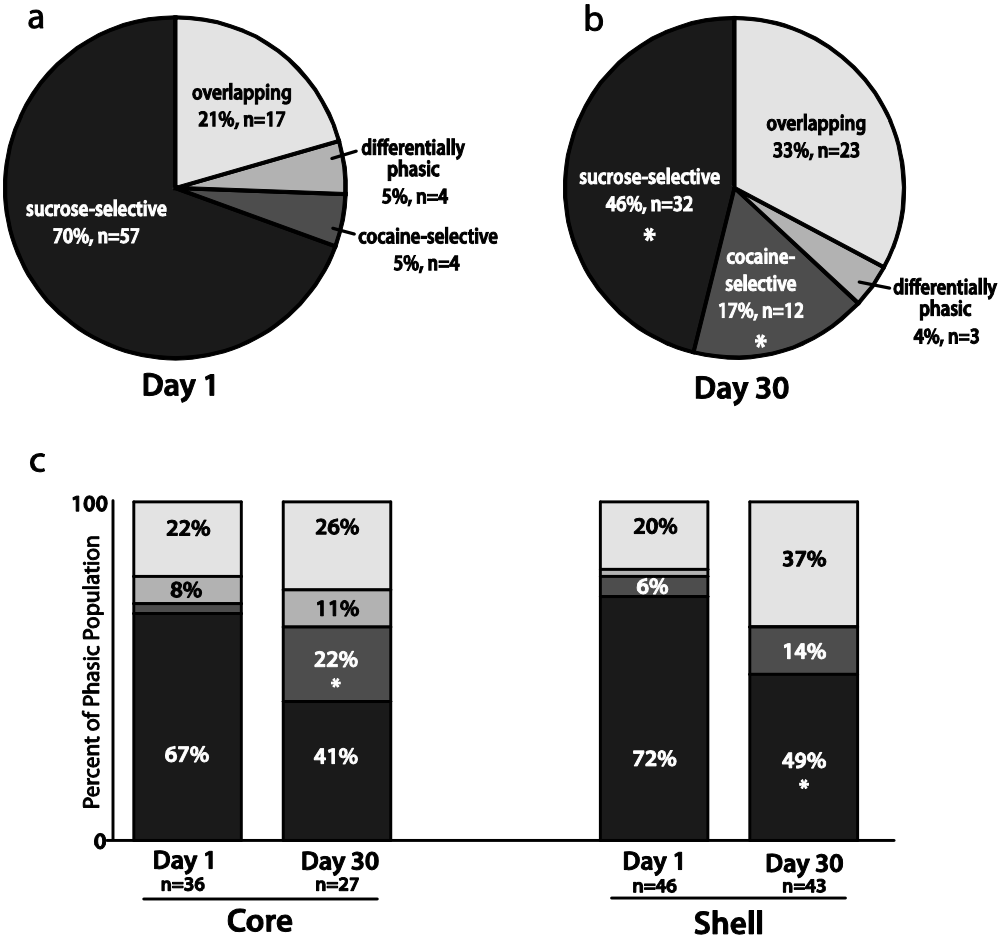

Figure 5. Distribution of phasic activity of NAc neurons during sucrose/cocaine multiple schedule. *a*, Breakdown of phasic activity of NAc neurons (core and shell, combined) recorded before cocaine abstinence. *b*, Phasic activity of NAc neurons (core and shell, combined) recorded following 30 days of cocaine abstinence. After abstinence, the percentage of Sucrose-Selective cells significantly decreased while the percentage of Cocaine-Selective cells significantly increased. *p<0.05. *c*, Phasic activity of neurons in the NAc core vs. shell. In the core, the percentage of Cocaine-Selective cells significantly increased from Day 1 to Day 30. In the shell, the percentage of Sucrose-Selective cells significantly decreased from Day 1 to Day 30. *p<0.05.

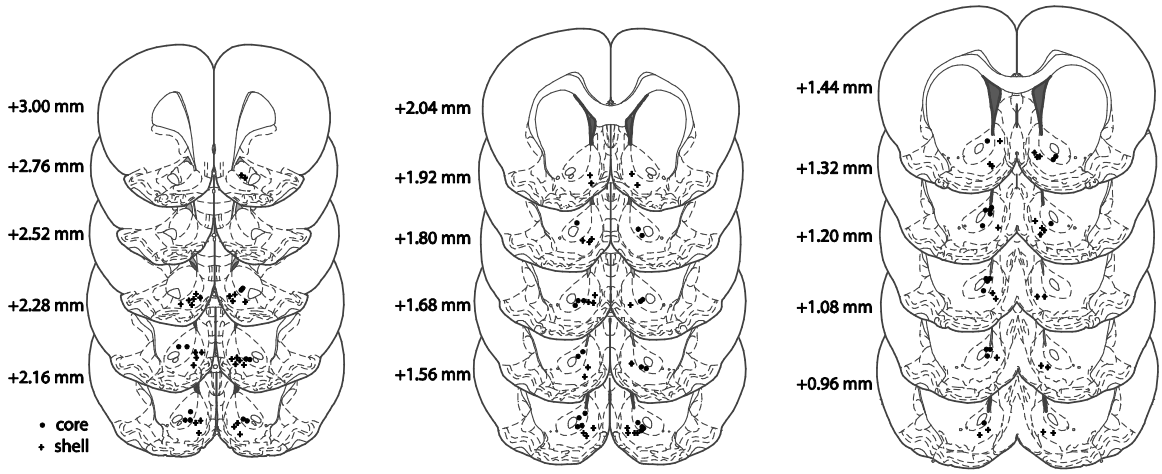

Figure 6. Schematic representation of electrode tip placements in the NAc core (dots) and shell (crosses). Numbers to left of coronal sections indicate distance anterior to bregma (Paxinos & Watson, 2007).

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