NUCLEUS ACCUMBENS NEURONS DIFFERENTIALLY ENCODE INFORMATION ABOUT AVERSIVE CUES THAT PREDICT COCAINE AVAILABILITY AND COCAINE SELF-ADMINISTRATION FOLLOWING EXTENDED TASTE-DRUG PAIRINGS.

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ABSTRACT

JENNIFER LYNN GREEN: Nucleus accumbens neurons differentially encode information about aversive cues that predict cocaine availability and cocaine self-administration following extended taste-drug pairings
(Under the direction of Regina M. Carelli)

Nucleus accumbens (NAc) neurons differentially respond to rewarding and aversive gustatory stimuli: rewarding tastants elicit mostly inhibitions while aversive stimuli elicit excitations. A switch from inhibitory to excitatory responses occurs when a natural reward, saccharin, predicts delayed cocaine availability [1]. Changes in the behavioral response to tastants (termed taste reactivity) track this alteration in NAc activity. Here, we examined: 1) the dynamics of NAc activity and taste reactivity across 5 and 10 taste-drug pairings, and 2) if NAc cells that are responsive to the 'aversive' tastant are the same cells that process information about lever pressing for cocaine. Results showed that the switch from inhibitory to excitatory responses (and taste reactivity) occurred following 5 and 10 taste-drug pairings. Further, neurons responsive to tastant infusion were generally not the same cells that encode goal-directed behaviors for cocaine. Thus, distinct subsets of NAc neurons differentially encode cocaine versus natural rewards even when the natural reward predicts delayed cocaine availability.

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TABLE OF CONTENTS

LIST OF FIGURES

Figure

LIST OF ABBREVIATIONS

BLA	Basolateral Amygdala
DSPs	Digital Signal Processors
FR	Fixed Ratio
INT	Inter-trial Interval
MAP	Multichannel Acquisition Processor
NAc	Nucleus Accumbens
NP	Nonphasic
PEH	Perievent Histogram
PR	Pre-response
RFe	Reinforcement-excitation
RFi	Reinforcement-inhibition
VTA	Ventral Tegmental Area

CHAPTER 1

INTRODUCTION

Cocaine is a psychostimulant that directly affects regions of the brain that process reward related information such as the ventral tegmental area (VTA) and its dopaminergic projections to the nucleus accumbens (NAc) [2, 3]. Interestingly, these are the same neural regions that are activated during goal-directed behaviors for natural rewards such as food and water [3, 4]. As such, numerous studies have focused on this neural system in their examination of the neurobiological mechanisms underlying drug (i.e. cocaine) and natural rewards.

The NAc is anatomically situated to process reward-related information and influence goal-directed behaviors. The NAc receives limbic information from a variety of cortical and subcortical structures including the basolateral amygdala, prefrontal cortex, and hippocampus [3, 5]. In turn, the NAc sends efferent projections through the ventral pallidum to thalamic, brainstem somatomotor, and autonomic effector sites [5]. Given this anatomy, the NAc has been described as a 'limbic motor interface', that integrates information about memory, drive and emotion and influences goal-directed behavior [6]. The NAc is comprised of two primary subregions that include the Core and Shell that have slightly different afferent and efferent projections indicating that they contribute differently to reward related behaviors. For example, the NAc shell receives limbic inputs from the basolateral amygdala (BLA) and ventral subiculum (major output region

of the hippocampus) while the NAc core receives similar inputs from the BLA and parahippocampal regions of the hippocampus [7].

Electrophysiology studies conducted in behaving animals show that NAc neurons encode information about goal-directed behaviors for cocaine and natural (i.e. food, water) rewards [8]. In an initial study conducted in the Carelli laboratory, NAc cell firing was recorded in different animals during lever pressing for either a natural reward (water) or intravenous infusion of cocaine (self-administration) [4]. NAc neurons exhibited specific types of patterned discharges within seconds of the reinforced lever press for cocaine or natural rewards [4, 9, 10]. One group of NAc neurons was activated immediately before the lever press (termed pre-response or PR cells), believed to represent 'anticipation' of reward. A second group of NAc neurons was activated immediately after the lever press (exhibiting either an inhibitory or excitatory response, termed reinforcement-excitation, RFe or reinforcement-inhibition, RFi). Importantly, RFe and RFi NAc neurons also respond to conditioned stimuli paired with cocaine infusion during self-administration sessions [11]. Collectively, initial electrophysiology studies showed that distinct subsets of NAc neurons encode the important features of reward directed behaviors including response initiation, response completion, and reward delivery.

Subsequent studies from the Carelli laboratory determined if cocaine 'taps into' the same neural circuit that normally processes information about natural rewards. A series of studies were completed that examined the activity of the *same* NAc neurons in rats responding on multiple schedules for either two distinct 'natural' reinforcers (water and food), or one of those 'natural' reinforcers and the intravenous self-administration of

cocaine [12]. Although the same types of NAc cell firing patterns as described above (types PR, RFe, RFi) were observed in this study, important differences were noted in firing dynamics in the multiple schedule. Specifically, the majority of neurons tested exhibited similar, overlapping neuronal firing patterns across the two 'natural' reinforcer conditions. In contrast, the majority of NAc cells exhibited differential, nonoverlapping firing patterns relative to operant responding for water (or food) vs. cocaine reinforcement. This finding was observed regardless of which reinforcer (i.e. natural or cocaine) occurred first in the multiple schedule. Further, the selective encoding of cocaine and water rewards by NAc cells was not due to repeated cocaine exposure [13]. These findings provided novel insight into the functional organization of the NAc by indicating that discrete microcircuits exist in this structure that selectively process information about the distinct features of goal-directed actions for drug versus 'natural' reward.

Delayed cocaine availability induces devaluation of a natural reward: an animal model

Importantly, members of the Carelli laboratory noted another interesting feature of natural versus drug reward-seeking in their early work that incorporated multiple schedules mentioned above. In those studies, animals were initially trained to press one lever for water, and a second lever for intravenous cocaine, and only a few sessions involved both reinforcers in the multiple schedule design. On occasions when the task was run across several days and the natural reward lever consistently preceded the drug lever, rats would actually stop pressing the lever for the natural reward (unpublished findings). This was a fascinating observation given the work by others that showed that

animals will respond less for a previously palatable natural reward when it comes to predict an abused drug such as cocaine or amphetamine [14, 15]. Given the potential clinical relevance of this effect, members of the Carelli lab were eager to explore those findings further and examine a potential role of the NAc in the development of natural reward devaluation by cocaine.

For our electrophysiology studies, we needed a behavioral design that incorporated discrete tastant infusions (trials) in order to generate perievent histograms (PEHs) of neural activity time-stamped to tastant delivery. To do this, we incorporated intraoral infusions of the tastants instead of lever pressing for the sweet. Our model was based on the finding that rats exhibit stereotyped oromotor responses to palatable and unpalatable taste stimuli when infused directly into the oral cavity that correspond to the hedonic valence of the stimulus [16]. Importantly, oral facial responses, termed taste reactivity, reflect not only innate taste preferences but also conditioned changes in affect [1, 17]. Rats exhibit appetitive taste reactivity (i.e., licks) during infusion of a sweet tastant such as saccharin, and aversive taste reactivity (i.e., gapes) during intraoral infusion of a bitter tastant such as quinine. In our initial electrophysiology study using this approach [1], a sweet taste cue (saccharin) was intraorally delivered in discrete intervals (i.e., 30, 3.5 s infusions, given every minute across 30 minutes). Immediately after the tastant infusion phase, a lever was inserted in the chamber and animals could press it for intravenous cocaine (i.e. during a 2 hr self-administration phase). Thus, the taste cue signaled impending but delayed cocaine availability. We believe that this ‘‘drug waiting’’ period when the tastant was infused allowed for a strong association to develop

between the taste and *delayed* drug, and enabled the emergence and expression of a negative affective state as measured by taste reactivity.

To test this possibility, members of the Carelli laboratory used videotape analysis to detect and quantify those responses, and examined EMG activity of the anterior digastric muscle, a muscle coupled to licking [1, 16, 18]. They showed that the repeated pairing of a sweet tastant with cocaine results in an aversive state that is reflected in behavior. That is, animals exhibited aversive taste reactivity to the sweet which corresponded to aversive movements (gapes) evident in EMG recordings. Critically, this negative affective state increased motivation to consume cocaine. That is, aversive taste reactivity (gapes) was significantly correlated with cocaine loading responses (presses during the first 5 min of the session) and latency to the first press during self-administration. Rats that exhibited the most gapes showed the greatest number of load up responses and the fastest latency to initiate responding for cocaine once self-administration was accessible. Importantly, this aversive state was reflected in a shift in the activity of distinct populations of NAc activity. Specifically, NAc neurons normally show decreased cell firing during infusions of a sweet and increased activity during infusion of aversive tastants [18]. However, following repeated taste-drug pairings a population of NAc neurons shifted from predominately inhibitory activity to predominately excitatory firing during infusion of the sweet tastant paired with impending cocaine availability. Collectively, these findings suggest that cocaine-conditioned taste cues elicit a cocaine-need state that is aversive, is encoded by a distinct subset of NAc cells, and promotes cocaine seeking.

The objective of the present study was twofold. First, we sought to determine if differences in the number of saccharin-cocaine pairings in our model would have varying results in taste reactivity and NAc activity. Two groups of rats were tested; one group had five saccharin-cocaine pairings while the other group had ten saccharin-cocaine pairings in our model. Given prior findings, it is hypothesized that on the first day of saccharin-cocaine pairings (naïve situation) rats will elicit an appetitive response to saccharin. On the last day (Days 5 or 10) of saccharin-cocaine pairings, rats will elicit aversive taste reactivity during infusion of the same concentration of saccharin and that this negative state will be reflected in shifts in NAc cell firing properties reported by Wheeler et al [1]. Importantly, in Wheeler et.al.[1], NAc cell firing was only recorded during intra-oral infusions and never during the cocaine self-administration phase. Thus, the second objective of this experiment was to determine if neurons that are responsive during intraoral saccharin infusions following 5 or 10 taste-drug pairings, are the same neurons that are activated during cocaine self-administration (types PR, RFe or RFi cells). The results of this objective will provide critical insight into how NAc neurons encode information about aversive cues that predict delayed cocaine availability versus neurons that encode goal-directed behaviors for drug reward during self-administration.

CHAPTER 2

METHODS

Subjects

Seventeen male Sprague-Dawley rats (300-350g), approximately 8 weeks old at the time of arrival, were individually housed with ad libitum food and placed on a 12h/12h light/dark cycle (lights on at 7 am). Water was available ad libitum except for 24 hours before the start of the training sessions when the animals were water-deprived (25ml). All experiments were conducted between 9 am and 3 pm during the light phase. All procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

Surgeries

Rats were anesthetized with a ketamine hydrochloride (100mg/kg) and xylazine (20mg/kg) mixture and surgically implanted with an intra-oral cannulae, intravenous catheter and microelectrode arrays in a single surgery. The intra-oral cannulae was composed of polyethane-100 tubing that was 6 cm in length and attached at one end with a Teflon washer. Briefly, the catheter was inserted laterally to the first maxillary molar with the Teflon washer placed against the molar. The other end of the catheter protruded from an incision at the top of the head and was held in place with dental acrylic. Nine rats were implanted with bilateral intra-oral catheters while separate group of eight rats were implanted with unilateral intra-oral catheters. A detailed description of the cannulae and surgical implantation procedure has been described previously [1, 19].

Intravenous catheters for self-administration were purchased from a commercial source (Access Technologies, Skokie, IL). Surgical procedures for catheter implantation have been described previously [20]. Briefly, the catheter was inserted into the jugular vein and then subcutaneously routed to the animal's back where it was attached to a coupling assembly. The syringe pump (fluid injection assembly) was connected to a swivel system in the experimental chamber which enabled intravenous infusion of cocaine during self-administration sessions. In addition, two eight-wire microelectrode arrays (50 µ diameter; tip separation 0.25-0.50 mm; span 1 x 1 mm) were permanently implanted bilaterally into the NAc in all rats, using established procedures [11]. Arrays were custom-designed and obtained from a commercial source (NB Labs, Dallas, TX). One array in each rat was aimed at the NAc core (coordinates AP: +1.7mm, ML: +/-1.3, DV: -6.3) and the other at the NAc shell (coordinates AP: +1.7mm, ML: +/-0.8, DV: -6.3).

Behavioral task

Training and testing sessions were conducted in a plexiglas chamber (43 x 43 x 53 cm^3; Med Associates, Inc., St Albans, VT) housed within a commercial sound-attenuated cubicle equipped with two retractable levers. Figure 1 shows a diagram of the experimental regimen. Initially, mildly water-deprived (25ml/day) rats were trained to press a lever for water. Once trained, animals were surgically implanted with intra-oral cannulae, intravenous catheters, and microelectrode arrays as described above. Animals were given a 1 week recovery period then conditioning sessions began. Before the start of each session, animals were connected to a flexible cable for extracellular recording. Behavioral sessions were conducted in two phases per day: 1) intra-oral tastant infusions

(over 45 min) and 2) cocaine self-administration (2 hrs). In phase 1, infusion lines were connected to the intraoral cannulae to permit uninhibited movement within the chamber during the session [19]. Each trial consisted of a 3.5 s delivery of 200µl of a 0.15% saccharin solution. A total of 45 discrete tastant infusions (trials; approximately 1 trial/min) were given on a variable interval 45 s schedule (VI45). Immediately thereafter the intravenous catheter line was attached and phase 2 (cocaine self-administration) was initiated, as described previously [19]. Briefly, rats were trained to self-administer cocaine on a fixed ratio 1 (FR1) schedule of reinforcement over a 2h period. Each lever press resulted in intravenous infusion of cocaine (0.33mg/kg/infusion, over 6 s) termination of the cue light positioned above the lever, and onset of a tone (67db, 1 kHz) and house light (25W) stimulus. Lever presses during the 20 s post response period had no programmed consequences. One group of rats underwent 5 days of taste-drug pairings (5-day group; Fig. 1A) while a second group experienced 10 days of taste-drug pairings (10-day group; Fig. 1B). Taste reactivity was only recorded and measured during the saccharin infusion phase on days 1 and 5 for the 5-day group, and on days 1 and 10 for the 10-day group. Likewise, electrophysiological recordings were completed during the entire session but only on days 1 (all rats), 5 (5-day group) or 10 (10-day group). For the 10-day group, only a subset of rats (n=3) were used to analyze taste reactivity data due to video equipment malfunctions.

Electrophysiological recordings

Electrophysiological recording procedures have been described in detail previously [1, 16]. The headstage of each recording cable contained 16 miniature unity-

gain field effect transistors connected to the microelectrode arrays. The neuronal activity was recorded differentially between each active and inactive (i.e. reference) wire. The reference wire was chosen based on the absence of neuronal activity [1]. Analysis of NAc neurons was carried out using a commercially available neurophysiological system (multichannel acquisition processor [MAP] system; Plexon Inc., Dallas TX). Neuronal activity was sorted using high-speed-analog-to-digital signal processing in combination with computer software that enabled isolation of neuronal signals on the basis of waveform analysis. This system utilizes digital signal processors (DSPs) for continuous spike recognition. Behavioral events were recorded through another system (Med Associates, Inc) that sent digital outputs for each behavioral event to the MAP box to be time stamped along with the neural data. Distinguishing between the individual waveforms corresponding to a single neuron was achieved using template and principle component procedures supplied by the MAP system. The template procedures required taking sample waveforms and constructing a template for that extracellular waveform. After the experiment, cells were differentiated further using principle component analysis in Offline Sorter (Plexon Inc., Dallas, TX). NAc cell firing was aligned to solenoid opening onset for the intraoral infusion (phase 1) and to the lever press responses during cocaine self-administration (phase 2).

Data analysis: behavior

Taste reactivity was analyzed frame-by-frame using digital video-recordings. Appetitive and aversive taste reactivity was measured using the technique of Grill and Norgren [17] as described previously [1, 21] Taste reactivity was measured during a 6 s

interval following the start of the saccharin infusion. When the tongue protruded and crossed the midline, it was regarded as an appetitive response which was counted and defined as one lick. Aversive taste reactivity was defined as a facial movement that matched the "triangle" shape for a duration exceeding 90 ms which was counted and defined as one gape. For each intra-oral saccharin delivery trial, the number of elicited responses to each saccharin infusion was counted as either appetitive or aversive. Behavioral responding during cocaine self-administration was quantified via generation of cumulative lever press records, calculation of the number of lever press responses/session, and inter-trial intervals (INT).

Data analysis: neuronal activity

Saccharin Intra-oral Infusions: NAc neurons typically exhibit a change (increase or decrease) in cell firing during gustatory stimulation that occurs 1 s after the solenoid opening and then typically resolves within 5 s [19, 21]. Thus, examination of the change in NAc neuronal activity was confined to the 5 s period after the solenoid opened in Phase 1. NAc neurons were classified as exhibiting phasic activity (i.e. excitation or inhibition) if during the saccharin delivery there was a greater increase or decrease in firing rate relative to the 99.9% confidence interval projected from the baseline period (i.e. 5 s before solenoid opening) for at least two 250 ms time bins. If there was not a significant change (increase and/or decrease) in cell firing during the 5 s post infusion period, neurons were classified as nonphasic (NP).

Cocaine self-administration: Changes in neuronal firing patterns relative to cocaine-reinforced lever press responses were analyzed by constructing raster displays

and perievent histograms (bin width, 250 ms) surrounding each response using commercially available software (Neuroexplorer, Plexon). Cell firing during cocaine self-administration was visually classified into one of three well-defined types of phasic neuronal firing patterns that occurred within seconds of the reinforced lever press response [4, 22-24]. Specifically, cells were classified as "type pre-response" (PR) if they displayed an 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 the reinforced response. Cells that showed no change in firing rate (increase and/or decrease) relative to the reinforced response were classified as nonphasic (NP). To confirm visual classification, individual units were statistically verified 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 (i.e. 10 s before lever press) for at least two 250 ms time bins [10, 25]. 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 [26].

Statistical analysis

Paired t-tests were used to determine if there was a significant change in the mean number of licks, and the mean number of gapes between the first and last days of conditioning. Chi-Square analyses were conducted on the number of inhibitory versus excitatory cells for the 5 (on days 1 and 5) and 10 (on days 1 and 10) day groups to examine if the population of NAc cells exhibited a significant shift from inhibitory to excitatory activity during saccharin infusion in phase 1. Repeated measures ANOVAs were used to determine if significant differences were observed in the mean percentage of cells exhibiting phasic activity across training days 1, 5, and 10 in phase 1 (types inhibitory or excitatory) or during self-administration (types PR, RFe and RFi) in the two groups of animals. Statistical data was analyzed using Prizm 4 and Statistica.

Histology

After the completion of experiments, all animals were deeply anesthetized with a ketamine (100mg/kg) and xylazine (20mg/kg) mixture. To determine electrode placement, each electrode tip was marked by passing a current (13.5 μA) for 5 s through the electrodes. Rats were transcardially perfused with saline followed by 10% formalin and 3% potassium ferracynaide and then the brains were removed and placed in a fixing agent (10% formalin and 3% potassium ferracynaide). After fixing and freezing, brains were sliced into 40 μm sections through the NAc. These sections were mounted on slides and then analyzed for electrode tip placement [1]. Only neurons recorded from wires that were verified to be in the NAc core or shell were used in the analysis.

CHAPTER 3

RESULTS

Taste reactivity

An important aspect of the first objective was to determine if aversive taste reactivity develops in 5 or 10 taste-drug pairings. Across all rats, there was a significant decrease in the mean number of appetitive licks during the first day compared to the last (days 5 or 10) day of training (Fig. 2A; t_{10}= 3.53, $p < 0.01$). This corresponded to a significant increase in the mean number of aversive gapes (Fig. 2B; t_{10}= 4.29, $p < 0.01$) for all rats on day 1 versus the last conditioning day. Examination of taste reactivity measurements as a function of number of training days (5 versus 10) revealed a similar trend. Specifically, there was a significant decrease in appetitive taste reactivity on the first conditioning day compared to last day in the rats that underwent 5 (t_7=2.69, $p<0.05$) or 10 taste-drug pairings (t_2=6.53, $p<0.05$). There was also a significant increase in aversive taste reactivity (gapes) following 5 taste-drug pairings t_7=3.56, $p<0.01$), and a trend toward a similar finding after 10 taste-drug pairings, although this was not significant (t_2=2.58, $p>0.05$).

Aversive taste reactivity predicts drug taking

Wheeler et al [1] demonstrated that cocaine-induced devaluation of saccharin was correlated with an increased motivation to seek cocaine. In the current study, the motivation to seek cocaine was examined via cumulative lever press records, number of

lever press responses/session, and the inter-trial intervals (INT). Behavioral responding was measured across training days for the 5 and 10-day groups. On the first day for cocaine self-administration, all rats exhibited erratic (i.e. not regularly spaced) behavioral responding. Similar erratic lever pressing behavior was also observed following 5 days of training. An example of one representative rat's lever press performance on day 5 is shown in Figure 3A. Behavioral responding became stabile (i.e. regularly spaced) in the 10-day group by their last day of training (mean responses ± SEM, 17.25 ± 2.16, mean INT ± SEM, 3.06 ± 1.33 min). Figure 3B shows an example of a representative rat's lever pressing performance on day 10.

In previous studies from our lab [1, 12, 13] and others [27, 28] rats typically exhibit rapid drug intake at the beginning of the session (i.e. called drug loading or load-up behavior), then transition to more regularly spaced responding with greater INTs for the remainder of the session.. This 'load-up' behavior is illustrated in the first 3 responses in the session shown in Figure 3B. We previously showed that after repeated saccharin-cocaine pairings in our model, drug loading was correlated with aversive taste reactivity [1]. Likewise, latency to first lever press for cocaine was correlated to aversive taste reactivity. These findings indicated that the more aversive gapes exhibited by rats, the faster they are to initiate lever press responding and the larger the number of load-up responses were observed once cocaine was available to self-administer. In the current study, drug loading was not significantly correlated to aversive taste reactivity ($r=0.281$, $p>0.05$), or latency to first press ($r=0.428$, $p>0.05$). However, across all rats we found there was a positive correlation ($r= 0.555$, $p<0.05$) between the total number of lever presses in the session and aversive taste reactivity (Figure 3C). Thus the more averse the

saccharin solution was to the rat the greater the number of lever presses for cocaine during the self-administration phase.

NAc neurons exhibit patterned cell firing relative to intra-oral saccharin infusions

NAc neurons exhibited two types of firing patterns relative to intra-oral saccharin infusions, as previously described (1,4). The perievent histograms (PEH) in Figure 4 show examples of the types of NAc responses during the saccharin intra-oral infusion session. The PEHs consist of NAc cell firing during a 20 s time interval surrounding the intra-oral infusions (represented by the dashed line at time I). One type of NAc neuron exhibited a decrease (i.e., termed 'inhibitory' cell) in firing rate immediately after the start of the saccharin infusion that resolved within 5 s (Figure 4A). Another type of NAc neuron exhibited an increase (i.e., termed 'excitatory' cell) in firing rate 1 s after the solenoid opening that resolved within approximately 8 s (Figure 4B). The last cell type exhibited no change in firing rate during saccharin infusion (i.e., termed 'nonphasic' cell; Figure 4C).

NAc neurons track the conditioned shift in palatability

As in our prior report [1], the learned aversion to the taste stimulus was reflected in the electrophysiological activity of distinct populations of NAc neurons. Similar to responses in naive rats [1, 19], infusions of the tastant on day 1 elicited a predominantly inhibitory profile in rats destined for either 5 (n = 30, 73%, Figure 5A, top) or 10 (n = 34, 67%, Figure 5B, top) taste-drug pairings. That is, of all phasically active cells recorded on day 1, the majority displayed inhibitory activity during saccharin infusion regardless

of whether they later experienced 5 or 10 taste-drug pairings. In contrast, primarily excitatory responses were elicited by infusion of the taste stimulus paired with impending cocaine availability following both 5 (n = 25, 60%, Figure 5C, bottom) or 10 (n=32, 60%, Figure 5D, bottom) taste-drug pairings. To confirm if this shift in the population response was significant, Chi-square analysis was conducted. Using a chi-square 2x2 contingency table, the shift from predominately inhibitory to predominately excitatory cell firing was significant for both 5 (χ^2= 9.029, p<0.003, df=1) and 10 (χ^2=7.629 , p<0.01, df=1) day groups.

NAc neurons exhibit patterned cell firing relative to reinforced responding during cocaine self-administration

During the self-administration phase of the session, NAc neurons exhibited three types of patterned discharges relative to the lever press. The PEHs in Figure 6 show examples of the activity of representative phasically active neurons recorded during cocaine self-administration. One neuron (Figure 6A) exhibited an anticipatory increase in cell firing within seconds before the lever press, classified as a 'Pre-response' or PR cell. A different NAc neuron (Figure 6B) exhibited an increase in firing rate following the lever press, termed 'Reinforcement-excitation', or RFe cell. A third neuron exhibited a decrease in firing rate immediately after the lever press, termed 'Reinforcement-inhibition' or RFi (Figure 6C). Finally, the remaining neurons exhibited no change in firing rate relative to the reinforced response, termed 'nonphasic' or NP (Figure 6D).

NAc neurons differentially encode information about saccharin infusion versus cocaine self-administration

The second primary objective of the current experiment was to determine if the same NAc neurons that exhibit phasic cell firing during saccharin intra-oral infusions in Phase 1 are the same cells that process information about lever press responding for cocaine during self-administration in Phase 2. Figure 7 shows the distribution of NAc phasically active neurons during both phases of the session on Days 1, 5, and 10. For recordings conducted on animals destined for 5 and 10 taste-drug pairings, on day 1, the majority of neurons differentially encoded information about cocaine self-administration (6%) and saccharin infusion (41%), or exhibited nonphasic activity (41%). Only a minority of cells (11%) exhibited phasic cell firing in both phases of the study (i.e., during saccharin infusion and cocaine self-administraiton). This distribution of neural activity remained largely the same following 5 taste-drug pairings (Figure 7B). However, following 10 taste-drug pairings, more NAc neurons were recruited to encode goal-directed behaviors for cocaine during the self-administration phase (from 6% and 7% on days 1 and 5, to 16% on day 10; Figure 7C). Regardless, there remained a low percentage of cells that were phasically active during both saccharin intraoral infusions in phase 1, and cocaine self-administration in phase 2 following 10 taste-drug pairings.

Histology

The locations of the microwires from which NAc neurons were recorded are presented in Figure 8. One hundred and sixteen microwires were implanted in the NAc of rats receiving 5 taste-drug pairings while one hundred and seven microwires were

implanted in the NAc of rats receiving 10 taste-drug pairings. The majority of the microwires (5 day: n=96, 83% of microwires, 10 day: n=76, 71% of microwires) were in the NAc shell for both groups. Forty-nine microwires were implanted outside of the NAc border and recordings from these wires were not included in the analyses.

CHAPTER 4

DISCUSSION

Here, two objectives were examined using a behavioral model developed in the Carelli lab in which delayed cocaine availability induces devaluation of a natural reward. First, it was determined if differences in the number of saccharin-cocaine pairings in our model would have varying results in taste reactivity and NAc activity observed previously [1]. Two groups of rats were tested; one group had five saccharin-cocaine pairings while the other group had ten saccharin-cocaine pairings. As expected, all rats elicited appetitive taste reactivity on the first day of saccharin-cocaine pairings (i.e., naïve situation). Importantly, on the last day of taste-drug pairings, rats elicited aversive taste reactivity during infusion of the same concentration of saccharin regardless of the number of pairings (i.e., 5 or 10). Further, this negative affective state was reflected in shifts in NAc cell firing, as reported by Wheeler et al [1]. Second, we showed that the majority of neurons that were responsive during intraoral saccharin infusions following 5 or 10 taste-drug pairings were not the same neurons that were activated during cocaine self-administration (types PR, RFe or RFi cells). These findings indicate that populations of NAc neurons differentially encode information about a predictive cue signaling impending but delayed cocaine availability versus goal-directed behavior for the drug. In the next sections I will discuss the implications of these findings in more detail.

The effects of the number of saccharin-cocaine pairings (5 versus 10) on taste reactivity and NAc activity

As noted above, our first objective was to determine if differences in the number of saccharin-cocaine pairings in our model would have varying results in taste reactivity and NAc activity to that observed previously [19]. In a previous study from our lab, the same reward comparison paradigm was used in which intra-oral saccharin delivery predicted delayed access to cocaine. In this case, rats were exposed to a total of 5 taste-drug pairing sessions. On test day (i.e. day 5) the investigators measured taste reactivity and dopamine release (using fast scan cyclic voltammetry, FSCV) during tastant infusion. FSCV is an electrochemical technique in which rapid (subsecond) dopamine release events are measured in 'real time' during behavior. The authors reported aversive taste reactivity and a decrease in dopamine release during saccharin infusion on day 5. In contrast, a saccharin tastant that was paired with access to saline induced appetitive taste reactivity and an increase in NAc dopamine release after 5 pairings. Therefore, the decrease in dopamine release after 5 taste-drug pairings reflected an aversive response to saccharin. The behavioral findings are similar to the results found here; an aversion to saccharin developed after it was repeatedly paired with the delayed access to cocaine self-administration. Thus, a negative affective state develops after as little as 5 taste-drug pairings.

In another study conducted in our lab, a behavioral model incorporating similar taste-drug pairings was used but involved a within subject design. [1]. In this case, rats learned to discriminate between intra-oral infusions of an orange or grape flavored 0.15% saccharin solution. One tastant was paired with the delayed self-administration of saline

while the other flavored saccharin was paired to the delayed self-administration of cocaine. Tastant pairing with saline or cocaine occurred on alternating days. Taste reactivity and NAc cell firing was examined following 14 sessions (i.e., after 7 taste-drug pairings that alternated with 7 taste-saline pairings). On the test day, taste reactivity was measured during 30 intra-oral infusions of the tastant previously paired with saline followed by 30 intra-oral infusions of the tastant paired with cocaine. The results showed that animals exhibited appetitive taste reactivity to the flavored saccharin solution paired to delayed access to self-administer saline. However, the same rats exhibited aversive taste reactivity to the flavored saccharin solution paired with delayed access to cocaine self-administration. The emergence of this aversive state was reflected in a shift in NAc cell firing dynamics, similar to the present study (from primarily inhibitory to excitatory activity during tastant infusion). Further, aversive taste reactivity was significantly correlated to both drug loading (i.e. number of lever presses within the first 5 minutes of the session) and the latency to make the first press [1]. Specifically, aversive taste reactivity was positively correlated to rapid drug intake (loading) at the beginning of the session while aversive taste reactivity was negatively correlated to the latency to first press. These findings indicate that the aversive state lead to a greater motivation to consume cocaine, once available.

Here, we were able to demonstrate that after repeated taste-drug pairings the rats elicited an aversive response to saccharin and that populations of NAc neurons shifted how this information was encoded (i.e. exhibited primarily excitatory as opposed to primarily inhibitory activity during tastant infusion), similar to the previous study [1]. However, unlike Wheeler et. al [1], we were not able to find a significant correlation

between aversive taste reactivity and drug loading or latency to make the first press. The previous study compared aversive taste reactivity to drug loading and latency to first press in well trained rats (i.e. tested after the 14th sessions) [1]. However, in the current study, the 5 day group was not well-trained, reflected in less than stabile responding (Figure 3A) for intravenous cocaine by their last day of training. This lack of training may explain why we were not able to replicate the correlational results for this group. Even though the 10 day group had stabile responding by their last day of training, only a subset (n=3) of aversive taste reactivity data was measured due to equipment failure. Thus, a correlation between these variables may have been evident in the 10 day group but was not measured. However, we did demonstrate a correlation between overall lever pressing during the 2 hours self-administration phase and aversive taste reactivity (Figure 3C). Thus, the more averse the saccharin solution was the more the rat lever pressed for cocaine. This result indicates, as in the previous study, that the aversive state reflected in aversive taste reactivity increases motivation for the drug.

NAc neurons encoded Cocaine seeking after 10 saccharin-cocaine pairings

Although a shift in taste reactivity and NAc neural activity occurred in as little as 5 taste-drug pairings, the emergence of NAc neural activity that encoded goal-directed behaviors for cocaine (i.e., cell types PR, RFe, RFi) was only evident after 10 taste-drug pairings. That is, although there were a small portion of NAc neurons that encoded cocaine seeking on day 5, there were significantly more NAc cells that did so following 10 taste-drug pairings. Why do shifts in taste activity occur long before NAc neurons encode cocaine self-administration? One possibility is that specific cells that encode

information about cocaine seeking behavior only begin to do so once the behavior is well learned. As such, we can hypothesize that NAc encoding of cocaine seeking behavior is related to the rat's lever pressing stability during self-administration. As mentioned earlier, behavioral responding was not regularly spaced during cocaine self-administration following 5 taste-drug pairings, however responding became regularly spaced by the 10^{th} taste-drug pairing. The development of stabile lever pressing behavior following 10 sessions parallels the increase in the number of cells encoding cocaine self-administration.

NAc neurons differentially encode information about aversive taste cues that predict cocaine versus goal-directed behaviors for the drug following extended taste-drug pairings

In prior work from the Carelli lab, it was revealed that distinct populations of NAc neurons differentially encode information about goal-directed behaviors for natural (food/water) and cocaine rewards [4, 12]. In those studies, a multiple schedule of reinforcement was used in which rats initially lever pressed for a natural reward (food or water); this was immediately followed by a self-administration session in which rats lever pressed for intravenous cocaine. The authors reported that NAc neurons that were phasically active during goal-directed behaviors for the natural reward were not activated during cocaine seeking behavior (and vice versa) [12]. These findings were not the results of repeated cocaine exposure because similar separate neural encoding was observed as early as the first day of cocaine self-administration training [13]. Collectively, these

findings indicate that separate neural circuits exist in the NAc that differentially encode information about goal-directed behaviors for cocaine versus natural rewards.

Given these findings, a second objective of the current study was to determine if the same neurons that are responsive during intraoral saccharin infusions following 5 or 10 taste-drug pairings, are the same neurons that are activated during cocaine self-administration (types PR, RFe or RFi cells). This issue was not addressed in our prior papers [1, 19], since NAc cell firing was only recorded during intra-oral infusions and never during the cocaine self-administration phase. Here, we found that NAc neurons that were responsive to the non-contingent tastant infusions were generally not the same cells that encode goal-directed behaviors for cocaine. In the current study, few cells were activated during cocaine self-administration on days 1 and 5 so we could not determine if separate neural circuits are recruited to encode the natural reward versus cocaine. However, by the 10th taste-drug pairing more NAc cells were activated during cocaine seeking. Interestingly, these were generally not the same cells that were responsive during intraoral infusions of the tastant. These findings indicate that populations of NAc neurons differentially encode information about a predictive cue signaling impending but delayed cocaine availability versus goal-directed behavior for the drug.

Theoretical considerations: implications for addiction

These findings may be clinically relevant and model an important aspect of addiction in humans since one of the defining features of drug addiction is the establishment of a negative affective or emotional state. This negative state is believed to be a primary motivator for drug intake and relapse and is believed to reflect the stage of

motivational withdrawal when the drug is not available [3, 29]. As such, this often leads addicts to devalue various natural rewards they once viewed important such as relationships and employment. Therefore, understanding the neurobiological mechanisms mediating the emergence of negative affective states and associated devaluation of natural rewards by cocaine could lead to novel pharmacological treatments that decrease this state, help refocus the addicts' interest toward natural reward procurement, and decrease drug craving.

Koob et. al. [30] proposed the allosteric model for drug addiction which states the brain changes during the addiction cycle and these neurological changes explain the susceptibility to relapse long after drug taking ceases. With this model it is believed that allosteric regulation decreases the hedonic value set for natural rewards by increasing tolerance to the hedonic value of cocaine [1, 27, 31, 32]. As a result, the negative emotional state that develops is predicted to be reduced by repeated drug administration. Thus the more intense the negative emotional state the more drug will be self-administered. In this study, we created a situation in which rats anticipated the access to self-administer cocaine by receiving several non-contingent intra-oral infusions of saccharin solution before they had access to self-administer cocaine. After repeated taste-drug pairings, a negative emotional state developed during the first phase (i.e. saccharin intra-oral infusions) of the behavioral task. This development was correlated with higher drug intake strengthening the concept of the allosteric model.

Future directions: role of environmental enrichment

Recently, research has examined the effects of environmental enrichment to prevent addiction. In enriched environments, animals are exposed to social cohorts and/or inanimate objects such as toys or a running wheel. This exposure enhances sensory, cognitive, and motor behaviors [33]. An isolated environment, in which the rat is individually housed, induces stress, particularly if the rats are water or food restricted [33]. This enhanced stress can lead to the acquisition of drug-seeking and chronic drug self-administration. Environmental enrichment produces beneficial effects on several brain functions and prevents or reverses symptoms of neurodegenerative diseases and psychiatric disorders as well as reduces drug-seeking and the reinforcing effects of psychostimulants [33-36]. Thus an enriched environment may provide protection against neurological changes and drug relapse due to repeated drug exposure. For example, mice exposed to an enriched environment during adolescence showed molecular changes in the striatum that resulted in the reduction of the rewarding effects of cocaine [37]. In addition, housing mice in an enriched environment following repeated cocaine exposure, eliminated later cocaine-induced sensitization and conditioned place preference (CPP) [33]. The authors also reported that enrichment prevented reinstatement of cocaine CPP in mice, suggesting that enrichment may decrease sensitivity to stimulant drugs in individuals with a previous drug history, thus making an enriched environment potentially useful for preventing relapse.

Given the reported protective effects of environmental enrichment on drug addiction behaviors, devaluation of natural rewards by cocaine and the aversive state that develops may be prevented by environmental enrichment [33, 37, 38]. One approach in future studies could be to use the same paradigm used in the present report and examine

if voluntary wheel running would reduce devaluation of a natural reward. It is predicted that wheel running, an important form of environmental enrichment, would reduce aversive taste reactivity and subsequent cocaine seeking in our model.

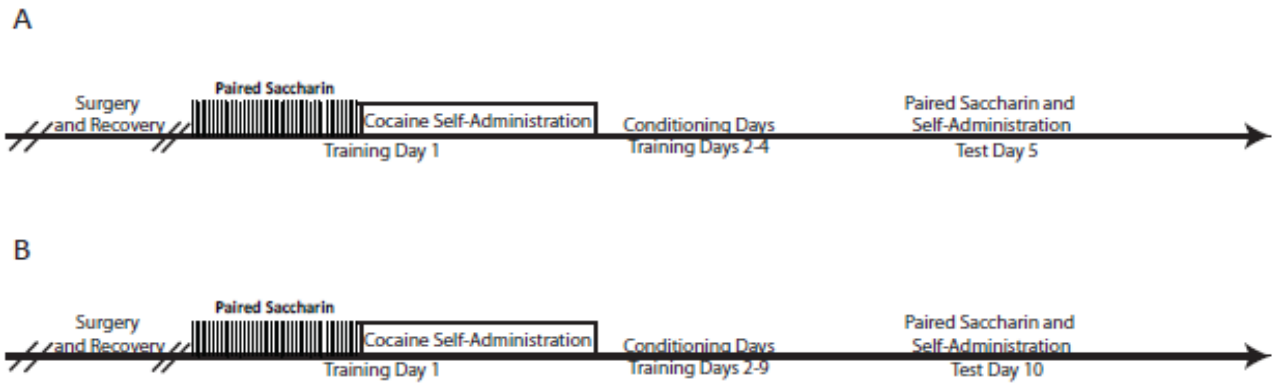

Figure 1. Schematic of *behavioral task.* All rats were first trained to press a lever for water reinforcement then surgically implanted with an intravenous cannula, intra-oral catheter and microelectrode arrays. After recovery (1 week), rats underwent either 5 (Figure 1A) or 10 (Figure 1B) conditioning days during which the behavioral task was conducted in two phases: 1) intra-oral tastant infusions and 2) cocaine self-administration.

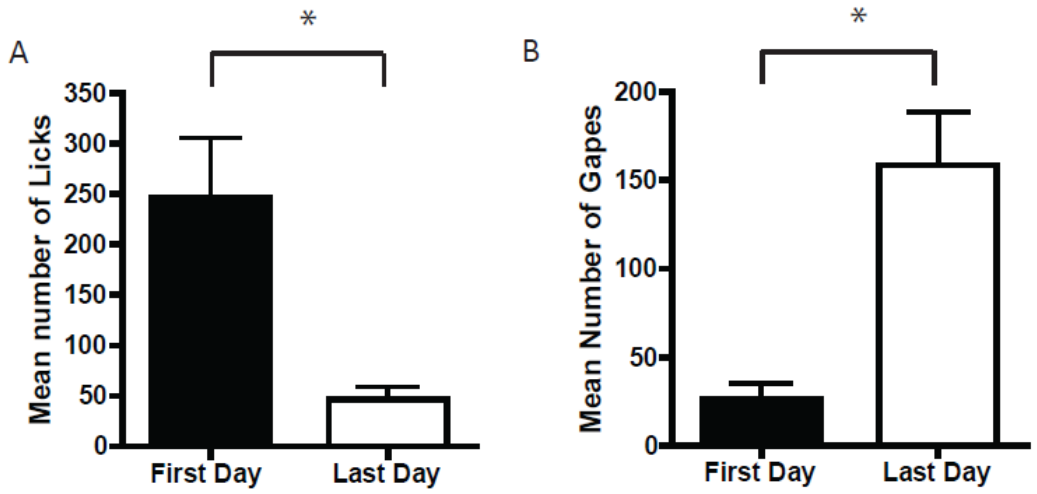

Figure 2. *Appetitive and aversive taste reactivity for 5 and 10 taste-drug pairings groups.* (A) On the first day of the taste-drug pairing animals predominately elicited appetitive taste responses (i.e. licks) in the 5 and 10-day groups. This appetitive taste reactivity significantly decreased from the first to the last day of the taste-drug pairings. (B) Those same animals elicited predominately aversive taste responses to the same saccharin solution after 5 or 10 taste-drug pairings (last day). This was reflected in a significant increase in aversive taste reactivity from the first to last day of the taste-drug pairings.

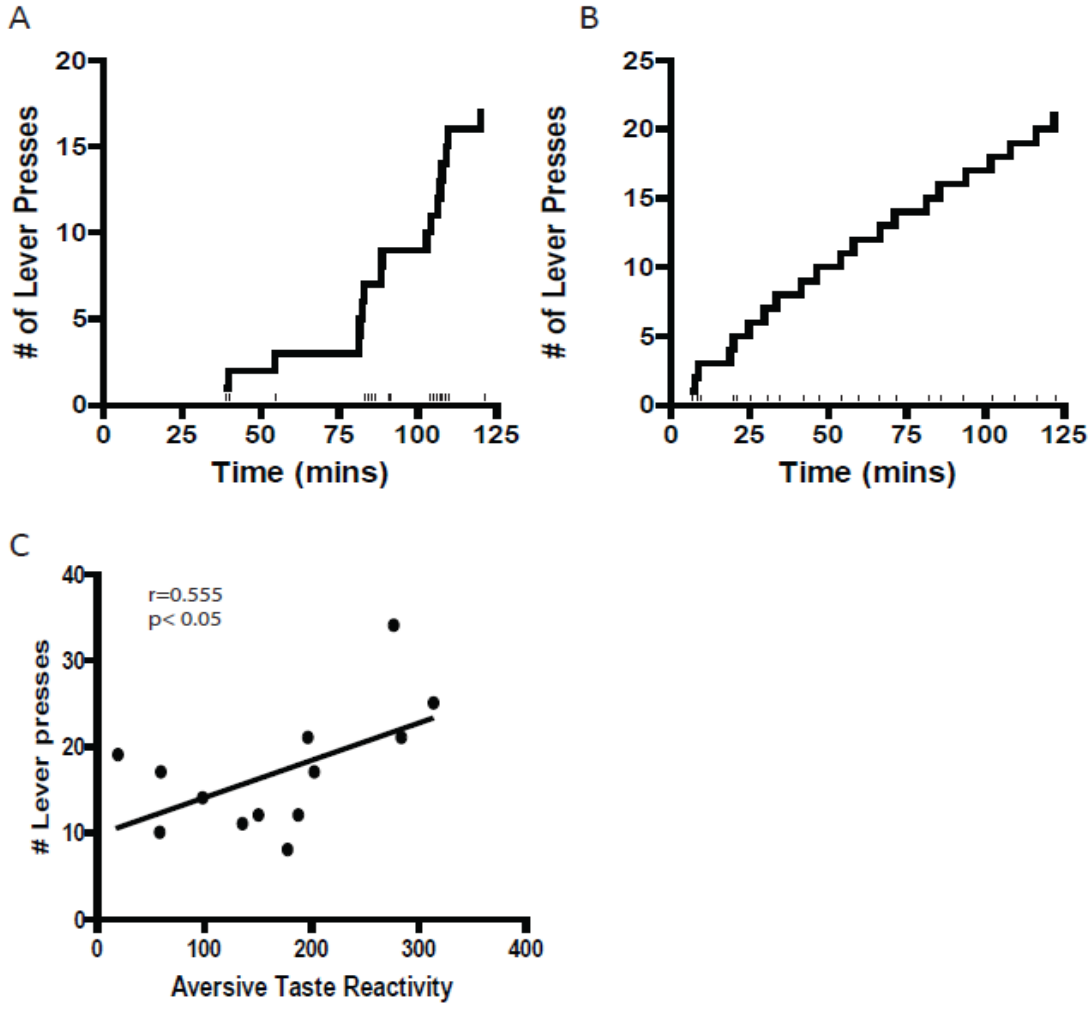

Figure 3. *Aversive taste reactivity predicts drug taking.* (A) A cumulative record showing the behavior of a representative animal during the self-administration phase after 5 taste-drug pairings. Behavioral responding was not regularly spaced across the cocaine self-administration phase. (B) A cumulative record showing the behavior of a representative animal during the self-administration phase following 10 taste-drug pairings. Typically behavioral responding was stable across the cocaine self-administration session on the 10th last day of training. (C) Number of lever presses for cocaine was correlated (r^2= 0.3077, $p<0.05$) to aversive taste reactivity across all rats.

Thus the more averse the saccharin solution was to the rat the more the rat lever pressed for cocaine, once available.

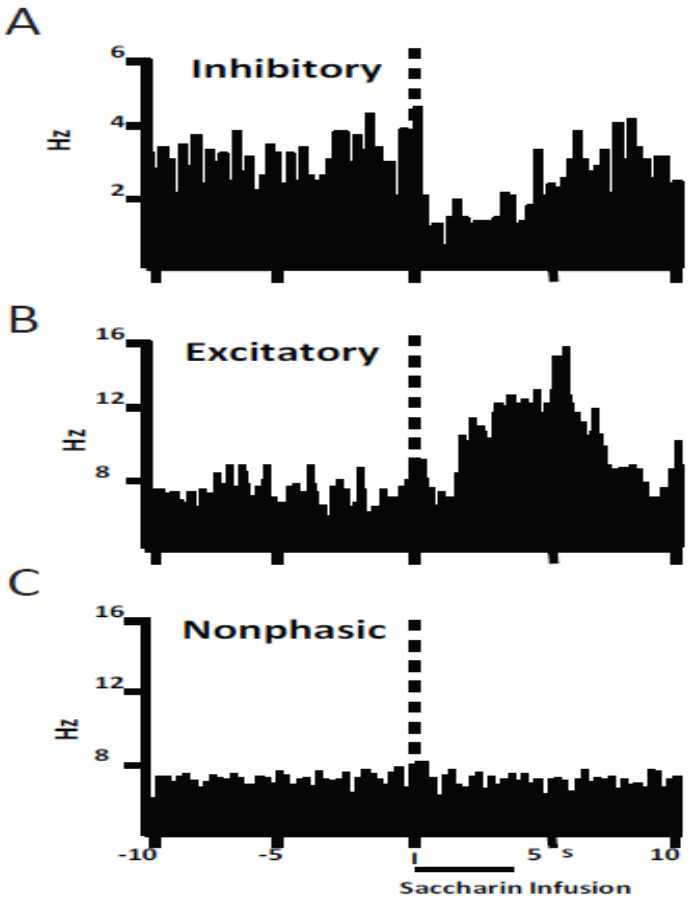

Figure 4. *NAc neurons exhibit patterned cell firing relative to intra-oral saccharin infusions.* (A) The PEH shows an example of one type of NAc neuron that exhibited a phasic inhibition in cell firing during the saccharin infusion. (B) Another population of NAc neurons exhibited a phasic excitation in cell firing during saccharin infusion, illustrated in the representative PEH. (C) Other cells exhibited no change in activity (type nonphasic neurons) during the saccharin infusion.

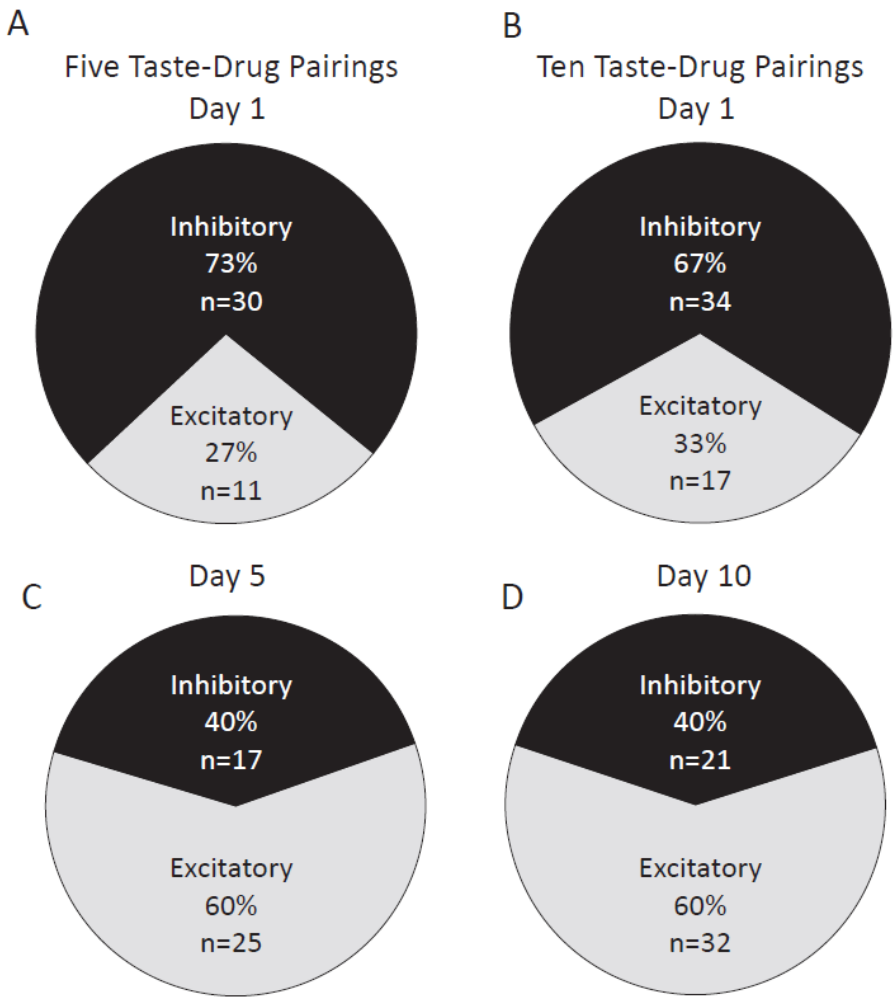

Figure 5. *NAc neurons encoding of saccharin infusions (inhibitory versus excitatory).* Pie charts show a summary of electrophysiological response profiles of NAc neurons to tastant infusions on day 1 versus 5 or 10 taste-drug pairings. On day 1, infusions of saccharin solution elicited primarily inhibitory responses for rats destined to receive 5 (A) or 10 (B) taste-drug pairings. However, the population response shifted such that infusions of the same flavored saccharin solution elicited primarily excitatory response profiles following 5 (C) or 10 (D) taste-drug pairings.

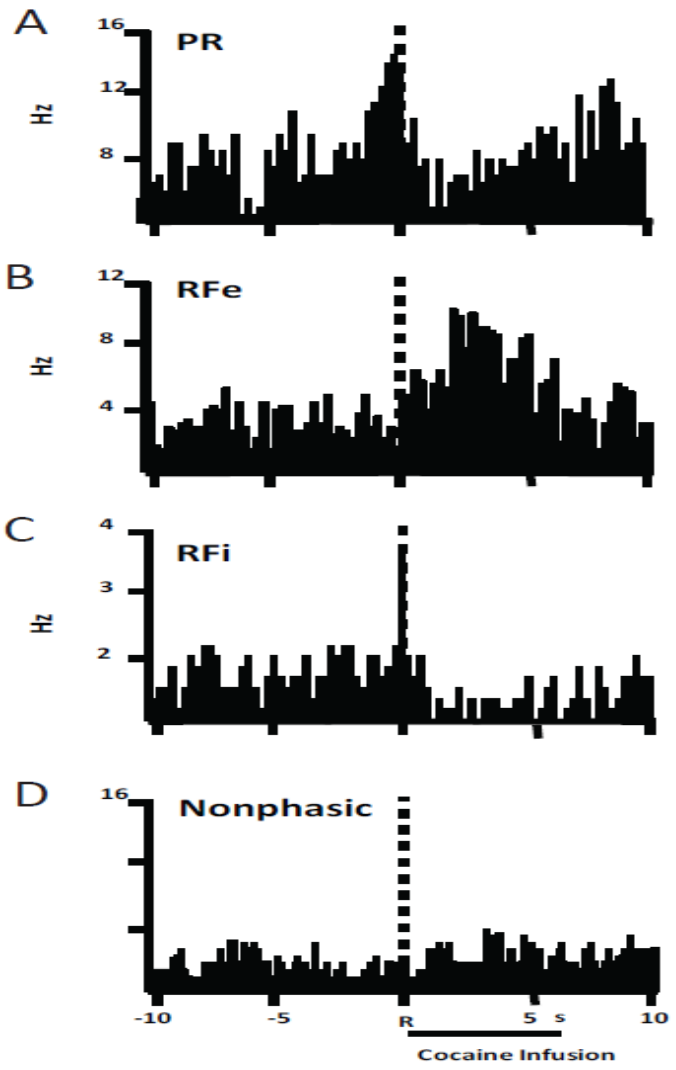

Figure 6. *NAc cell firing profiles relative to reinforced responding during cocaine self-administration.* PEHs show examples of NAc neurons exhibiting increased firing within seconds preceding the lever press response (A), increased activating within seconds following the lever press (B) or decreased activity following response completion (C). Another subset of cells exhibited no change in firing rate relative to the response for intravenous cocaine (D).

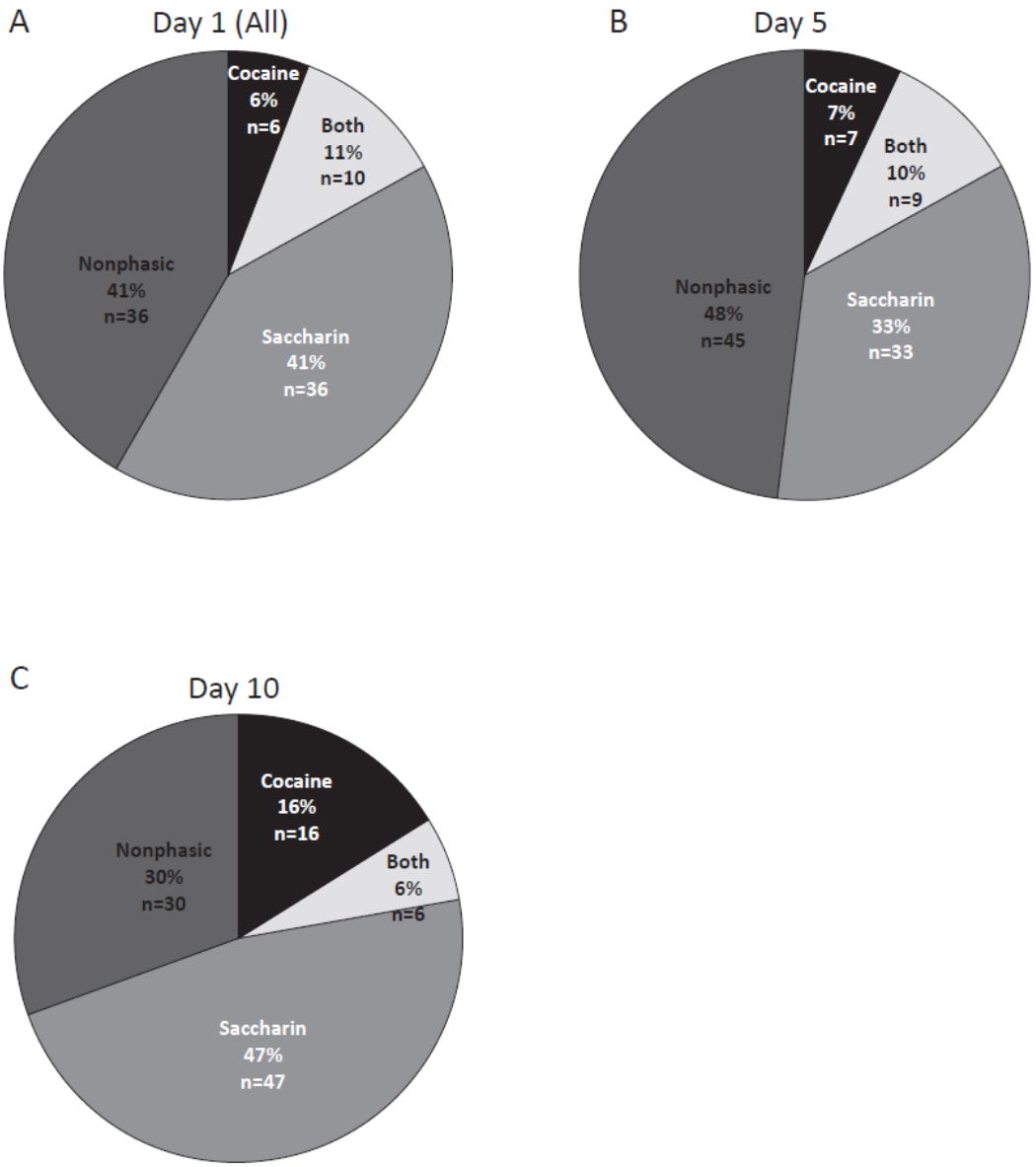

Figure 7. The pie charts illustrate that *NAc neurons differentially encode information about saccharin infusion versus cocaine self-administration following 5 or 10 taste-drug pairings.* Neurons were classified as exhibiting phasic activity only during saccharin infusions in phase 1 ('saccharin'), only during cocaine self-administration in phase 2 ('cocaine'), exhibiting phasic firing in both phases of the session ('both') or showing

nonphasic activity in both phases (‘nonphasic’). (A) The distribution of activity on the first day of training for animals destined for 5 or 10 taste-drug pairings (combined). (B) The distribution of activity after 5 taste-drug pairings. (C) The distribution of activity after 10 taste-drug pairings.

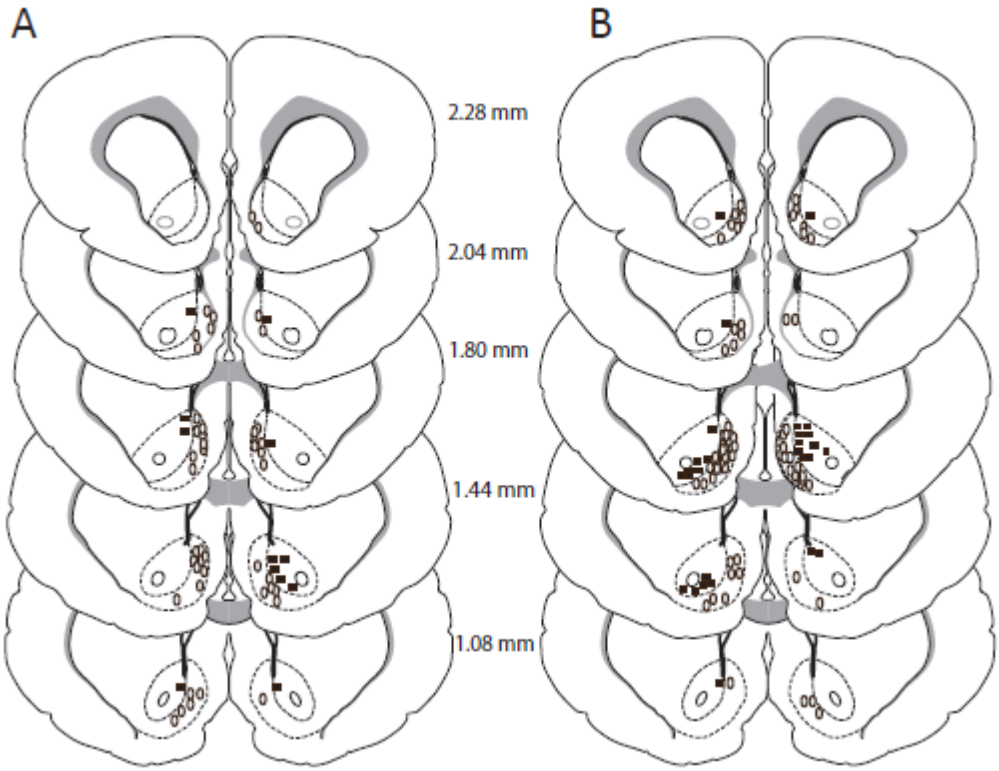

Figure 8. *Histology*. Closed squares symbols represent placement in the NAc core while open oval symbols represent placement in the NAc shell. (A) Microwire placement for animals that received 5 taste drug pairings. (B) Microwire placement for animals that received 10 taste drug pairings.

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