Behavioral and Electrophysiological Indices of Negative Affect Predict Cocaine Self-Administration

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SUMMARY

The motivation to seek cocaine comes in part from a dysregulation of reward processing manifested in dysphoria, or affective withdrawal. Learning is a critical aspect of drug abuse; however, it remains unclear whether drug-associated cues can elicit the emotional withdrawal symptoms that promote cocaine use. Here we report that a cocaine-associated taste cue elicited a conditioned aversive state that was behaviorally and neurophysiologically quantifiable and predicted subsequent cocaine self-administration behavior. Specifically, brief intraoral infusions of a cocaine-predictive flavored saccharin solution elicited aversive orofacial responses that predicted early-session cocaine taking in rats. The expression of aversive taste reactivity also was associated with a shift in the predominant pattern of electrophysiological activity of nucleus accumbens (NAc) neurons from inhibitory to excitatory. The dynamic nature of this conditioned switch in affect and the neural code reveals a mechanism by which cues may exert control over drug self-administration.

INTRODUCTION

The emotional impact of substance abuse includes the emergence of negative affect (including dysphoria, irritability, and anhedonia) that manifests itself in the tangible examples of employment, social, and recreational neglect (American Psychiatric Association, 1993; Dackis, 2004; Jones et al., 1995; Nair et al., 1997; Santolaria-Fernandez et al., 1995). In fact, the negative feelings of drug abusers predict the euphoric effects of cocaine, and these negative feelings play a prominent role in craving and relapse (Fox et al., 2007a, 2007b; Harris et al., 2005; Newton et al., 2003; Sofuoglu et al., 2003). The hypothesis that negative reinforcement mechanisms can elicit drug seeking by inducing a negative-affective withdrawal state was put forth decades ago

(Solomon and Corbit, 1973, 1974) and was subsequently supported (Baker et al., 2004; Koob and Le Moal, 1997, 2001).

However, cocaine addiction is a disorder of chronic relapse that resists therapeutic intervention, not because of the acute motivational properties of the drug, but because learned associations exert strong control over the motivation to seek drugs (Hyman et al., 2006; Kalivas and McFarland, 2003; Kelley, 2004a; See, 2005; Stewart, 1983; Weiss et al., 2000). It is unknown whether the negative affective state induced by the presentation of drug-associated stimuli will promote drug seeking. Solomon's hypothesis predicts this (Solomon and Corbit, 1973, 1974). Unfortunately, most animal models of drug seeking (Ahmed et al., 2002; Deroche-Gamonet et al., 2004; Robinson and Berridge, 1993; Vanderschuren and Everitt, 2004) are not designed to assess the emotional impact of drug taking and, more importantly, the impact of cue-induced negative affect on drug taking. Thus, rigorous testing of the hypothesis is difficult but critical to accurately model how conditioned cues contribute to the persistent nature of human cocaine addiction and to understand its neural foundation. Here we demonstrate a model by which a drug-associated taste cue elicits a conditioned aversive state that is both behaviorally and neurally quantifiable and predicts subsequent cocaine consumption.

In this report, affective responses to a saccharin taste cue were assessed in a rodent model of cocaine self-administration. 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 (Berridge, 2000) and are detectable by videotape analysis and examination of electromyographic (EMG) activity of the anterior digastric muscle, a muscle coupled to licking (Kaplan et al., 1995; Roitman et al., 2005). These responses, termed taste reactivity, reflect not only innate taste preferences (Grill and Norgren, 1978) but conditioned changes in affect (Berridge, 2000) that are dissociable from simple ingestive behavior and can be elicited by conditioned stimuli that engage other sensory modalities (Delamater et al., 1986; Kerfoot et al., 2007). Taste preferences may be assessed neurophysiologically as well. For instance, electrophysiological patterns of activity in the nucleus accumbens (NAc), an area of the brain essential for the expression of a broad
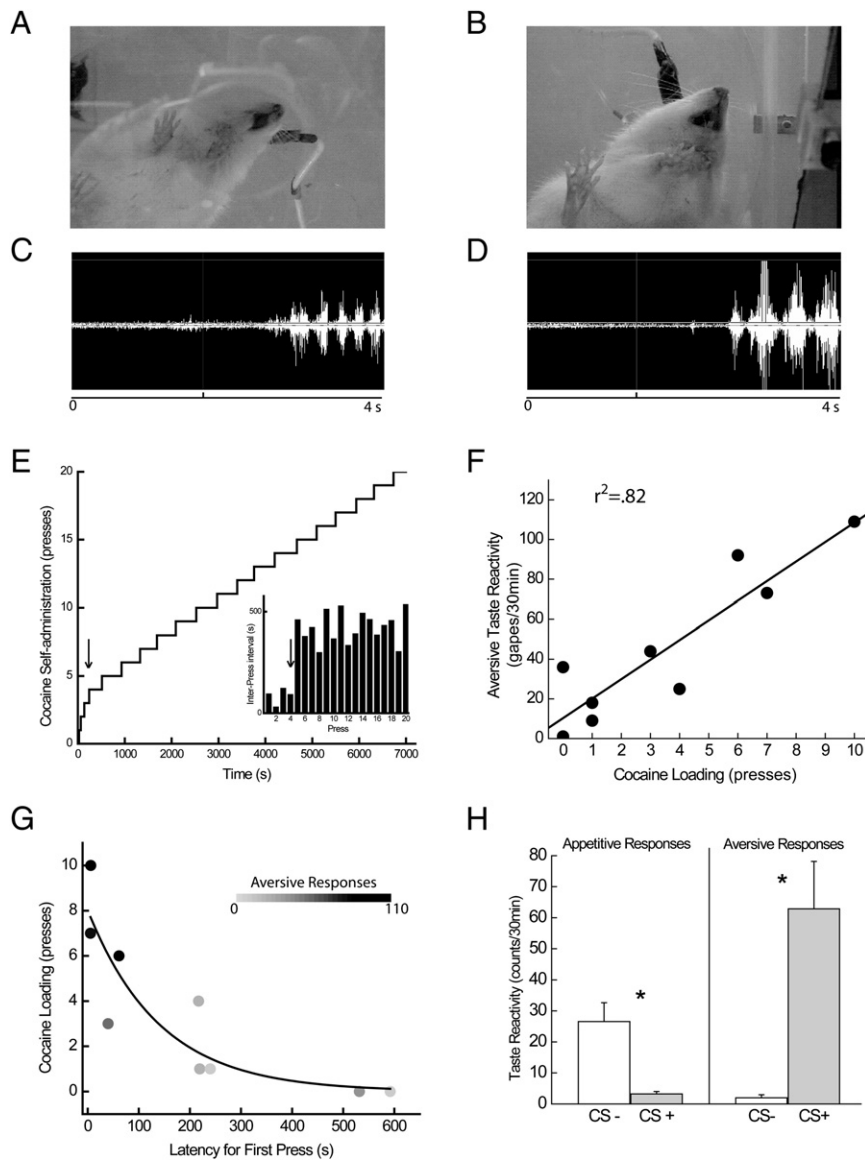
Figure 1. Behavioral Responses to Saccharin that Predict Cocaine Access

During training, rats exhibited predominantly appetitive orofacial reactions to infusion of the CS− (A) but aversive reactions to the CS+ (B). These responses were reflected in the patterns of EMG activity. EMG activity occurred in rapid, low-amplitude bursts indicative of licking shortly after CS− infusion (C). These responses occurred during the infusion of the CS+ as well, but longer-duration, higher-amplitude contractions indicative of aversive taste reactivity also were observed (D). During training, cocaine-induced devaluation was correlated with motivation to take cocaine. Some rats exhibited drug loading (rapid drug intake early in the session). The cumulative record of one rat's presses for cocaine demonstrates this (E). (Inset) Inter-press interval for each cocaine reinforcer further illustrates drug-loading behavior. Arrows indicate the last trial of drug loading. Aversive taste reactivity scores were highly correlated with drug loading in the same session (F). Drug loading was correlated both to the latency to make the first cocaine press and the expression of aversive taste reactivity (plotted in shading, [G]). An analysis of oromotor behavior (mean ± SEM) during the test session revealed appetitive taste reactivity expressed for the CS− and aversive taste reactivity for the CS+ (H). Asterisks denote significant differences, p < .05.

RESULTS

Gustatory Cues that Predict Cocaine Access Elicit Aversive Taste Reactivity

Mildly water deprived rats (n = 9) were trained to discriminate intraoral infusions of an orange or grape flavored saccharin solution. One flavor predicted the opportunity to self-administer cocaine (paired conditioned stimulus, CS+), while the other flavor predicted the opportunity to self-administer saline (unpaired conditioned stimulus, CS−). Commensurate with intraoral delivery, rats consumed both the paired and unpaired solutions (as indicated by a review of videotaped ingestive behavior). However, oromotor behavior and associated EMG activity were different for CS− and CS+. The unpaired CS− elicited mouth movements that were more rapid and of lower amplitude than were those elicited by the paired CS+. The more rapid, lower-amplitude mouth movements elicited by the CS− correspond to ingestive licks and/or positive lateral tongue movements (Figures 1A and 1C), while the slower, larger-amplitude mouth movements elicited by the CS+ correspond to aversive rejection responses, referred to as gapes (Figures 1B and 1D). The successful completion of training depended upon the selective expression of aversive taste reactivity (i.e., gaping) for the CS+. Aversive taste reactivity was most pronounced during the 3.5 s infusion periods but also occurred at seemingly random intervals between taste trials. Twenty-two

range of motivated behaviors (Aragona et al., 2006; Berridge and Robinson, 2003; Kelley, 2004b; Wise, 2002), also reflect the hedonic valence of palatable and unpalatable taste stimuli (Roitman et al., 2005; Wheeler et al., 2005).

It is well known that rats will avoid intake of a taste cue that has been paired with a self-administered drug of abuse (Wise et al., 1976), and greater avoidance of the taste cue is associated with greater drug self-administration (Grigson and Twining, 2002), but the mechanism and involvement of the NAc in this process is far less clear (Di Chiara et al., 2004; Grigson and Hajnal, 2007; Nader et al., 2006; van der Kooy et al., 1983). Given increasing evidence for the role of negative affect in addiction and relapse (Baker et al., 2004), we hypothesized that a sweet taste cue would be devalued by its association with cocaine and would, thereby, elicit a negative affective state (as determined by both behavior and neurophysiology) that would be predictive of cocaine self-administration.

percent of the aversive events recorded occurred after the infusion-period lick bout and during the intertrial interval.

Aversive Taste Reactivity Predicts Drug Taking

The degree of hedonic devaluation of the drug-associated taste cue (CS+) predicted specific aspects of subsequent cocaine intake in the same session. That is, aversive taste reactivity scores on the last training day correlated positively with rapid early cocaine intake, termed drug loading, immediately following the taste session. Drug loading is the primary drug-taking measure that is elevated in extended access models that promote escalated drug use (Ahmed et al., 2002; Ahmed and Koob, 1998). Here, drug loading was defined as the number of lever presses for cocaine early in the session that occurred below one-half of the inter-press-interval for the entire session. Figure 1E illustrates a cumulative record of cocaine self-administration with early-session drug loading. Figure 1F demonstrates the predictive relationship between aversive taste reactivity and subsequent drug loading, $r^2 = .82$, p < .05. Aversive taste reactivity also was correlated with a reduced latency to make the first self-administration response in the subsequent self-administration session, $r^2 = .50$, p < .05. Moreover, the number of drug-loading presses was correlated to the latency to make the first cocaine press, and Figure 1G illustrates their relationship to aversive taste reactivity, $r^2 = .63$, p < .05. Specifically, more aversive taste reactivity related to more cocaine loading and reduced latency to respond for drug. It is important to note that latency to press and drug loading are highly related, as drug loading depends on rapid responses. Interestingly, other factors more closely linked to drug taking (e.g., the amount of cocaine self-administered on day 1 of training, $r^2 = .04$; the latency to acquire stable self-administration, $r^2 = .50$; the total number of presses for cocaine on the last training day, $r^2 = .02$; and the latency to make the first press for cocaine on that day $r^2 = .63$) were not as strongly correlated with drug loading as the expression of aversive taste reactivity. In fact, the stepwise multiple regression (multiple R = .96) of all measured aspects of conditioning revealed that the expression of aversive taste reactivity during training was the only significant variable in the model predicting variance in drug loading, $F_{(4,22)} = 10.84$, p < .05, adjusted R square = .83, significant variable: aversive taste reactivity, $\beta = .665$, p < .05.

Following the final training day, rats were given a test session in which the CS−, then the CS+, were intraorally infused while electrophysiological and EMG recordings were conducted. Frame-by-frame video analysis of the test session revealed that the rapid, low-amplitude responses were elicited by the CS− and were due to neutral ingestive responses and appetitive taste reactivity [i.e., licks and lateral tongue protrusions, $t_{(8)} = 4.33$, p < .05]. Conversely, aversive taste reactivity was exhibited for the cocaine-paired saccharin solution, the CS+ [Figure 1H, $t_{(8)} = 4.08$, p < .05].

Additionally, following the test session, all rats preferred the CS− to the CS+ in a two-bottle test (mean ± SEM; CS− 10.3 ± 1.5 ml/30 min, CS+ 2.8 ± 0.5 ml/30 min, p < .05). The strength of the aversion was indexed using a preference ratio (CS+ intake/CS− + CS+ intake), and results showed that the degree of aversive taste reactivity exhibited to the CS+ during the

preceding intraoral test was significantly correlated with the strength of the aversion demonstrated in the two-bottle intake test ($r^2 = .50$, p < .05). Together these data demonstrate a robust reversal of palatability (from rewarding to aversive) for a taste cue that predicts cocaine availability. The magnitude of this affective response was proportional to subsequent cocaine self-administration behavior.

NAc Neurons Are Predominantly Inhibited by Palatable Gustatory Stimuli in Naive Rats

Consistent with previous reports (Roitman et al., 2005), the brief infusion of orange and grape saccharin solutions in naive rats (n = 3) elicited transient inhibitory responses in most responsive NAc neurons (n = 17, 77% of responsive neurons). The number and magnitude of inhibitory responses were identical for orange and grape flavored saccharin solutions, $F_{(4,13)} = 0.90$, p > .05 (Figures 2A, 2B, 3A, and 3B). A smaller proportion of neurons (n = 5, 23%) exhibited excitatory responses. Again, the number and magnitude of responses were identical for the two tastants $F_{(4,13)} = 0.55$, p > .05 (Figures 2C, 2D, 3A, and 3B). Intake of these solutions in a two-bottle test administered to the naive rats the following day also did not differ (orange 9.2 ± 0.4 mean/SEM, grape 8.7 ± 1.2 ml/30 min, p > .05). These data are consistent with the previous finding that palatable gustatory stimuli elicit predominantly inhibitory responses in NAc neurons (Nicola et al., 2004; Roitman et al., 2005).

NAc Neurons Track the Conditioned Shift in Palatability

The learned aversion to the taste stimulus was evident in the electrophysiological activity of NAc neurons. Similar to responses in naive rats, infusions of the CS− in the nine cocaine-trained rats also elicited a predominantly inhibitory profile (n = 34, 74%, Figure 3C). In contrast, more excitatory responses were elicited by infusion of the CS+, the taste stimulus paired with impending cocaine availability (n = 28, 61%, Figure 3D). This pattern of activity is consistent with the predominant response to the intraoral delivery of an aversive tastant, quinine (Roitman et al., 2005). The ratio of excitatory electrophysiological responses compared to inhibitory responses for the CS+ compared to the CS− was correlated to the magnitude of the expression of aversive taste reactivity ($r^2 = 0.45$, p < .05, Figure 3E). The excitatory response profile and corresponding aversive taste reactivity were inversely correlated to the latency to make the first press for cocaine in the previous cocaine session ($r^2 = 0.64$, p < .05, Figure 3F).

The switch in the activity of NAc neurons resulted from two populations. The first group (n = 12, 26% of responsive neurons) responded with inhibitions for the CS− and excitations for the CS+, $F_{(4,8)} = 6.78$, p < .05 (Figures 4A and 4B). The second group (n = 7, 15% of responsive neurons) exhibited no response for the CS− but transiently increased firing rates for the CS+, $F_{(4,8)} = 7.63$, p < .05 (Figures 4C and 4D). These data demonstrate a shift in patterned NAc neuronal firing corresponding to a learned shift in affect.

Subregion Distribution of Phasic Responses

One hundred ninety-two microwires (16 per animal) were implanted in 12 rats. Forty neurons with phasic responses (inhibitory or excitatory) to infusions of the palatable saccharin solution

Orange 0.15% Saccharin   Grape 0.15% Saccharin
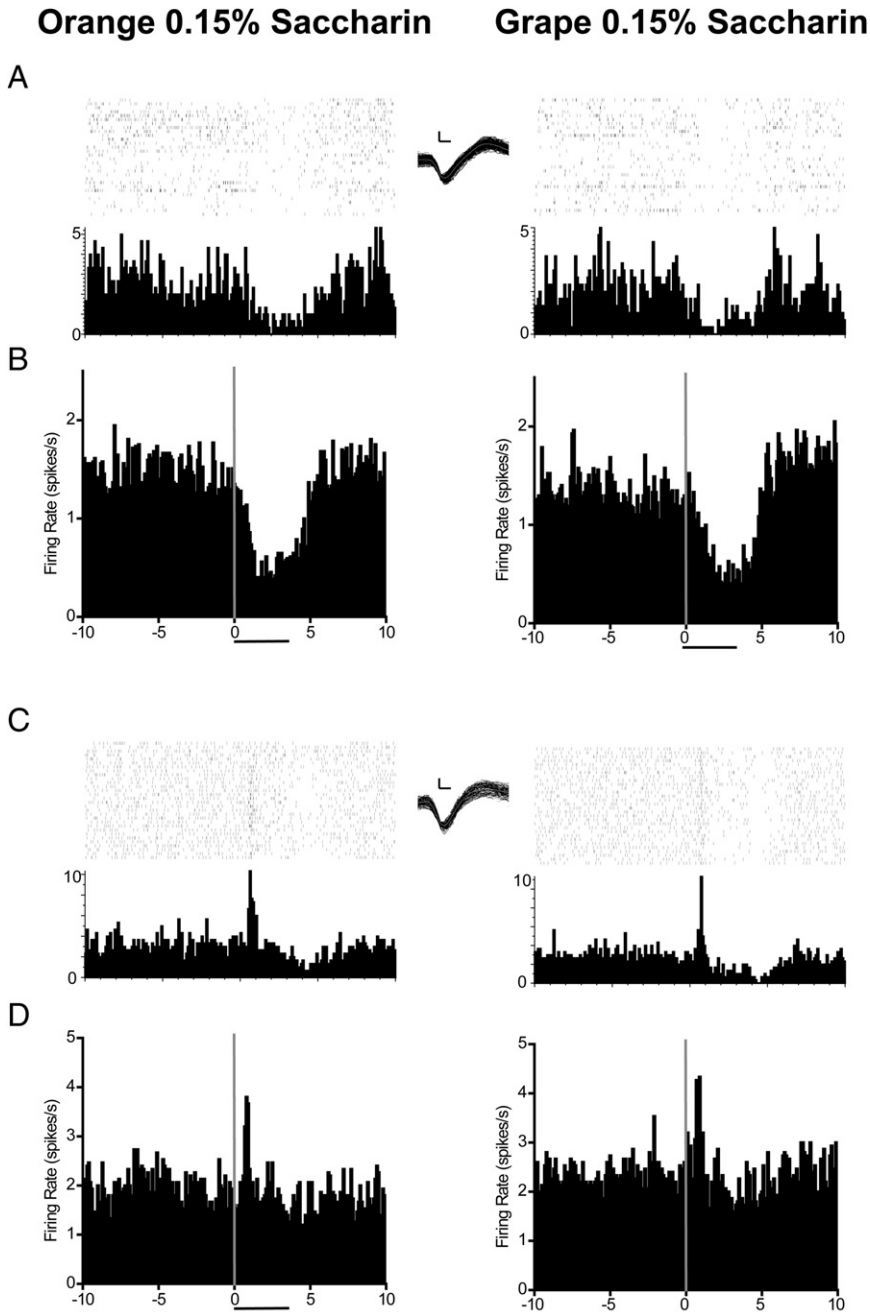
Figure 2. NAc Neurons Exhibit Phasic Responses to Palatable Taste Stimuli in Untrained, Naive Rats

Infusions of orange ([A], left) and grape ([A], right) flavored saccharin solutions primarily elicited inhibitory responses, as shown in the perievent raster for one representative NAc neuron. (Inset) Superimposed extracellular waveforms for this neuron sampled randomly during the session (positive up; scale calibration: 26 μV, 100 μs). The average perievent histograms for all neurons of the same type (n = 17 cells) show the same pattern of inhibitory responses during infusion of orange ([B], left) and grape ([B], right) solutions. Other NAc neurons exhibited excitatory responses to both saccharin solutions, as illustrated for one representative neuron ([C], left and right). (Inset) Positive up; scale calibration: 26 μV, 100 μs. This same pattern of activity was observed across the population of neurons of this type (n = 5 cells), as shown in (D).

responses to saccharin infusions were inhibitory. In the core, 22 of 28 (78%) were inhibitory, ($X^2 = 0.08$, p > .05). There also were no regional differences in phasic responses to the unpalatable CS+. In the shell, 10 of 24 (42%) of the phasic responses to the CS+ infusions were inhibitory. In the core, 8 of 22 (36%) were inhibitory, ($X^2 = 0.004$, p > .05). Therefore, infusions of the devalued taste cue that predicted cocaine access reduced the proportion of inhibitory responses in both the shell and core.

DISCUSSION

Here we show that an initially palatable saccharin solution becomes unpalatable as it comes to predict the opportunity to self-administer cocaine. This switch in the hedonic valence of a tastant is accompanied by a switch in the responsiveness of NAc neurons, also indicative of a reversal of palatability. Aversive taste re-

were recorded from 102 wires histologically verified to be in the NAc shell. Twenty-eight neurons with phasic responses to the palatable saccharin stimulus were recorded from 70 wires verified to be in the NAc core. Twenty wires were verified to be placed outside of borders of the NAc, and recordings from these neurons were not included in analyses. Figure 5 shows the distribution of the electrode placements with phasic responses. From the histological boundaries of the NAc core and shell, there were no differences in the regional distribution of response profiles to infusions of the palatable tastants. Responsive neurons from all rats that received palatable tastants (Naive and CS−) were combined for the analysis. In the shell, 29 of 40 (72%) of the phasic

activity was the single best predictor of rapid drug loading, which has previously been interpreted as a behavioral correction for an anhedonic state (Ahmed and Koob, 1998). Together, these data provide compelling evidence that cues that predict impending cocaine availability can induce a conditioned negative affective state that is reflected in the activity of NAc neurons and is corrected by cocaine self-administration.

Cocaine-Induced Aversive Taste Reactivity

We observed the emergence of pronounced aversive taste reactivity to a gustatory cue that predicts access to cocaine. It has long been known that psychostimulants paradoxically support both
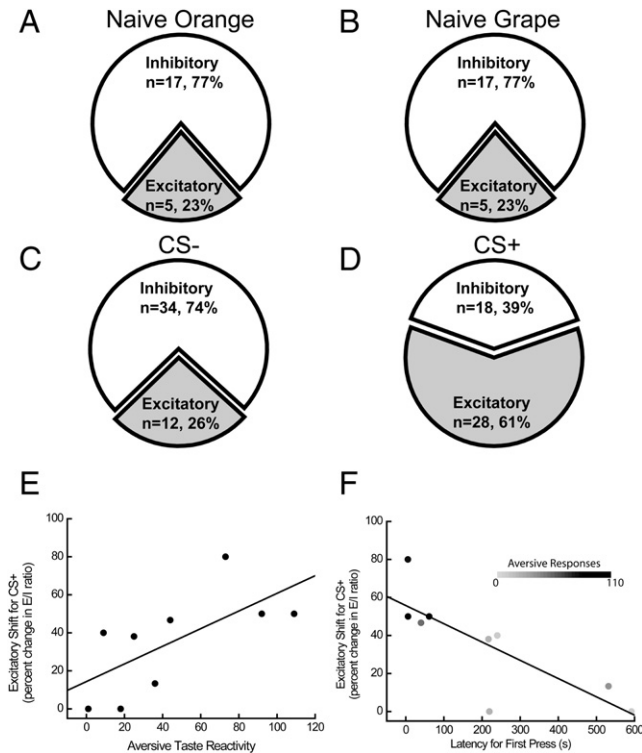
Figure 3. Summary of Electrophysiological Response Profiles of NAc Neurons to Palatable and Conditioned Unpalatable Tastants

Infusions of orange and grape flavored saccharin solutions in naive rats primarily elicited inhibitory responses (A and B). Infusions of the CS− in trained rats at test primarily elicited inhibitory responses (C), while infusions of the CS+ primarily elicited excitations (D). The magnitude of the neurophysiological change correlated to the magnitude of the aversion to the CS+ (E). These related factors, the excitatory change and aversion (plotted in shading), also were inversely related to the latency to self-administer cocaine during training (F).

conditioned taste avoidance and self-administration (Wise et al., 1976). However the mechanism by which cocaine causes avoidance of a taste cue that predicts it is unclear. The phenomenon has been interpreted as conditioned taste aversion, conditioned avoidance, and reward comparison (Grigson, 1997; Grigson and Twining, 2002; Parker, 1995; Riley and Tuck, 1985). The observation of aversive taste reactivity in this report could suggest that the mechanism is due to the aversive properties of cocaine. In fact, the mechanism that appears to be most consistent with aversive taste reactivity is the development of a conditioned taste aversion (i.e., aversion to a taste stimulus that predicts illness). The shift toward excitatory responses in the NAc for the cocaine-paired tastant, similar to those observed for an aversive tastant (Roitman et al., 2005) or a tastant paired with illness (unpublished data), supports this interpretation as well. However, the strong correlation between indices of the reinforcing properties of cocaine (i.e., drug loading and latency to respond) and aversive taste reactivity negates the simple conditioned taste aversion interpretation. In fact, it may be that neither the acutely rewarding nor the acutely aversive properties of cocaine are directly responsible for the development of aversive taste reactivity observed here. The present data suggest an alternative mechanism.

The strong correlations of aversive taste reactivity with drug loading and latency to respond for drug appear paradoxical. One would not expect signs of aversion to predict increased responding for cocaine. One possibility is that increased motivated behavior for cocaine may be a consequence of the development of a negative affective state. Given sufficient drug access, chronic cocaine self-administration alters the rat's hedonic set point, reducing responsiveness to rewarding stimuli (Ahmed and Koob, 1998). As a modified form of the opponent process theory, it is thought that allosteric regulation reduces the hedonic set point by increasing the function of "antireward" brain systems, thereby increasing tolerance to the hedonic effects of cocaine (Ahmed et al., 2002; Ahmed and Koob, 1998; Koob et al., 2004). This negative state is thought to be alleviated by drug loading (a phenomenon observed in this report in rats that show pronounced aversion to the CS+). A hallmark of the extended access model is an increase in drug intake early in the session that may be mediated by negative reinforcement mechanisms (Ahmed et al., 2002; Ahmed and Koob, 2005). Here, we used a 2 hr access period rather than a 6 hr access period used in the aforementioned studies. Even so, some rats exhibited an increase in early drug intake, or drug loading, and a reduced latency to make the first press for cocaine. This may be a necessary antecedent to a more dramatic shift in hedonic set point that occurs with extended access and may involve the onset of a negative affective state. Of course, while all rats may have experienced some manner of a broad reduction in hedonic set point related to drug exposure, this experiment examined the role of conditioned cues in this process. That is, the expression of aversive taste reactivity was specific to the taste cue that predicted cocaine availability and was not evident during exposure to the CS−.

We hypothesize that the cocaine-paired taste served as a predictive cue of cocaine's impending availability and precipitated the expression of a conditioned aversive state in learned anticipation of the future opportunity to self-administer cocaine. The finding that 22% of the aversive events recorded occurred during the intertrial interval suggests that the aversive state persisted throughout the taste session. Candidates for the learned aversive state include the onset of conditioned anxiety, craving (a strong subjective drive to use the drug), and/or withdrawal (i.e., a cocaine-need state). Cocaine administration is acutely reinforcing but is aversive shortly after the initial experience (Ettenberg et al., 1999). Thus, rats approach a context associated with cocaine administration but avoid a context associated with the postmetabolic consequences. Similarly, it is thought that the progressive increase in runway times for cocaine is due to an opponent process causing the emergence of a conditioned negative affective state in preparation for cocaine administration (Ettenberg, 2004). This negative affective state may be akin to a cocaine-need state. Withdrawal has been described as being mediated by compensatory neural adaptations experienced in the absence of the drug (Rasmussen et al., 1990; Russo et al., 2007). That drug-associated stimuli can elicit strong physiological conditioned responses is well established (Ehrman et al., 1992; Eikelboom and Stewart, 1979, 1981). The mechanism by which withdrawal can emerge as a conditioned response is less clear. To that end, nongustatory cues associated with naloxone-precipitated heroin withdrawal significantly increase both
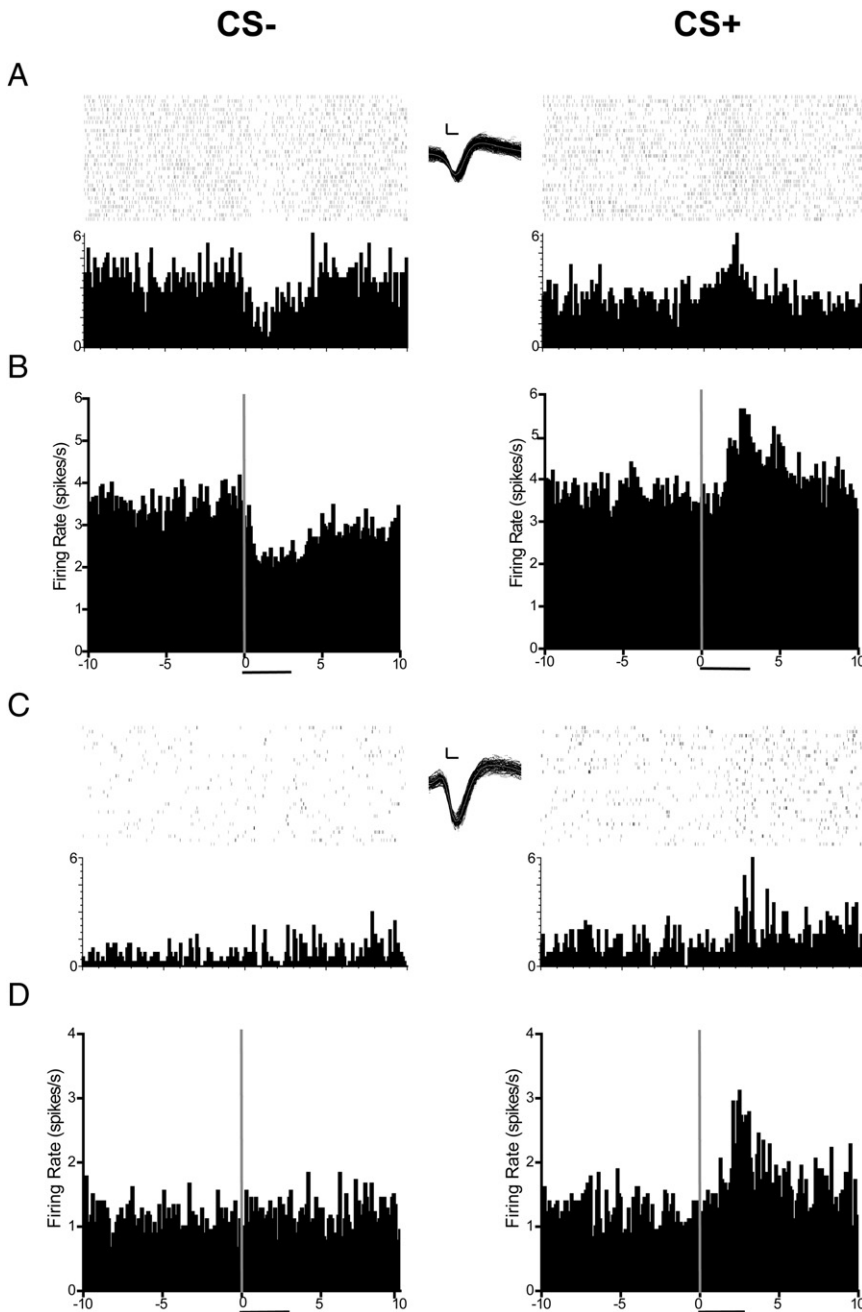
Figure 4. NAc Neurons Track the Learned Aversion to Saccharin

Infusions of the CS− at test elicited inhibitory responses, as shown in the perievent raster for one representative neuron ([A], left), while infusions of the CS+ elicited an excitatory response in the same cell ([A], right). (Inset) Scale calibration: 26 μV, 100 μs. The same pattern of responses was observed across the population of neurons of the same type (n = 12 cells; [B], left and right). Another population of cells exhibited no change in firing rate during the CS− but a shift to an excitatory response during CS+ infusions. This firing pattern is shown for one representative NAc neuron in (C), (scale calibration: 26 μV, 100 μs) and for the population of neurons displaying the same response properties (n = 7) in (D).

cocaine self-administration session. This type of response has been previously termed a conditioned compensatory response (Ramos et al., 2002). By this analysis, the drug is not aversive, but the drug-associated taste cue when infused directly into the oral cavity would elicit a conditioned compensatory response (i.e., conditioned withdrawal), which is aversive. Thus, while the taste cue may initially be avoided because it is devalued by the availability of a more preferred drug of abuse (Grigson, 1997), ultimately it may be avoided and even rejected because it elicits a negative affective state (Grigson et al., 2008).

This interpretation reconciles a longstanding paradox by explaining why avoidance behavior has been observed for a palatable taste cue that predicts a more rewarding cocaine stimulus. Specifically, intraoral delivery of a taste (making it inescapable) and high doses of investigator-delivered cocaine cause avoidance of a sucrose cue but are insufficient to elicit clear signs of aversion (Parker, 1995). It is known, however, that self-administered cocaine engages

intracranial self-stimulation thresholds (indicative of negative affect) and heroin self-administration (Kenny et al., 2006). In addition, taste cues associated with naloxone-precipitated opiate withdrawal are known to elicit aversive taste reactivity (McDonald et al., 1997).

In human drug abusers, conditioned responses also modulate the expression of tolerance. If a preparatory response is eliminated by the omission of a predictive cue, tolerance "fails" and can result in death by apparent overdose (Siegel and Ramos, 2002). In the present report, we hypothesize that the taste cue that predicted cocaine availability induced a negative affective state as a preparatory response in anticipation of the impending

midbrain dopamine systems differently than investigator-delivered cocaine (Hemby et al., 1997; Stuber et al., 2005) and is more likely to lead to compulsive use (Kippin et al., 2006; Wise, 2002). One possibility is that drug self-administration is a necessary component of the development of a pronounced learned aversion. This is unlikely, as we observe robust aversive taste reactivity when the drug is investigator delivered (unpublished data). What is unique about the current study, then, is that the taste cue was intraorally delivered within 3.5 s intervals across a 30 min period prior to cocaine availability. This may constitute a long "drug waiting" period that may allow for a strong association to develop between the taste and the negative affective
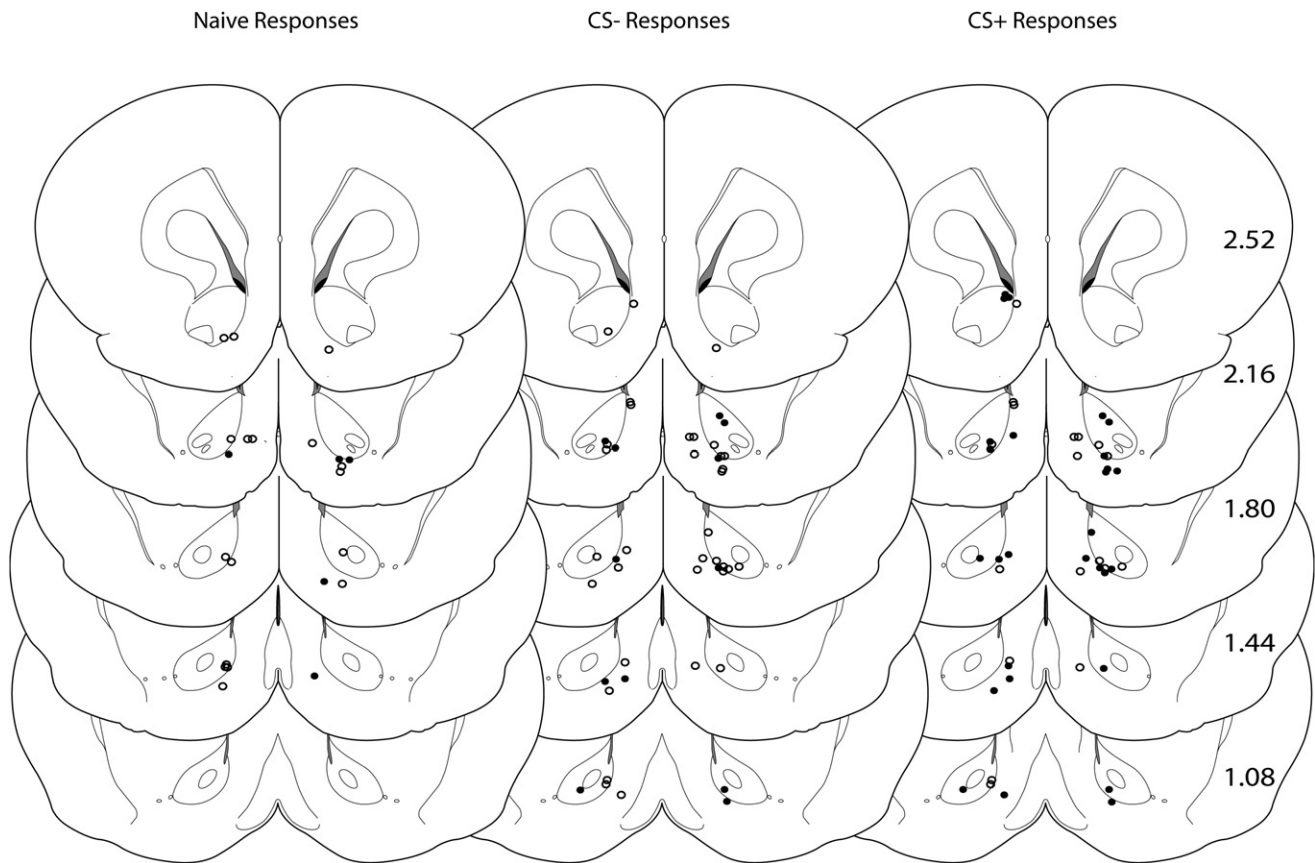
Figure 5. Regional Distribution in the NAc of Responsive Neurons
Coronal sections of the rat brain, depicting electrode placements in control (naive responses) and well-trained (CS− and CS+ responses) rats. The locations of electrode placements that recorded inhibitory responses are shown as open circles, while placements that recorded excitatory responses are shown as closed circles.

state elicited by the taste, as well as providing the opportunity for the behavioral expression of the magnitude of the aversion.

The relationship between negative affect and rapid drug responding is consistent with a view that negative reinforcement exerts motivational control in addiction, but not to the exclusion of other processes involving, for example, sensitization. Drug experience induces sensitization of the mesolimbic dopamine system as well as descending glutamatergic systems into the midbrain (Kalivas et al., 2003; Robinson and Berridge, 1993, 2001, 2003). These alterations may underlie addicted states, giving rise to theories of compulsive drug seeking as a result of compulsive habit formation (Everitt and Robbins, 2005) and incentive sensitization (Robinson and Berridge, 1993). Behaviorally sensitized animals exhibit greater motivation to consume drugs than nonsensitized animals, as demonstrated by several behavioral measures, including more rapid acquisition of self-administration, higher break points in progressive ratio self-administration, and potentiated drug-primed relapse (for a review see Robinson and Berridge, 2003). Recent data demonstrate that animals exhibiting tolerance also show augmented motivation in similar behavioral tests (Lenoir and Ahmed, 2007; Knackstedt and Kalivas, 2007; Lack et al., 2008). While tolerance and sensitization, by definition, occur in opposite physiological directions, the

processes are not mutually exclusive and may even occur simultaneously (Izenwasser and French, 2002). These phenomena could potentially lead to the same behavioral endpoint, at least regarding drug seeking. In other words, it is possible that the animals in the current study exhibited signs of sensitization to the effects of cocaine (i.e., hyperlocomotion) that will be evaluated in future studies. Therefore, our model could prove useful in probing the affective state of the sensitized animal as well as its role in drug seeking and relapse.

Neural Evidence of Conditioned Negative Affect
NAc neuronal response profiles encode the hedonic valence of gustatory stimuli. In naive rats, intraoral infusion of the unpaired, flavored saccharin solution was associated, predominantly, with transiently reduced firing rates. As predicted, the saline-paired saccharin CS− also produced a reduction in NAc firing rates. The predominant response of these same NAc neurons, however, was an increase in firing rate in response to the infusion of the saccharin CS+ that had been devalued by the association with cocaine and, importantly, predicted the impending opportunity to take the drug. These observations of predominantly inhibitory responses to an appetitive stimulus, and excitatory responses to an aversive stimulus, are consistent with previous

studies (Nicola et al., 2004; Roitman et al., 2005; Taha and Fields, 2005, 2006). This type of response has been proposed to gate appetitive behavior through disinhibition of the ventral pallidum (Taha and Fields, 2006). Pharmacological manipulations of the NAc support this theory as well. Opioid and GABA agonist microinfusions into the NAc increase appetitive behavior and hedonic responses for gustatory stimuli (Kelley et al., 2002; Kelley and Swanson, 1997; Pecina and Berridge, 2000, 2005; Reynolds and Berridge, 2002). Generally, increases in motivated behavior for a given stimulus appear to be driven by decreased activity in the NAc (Carlezon and Wise, 1996).

The data presented here confirm and extend these findings. Most previous work has characterized NAc activity when the rats are engaged in goal-directed behavior (Day et al., 2006; Nicola et al., 2004; Yun et al., 2004). Here, rats were not engaged in motivated behavior during electrophysiological recording because the tastant was directly administered into the oral cavity. Therefore, this pattern of neural activity cannot be attributed simply to goal-directed behavior and more likely reflects the hedonic valence of the taste stimulus. The best predictor of subsequent motivated behavior was the expression of aversive taste reactivity. Therefore, the reduction obtained in inhibitory responses for the CS+, shown in Figure 3D, is best accounted for by the reversal of palatability for the CS+ (from rewarding to aversive). In support, this excitatory response profile matches the pattern of neural activity elicited following the infusion of an aversive, bitter tastant into the oral cavity (Roitman et al., 2005). As such, the present data provide evidence for a learned shift in the NAc signal commensurate with a shift in affect. The data could, in part, be attributed to Pavlovian conditioning, because previously neutral cues (i.e., tones and lights) that predict cocaine also elicit excitatory responses (Carelli and Wightman, 2004) while unpaired neutral stimuli elicit no response from NAc neurons (Hollander and Carelli, 2007). Some neurons showed this pattern (see Figure 4C). Indeed, the correlation between the magnitude of aversive taste reactivity expressed and the number of excitatory responses recorded in the NAc on the test day (Figure 3E) may in part reflect the associative strength of the conditioning. Both of these factors (aversive taste reactivity and phasic excitations) were correlated to subsequent rapid drug taking (Figure 3F), indicating that these factors are intimately related.

It is interesting that pharmacological manipulations that alter appetitive behavior have specific effects in the shell of the nucleus accumbens (Kelley et al., 2002, 1996; Kelley and Swanson, 1997; Pecina and Berridge, 2005), while we observe phasic changes (from inhibitions to excitations) in both the shell and core of the NAc in the present design. In line with this, we observed that the predominant response pattern of the shell (especially the dorsal shell) was inhibitory, but this trend did not rise to the level of significance. It is important to note that the core subregion has been implicated in mediating the associative aspects of motivated behavior (Day et al., 2006; Hollander and Carelli, 2007; Ito et al., 2004; Kelley and Swanson, 1997). In our design, the response to the cocaine-predictive taste cue involves both appetitive behavior and associative learning, potentially accounting for the broader distribution of phasic differences observed throughout the NAc.

In sum, it is not surprising that the animals that learned the taste-drug association best show a neurophysiological response profile that reflects association as well as increased motivated behavior. What is surprising about this finding is that these animals also show signs of conditioned aversion that predict subsequent motivated behavior and produce the predicted neurophysiological response profile. Distinguishing the neurophysiological contributions of association and aversion in this behavioral task will be the subject of further investigation.

Conclusion

These data demonstrate how conditioned cues can transiently influence behavior and neurophysiology linked to subsequent drug taking, a key aspect of cocaine addiction in humans. Additionally, the behavioral and electrophysiological data presented here provide a framework to consider the impact of cocaine experience on responding for natural rewards. Drugs devalue natural reward cues, and greater devaluation is associated with greater drug taking (Grigson, 1997; Grigson and Twining, 2002). Here, however, we observe severe devaluation of a previously palatable tastant due to its predictive association with impending cocaine availability. This devaluation is evidenced by a reversal of affective behavioral responses and a shift in NAc neuronal activity. The emergence of aversive taste reactivity to an appetitive stimulus that predicts the opportunity to self-administer cocaine provides compelling behavioral data that a conditioned negative affective state is rapidly inducible and pronounced. The magnitude of this conditioned aversive state is significant because it predicts the motivation to rapidly take cocaine. This finding also is provocative because it bridges two well-known drug-abuse phenomena. The first, that drug-associated cues elicit drug seeking, has been well documented in humans and animal models. The second, that negative affect drives drug seeking, has been well described by human addicts but is difficult to model in animals. The importance of this study lies in this animal model's potential to define and then ameliorate the motivational properties of negative affect evoked by drug-associated stimuli and thereby decrease the drive for the drug.

cannulae. Each cannula consisted of an ∼6 cm length of PE-100 tubing that was phalanged at one end with a Teflon washer. The cannula was inserted just lateral to the first maxillary molar with the Teflon washer flush against the molar. The other end was exteriorized out the incision at the top of the head and held in place along with the EMG connector and arrays with dental acrylic. The nine cocaine-trained animals were also surgically prepared for cocaine self-administration via implantation of a catheter into the jugular vein using established procedures (Carelli and Deadwyler, 1994). Rats were permitted at least 1 week to recover from surgery.

Experimental Design

Mildly water deprived rats were trained in daily sessions to press a lever for water. Upon acquisition of this behavior, surgery was conducted as described above. After recovery, rats were mildly water deprived (30 ml/day) and tested for retention of lever pressing for water. Rats were then given daily drug conditioning sessions. In each session, rats were intraorally infused with a 0.187% grape or orange Kool-Aid flavored 0.15% saccharin solution, the CS+ (0.2 ml delivered through a solenoid over 3.5 s/trial for 30 trials). Following this 30 min session, there was a brief (∼5 min) delay as an iv catheter line was attached and a cocaine self-administration session was initiated, 2 hr access to 0.33 mg/inf. Specifically, animals were trained to self-administer cocaine during daily 2 hr sessions conducted in a 43 × 43 × 53 cm^3 plexiglas chamber (Med. Associates, Inc., St Albans, VT) housed within a commercial sound-attenuated cubicle (Fibrocrete, Inc., Crandall, GA). The beginning of the self-administration session was signaled by the onset of a cue light positioned 6.5 cm above a lever and lever extension. Lever depression on an FR1 schedule resulted in intravenous cocaine delivery (0.33 mg/infusion, dissolved in sterile heparinized saline vehicle) over a 6 s period via a computer-controlled syringe pump (Model PHM-100, Med Associates, Inc., St. Albans, VT). Initiation of drug delivery was signaled by termination of the cuelight and simultaneous onset of a tone (67 db, 1 kHz) houselight (25 W) stimulus complex (20 s). The following day, the same rats were intraorally infused with the other flavored 0.15% saccharin solution, the CS−, that predicted iv saline access (2 hr) on a distinct lever. Rats received 8 to 15 taste-drug pairings, until behavioral oromotor evidence of the association (aversive taste reactivity) was observed and cocaine self-administration was stable. Criterion was defined by the selective expression of aversive taste reactivity for the CS+ during training, not the CS− on consecutive training days during which cocaine self-administration was stable. Variance in training resulted from individual differences in subject-controlled cocaine self-administration intentionally inherent in the design. On the test day, rats were placed in the taste reactivity chamber and were connected to EMG and electrophysiological cables as well as infusion lines to the intraoral cannulae. After neurons were sorted online, the experimental session began. Rats were infused, in the same session, with the CS− and then the CS+ (0.2 ml delivered over 3.5 s/trial for 30 trials each) while the hedonic impact was simultaneously evaluated using electromyographic (EMG) recordings of the anterior digastric muscle and verified by video analysis using the taste reactivity test. Three additional mildly water deprived rats were tested in the same way for naive responses to the flavored saccharin solutions.

Electromyographic and Electrophysiological Recordings

Electrophysiological procedures have been described in detail previously (Roitman et al., 2005). Briefly, before the start of the behavioral session, the rat was placed into a plexiglas chamber within a sound attenuating box. Rats were connected to a flexible recording cable (Plexon Inc., Dallas, TX) attached to a commutator (Crist Electronics, Hagerstown, MD) that permitted virtually unrestrained movement within the chamber. The headstage contained 16 miniature unity-gain field effect transistors. NAc activity was recorded differentially between each active wire and an inactive wire chosen for an absence of neuronal activity. Online isolation and discrimination were accomplished using a commercially available neurophysiological system (multichannel acquisition processor [MAP] system; Plexon Inc., Dallas, TX). Multiple window discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals on the basis of 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 IV computer. Another computer controlled behavioral events of the experiment (Med Associates, Inc.) and sent digital outputs corresponding to each event to the MAP box to be time-stamped along with the neural data. The neurophysiological system has the capability of recording up to four neurons per microwire using real-time discrimination of neuronal action potentials. However, in the present study, typically one or two neurons were recorded per microwire, as in previous reports (Roitman et al., 2005). Criteria for identifying different neurons on a single wire have been described in detail elsewhere (Roitman et al., 2005). Briefly, discrimination of individual waveforms corresponding to a single neuron was accomplished using template and principle component analysis procedures provided by the neurophysiological software system (MAP system). The template analysis procedure involves taking a sample of the waveform and building a template of that extracellular waveform. Subsequent neurons that match this waveform are included as the same neuron. Cell sorting was further accomplished after the experiment was over using additional principle components analysis in Offline Sorter (Plexon Inc., Dallas, TX). After sorting, firing rates of individual neurons were aligned to solenoid opening onset for the intraoral infusion. Peri-event histograms were constructed using commercially available software (NeuroExplorer, Plexon Inc., Dallas, TX). Firing rates were calculated for each neuron across five, 1 s intervals from −1 to +4 s relative to fluid delivery in 100 ms bins. Data were then imported into Excel.

For EMG recordings, rats were attached to a second flexible cable (Plexon Inc., Dallas, TX), and EMG potentials were recorded differentially. Briefly, wires were led to an amplifier (Plexon Inc., Dallas, TX) and signals were amplified (1000×). Processed analog signals were then led through a national instruments board to the same computer that collected electrophysiological data. The same program (Sort Client, Plexon Inc., Dallas, TX) that collected electrophysiological data also collected EMG data. To analyze EMG signals, a horizontal threshold was positioned higher than at least 6σ of the noise. Threshold crossings were time-stamped and examined relative to solenoid opening using NeuroExplorer.

Behavioral Analysis

Taste reactivity was analyzed in a frame-by-frame analysis using videotape recorded on the test day. Appetitive and aversive taste reactivity was counted using the technique of Grill and Norgren (Grill and Norgren, 1978). Briefly, mouth movements that matched the "triangle" shape shown in Figure 1B for a duration exceeding 90 ms were counted as aversive. These criteria excluded all neutral and ingestive mouth movements. Instances in which the tongue protruded and crossed the midline (as in Figure 1A) were counted as appetitive. Counts for each animal were analyzed with a paired t test for both appetitive taste reactivity and aversive taste reactivity. Intake of the CS− and CS+ during a two-bottle post-test also was analyzed with a paired t test. Taste reactivity scores were generated by two raters with an inter-rater reliability of 91%. Stepwise linear regression with partial correlation was performed on all behavioral data relevant to cocaine self-administration. Statistical analyses of all behavioral data were performed using commercially available software (SPSS, Tulsa, OK).

Determination of Phasic Responses

Consistent with other reports from our lab (Roitman et al., 2005), NAc neurons were classified as exhibiting a phasic excitation or inhibition in firing rate during saccharin delivery. The transient effect of gustatory stimulation is initiated ∼1 s after solenoid opening and typically is resolved within 5 s (see Figures 2A and 2C). Therefore, analyses were confined to each cell's firing rate across a 5 s period over the 30 trial test session. To this end, neural activity was characterized via the activity of each neuron 1 s prior to saccharin delivery (baseline period) and four 1 s bins following delivery (effect period). Specifically, NAc neurons were classified as phasic (excitatory or inhibitory) to tastant delivery if their averaged activity across all 30 trials/session during any of the four 1 s effect period bins (4 s postinfusion) differed significantly from the baseline period. A repeated-measures ANOVA was run on each neuron's activity in these five time bins, with planned contrasts between the four effect periods and the baseline.

Neural Representation of Devaluation

After determination of phasic responses to infusions of the CS− and CS+, groups of cells were averaged based on similar response profiles. These groups

(e.g., inhibitory for CS− and excitatory for CS+) were averaged and analyzed as above to determine significant differences in responsiveness for the stimuli in the subpopulations. It was hypothesized that neural representations of conditioned aversive gustatory stimuli would resemble intrinsically aversive gustatory stimuli. That is, rewarding taste stimuli produce a majority of inhibitory responses and aversive stimuli produce predominantly excitatory responses. Therefore, "inhibitory to excitatory" cell counts were calculated as percentages of the total number of phasic cells for each rat across stimuli (as illustrated in Figure 4B). Statistical analyses of all electrophysiological recordings were performed using commercially available software (SPSS, Tulsa, OK).

Histology

Following experiments, rats were deeply anesthetized with ketamine/xylazine, and electrode tips were marked by passing current (13.5 μA, 5 s) through the electrodes. Rats were then transcardially perfused with saline, then formalin, brains were removed, and, after postfixing and freezing, sliced into 40 μm sections through the forebrain. Sections were then mounted on slides and stained with potassium ferocyanide and counterstained with thionin to visualize electrode tips using well-established procedures (Roitman et al., 2005). χ^2 analyses were conducted on counts of phasic cells, inhibitory and excitatory, recorded in the NAc core and shell.

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