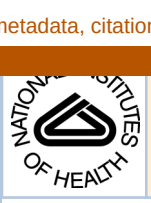

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Neural correlates of Pavlovian-to-instrumental transfer in the nucleus accumbens shell are selectively potentiated following cocaine self-administration

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Abstract

During Pavlovian-to-instrumental transfer (PIT), learned Pavlovian cues significantly modulate ongoing instrumental actions. This phenomenon is suggested as a mechanism under which conditioned stimuli may lead to relapse in addicted populations. Following discriminative Pavlovian learning and instrumental conditioning with sucrose, one group of rats (naive) underwent electrophysiological recordings in the nucleus accumbens core and shell during a single PIT session. Other groups, following Pavlovian and instrumental conditioning, were subsequently trained to self-administer cocaine with nosepoke responses, or received yoked saline infusions and nosepoked for water rewards, and then performed PIT while electrophysiological recordings were taken in the nucleus accumbens. Behaviorally, although both naive and saline-treated groups showed increases in lever pressing during the conditioned stimulus cue, this effect was significantly enhanced in the cocaine-treated group. Neurons in the core and shell tracked these behavioral changes. In control animals, core neurons were significantly more likely to encode general information about cues, rewards and responses than those in the shell, and positively correlated with behavioral PIT performance, whereas PIT-specific encoding in the shell, but not core, tracked PIT performance. In contrast, following cocaine exposure, there was a significant increase in neural encoding of all task-relevant events that was selective to the shell. Given that cocaine exposure enhanced both behavior and shell-specific task encoding, these findings suggest that, whereas the core is important for acquiring the information about cues and response contingencies, the shell is important for using this information to guide and modulate behavior and is specifically affected following a history of cocaine self-administration.

Keywords

electrophysiology; natural reward; operant; rat; striatum; sucrose

INTRODUCTION

Animals are faced with the necessity of seeking rewards in their environments. Whereas natural rewards such as food or mates motivate much goal-directed behavior, similar mechanisms appear to drive seeking for drugs of abuse such as cocaine (Parkinson et al., 2000a; Everitt et al., 2001; Robbins and Everitt, 2002). Further, through associations with the reward, environmental cues acquire motivational significance that can influence goal-

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directed behavior (Holland and Rescorla, 1975; Hyde, 1976; Rescorla, 1994; Arroyo et al., 1998). For example, food-related cues can induce feeding in rats that are completely sated, suggesting that such motivational cues have the ability to over-ride homeostatic satiety signals (Holland and Petrovich, 2005). Similarly, animal and humans will re-engage in drug-taking behaviors when presented with drug-associated cues after long periods of abstinence (Grimm et al., 2002; Kalivas and McFarland, 2003; Fuchs et al., 2004). These findings argue that Pavlovian cues provide powerful motivational features through their associations with various reinforcers. Given these common associative mechanisms, understanding the manner in which learning comes to guide goal-directed behavior for natural rewards can also provide insight into similar processes that become pathological in the drug-addicted state.

One setting in which these cues drive goal-directed behavior is in a task known as Pavlovian-to-instrumental transfer (PIT). In this behavioral model, previously learned Pavlovian cues are able to invigorate ongoing goal-seeking behavior (Estes, 1948; Rescorla and Solomon, 1967; Lovibond, 1983; Bray et al., 2008). Detailed studies have shown that this ‘PIT effect’ is dependent upon the associative value of the cue, and that this value can be of general motivational significance or specific to a single reinforcer (Blundell et al., 2001; Shiflett and Balleine, 2010). Indeed this paradigm has been proposed to model features of addiction as it highlights the importance of the conditioned aspects of drug-taking behavior (Everitt et al., 2001). Consistent with PIT as a model of addiction, microinfusions of amphetamine into the brain induced greater levels of PIT than in normal animals (Parkinson et al., 1999; Wyvell and Berridge, 2000), whereas repeated administration of drugs of abuse like amphetamine or heroin makes the PIT effect more sensitive during cue presentation (Wyvell and Berridge, 2001; Ranaldi et al., 2009). Further, blockade of the neurotransmitter dopamine (DA) (Dickinson et al., 2000; Lex and Hauber, 2008) or inactivation of DA-signaling neurons (Murschall and Hauber, 2006; Corbit et al., 2007) attenuates the ability of Pavlovian cues to potentiate instrumental responding.

The neural underpinnings of PIT are poorly understood, but have been shown to involve a host of limbic structures, such as the central and basolateral nuclei of the amgydala (Blundell et al., 2001; Hall et al., 2001; Holland and Gallagher, 2003) and dorsal regions of the striatum (Corbit and Janak, 2007; Homayoun and Moghaddam, 2009). Given the involvement of dopaminergic processes in modulating the transfer effect, it is not surprising that the nucleus accumbens (NAc) – a primary target of dopaminergic terminals arising from the ventral tegmental area – is also involved in supporting the PIT effect. Neurotoxic lesions of the NAc abolish PIT without affecting more general features of instrumental or Pavlovian conditioning separately (de Borchgrave et al., 2002), whereas delivery of amphetamine or CRF within the NAc enhances transfer (Wyvell and Berridge, 2000; Pecina et al., 2006). However, the specific roles that these accumbal regions contribute to the transfer effect remain controversial. For example, in one set of findings, lesions of the core but not the shell of the NAc selectively abolished PIT (Hall et al., 2001; Cardinal et al., 2002a), whereas the opposite finding demonstrating the selective involvement of the NAc shell in PIT has also been reported (Corbit et al., 2001). However, selective blockade of DA receptors at the time of transfer produced pronounced deficits in the PIT effect after infusion of the D1 antagonist SCH-23390 (and, to a lesser extent, the D2 antagonist raclopride) into either the core or shell (Lex and Hauber, 2008), suggesting that both regions may play an important role in this task.

This set of conflicting data argues that subregions of the NAc encode key features of learning that enable Pavlovian cues to modulate ongoing goal-directed instrumental behavior, and that this coding is critically dependent upon DA. However, the lack of temporal specificity inherent in the above techniques, such as permanent lesions or long-term blockade, may obscure the more subtle effects that these regions contribute to this task.

To address this, we recorded from single neurons in the NAc core and shell during the performance of PIT. Further, we assessed how neural encoding was altered by cocaine, a drug that acts by blocking DA reuptake in the synapse of NAc neurons, by comparing neural firing in animals with a history of cocaine self-administration with naive and saline-infused controls.

Materials and methods

Experiment 1: electrophysiological examination of nucleus accumbens cell firing during Pavlovian-to-instrumental transfer

Subjects—Experimentally naive male Sprague-Dawley rats (n = 10; Charles River Laboratories), aged between 8 and 12 weeks and weighing approximately 300 g at the time of arrival were used. The individually-housed rats were allowed to habituate to the vivarium for approximately 1 week, during which time they had *ad-libitum* access to food and water and were maintained on a 12 h light/dark schedule. Following habituation, rats were implanted with indwelling electrophysiological arrays in the core and shell of the NAc (see below). After 2 weeks recovery, rats were shifted to food restriction (unlimited water access) that maintained their weight at 85% of their free-feeding baseline weight. Rats remained on this restricted diet for the duration of the training and test procedures. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the guidelines of the University of North Carolina at Chapel Hill Institutional Care and Use Committee.

Surgical methods—Prior to all behavioral testing, rats were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg), and then placed in a stereotaxic apparatus (Kopf Instruments, Tijunga, CA, USA). The scalp was incised and retracted, and the head was adjusted to level in all planes. Holes were drilled in the skull above the NAc core (AP: +1.8 mm, ML: ± 1.4 mm, relative to Bregma) in one hemisphere, and the NAc shell (AP: +1.8 mm, ML: ± 0.8 mm) in the other hemisphere. The side of the NAc core and shell array placements was counterbalanced across subjects such that approximately equal numbers of recordings were taken from the left and right core and shell subregions, respectively. An eight-wire recording array (NB Labs, Denison, TX, USA) was slowly lowered into the NAc core or shell at a depth of −6.2 mm from the brain surface. The arrays consisted of two parallel rows of four stainless-steel Teflon-coated, 50 μm-diameter wires, tips spaced evenly 0.5 mm apart. A ground wire for each array was placed in the brain distal to the recording location in the same hemisphere. The apparatus was chronically secured with dental acrylic attached to screws placed on the skull surface. Animals were given an oral dose of 1.0 mg/kg meloxicam (Metacam, Boehringer Ingelheim Vetmedica, St Joseph, MO, USA) as a post-operative analgesic for 2 days, and at least 1 week to recover from surgery before beginning food restriction and behavioral training.

Apparatus—All training and testing took place in a custom-built behavioral chamber (43×43×53 cm; MED Associates, St Albans, VT, USA) housed in a sound-attenuating cabinet. The interior walls of the cabinet were covered in metal mesh to provide insulation from external electrical signals. Chambers were illuminated by a houselight located on the ceiling. Masking noise and ventilation were provided by a wall-mounted fan. A ceiling-mounted digital camera enabled digital recording on a computer (API Software), which was later scored by the experimenter. A centrally-located foodcup (approximately 4 cm above the floor) was mounted on the right wall of the chamber. Flanking the foodcup on either side were two retractable levers (Coulbourn Instruments, Whitehall, PA, USA), both 4 cm above the chamber floor. During Pavlovian training, the levers were retracted from the chamber, but remained extended into the chamber during instrumental training and the final transfer

session. Auditory cues consisted of either a tone (70 dB, 1500 Hz) or white noise (65 dB) delivered by a speaker 18 cm above the floor. A red LED was located behind the foodcup (not visible to the rats but recorded on a video camera to aid in behavioral scoring). The LED illuminated at 10s prior to auditory cue onset and remained illuminated for the duration of the auditory cues.

Electrophysiological recordings were taken on the final day of transfer, although the rats were connected to the recording apparatus for two sessions prior to transfer to habituate them to the tether. Details on electrophysiological recording have been reported previously (Carelli et al., 2000). Briefly, rats were connected to a recording harness that terminated in a headstage (Plexon Inc., Dallas, TX, USA). The harness was connected at the other end to a commutator (MED Associates and Crist Instruments) allowing free movement throughout the chamber during sessions. Amplified neural signals were then passed to a MAP system (Plexon Inc.) where they were captured by a neural analysis program (Sort Client, Plexon Inc.). A separate computer controlled external stimuli and captured behavioral events (TRANS IV, MED Associates). Neural data were acquired using techniques and apparatus similar to those described elsewhere (Roitman et al., 2005). Briefly, software was employed to sort neural waveforms by principal components analysis (Offline Sorter, Plexon Inc.). Finally, the resulting timestamps for valid waveforms were further analyzed in relation to behavioral markers using NeuroExplorer software (NEX Technologies, Littleton, MA, USA).

Behavioral training

Pavlovian training: An overview of all behavioral training appears in Table 1. Sessions began with the onset of the houselight and fan. Rats received 11 consecutive days of Pavlovian training (32 min/session). One auditory stimulus (either tone or white noise, counterbalanced across subjects) served as a Pavlovian conditioned stimulus (CS+). Each CS+ cue was presented for 120 s, and the time between cue presentations randomly varied between 2 and 6 min (average 4 min). For sessions 1-6, rats received four 45 mg sucrose pellets (Purina, Richmond, IN, USA) during the CS+ (on average every 30 s). For reasons specific to the transfer effect, the outcome value was gradually lowered over training such that cues did not overshadow the lever pressing when presented simultaneously. Thus, for sessions 7 and 8, three pellets were delivered during the CS+ (every ~40s), whereas for sessions 9-11, two pellets were delivered during each CS+. For sessions 1-10, rats received six CS+ presentations. For session 11, a 120 s non-reinforced CS− was introduced, which was the other auditory stimulus. In this session, rats received four CS+ and two CS− presentations.

Instrumental training: After completing Pavlovian training, rats were trained to press a single lever to obtain sucrose pellets. During the first instrumental training session, lever presses were reinforced on a fixed ratio 1 schedule, in which each lever press resulted in the delivery of a single sucrose pellet. Rats were allowed to press for 60 min or until they obtained 50 pellets, whichever came first. Following fixed ratio 1 acquisition, rats were moved to a leaner reinforcement schedule. Instrumental sessions 2 and 3 were on a variable interval (VI) 30 s schedule, i.e. the first lever press on the active lever in each VI block (from 5 to 55 s, mean 30 s) was reinforced with a single pellet, whereas subsequent presses in that block were not. During the third session, a second lever was introduced to the test chamber, but presses on this ‘inactive’ lever had no programmed consequences. In all subsequent sessions, the active and inactive levers were present in the test chamber for the duration of the session. Following the 2 days of VI30 training, rats had three sessions on VI60 and a final two sessions on a VI90 schedule.

Pavlovian-to-instrumental transfer: At 2 days prior to the final transfer session, rats were given a 'reminder' Pavlovian session that was similar to the 11th day of training, but with twice as many cues presented (eight CS+, four CS−). The following day, rats received a final reminder VI90 instrumental session that was identical to the last day of instrumental training. In both sessions, rats were connected to the electrophysiological cable to acquaint them with the recording apparatus prior to transfer.

On the day of transfer, the 2 h session proceeded similarly to a VI90 session. Similar to previous PIT studies (e.g. Holland and Gallagher, 2003; Holland, 2004), throughout the session, both the active and inactive levers were extended into the test chamber, although unlike those studies, presses on the active lever were still reinforced on the VI90 schedule. This was in order to maintain constant rates of operant performance throughout the session and to prevent extinction effects. In contrast to normal VI90 sessions, however, during transfer a series of thirty 1 min CS+ and CS− cues (average ISI: 2 ± 1 min) were presented throughout the session. In the transfer session, neither cue had any additional consequences; specifically, the CS+ cue was not associated with additional delivery of food pellets independent of the presses. Thus, any changes in behavior during the cues depended solely on the associative value of the CSs.

The behavioral PIT effect was assessed in this task by comparing the rate of active lever pressing in the 10 s prior to CS presentation (baseline phase) with lever pressing in the 10 s following CS onset (cue phase). The average rate of pressing in both baseline periods (CS+ and CS−) was compared with mean lever pressing in the cue periods for CS+ and for CS− for each subject.

Histology—Histological verification of electrode placements was accomplished using established procedures (e.g. Day et al., 2006). Briefly, after the experiments, animals were heavily anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg). A 15 μA current was then passed through each stainless-steel microwire for 5 s to leave an iron deposit in the tissue. To identify the wire tips, rats were perfused transcardially with saline (10 min, 20 mL/min), followed by a 3% potassium ferricyanide in 10% formalin solution. The brain was removed, frozen to −20 °C and coronally sliced (30 μm thick) throughout the extent of the NAc. Slices were mounted on slides, counterstained with thionin and electrode placement was confirmed within the NAc using a standard atlas (Paxinos and Watson, 1997).

Electrophysiological data analysis

Analysis of neural firing: The activity of all putative medium spiny neurons identified within the NAc core and shell was used for analysis. To determine whether a cell was 'phasic' (firing rates were transiently and significantly above or below baseline), a peri-event histogram was created for each neuron across each behavioral event, synched to event onset (100 ms bins). Phasic cells showed firing that was outside a 95% confidence interval (if fewer than 20 presentations of an event) or a 99% confidence interval (if more than 20 presentations of the event). Confidence intervals were created using the 10 s baseline period prior to event presentation. A cell was considered phasic if at least two consecutive bins were above (excitatory) or below (inhibitory) the confidence interval within 2 s of event presentation. Low-firing cells (baseline less than 1 Hz) were further classified as inhibitory if there were at least twice as many consecutive 'zero' bins (i.e. bins in which there was no spiking activity) in the effect period as in the 10 s baseline period. For analysis of neural firing related to lever presses, neural activity was examined from 5 s pre-press to 5 s post-press, and compared with a baseline of activity from 10 s pre-press to 5 s pre-press. To determine phasic firing for reward-related activity, we first aligned histograms to the rewarded lever presses, and then subtracted from that response the average firing pattern of

that cell during unrewarded responses. Sustained (>200 ms) residual activity within the first 5 s following a rewarded press compared with a 99% CI constructed around the baseline was considered phasic.

Next, it was important to determine whether phasic activity during the cue period was selective for one cue compared with the other. To determine selectivity, the firing rate in each bin was calculated using the trial-by-trial average. Each cell was thus subjected to a three-way repeated-measures ANOVA, with bin (±1000 ms), cue onset (pre-onset vs. post-onset), and cue type (CS+, CS−) as factors. Selective cells (as demonstrated by a significant cue × onset interaction) were significantly different between cues after onset, but not different during the baseline.

It was hypothesized that as PIT modulated the vigor of lever pressing, it would be possible to see changes in the lever press-related neural activity as a function of whether Pavlovian cues were present, i.e. a PIT-encoding neuron would show firing that was significantly different around the time of press when the CS+ was presented compared with the CS− and baseline, but that the response would be similar during the CS− and baseline. To assess PIT selectivity, the response of each neuron was sorted by whether it was made in the 60 s pre-cue onset (baseline), or the 60 s epoch containing the CS+ and the CS−. The average firing rate in each 250 ms bin across all presses was thus compared across conditions (baseline, CS + and CS−) in a 4 s window time-locked to the press using a two-way repeated-measures ANOVA.

It was further predicted that encoding information about cues was critical to supporting successful transfer behavior during test. Specifically, it was hypothesized that the degree to which cells developed cue selectivity would correlate with performance on the task. To assess this, a PIT selectivity index was developed, which was calculated as the difference in the lever-pressing rate between CS+ and CS− as a ratio of the average baseline lever-pressing rate, or

$$\text{PIT}\quad\text{selectivity}\quad\text{index} = (\text{CS}+ - \text{CS}-)\,/\text{baseline}$$

This index depicts the elevation of responding selective to the CS+ relative to baseline. Importantly, by incorporating the difference of the CS+ and CS−, this index will approach 0 if rates are elevated above baseline similarly in both CS+ and CS−, and increase as rats selectively increase responding during the CS+ period exclusively. As such, this index allowed us to correlate specific patterns of neural firing with behavior.

Experiment 2: effects of cocaine exposure on Pavlovian-to-instrumental transfer

Subjects—The subjects were 11 male Sprague-Dawley rats (Charles River Laboratories), aged 8-12 weeks and approximately 350 g at the time of training, housed and maintained identically to those in Experiment 1. Prior to appetitive training (Pavlovian, instrumental and transfer sessions), rats were food restricted to 85% of their *ad-libitum* weight and maintained this weight. During the 14 days of cocaine self-administration training, rats were allowed *ad-libitum* access to food but were allowed 30 min access to water following each session. For the reacquisition transfer sessions, rats were returned to the food-restricted diet (85% *ad libitum*) with free access to water.

Surgical methods—After Pavlovian and instrumental training, but prior to cocaine self-administration, rats were prepared for surgery as in Experiment 1. All rats were implanted with a custom-made chronic indwelling catheter into their right jugular vein under aseptic conditions. Catheter construction and surgical implantation have been described previously

(Carelli and Deadwyler, 1994). During the same surgery, a subset of rats (n = 9) were then chronically implanted with bilateral electrophysiological arrays aimed at the NAc core in one hemisphere and the NAc shell in the contralateral hemisphere, as described in Experiment 1. Two rats were prepared for self-administration but did not receive arrays. All rats were allowed at least 7 days to recover before self-administration training.

Apparatus—Rats were run in two different contexts. For appetitive training (Pavlovian, instrumental and transfer sessions), rats were run in the same behavioral test chambers as described in Experiment 1, except that an infrared beam (MED Associates) was positioned on either side of the foodcup to allow precise detection of the timing of foodcup entries and exits. For cocaine self-administration, rats were trained in a separate context in another room in the laboratory. These smaller test chambers (25×25×30 cm; MED Associates) were comprised of two clear Plexiglas walls in the front and rear, and two stainless-steel walls on the left and right side of the chamber. Each behavioral chamber was housed in a larger sound-attenuating cabinet equipped with a fan to mask noise. Unlike the solid plastic floor in the appetitive test chambers, the floorgrid in these contexts was comprised of evenly-spaced stainless-steel bars (0.5 cm diameter, 1.5 cm apart). On the left wall a centrally-located houselight was positioned 1cm below the Plexiglas ceiling. On the right wall, 5 cm below the ceiling, two jewel lights were spaced 14 cm apart. An illuminated nosepoke hole (2.5 cm diameter) was located 1 cm above the floorgrid in the middle of the left wall, and a recessed foodcup was located on the opposite wall. Cocaine was administered via an intrajugular catheter attached to a syringe. Cocaine infusion was controlled via a motor-driven syringe pump (MED Associates), and tubing was tethered using a counterweighted arm to provide for animal mobility.

Behavior

Pavlovian training: Rats began training on a Pavlovian schedule similar to those described in Experiment 1 (Table 1). Briefly, rats received 8 days of 30 min auditory Pavlovian conditioning. During the first six sessions, the rats received six 2 min auditory cues that served as the CS+, during which four pellets were pseudorandomly delivered on average every 30 s. During the last 2 days of conditioning, rats received four presentations of the CS + and two CS− presentations. Equal numbers of rats received tone or noise for the CS+, and assignments were completely counterbalanced across subject and test chamber.

Instrumental training: Following Pavlovian training, rats were trained on 7 days of instrumental conditioning to obtain sucrose pellets, identical to those in Experiment 1. Briefly, rats received 1 day of fixed ratio 1 training, followed by 2 days at VI30, then 3 days at VI60 and finally 2 days at VI90. As before, an inactive lever was present from day 3 until the conclusion of instrumental training.

Cocaine self-administration: At 1 week following the catheter surgery (and following Pavlovian and instrumental training), a subset of animals (n=6) were trained to self-administer cocaine during 2 h daily sessions, lasting for 14 days. During each session, a houselight illuminated the chamber, and a single white LED lamp recessed in the rear of the nosepoke receptacle indicated that entries would be rewarded. Upon a successful entry into the nosepoke receptacle, rats received an intravenous injection of cocaine (0.33 mg/inf over 6 s). For 20s following the nosepoke, the houselight was extinguished and the two panel lights on the right wall flashed intermittently (1 Hz). During this period, subsequent nosepokes did not result in cocaine reinforcement. At the end of the 20 s period, the panel lights were turned off and the houselight turned back on. Control rats (n=5) received the same treatment, except only vehicle (0.2 mL saline, 6 s) was injected into the catheter. Control rats were yoked to the delivery schedule of rats in the cocaine self-administering

group such that successful nosepokes by a self-administering rat in one box delivered saline infusions to the paired yoked control rat in an adjacent box. To better equate for learning a self-administration operant behavior in the control group, these thirsty rats were reinforced for successful nosepokes by receiving a bolus of water at the foodcup on a VI30 schedule.

Pavlovian-to-instrumental transfer: Following cocaine self-administration, rats were returned to *ad-libitum* water daily, but food restricted to 85% of the free-feed weight as before self-administration training. At 1 week following self-administration, rats were run on the PIT test as in Experiment 1. Briefly, all rats received 'reminder' sessions in the original operant chambers that were used for Pavlovian and instrumental training while being connected to the electrophysiology recording wire harness. For Pavlovian reminder sessions, the rats received twice as many cues (60 min, eight CS+ and four CS− presentations; ISI average: 3 min), whereas instrumental sessions were identical to the last day of instrumental training. Following the reminder sessions, NAc cell firing was recorded during 1 day of a Pavlovian-to-instrumental (PIT) test identical to that described in Experiment 1. In addition to the behavioral and neural response analyses, which were performed identically to those in Experiment 1, foodcup entry behavior was examined. This behavior was analyzed for the subset of animals (n = 5 saline, n = 3 cocaine) in which it was automated (detected by infrared beam break). The number of foodcup entries was examined during a 20 s interval immediately following the CS−, CS+ and a baseline period. The baseline was defined as foodcup entries made during a 20 s epoch at 60 s prior to each CS+ and CS− onset. In addition, we assessed whether neural responses during foodcup entries showed a PIT-modulated response similar to those seen during lever pressing by comparing phasic firing during foodcup entries in the presence of CS+ with that during the baseline and CS− epochs.

Results

Experiment 1: electrophysiological examination of nucleus accumbens cell firing during Pavlovian-to-instrumental transfer

Behavior

Pavlovian behavior: Rats rapidly learned to acquire the Pavlovian discriminations. Rats spent significantly more time in the foodcup during the cue period compared with baseline ($F_{1,10} = 55.36$, $p < 0.0001$), and showed a reliable increase in total time spent in the foodcup across sessions ($F_{9,90} = 6.73$, $p < 0.0001$) (Fig. 1A). This effect was carried by a selective increase in foodcup time only during the CS+ but not baseline, as indicated by a significant cue × day interaction ($F_{9,90} = 4.35$, $p < 0.002$). Specifically, rats failed to discriminate between the baseline and cue period on days 1 and 2 (Tukey, $p > 0.5$), but reliably showed a greater percentage of time in the foodcup during the CS+ compared with baseline in all subsequent sessions (Tukey, $p < 0.005$ for each session).

On days 11 and 12, the CS− cue was introduced (Fig. 1A). On both days, rats displayed significantly more time in the cue period for the CS+ compared with both the CS− (Tukey, $p < 0.0002$) and baseline (Tukey, $p < 0.0002$). In contrast, rats showed no differences in foodcup behavior during the CS− and baseline on either day (Tukey, $p > 0.5$).

Instrumental behavior: All rats learned to press the active lever on a fixed ratio 1 schedule within a single session (Fig. 1B). A main effect of day ($F_{7,42} = 13.35$, $p < 0.0001$) was due to a lower rate of pressing on day 1 than on all subsequent VI sessions (Tukey, all $p < 0.001$). Rates were temporarily dampened when the schedule shifted from VI60 to VI90 (day 6 vs. day 7; Tukey HSD, $p < 0.05$), but no other sessions were significantly different. Finally, despite the presence of the inactive lever on days 3-8, rats easily discriminated

between the responses. Lever presses for the active lever were consistently higher than the inactive lever ($F_{1,9} = 81.05$, $p < 0.00001$), a pattern that was consistent for all sessions (Tukey; all p-values < 0.0001).

Transfer: During transfer, we assessed the ability of the Pavlovian cues (CS+ or CS−) to potentiate ongoing lever pressing compared with baseline. In this session, there was a significant main effect of cue ($F_{2,18} = 4.16$, $p < 0.03$). Specifically, although there was a significant increase in lever pressing during the CS+ compared with the baseline (Tukey, $p < 0.05$), there was no such difference in pressing rate between the CS− and baseline (Tukey, $p = 0.29$) (Fig. 1C). However, the numerical increase in pressing during the CS+ compared with the CS− showed only a trend towards significance ($p = 0.08$).

Neural data

Pavlovian cues: First, we assessed the level of neural encoding during the presentation of either the CS+ or CS− by determining the percent of cells phasic in the cue period. An example of a phasic neuron encoding the CS+ is shown in Fig. 2A. Note that the cell showed a significant increase in firing rate during CS+ (left) but not CS− (right) presentation. There were no significant differences in the percent of phasic cells in the core and shell [32% (16/50) and 25% (10/40), respectively]. Of phasic cells, a majority in both the core and shell encoded information about the CS+ [75% (12/16) in core and 80% (8/10) in shell] compared with the CS− (25% and 20%, respectively). Further, cue-encoding cells were reliably more likely to be excitatory than inhibitory, and this difference was similar in the core (57% excitatory vs. 43% inhibitory) and shell (80% excitatory vs. 20% inhibitory) (Fig. 2B, inset).

Finally, we specifically investigated whether cells selectively encoded information about a particular cue. Indeed, nearly all of the cells that were phasic for one cue were non-phasic for the other, suggesting cue-selective encoding (e.g. Fig. 1A). Further, this selectivity in cue-related activity differed across the core and shell (Fig. 2B). In the core, 42% of the neurons (21/50) encoded selective information about at least one of the cues and, of those, the great majority encoded information about the CS+ (86%; 18/21) rather than the CS− (14%; 3/21). Shell neurons were less likely to encode information about the cues. Only 13% of shell neurons (5/40) encoded specific information about one of the cues, a proportion that was significantly less than in the core ($\chi^2 = 9.41$, $p < 0.005$). However, similar to those in the core, shell neurons preferentially encoded information about the CS+ (80%; 4/5) compared with the CS− (20%; 1/5), and the relative proportion of CS+ to CS− in the core and shell was not statistically different ($\chi^2 = 0.1$, $p = 0.7$).

Animals with a greater percentage of cue-selective neurons were significantly positively correlated with PIT performance as measured by the PIT index ($r^2 = 0.65$, $p < 0.005$) (Fig. 2C). This did not appear to be specific to either the core or shell regions, as both regions showed strong positive correlations between selectivity and performance ($r^2 = 0.37$ in core; $r^2 = 0.43$ in shell), although both of these only showed a significant trend towards significance ($p = 0.084$ in core; $p = 0.055$ in shell) individually.

Reward: Selective reward encoding was seen in 56% of core and 38% of shell neurons, although there was only a trend towards a statistical difference between regions ($\chi^2 = 3.0$, $p = 0.08$). Phasic responses developed shortly after the rewarded lever press. An example of a representative neuron that showed reward-related firing is shown in Fig. 3A.

Previous studies have shown that cells that encode information about both cues and outcomes may be particularly important for supporting normal goal-directed behavior (Schoenbaum et al., 2003a). Given this, it was possible that there would be a population of reward-encoding neurons that also expressed cue selectivity. Overall, there were

significantly more neurons encoding this conjunction in the core (28%) than in the shell (5%) ($\chi^2 = 8.04$, $p < 0.005$) (Fig. 3B). Thus, despite similar rates of cue and outcome encoding separately in both regions, core neurons were more likely to encode more explicit stimulus-outcome representations than shell neurons.

Instrumental responding: Next, the neural correlates of lever-pressing behavior were investigated. In the first analysis, active lever presses were examined regardless of whether there was a cue present or not. A large percentage of neurons were involved in encoding some aspect of lever-pressing behavior. Specifically, 72% (36/50) of core neurons were phasic around the press, whereas 85% (34/40) of shell neurons were phasic. As in previous work, some cells were phasic prior to the press (e.g. Fig. 4A), some following the press (e.g. Fig. 4B) and some encoded both approach and response (not shown). The majority of phasic neurons encoded both approach and response in both regions (55% in core; 58% in shell). A much smaller proportion in both regions (14% core; 18% shell) was only active during the approach, and a slightly larger proportion was selectively phasic following the response (31% core; 24% shell).

Next, lever pressing between the active and inactive lever was assessed. Although the majority of cells recorded showed some form of phasic press-related activity, there was little evidence that these same neurons showed similar phasic firing on the inactive lever (Fig. 4C). Both core and shell neurons showed significantly greater phasic activity for the active compared with the inactive press, but there were no reliable differences between the core and shell in the percentage of phasic neurons encoding active and inactive lever presses ($\chi^2 = 1.01$, $p = 0.31$) (Fig. 4C). Further, whereas the population for active lever pressing was inhibitory and locked to the time of press, there was no such general pattern for the population of inactive presses (Fig. 4D). These findings together suggest that phasic press-related activity is related to tracking the goal instead of merely encoding the motor response alone.

Pavlovian-to-instrumental transfer-modulated lever pressing: PIT-modulated lever pressing occurred in cells where phasic activity during the press was significantly different in the presence of the CS+ compared with the baseline and CS−. An example of such a PIT-modulated neuron is shown in Fig. 5A. Across all animals, neurons in both the core and shell encoded significant changes in lever-press firing selectively in the presence of the CS+ cue. However, there was not a significant difference in the average expression of these cells between the core (32%; 16/50) and shell (35%; 14/40) ($\chi^2 = 0.09$, $p = 0.72$, Fig. 5B).

There was a trend towards more cells in the core (24%) than shell (10%) that were jointly selective for cue and PIT selectivity ($\chi^2 = 2.89$, $p = 0.08$). However, the behavioral function of these PIT-selective cells varied across region. In the core, cue-selective neurons that developed PIT selectivity failed to correlate with behavior ($r^2 = 0.18$, $p = 0.25$), whereas cue-selective neurons that were not also PIT-selective were positively correlated with PIT behavior, a trend that was nearly significant ($r^2 = 0.40$, $p = 0.07$) (Fig. 5C). In contrast, in the shell, the cue-selective cells that developed PIT selectivity were significantly positively correlated with PIT performance ($r^2 = 0.42$, $p < 0.05$), whereas cue-selective neurons that did not develop PIT selectivity were not ($r^2 = 0.10$, $p = 0.4$) (Fig. 5D).

Experiment 2: effect of cocaine exposure on Pavlovian-to-instrumental transfer

Behavior

Pavlovian training: All rats (n=11) readily acquired the Pavlovian discrimination (Fig. 6A). To ensure that the groups were equal before drug exposure, rats that were destined for cocaine or saline were analyzed separately for the Pavlovian discrimination and instrumental

responding. Similar to Experiment 1, a repeated-measures ANOVA of treatment (saline vs. cocaine), cue (CS+ vs. baseline) and day (1-6) revealed a significant main effect of cue ($F_{1,9}$ =232.6, $p < 0.0001$), with rats responding significantly more during the CS+ than baseline, and a main effect of day ($F_{5,45} = 7.1$, $p < 0.0001$) that showed that rats spent significantly more time in the foodcup on days 2-5 than on day 1 (Tukey; all p-values < 0.05). A significant interaction between cue and day ($F_{5,45} = 11.3$, $p < 0.0001$) was due to a failure to discriminate between the cue and baseline on day 1 (Tukey; $p = 0.99$), but there were robust increases for the CS+ compared with the baseline on all subsequent days (Tukey; all $p < 0.005$). Importantly, there was no significant main effect of future cocaine treatment, nor any interactions between treatment and cue or day. For the last 2 days of Pavlovian discrimination a CS− was introduced. A separate three-way ANOVA on those days (days 7 and 8) revealed a significant main effect of cue ($F_{2,18} = 28.82$, $p < 0.0001$). Specifically, rats spent significantly more time in the foodcup during the CS+ than either the baseline or CS− (Tukey; $p < 0.0002$ for each comparison), but there was no difference between the CS− and baseline ($p = 0.29$). There were no other significant main effects of day, treatment or interactions between factors.

Instrumental training: Mean lever pressing across days is shown in Fig. 6B. All animals acquired instrumental responding as shown by a significant effect of day ($F_{7,63} = 10.51$, $p < 0.0001$). Although the rate of responding was significantly lower on day 1 than all other days of operant conditioning (Tukey; all p-values < 0.001), responding rapidly leveled off and was maintained at this rate for the remaining 7 days of training. There was no main effect of future cocaine treatment, nor an interaction of treatment by day.

Cocaine self-administration: Following Pavlovian and instrumental conditioning, rats were trained on either a cocaine or water self-administration procedure over 14 days. During training, complications with catheter patency prevented some cocaine-administering rats from completing all days of training (n=3), and these rats were not used in subsequent analyses. Across the last 3 days of training, successful cocaine self-administering rats (n=3) showed stable responding, completing 35.8 ± 4.9 responses with a mean intertrial interval of 3.7 ± 0.4 min. Yoked control rats equipped with electrophysiological arrays (n = 3) received the same amount of saline via the catheter as the paired cocaine self-administering rats. However, rats in the control group nosepoked to receive water reinforcements. Due to the large variability across saline-treated animals, a two-way ANOVA indicated no significant differences between the cocaine and water self-administering groups for the number of all nosepokes ($F_{1,4} = 2.72$, $p = 0.17$), nor an effect of day ($F_{13,52} = 1.6$, $p = 0.10$) or interaction of group × day ($F_{13,52} = 1.6$, $p = 0.10$).

Pavlovian-to-instrumental transfer: Finally, rats were run on PIT (Fig. 6C). Across all subjects, there was a main effect of cue ($F_{2,5} = 17.66$, $p < 0.001$). A Tukey HSD test showed that lever pressing during the CS+ was significantly greater than during the CS− ($p < 0.002$) and the baseline ($p < 0.001$). A significant interaction of treatment × cue ($F_{1,6} = 5.48$, $p < 0.001$) revealed that there was a modest trend towards an increase in the rate of lever pressing during the CS+ compared with the baseline in the saline control group (Tukey; $p = 0.07$; other comparisons not significant), whereas, in contrast, cocaine-treated animals showed a significant difference between the CS+ and baseline (Tukey; $p < 0.005$) and between CS+ and CS− (Tukey; $p < 0.01$). Further, although there were no differences in lever-pressing rates between the treatment groups during baseline (Tukey; $p = 0.23$), the cocaine group pressed significantly more during the CS+ than the saline group (Tukey; $p < 0.001$).

Similar to lever-pressing behavior, rats showed an enhanced foodcup response during the CS + compared with the CS− and baseline. Specifically, a main effect of cue ($F_{2,12} = 7.88$, $p <$

0.01) revealed a significant increase in foodcup entries during the CS+ compared with the CS− (Tukey; $p < 0.02$) and baseline (Tukey; $p < 0.01$), but showed no difference between CS− and baseline ($p = 0.85$). However, unlike the lever-pressing PIT effect, cocaine exposure had no effect on increased foodcup behavior (main effect exposure and interaction of exposure × cue, both $F < 1$).

Neural analysis

Pavlovian cue encoding: Similar to the results for Experiment 1, rats in the saline-treated control group showed a bias towards encoding cue-selective information in the core (37%) compared with the shell (16%) (Fig. 7A). Indeed, there was no difference in overall cue-selective encoding between the core and shell in saline-treated and naive populations ($\chi^2 = 0.02$, $p = 0.96$). However, in the rats with a history of cocaine self-administration, there was an increase in the percentage of core (50%) and shell (39%) neurons encoding cue-selective information, an increase that was marginally greater than both the saline controls and naive animals from Experiment 1 ($\chi^2 = 3.96$, $p = 0.051$). Tests restricted to core and shell subregions (Fig. 7A) revealed that there was no difference in cue-encoding rates in the core between the cocaine-treated group and either the saline-treated ($\chi^2 = 1.03$, $p > 0.10$) or naive ($\chi^2 = 0.12$, $p > 0.10$) groups. In contrast, in the shell, there was a significant increase in cue encoding in the cocaine group compared with the saline-treated and naive groups ($\chi^2 = 5.34$, $p < 0.03$), but no difference between the naive and saline-treated groups ($\chi^2 = 0.08$, $p = 0.77$).

Phasic activity during the reward: Next, reward-related encoding was analyzed for this population of neurons. Saline-treated controls again showed a similar pattern of activity in both the core (36%) and shell (17%) compared with the untreated naive population in Experiment 1. There was no statistical difference in the overall rate of reward encoding between the saline-treated and naive group ($\chi^2 = 0.05$, $p = 0.82$), nor any differences between the control groups in either the core ($\chi^2 = 1.39$, $p = 0.23$) or shell ($\chi^2 = 0.98$, $p = 0.32$).

In contrast, cocaine-treated rats showed a different pattern of reward encoding. There was an overall increase in reward encoding in cocaine-exposed animals compared with saline-treated controls ($\chi^2 = 3.92$, $p < 0.05$). This difference was carried by a selective increase in the shell, whereas there were no differences between the percentage of reward encoding in the core of cocaine-treated animals compared with either control group (saline: $\chi^2 = 0.49$, $p = 0.48$; naive: $\chi^2 = 0.18$, $p = 0.67$); shell neurons in the cocaine-treated rats were significantly more likely to code for reward than either the saline ($\chi^2 = 4.53$, $p < 0.05$) or naive control ($\chi^2 = 7.43$, $p < 0.01$) group (Fig. 7B).

Lever press encoding: As in naive controls, the majority of neurons in both the core and shell showed phasic activity aligned to the lever press regardless of treatment. Replicating the results from Experiment 1, rats in the saline-treated group showed a bias towards lever-press encoding in the core (82%) compared with the shell (50%). Of these, in the core, 9% encoded information exclusively about the approach to the lever, 55% exclusively encoded information following the press and 18% of neurons encoded both the approach and post-press response. Shell neurons in the saline controls showed less phasic activity, as 17% encoded the approach, 33% encoded the post-press response, but no cells showed encoding for both. These rates were statistically similar to those seen in Experiment 1.

Cocaine-treated rats showed slightly higher rates of lever press encoding in the core than the saline-treated controls, as there was a marginal increase in the overall rate of lever press encoding following cocaine exposure ($\chi^2 = 3.63$, $p = 0.056$). This increase was not seen in

the core, where similar rates of lever press encoding were observed in both the saline (81%) and cocaine-treated (93%) groups ($\chi^2 = 0.94$, $p = 0.33$). In the shell, there was a significant increase in the total percentage of neurons encoding the press for cocaine-treated animals (89%) compared with the saline-treated controls (50%) ($\chi^2 = 4.13$, $p < 0.05$) (Fig. 8A).

Pavlovian-to-instrumental transfer-selective encoding: Finally, the development of PIT-selective neural encoding during lever press was assessed in both the core and shell following self-administration. The rate at which PIT-selective neurons developed in the saline-treated controls (29%) was similar to that seen in the naive population (33%) in Experiment 1, and there were no differences in this rate in the core (36% saline, 32% naive; $\chi^2 = 0.08$, $p = 0.78$) or shell (17% saline, 35% naive; $\chi^2 = 0.35$, $p = 0.55$).

Cocaine exposure induced a dramatic increase in the total number of PIT-selective lever press neurons. There was almost a doubling in the total percentage of PIT-selective neurons in the cocaine-treated rats (62%) compared with the saline-treated ($\chi^2 = 4.75$, $p < 0.03$) and naive controls ($\chi^2 = 8.24$, $p = 0.005$). Unlike encoding for cues, rewards and simple lever presses that showed selective enhancement of encoding in the shell, PIT-selective encoding was increased in both the core and shell of cocaine-exposed animals. The core (69%) was greater than either control group (saline: $\chi^2 = 4.89$, $p < 0.05$; naive: $\chi^2 = 11.67$, $p < 0.001$). Similarly, there was a trend towards more PIT-selective encoding in the shell (56%) of cocaine-treated rats compared with the control groups (saline: $\chi^2 = 2.71$, $p = 0.09$; naive: $\chi^2 = 2.82$, $p = 0.09$) (Fig. 8B).

In contrast to the changes in lever-press-related PIT-modulated encoding, there were similar numbers of PIT-modulated foodcup responses in the core and shell. Further, there was no difference in the percentage of cells that encoded such PIT-modulated responses in the cocaine compared with the saline-treated groups, nor was there any interaction between regions (core and shell) and cocaine treatment (all p-values > 0.35).

Histology—The placement of all recording wires histologically confirmed in the NAc for both Experiments 1 and 2 are shown in the Supporting Information (Fig. S1). Cells recorded from wires located outside the core and shell, or on the border between the structures were excluded from the analysis.

Discussion

The present data provide an important insight into the specific roles of NAc subregions during PIT. In all groups tested, there was a selective behavioral enhancement in lever pressing in the presence of the CS+ cue that was not seen in the presence of the CS− cue. However, rats with a history of cocaine self-administration showed transfer that was significantly more robust than either control group. At the neural level, evidence was found that both the core and shell contributed important facets of encoding critical to supporting successful transfer. In all groups, core neurons were reliably biased in encoding information about cues, rewards and operant task performance compared with the shell, and cue-related encoding in the core was correlated with the degree of behavioral transfer. In contrast, in naive rats, only shell neurons showed cue-modulated responses during lever press (PIT-modulated neurons) that were correlated with task performance. However, following chronic cocaine taking, shell but not core neurons showed enhanced encoding for all task-related events compared with controls, whereas both core and shell showed a dramatic increase in the percentage of PIT-modulated neural activity to the press.

In contrast, the analysis of foodcup entries and neural activity that encoded these responses highlights the specificity of the instrumental transfer feature of the PIT task. Although

cocaine experience resulted in a significant potentiation of the PIT effect for lever pressing, it did not translate into more general behaviors in the task such as foodcup activity. These findings indicate that psychostimulant experience did not simply increase hyperactivity in the box, nor did it lead to a differential response conflict between the instrumental and Pavlovian responses during transfer. Instead, cocaine experience selectively enhanced the instrumental response in the presence of the CS+, a feature that was reflected in both the behavior and neural response.

In the present study, encoding information about Pavlovian cues in naive animals was largely a function of the NAc core, although a few shell neurons encoded this associative information. This pattern of encoding has been demonstrated reliably in previous studies, whether the cues predict natural rewards such as sucrose (Setlow et al., 2003; Day et al., 2006; Jones et al., 2008) or drugs of abuse such as cocaine (Hollander and Carelli, 2007). These neural representations encode not only the identity of these cues, but also the motivational significance and predictive value of the associated outcome. For example, studies from this laboratory have repeatedly demonstrated that NAc core neurons show little overlap between cues predictive of cocaine and cues predictive of natural reward (Carelli et al., 2000; Carelli and Wondolowski, 2003). Further, in a go/no-go task, NAc core neurons rapidly encoded new associations, and rapidly switched or lost this cue selectivity when response contingencies were reversed (Setlow et al., 2003). Studies employing neurotoxic lesion support these correlational findings; post-training core but not shell lesions impair performance on simple Pavlovian conditioning (Parkinson et al., 1999; Cardinal et al., 2002b), whereas lesions of the NAc centered on the core during a cued go/no-go task resulted in behavioral deficits suggestive that rats were insensitive to cued outcome value (Schoenbaum and Setlow, 2003). Further, reversible inactivation of the NAc core but not shell has been shown to selectively disrupt cue-induced reinstatement of cocaine self-administration (Fuchs et al., 2004). These data argue for a specific role for the NAc core for acquiring critical cue-related information for guiding behavior.

Interestingly, although much cue encoding was dependent on the core, only shell neurons in naive animals showed cue-modulated operant encoding that was correlated with the behavioral performance of PIT. Several studies have now suggested that the shell is critical for the transfer effect. For example, Corbit et al. (2001) showed that lesions of the NAc shell made prior to conditioning failed to impair either Pavlovian or instrumental conditioning, but selectively abolished cue-potentiated transfer, whereas NAc core lesions had no effect on transfer. Similarly, intrashell infusions of amphetamine (Wyvell and Berridge, 2000) or CRF (Pecina et al., 2006) results in potentiating the transfer effect, whereas lesions of the shell but not the core block this amphetamine potentiating effect (Parkinson et al., 1999).

These findings are somewhat at odds with other work that has shown specificity for the NAc core in PIT (Hall et al., 2001; de Borchgrave et al., 2002). In these studies, normal Pavlovian and instrumental conditioning were largely unaffected, but transfer was impaired. Importantly, in these studies, lesions of the core were made prior to any conditioning, whereas the above work showing the importance of the shell was performed in experiments where the lesion was administered after first-order conditioning but prior to transfer (Parkinson et al., 1999). This suggests an important distinction between the acquisition of Pavlovian information vs. the potentiation of instrumental responding in the presence of learned cues.

In line with this finding, the enhancement of PIT following a period of prolonged drug-taking was accompanied by a concurrent increase in shell-specific neural encoding. These results mirror the findings from Parkinson et al. (1999) in which post-training shell lesions abolished the ability for amphetamine to potentiate already-learned responses. As in their

study, this suggests that the shell acts to modulate previously learned Pavlovian and instrumental information, specifically those for which the drug inducing the alteration in behavior (either amphetamine or cocaine) was not the reinforcer being used to guide PIT. Similarly, amphetamine infusions into the NAc shell at the time of PIT significantly enhanced the transfer effect (Wyvell and Berridge, 2000). However, in both of these circumstances, the drug was present at the time of transfer, whereas in the present study and others (Ranaldi et al., 2009), animals were drug abstinent for 1 week prior to testing. Thus, the present findings suggest that repeated cocaine exposure may change the sensitivity of shell neurons to PIT-related stimuli, a mechanism that may be gated by prolonged exposure to phasic DA release. Intriguingly, previous studies have shown that DA release in the NAc following cocaine infusions is largely confined to the shell (Aragona et al., 2008). Cocaine self-administration may thus result in inducing a shell-specific DA-dependent process in which animals become exquisitely sensitive to task-related stimuli and rewards, and thus may be at greater risk for subsequent relapse.

Given these converging data, one model for these results that is in line with the present findings suggests a role of the NAc core neurons in learning the motivational significance of cues early in learning, whereas the core may become less important after the associations are fully learned. The naive animals reported here show such a pattern; core neurons reliably encoded cue-related information and, further, the degree to which this was learned predicted success on later transfer. However, these neural representations did not appear to modulate lever-pressing activity during PIT, suggesting a less essential role in expressing that behavior. Shell neurons showed a different pattern of activity in line with this model. Although not as involved with the encoding of cue-related information as the core, cells that were cue-modulated at the time of press were significantly correlated with performance on transfer. If this model is correct, we would predict that transient inactivation of the core, but not shell, during learning would impair subsequent transfer, whereas inactivation of the shell, but not core, at the time of transfer would have a similar transfer-inhibiting effect.

Previous work in this laboratory has also shown that, following cocaine abstinence, cue and task-related encoding are selectively potentiated in the core, but not the shell (Hollander and Carelli, 2005, 2007). However, in those studies, modulation was found for drug-related stimuli and responses, whereas in the present study, drug exposure altered encoding for non-drug (natural) reward during novel learning. Notably, in the earlier study, associative encoding for drug-related stimuli necessarily occurred while the cocaine was onboard, whereas in the present study, all animals had the opportunity to learn about Pavlovian and instrumental responses for natural reward while drug naive. Thus, in the earlier studies, these factors may strongly contribute to biasing rats towards core-specific encoding during learning, whereas in the present study, cocaine exposure may potentiate already-learned representations that may be more shell-dependent.

Thus, we predict that the role of repeated cocaine exposure would have differing effects from the present findings if presented prior to training. A series of work has now suggested that repeated cocaine exposure prior to learning can result in profound deficits in acquisition. For example, cocaine-treated rats have been shown to have impairments in acquiring normal Pavlovian (Schoenbaum and Setlow, 2005; Saddoris et al., 2010) and operant task (Schoenbaum et al., 2004; Calu et al., 2007; Roesch et al., 2007) performance. If animals are unable to learn about cue-outcome or response-outcome associations normally as a result of cocaine exposure (a putatively core-dependent process), then such cocaine exposure should result in impaired, not enhanced, PIT due to poor initial learning, but not because of poor transfer specifically.

Given that both the core and shell appear to coordinate activity to produce the PIT effect, it is not known how the core and shell subregions would coordinate activity in the course of learning to produce this phenomenon. Interestingly, many facets of NAc encoding presented here mirror results previously found in the amygdala. For example, similar to the core, lesions of the BLA disrupt behavior sensitive to Pavlovian cue encoding in similar tasks (Schoenbaum et al., 1998; Balleine et al., 2003; Pickens et al., 2003; Schoenbaum et al., 2003b), while also causing aberrant cue encoding in distally connected regions such as the prefrontal cortex (Schoenbaum et al., 2003a) and NAc (Ambroggi et al., 2008; Jones et al., 2010). In contrast, the central nucleus of the amygdala (CN) has been shown to be important for attention for learning (Gallagher et al., 1990; Hatfield et al., 1996; Parkinson et al., 2000b; Haney et al., 2010), but less important for detailed cue-outcome associative learning. Consequently, similar to differences between the core and shell in the NAc, BLA and CN show a similar dissociation in PIT. CN lesions abolish potentiating transfer effects, whereas BLA lesions only appear to abolish the behavioral selectivity (i.e. only pressing the CS+-associated lever) of the PIT (Blundell et al., 2001; Hall et al., 2001; Holland and Gallagher, 2003; Corbit and Balleine, 2005).

These core/BLA and shell/CN parallels suggest a larger system by which the amygdala and NAc coordinate activity to produce cue-modulated instrumental behavior. Indeed, BLA inputs to the NAc (Heimer et al., 1991; Brog et al., 1993) appear to be critical for supporting cue-related learning, as asymmetric lesions of the BLA and NAc block the ability for rats to use Pavlovian cues to support new learning (Setlow et al., 2002), whereas inactivation of the BLA selectively alters NAc core encoding during appetitive conditioning (Ambroggi et al., 2008; Jones et al., 2010). However, CN fibers do not terminate in the NAc shell; instead they presumably influence NAc activity via an indirect pathway through midbrain DA-expressing neurons. Consistent with this, inactivation of the ventral tegmental area abolished PIT (Corbit et al., 2007), whereas dopaminergic receptor blockade in the NAc attenuated transfer (Lex and Hauber, 2008). Conversely, amphetamine, which increases DA vesicular release, potentiates PIT after being selectively infused into the shell (Parkinson et al., 1999; Wyvell and Berridge, 2000). Thus, as the anatomical projections from the amygdala complex at the level of the ventral striatum (whether direct or indirect) are heavily intermixed, these functional parallels suggest that there is probably a necessary interplay between glutamatergic and dopaminergic processes that may differentially impact the ways in which motivational and detailed sensory information is coded within the NAc.

In conclusion, these results present an important basis for understanding the neural underpinnings of PIT in the NAc, and how this neural circuit is fundamentally altered following repeated exposure to cocaine and its resultant modulation of DA action in the NAc. Future work will need to investigate how this neural encoding acts within larger circuits of the limbic system such as the amygdala and dorsal striatum, and how such circuits are modulated by DA inputs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CN	central nucleus of the amygdala
CS+	conditioned stimulus
DA	dopamine
PIT	Pavlovian-to-instrumental transfer
VI	variable interval

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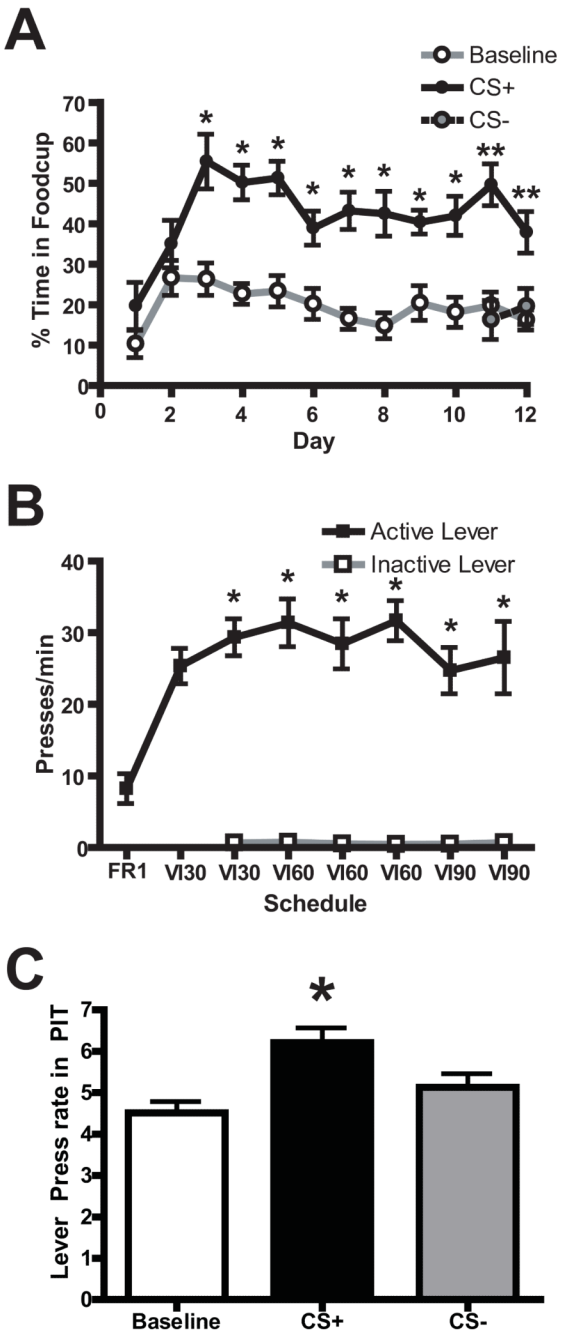

Fig. 1.

Behavioral results for PIT. (A) Rats rapidly learned the significance of the CS cues during Pavlovian training. Behavior was measured by percent time spent in the foodcup. Rats spent significantly more time in the foodcup during the first 10 s of cue onset for the CS+than the 10 s baseline period starting on day 3, and maintained this difference until the end of training. On days 11 and 12, rats readily discriminated between the CS+ and CS− cues, as they spent more time in the foodcup during the CS+ than either the CS− or baseline. *p<0.05, CS+ vs. baseline; **p<0.05, CS+ vs. baseline and CS+ vs. CS−. (B) Lever presses during training. The rate of pressing was consistent across days despite increasingly demanding VI schedules. Inactive lever presses were minimal, and lower than active presses

for all sessions. *p<0.05 active vs. inactive lever. (C) PIT effect on the day of transfer was assessed by comparing the rate of lever presses during the baseline with that during the CS+ and CS−. Rats showed significantly greater rates (presses per 10 s bin) of lever pressing during the CS+ compared with the baseline (*p <0.02); there was no difference between the CS− and baseline (p > 0.1). FR1, fixed ratio 1.

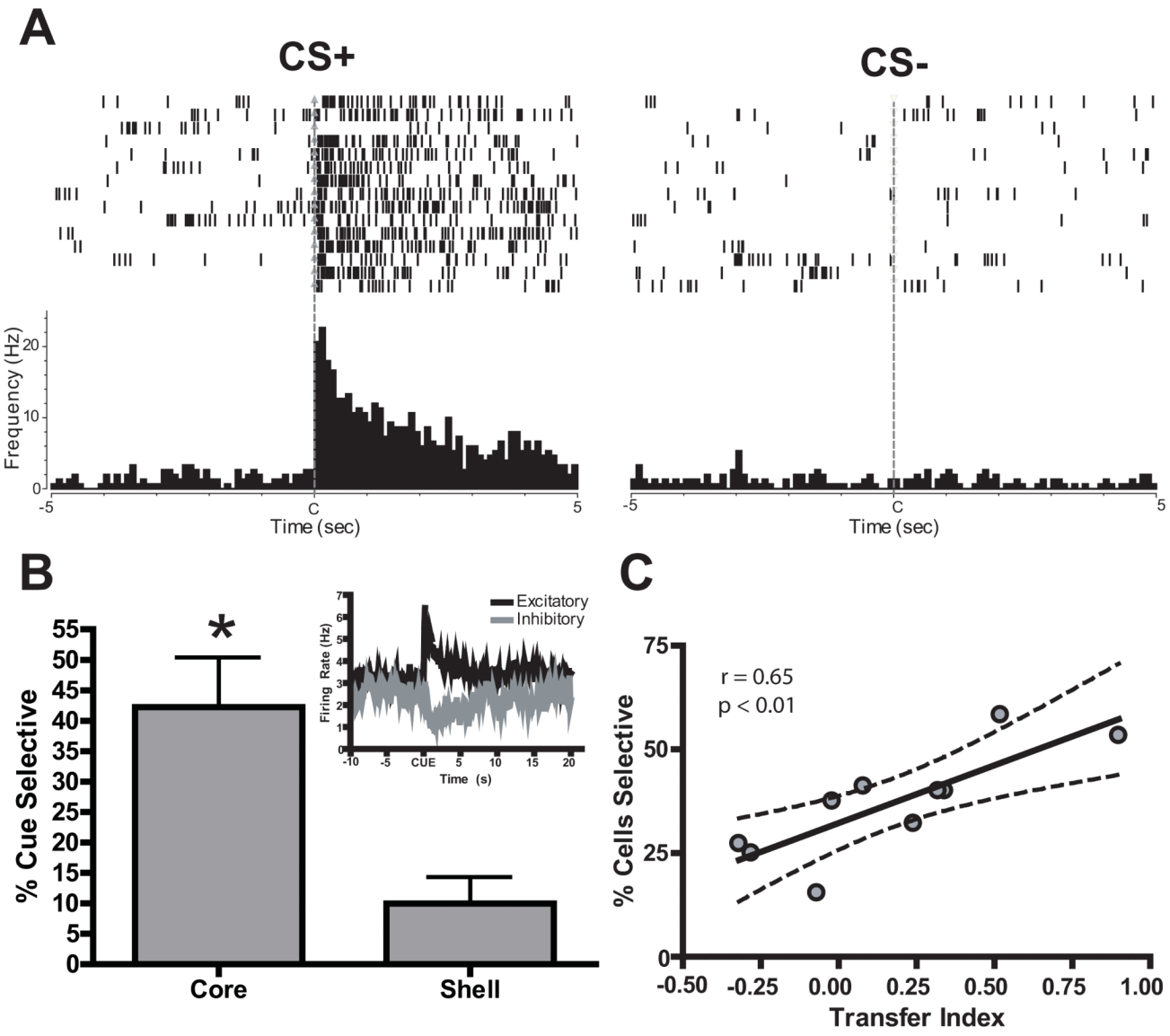

Fig. 2.

NAc neurons showed activity during the presence of Pavlovian cues during transfer sessions. (A) Example of a cue-selective cell recorded in the NAc core during PIT. The cell selectively increased firing at the onset of the CS+ (left) but not the CS− (right). (B) Cue-selective encoding was significantly greater in the core than the shell. Inset: population average of all phasic cue-encoding cells (core and shell), separated by whether the phasic activity was inhibitory or excitatory. Both excitatory and inhibitory neurons showed activity that was rapid at cue onset and then declined back to baseline within 2-10 s. *p<0.05. (C) Correlation between the percentage of cue-selective neurons per subject and that subject's transfer index (see Materials and methods). A higher index score indicates better transfer during PIT. Rats with a greater percentage of cue-selective neurons showed significantly better transfer.

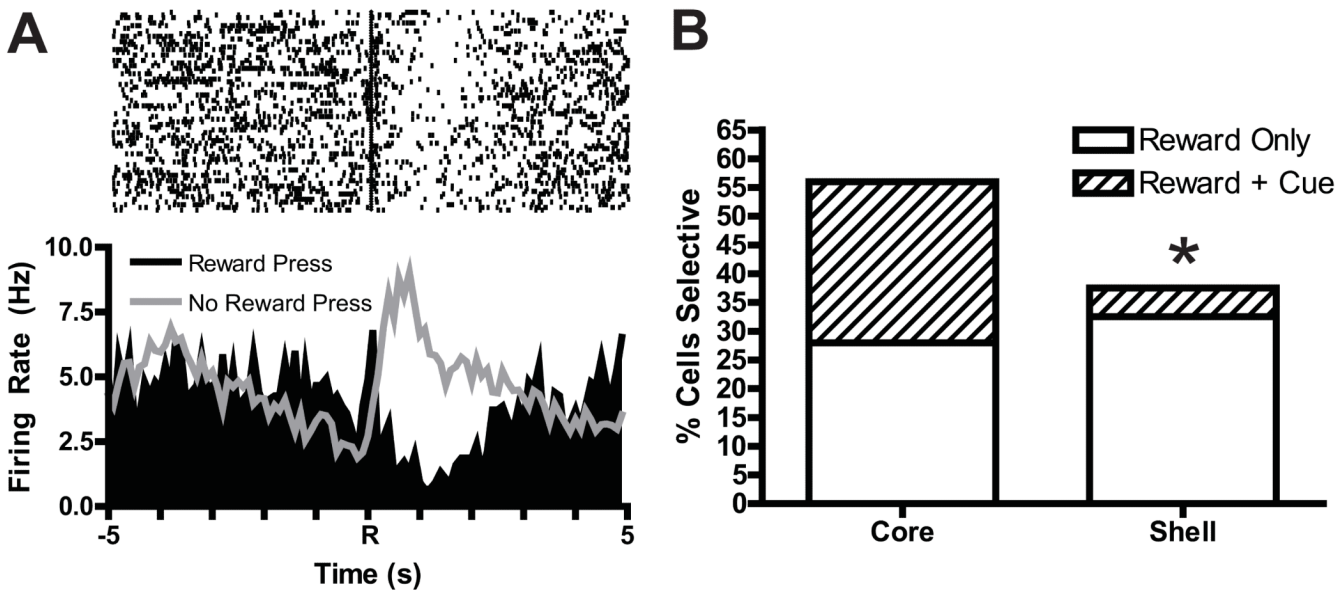

Fig. 3.

Reward encoding during transfer. (A) Peri-event histogram shows an example neuron exhibiting a consistent inhibition of firing within 500 ms of reinforced lever presses (indicated by R). This inhibition was not due to the press itself, as presses on the same lever that were not reinforced (gray line) led to transient post-press excitations. (B) Similar numbers of cells encoded information about the reward only in the core and shell (open bars) but neurons in the core (compared with shell) encoded both reward and cue more (hatched bars). *$p < 0.01$.

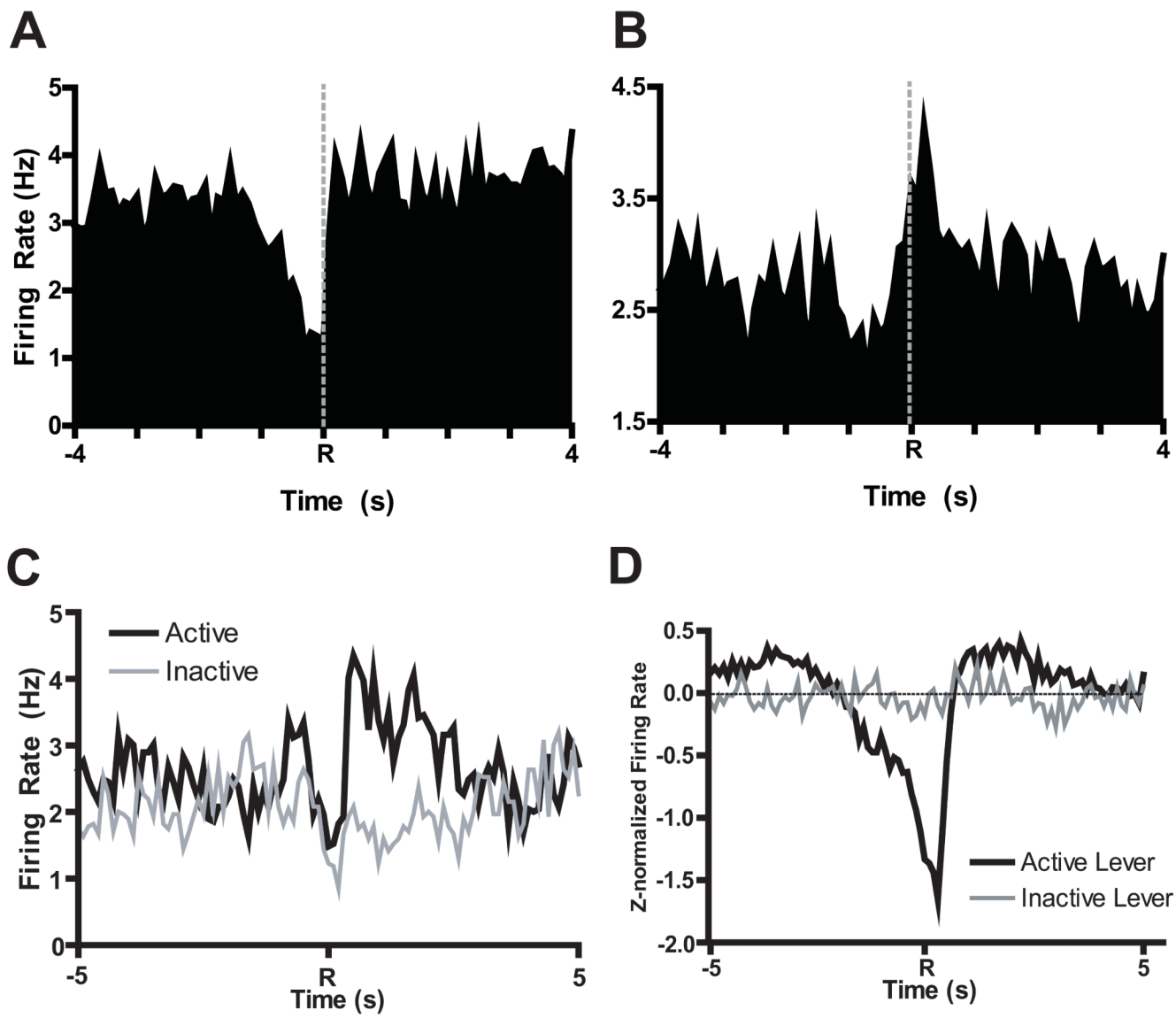

Fig. 4.

Lever-pressing-induced phasic changes in firing for the active, but not inactive, lever. (A) Example cell showing representative inhibitory 'approach-encoding' activity relative to the lever press response at R. (B) Example neuron showing 'response-encoding' activity characterized by a phasic excitation immediately before/following the press. (C) A representative neuron showing a phasic post-response excitation after pressing the active lever, but no change relative to the inactive lever (gray line). (D) Population response (n=34 cells) showing that, across regions, normalized firing to the lever was predominantly inhibitory only for the active lever. Inactive lever presses were not correlated with population-wide changes in firing in the NAc.

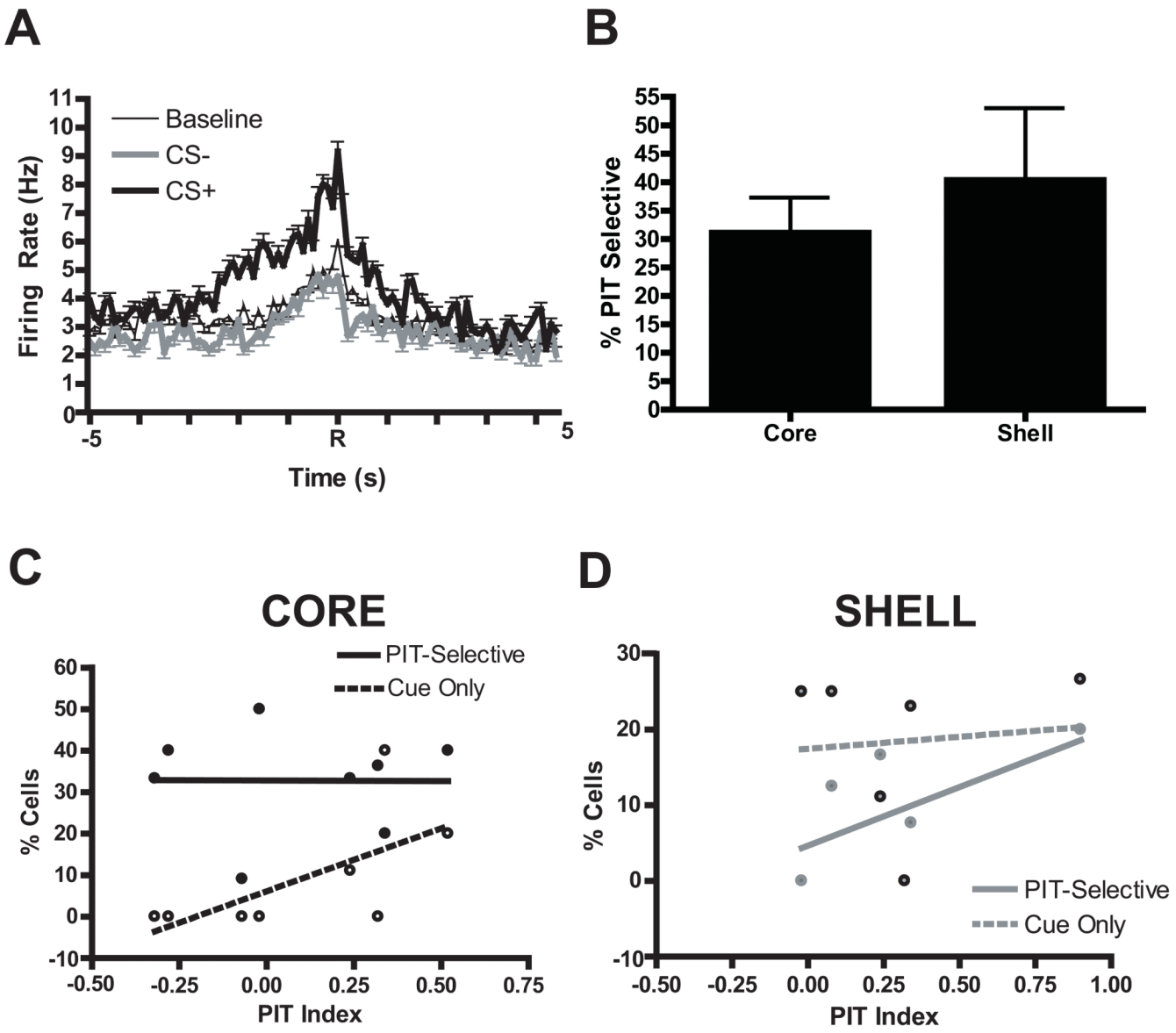

Fig. 5.

PIT-selective encoding emerged when instrumental responding was enhanced in the presence of the CS+ cue. (A) An example neuron showing increased activity preceding the lever press during baseline and CS− conditions that was enhanced during CS+presentations. (B) Percentage of PIT-selective neurons in the core and shell. Correlation between percentage of cells showing PIT-selective or cue-only activity as a function of PIT index for neurons in the core (C) or shell (D).

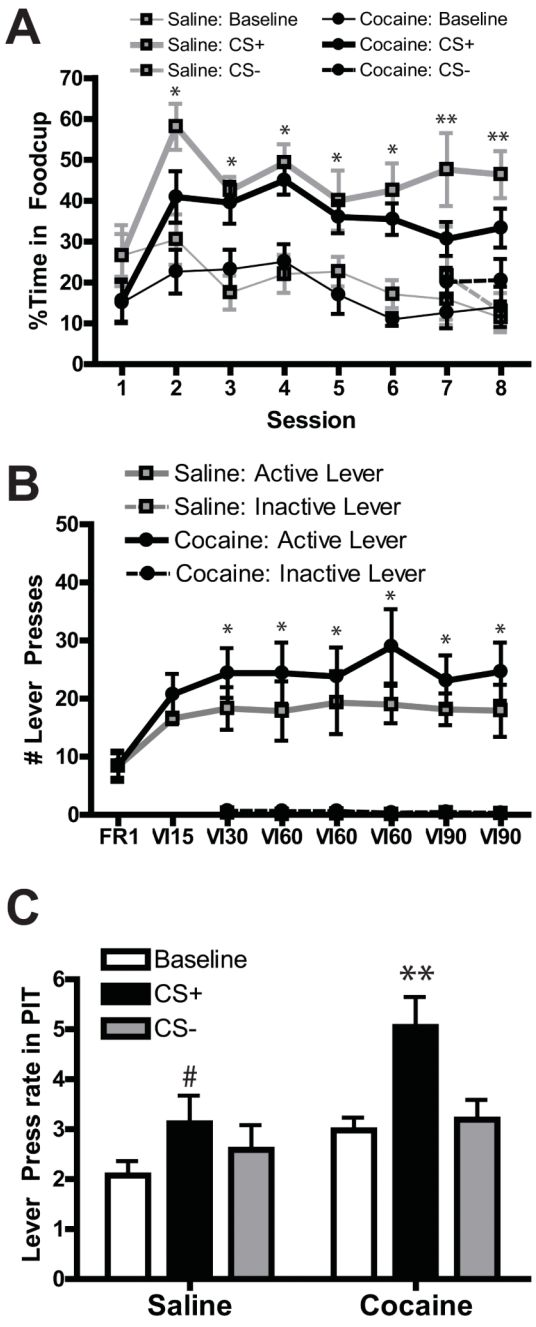

Fig. 6.

Cocaine intake resulted in a behavioral enhancement of PIT. (A) Prior to drug self-administration sessions, there were no differences between the rate of learning and cue discrimination between rats destined for cocaine or saline self-administration. *$p < 0.05$ for CS+ compared with baseline; **$p < 0.05$ for CS+ compared with both baseline and CS−. (B) Similarly, rats destined for either cocaine or saline self-administration showed no differences in the ability to learn operant lever pressing for food. *$p<0.05$ active lever compared with inactive lever. (C) Rate of lever presses during the transfer test (PIT session) as a function of saline or cocaine treatment. # $p = 0.07$ compared with baseline and CS+ periods; **$p < 0.01$ compared with baseline and CS−.

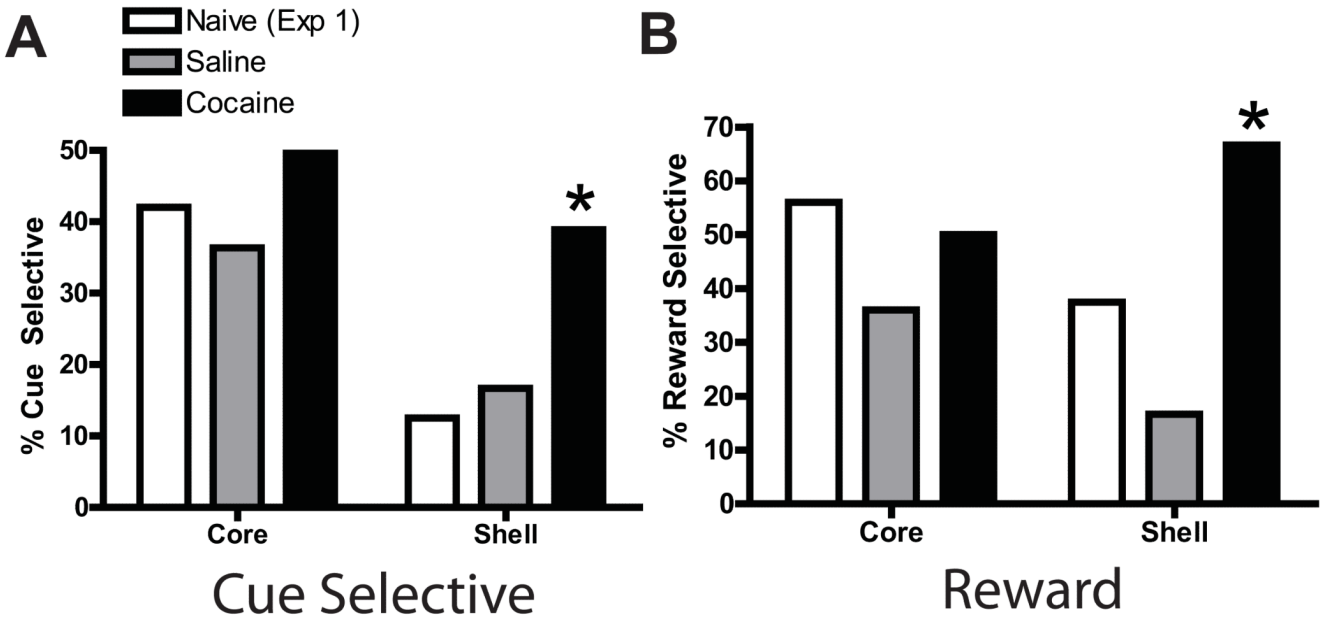

Fig. 7.

Following cocaine exposure, rats showed altered neural encoding that was specific to the shell. Here and in subsequent figures, data from Experiment 1 (white bars) are shown for comparison with saline-treated controls. (A) Cocaine exposure had no effect on the percentage of cue encoding in the core. However, shell neurons showed a significant increase in cue encoding after exposure. (B) Similarly, increases in reward-selective encoding were specific to the shell following cocaine exposure. *$p < 0.05$.

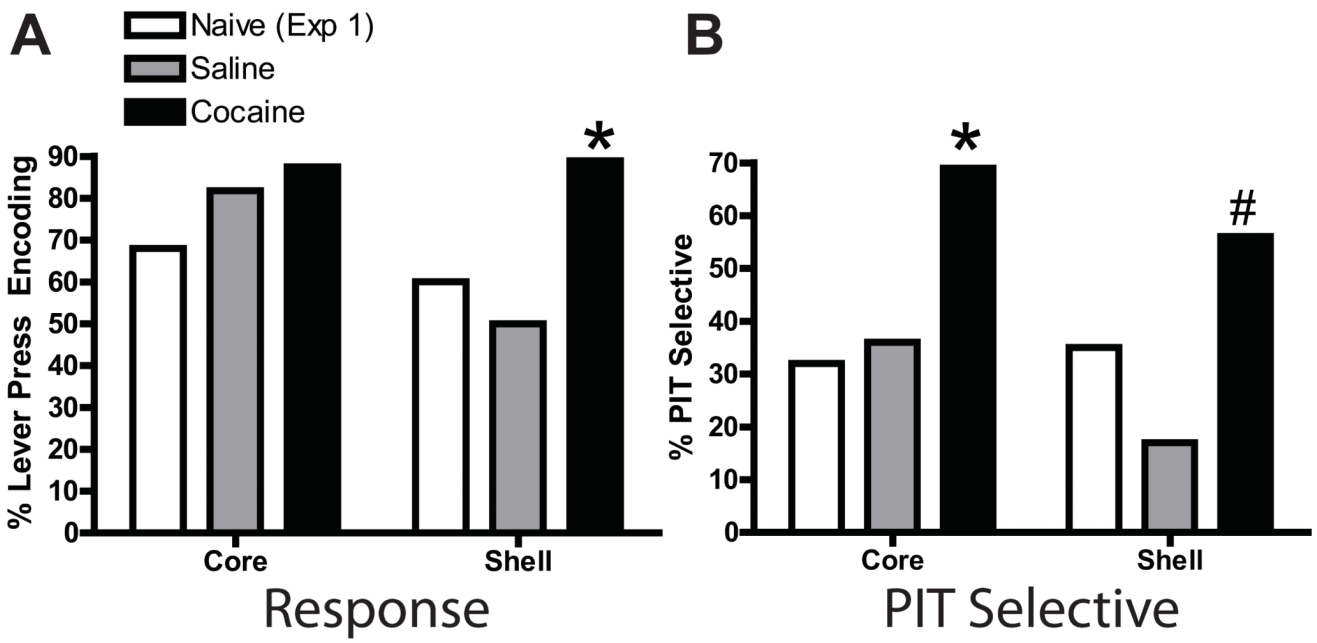

Fig. 8.

Lever press encoding was also altered following cocaine exposure. (A) Shell, but not core, neurons showed potentiated rates of encoding following cocaine exposure. (B) PIT-selective encoding was enhanced in both the core and shell for the cocaine-treated animals compared with either drug-naive population. *$p < 0.05$; #$p < 0.10$.

Table 1

Behavioral design for PIT training

	Group	Pavlovian	Instrumental (lever)	Cocaine (nosepoke)	PIT
Experiment 1	Naive	CS+ → food	FR1 → food (D1)		VI90 → food
		CS− → no food	VI30 → food (D2–3)		(CS+) VI90→
			VI60 → food (D4–6)		(CS−) VI90→
			VI90 → food (D7–8)		
Experiment 2	Saline	CS+ → food	FR1 → food (D1)	NP(FR1) → oral water	VI90 → food
		CS− → no food	VI30 → food (D2–3)	→0.9% saline i.v.	(CS+) VI90 →
			VI60 → food (D4–6)		(CS−) VI90 →
			VI90 → food (D7–8)		
Experiment 3	Cocaine	CS+ → food	FR1 → food (D1)	NP(FR1) → 0.33 mg cocaine i.v.	VI90 → food
		CS− → no food	VI30 → food (D2–3)		(CS+) VI90 →
			VI60 → food (D4–6)		(CS−) VI90 →
			VI90 → food (D7–8)		

CS+/CS−, tone or noise; FR1, fixed ratio 1, one lever press for food; VI, variable interval reinforcement for lever press; NP, nosepoke.