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Effects of nucleus accumbens core and shell lesions on autoshaped lever-pressing

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Abstract

Certain Pavlovian conditioned stimuli (CSs) paired with food unconditioned stimuli (USs) come to elicit approach and even consumption-like behaviors in rats (sign-tracking). We investigated the effects of lesions of the nucleus accumbens core (ACbC) or shell (ACbS) on the acquisition of sign-tracking in a discriminative autoshaping procedure in which presentation of one lever CS was followed by delivery of sucrose, and another was not. Although we previously found that bilateral lesions of the whole ACb disrupted the initial acquisition of sign-tracking, neither ACbC or ACbS lesions affected the rate or percentage of trials in which rats pressed the CS+. In addition, detailed video analysis showed no effect of either lesion on the topography of the sign-tracking conditioned response (CR). These and other results from lesion studies of autoshaping contrast with those from previous sign-tracking experiments that used purely visual cues (Parkinson, Robbins, and Everitt, 2000a; Parkinson, Willoughby, Robbins, and Everitt, 2000b), suggesting that the neural circuitry involved in assigning incentive value depends upon the nature of the CS.

Keywords

nucleus accumbens core; nucleus accumbens shell; incentive salience; autoshaping

1. Introduction

Environmental cues associated with food can become powerful elicitors of a variety of learned behaviors. For example, in an autoshaping procedure in which insertion of a lever conditioned stimulus (CS) in the chamber signals the delivery of a food unconditioned stimulus (US), the lever may control approach and contact responses directed to the lever ("sign-tracking") and/or to the food delivery site ("goal-tracking") [4,5,11,16]. These conditioned responses (CRs) develop despite the absence of any explicit response-US response contingency [26]. Some investigators have argued that CS-directed sign-tracking behaviors reflect the acquisition of incentive salience to the CS, by which the incentive motivational value of the US is transferred to the CS [1,2]. As a result of the attribution of incentive salience to a lever, rats may try to "consume" it by biting or chewing [1,2,30]. Moreover, the topography of the CR is also dependent on the type of US that is used: Rats given a food US tend to nibble and bite the lever CS, whereas rats given a liquid US (20% sucrose or 25% condensed milk) tend to lick the lever CS [8,9]. Consistent with previous

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findings that implicate ACb and its midbrain dopamine (DA) projections from the ventral tegmental area (VTA) in the transfer of incentive value from the US to the CS [10,15,24,25], Chang et al., (2012b) [7] found that whole ACb lesions impaired the initial acquisition of sign-tracking, although not its terminal levels.

To further characterize the role of ACb and its subnuclei in incentive learning, here we investigated the effects of separate bilateral lesions of the nucleus accumbens core (ACbC) or medial shell (ACbS) on autoshaped lever-pressing in rats. Parkinson et al. (2000b) [20] showed that bilateral lesions of ACbC, but not ACbS, disrupted the acquisition of conditioned approach to visual CSs (white rectangles displayed on a monitor). Although approach to a visual CS is considered a form of sign-tracking, we hypothesized that the neural circuitry involved in assigning incentive salience to a CS that can only be approached (white rectangle) may be different from that engaged by a CS that can be approached, manipulated, and “consumed” (lever). Specifically, autoshaping with lever CSs may allow for the transfer of hedonic properties from the US to the CS, referring to the pleasure associated with consuming a sweet and energy-rich reward (e.g. “liking”; [3]). Previous taste reactivity studies by Berridge and colleagues have shown that microinjections of the b5 opioid agonist DAMGO ([D-Ala², N-MePhe⁴, Gly-ol]-enkephalin) or the endogenous cannabinoid anandamide into particular “hotspots” into the medial ACbS can enhance the hedonic impact of a sweet US [18,22]. In contrast, no such hedonic enhancements were observed with comparable treatment in the ACbC. If autoshaping with lever CSs allows for the transfer of hedonic properties (e.g. conditioned “liking”; [1]), then we would expect ACbS but not ACbC lesions to alter sign-tracking, particularly in how rats interact with the lever CS. However, if sign-tracking with lever CSs is similar to sign-tracking with visual CSs, then ACbS lesions would have no effect and ACbC lesions would produce deficits in sign-tracking.

2. Materials and methods

2.1 Animals

The subjects were male Long-Evans rats (Charles River Laboratories, Raleigh, NC, USA), which weighed 300–325 g on arrival. Rats were individually housed in a climate controlled colony room that was illuminated from 7:00 A.M. to 7:00 P.M. Rats were provided *ad libitum* access to food and water before and continuing until after two weeks of recovery from surgery. They were then placed on food restriction and were maintained at 85% of their *ad libitum* weights throughout the autoshaping procedure. The research was approved by the Johns Hopkins University Institutional Animal Care and Use Committee and carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2 Surgical procedures

Surgery was performed under aseptic conditions with isoflurane anesthesia, and all infusions were made with a Hamilton 2.0- μ l syringe equipped with a 26-gauge needle. As with Shiflett and Balleine (2010) [29], ACbC lesions were made with *N*-methyl-D-aspartate (NMDA; Sigma, St. Louis, MO, USA) in PBS at a concentration of 10 mg/ml, 0.3 μ l/site, using the coordinates 1.4 mm anterior of bregma, 2.1 mm from the midline, and 7.2 mm ventral from the skull surface at the injection site. As with Parkinson et al. (2000b) [20], ACbS lesions were made with ibotenic acid (Sigma) in PBS at a concentration of 10 mg/ml using the coordinates 1.6 mm anterior of bregma, 1.1 mm from the midline, and 7.9 mm (0.15 μ l/site) and 6.9 mm (0.075 μ l/site) and 6.4 mm (0.075 μ l/site). Rats that received sham lesions underwent the same surgical procedures as their lesioned cohort, but no infusions were made once the needle was in position. Bilateral ACbC lesion surgery was performed

on 17 rats, and bilateral ACbS lesion surgery was performed on 17 rats. Bilateral ACbC sham and ACbS sham lesion surgeries were performed on 5 rats each.

2.3 Apparatus

The behavioral training apparatus consisted of eight individual chambers (20.5 cm×22.0 cm×22.5 cm) with stainless steel front and back walls, clear acrylic sides, and a floor made of 0.48-cm stainless steel rods spaced 1.9 cm apart. A 2-cm illuminated clear acrylic food cup was recessed in an opening of the front wall, and photocells at the front of the food cup recorded entries and time spent in the cup. Liquid sucrose was delivered to the cup by an infusion pump through an opening at the bottom of the slightly concave food cup, forming a small puddle. Locally-fabricated retractable levers, which were operated quietly by pneumatic controls, were located on either side of the food cup. Each chamber was enclosed inside a sound attenuating shell; the infusion pump was mounted outside the shell. An infrared light was located outside of each chamber, and cameras mounted within the shell allowed for television viewing and behavioral scoring.

2.4 Autoshaping

Rats first received two 64-min sessions in which they were trained to eat from the food cups. In each of these sessions, rats received 16 0.1-ml deliveries of 8% (w/v) sucrose solution, with a mean intertrial interval (ITI) of 240 s. Next, rats underwent 12 sessions of the autoshaping procedure. Within each 64-min session, there were 25 CS+ and 25 CS– trials (mean ITI = 77 s), with trial sequences randomized each day. On CS+ trials, one lever was extended for 10 s and reinforced with 0.1 ml of 8% sucrose upon retraction and on CS– trials, the other lever was extended for 10 s, but no sucrose was delivered. For half the rats, the CS+ lever was the left lever and the CS– lever was the right lever. For the other half, the sides of the CS+ and CS– levers were reversed.

2.5 Automated response measures

We reported the percentage of time rats spent with their heads in the food cup (goal-tracking), as well as the rate of lever pressing and the percentage of trials on which at least one lever press occurred, which we have previously reported can be differentially affected by various brain lesions [7]. Although we have previously shown that the rate of autoshaped lever pressing peaks during the last 5 s of CS lever presentations [6,7], here we analyzed the rate of lever pressing and the percentage of trials on which at least one lever press occurred across the entire 10-s CS interval, to permit fair comparisons with the video-scored data (described next).

2.6 Behavioral video scoring

In order to elucidate any differences between sham and lesioned rats in how they responded to the CS levers during the autoshaping procedure, videotapes from sessions 4, 8, 10, and 12 were analyzed. Behaviors towards the lever CSs that were scored included approach without contact (orienting and sniffing), consummatory contacts (biting and grasping), and non-consummatory contacts. In addition, we analyzed the total number of contacts rats made with the lever CSs (consummatory and non-consummatory contacts combined). We considered consummatory behaviors as a measure for the degree to which the hedonic properties of the US were transferred to the CS. Although rats had to first approach the lever to show consummatory behaviors, an approach behavior was not counted if a rat was simultaneously contacting the lever. In addition, orienting to the food cup and presence of the head in the food cup (food cup behaviors) were scored. Similar to Mahler and Berridge's (2009) [17] procedure, every 5th CS+ and CS– trial was scored in each session. Behaviors

were recorded by sampling every 2 s within each CS trial, paced by a digital clock recorded on videotapes.

2.7 Data analysis

Each measure (including the video-scored data) was subjected to a 3-way mixed analysis of variance (ANOVA) with the between-subjects variable of lesion condition (ACbC, ACbS, or sham), and repeated measures on the within-subject variables of cue type (CS+ vs. CS-) and session. For comparison with the results of our previous studies (e.g., [6,7]), we also reported the results of a 4-way ANOVA of the automated measures, which included evaluation of those responses during the first and second 5-s intervals of the 10-s CSs as an additional repeated measure. In all of these ANOVAs, the occurrence of discriminative autoshaping was indicated by significant effect of cue, and effects of lesions on autoshaping were revealed in significant interactions of lesion with cue.

2.8 Histological procedures

After behavioral testing, rats were anesthetized with sodium pentobarbital (100 mg/kg) and perfused intracardially with 0.9% saline, followed by 10% (v/v) Formalin in 0.1 M PBS. Brains were removed and stored in 0.1 M PBS and 20% (w/v) sucrose. Forty- μ m slices were collected and Nissl stained to verify lesion placements. In order to determine the percentage of damage of each lesion, a matrix of dots was digitally placed over the entire ACb on each figure of the Paxinos and Watson (1998) [21] atlas. After viewing each brain slice under a microscope, lesions were drawn on the appropriate figure. The percentage of damage to each subnucleus of ACb was calculated by dividing the number of dots within the damaged area over the total number of dots within each subnucleus.

3. Results

3.1 Histological results

Figures 1a and 2a show schematic representations of neural damage in accepted ACbC- ($n = 13$) and ACbS- ($n = 8$) lesioned rats. On average, $80.0 \pm 3.1\%$ (mean $\pm$ SEM) of ACbC was eliminated in ACbC-lesioned rats, whereas only $13.9 \pm 2.6\%$ of ACbS was damaged. In ACbS-lesioned rats, $70.3 \pm 5.9\%$ of ACbS was eliminated while only $13.3 \pm 1.9\%$ of ACbC was damaged. Data of rats with less than 48.9% damage to the intended lesion region were discarded. In addition, $33.13 \pm 5.29\%$ of lateral nucleus accumbens shell (lACbS) was damaged in ACbC-lesioned rats, and $17.9 \pm 5.09\%$ of lACbS was damaged in ACbS-lesioned rats. This difference in lACbS damage was marginally significant ($F_{1,19} = 3.75$, $p = 0.07$). Sham-lesioned rats ($n = 10$) had no observable damage other than near the needle track. Figures 1b–c and 2b–c show sample neurotoxic and sham ACbC and ACbS lesions, respectively.

3.2 Behavioral results

3.2.1 Autoshaped lever pressing—Figures 3a and b present the number of lever presses per minute and percentage of trials with a lever press during the entire 10-s CS interval, respectively, over the course of training. All groups acquired the sign-tracking CR and at comparable rates, responding increasingly more to the CS+ and minimally to the CS-. For both measures, ANOVAs for all of training confirmed main effects of cue ($F_{s1,31} > 61.26$, $ps < 0.01$) and session ($F_{s11,341} > 24.74$, $ps < 0.01$). However, there was no effect of lesion ($F_{s2,31} < 1.01$, $ps > 0.38$) or lesion $\times$ cue interaction ($F_{s2,31} < 0.87$, $ps > 0.43$).

As in our previous autoshaping studies, rats showed peak levels of sign-tracking during the last 5 s of CS presentations regardless of lesion condition. For comparison with those

studies, Table 1 shows the mean lever press rate and percentage of trials with a lever press of these rats during the first and second 5-s intervals of CS+ and CS- presentations, averaged over the entire training period. For both measures, 4-way ANOVAs with variables of lesion, cue, interval (first and second 5-s of CS interval) and session showed a main effect of interval (responding was greater in the second interval; $F_{s1,31} > 71.19$, $ps < .01$) with no lesion $\times$ interval interactions ($F_{s2,31} < 0.40$, $ps > 0.67$).

3.2.2 Food cup responding—Figure 3c presents the percentage of time spent in the food cup during the entire 10-s CS interval over the course of training. As training progressed, food cup behavior decreased in all groups, presumably as a consequence of increased sign-tracking. Neither sham-, ACbC-, or ACbS-lesioned rats showed greater amounts of food cup responding to the CS+ than the CS-. ANOVA confirmed a main effect of session ($F_{11,341} = 26.36$, $p < 0.01$) and lesion ($F_{2,31} = 7.98$, $p < 0.01$), as well as a lesion $\times$ session interaction ($F_{22,341} = 4.92$, $p < 0.01$). However, there was no significant effect of cue ($F_{1,31} = 0.65$, $p = 0.43$) or lesion $\times$ cue interaction ($F_{2,31} = 2.20$, $p = 0.13$). In contrast to autoshaped lever pressing, rats showed greater amount of food cup responding during the first 5 s of CS presentations regardless of lesion condition. A 4-way ANOVA confirmed a main effect of interval ($F_{1,31} = 6.06$, $p = 0.02$) but no lesion $\times$ interval interaction ($F_{2,31} = 0.35$, $p = 0.71$).

3.2.3 Behavioral video scoring

3.2.3.1 Approach behaviors: Figure 4a presents the number of approach behaviors (orienting and sniffing) directed towards the CS levers (in the absence of lever contact) over the course of training. Regardless of lesion, rats made very few approach behaviors without contacting either the CS+ or CS- throughout the entire course of training. ANOVA confirmed a main effect of session ($F_{3,93} = 2.96$, $p = 0.036$), but no effect of cue ($F_{1,31} = 0.65$, $p = 0.43$), lesion ($F_{2,31} = 0.98$, $p = 0.39$), or lesion $\times$ cue interaction ($F_{2,31} = 0.42$, $p = 0.66$).

3.2.3.2 Consummatory behaviors: Figure 4b presents the number of consummatory behaviors (biting/nibbling, grasping) made towards the CS levers over the course of training. Regardless of lesion, rats made more consummatory behaviors towards the CS+ than the CS-. ANOVA confirmed a main effect of cue ($F_{1,31} = 54.5$, $p < 0.01$), but no effect of lesion ($F_{2,31} = 0.23$, $p = 0.80$) or lesion $\times$ cue interaction ($F_{2,31} = 0.23$, $p = 0.80$). Consummatory response trends over sessions showed differences across lesion groups, with ACbS-lesioned rats showing slightly elevated consummatory behaviors to CS+ early in training compared to ACbC- and sham-lesioned rats. ANOVA showed a significant effect of session ($F_{3,93} = 6.99$, $p < 0.01$) and a lesion $\times$ cue $\times$ session interaction ($F_{6,93} = 2.38$, $p = 0.035$). A planned comparison showed that the difference in consummatory behaviors between the CS+ and CS- was greater in ACbS-lesioned rats than ACbC- and sham-lesioned rats on day 4 but not on days 8–12 ($F_{1,31} = 6.61$, $p = 0.015$).

3.2.3.3 Non-Consummatory contact behaviors: Figure 4c presents the number of non-consummatory contact behaviors made towards the CS levers over the course of training. Although few non-consummatory contacts were made towards the CS levers, rats made more non-consummatory contacts towards the CS+ than the CS-. The number of non-consummatory contacts was unaffected by either lesion. ANOVA confirmed a main effect of cue ($F_{1,31} = 12.4$, $p < 0.01$), but no effect of session ($F_{3,93} = 1.30$, $p = 0.28$), lesion ($F_{2,31} = 0.79$, $p = 0.46$), or lesion $\times$ cue interaction ($F_{2,31} = 1.39$, $p = 0.26$).

3.2.3.4 All contacts: Figure 4d presents the total number of contacts (consummatory and non-consummatory) made towards the CS+ and CS-. Given that rats made very few non-consummatory contacts, the results of combining all contacts appear similar to plotting

consummatory behaviors alone. ANOVA confirmed a main effect of cue ($F_{1,31} = 85.82$, $p < 0.01$) and session ($F_{3,93} = 4.42$, $p < 0.01$), but no effect of lesion ($F_{2,31} = 0.16$, $p = 0.85$) or lesion $\times$ cue interaction ($F_{2,31} = 0.64$, $p = 0.54$). As with consummatory behaviors alone, there was a significant lesion $\times$ cue $\times$ session interaction ($F_{6,93} = 2.56$, $p = 0.024$). A planned comparison showed that the difference in all contacts between the CS+ and CS- was greater in ACbSlesioned rats than ACbC- and sham-lesioned rats on day 4 but not on days 8–12 ($F_{1,31} = 5.41$, $p = 0.027$).

4. Discussion

We showed that bilateral lesions of either ACbC or ACbS alone did not disrupt the acquisition of sign-tracking, as indexed by conventional measures of the rate or probability of lever pressing. In addition, neither ACbC or ACbS lesions disrupted the number of consummatory behaviors made towards the CS+.

Our failure to find significant effects of separate lesions of ACbC or ACbS separately on the acquisition of autoshaped lever pressing contrasts with our previous observation that lesions that encompassed both ACbS and ACbC substantially altered the rate at which such responding was acquired [7]. One account for this difference is that both ACb subregions are involved in incentive learning processes crucial to the establishment of autoshaped lever pressing. In support of this possibility, Flagel et al. (2011) [12] showed elevated c-fos expression in both ACbC and ACbS in sign-tracking rats relative to expression in rats that received unpaired presentations of a lever CS and food, or in rats that received lever-food pairings, but which were identified as goal-trackers. Given that VTA projects to both ACbC and ACbS [14] and that ACbC and ACbS share direct and reciprocal connections with each other [33], perhaps ACbC and ACbS together are involved in general incentive salience attribution critical to the emergence of autoshaped lever pressing.

A less interesting account for the difference in outcomes of the present study and our previous whole-accumbens study is that one of the subregions is critical for the acquisition of autoshaped lever-pressing, but that region was damaged more in our previous study [7] than in the present one. Notably, Saunders and Robinson (2012) [27] found that injections of the DA receptor antagonist flupenthixol into ACbC disrupted the acquisition of sign-tracking. Thus, we cannot rule out the possibility that the deficit in sign-tracking produced by whole ACb lesions in Chang et al.'s (2012b) [7] study was due to greater damage to ACbC than in the ACbC-lesioned rats of the current study. In fact, there was significantly more damage to ACbC ($92.2 \pm 3.2\%$) in our previous whole ACb lesion study [7] than in the present one ($80.0 \pm 3.1\%$; $F_{1,22} = 7.39$, $p = 0.01$). However, we found no correlation between the amount of ACbC damage and sign-tracking in rats with whole ACb lesions in terms of rate ($r = -.05$, $p = 0.89$) or probability ($r = 0.01$, $p = 0.97$) over the first half of training sessions in Chang et al.'s (2012b) [7] study when lesion effects were observed. ACbC-lesioned rats in the present study tended to be better sign-trackers with more damage, but correlations between ACbC damage and sign-tracking over the first half of training were also not significant in terms of rate ($r = 0.22$, $p = 0.46$) or probability ($r = 0.30$, $p = 0.31$). Thus, it is highly unlikely that the lack of a deficit in sign-tracking observed in ACbC-lesioned rats was due to less damage to ACbC alone as compared to rats with whole ACb lesions.

Although we initially hypothesized that consummatory behaviors made towards the CS+ may reflect the transfer of hedonic properties of the US to the CS (e.g. conditioned "liking"; [1]), the lack of an effect of ACbS lesions on this behavioral measure does not support our hypothesis. However, it is important to note that Pecina and Berridge (2005) [22] observed enhancements in the hedonic impact of reward when DAMGO was microinjected into

anterior ACbS, while hedonic impact was decreased when DAMGO was microinjected into posterior ACbS. Thus, it may not be surprising that a lesion encompassing the entire ACbS may not disrupt the number of consummatory behaviors towards the CS+. Notably, differences in the hedonic impact of sucrose were observed by stimulating various sites of ACbS rather than lesioning various sites of ACbS; in contrast to DAMGO microinjections, 6-hydroxydopamine lesions of whole ACb have been shown to have no effect on the hedonic impact of sucrose [1,2]. While lesions of ACbS would presumably have no effect on “liking” reactions to sucrose, the effects of ACbS lesions on conditioned “liking” reactions remains uncertain. Alternatively, it is also worth noting that the emergence of consummatory behaviors is also considered a general feature of incentive salience [1,2]. Therefore, the transfer of hedonic properties may not even be necessary for the development of sign-tracking.

Combined with the findings of previous studies, the results of this experiment suggest that the nature of the CS can determine not only the form of the CR but also the neural circuitry engaged in conditioning. Discrete visual cues (e.g., an illuminated rectangle on a monitor) paired with food delivery come to elicit approach to both the visual CS and the food source in rats. Parkinson et al. (2000a,b) [19,20] found that bilateral lesions of ACbC or amygdala central nucleus (CeA) disrupted acquisition of sign-tracking to such a visual CS, whereas lesions of ACbS and basolateral amygdala (BLA) had no effect. In contrast, we showed that lesions of whole ACb and BLA produced deficits in initial and terminal levels of sign-tracking with lever CSs, respectively [7] and that CeA lesions had no effect [6]. Our lesion data with autoshaped lever pressing are consistent with those of Flagel et al. (2011) [12], who found elevated c-fos expression in ACbC, ACbS, and (marginally) BLA, but not in CeA, after presentation of a lever CS+ in sign-trackers, compared to expression induced by a lever CS that had been unpaired with food, or to a food-paired lever in goal-trackers. Thus, the neural circuitry involved in assigning incentive value to a CS that can be approached, manipulated, and consumed may differ substantially from that engaged by CSs that can only be approached. We believe that the most important determinant is the nature of the CS, especially whether it can (or cannot) be manipulated and support consummatory responses. However, it is important to note that other variables such as the type of reinforcer used (liquid sucrose vs. sucrose pellets), rat strain (Long Evans vs. Lister hooded), box dimensions, or some other variable could also have contributed to the differences between our results and Parkinson et al.’s.

Autoshaping has been frequently used as a model for addiction [11,13,31,32], in part because it is highly sensitive to relapse. Given that only sign-trackers display both neurobiological (phasic DA release in ACbC to CS presentations) and behavioral (impulsivity) characteristics related to drug relapse, autoshaping seems to be a reasonable model in exploring why some people are more susceptible to relapse than others [31]. If autoshaping is considered a model of addiction, observations of different patterns of lesion effects with different types of autoshaping stimuli imply that the neural basis of incentive salience attribution may be different for a purely visual stimulus such as an advertisement for fast food or beer than for a stimulus that is more proximal to the actual consumption of these items, such as a French fries container or a beer bottle. Therefore, different treatments targeting the neural structures involved in assigning incentive value to these various stimuli should be considered in order to maximize the probability of preventing relapse.

Acknowledgments

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Abbreviations

ACbC	nucleus accumbens core
ACbS	nucleus accumbens shell
CS	conditioned stimulus
CR	conditioned response
US	unconditioned stimulus
DA	dopamine
VTA	ventral tegmental area
CeA	amygdala central nucleus
BLA	basolateral amygdala

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Highlights

- Separate lesions of nucleus accumbens core or shell had no effect on signtracking.
- Results contrast with the effects of whole accumbens lesions on sign-tracking.
- Neural circuitry of sign-tracking depends upon conditioned stimulus modality.

Fig. 1. Histological results (a) Schematic representation of bilateral nucleus accumbens core (ACbC) lesions showing the minimum (grey) and maximum (white) amount of neuronal damage. Coronal sections are 1.70 to 1.00 mm relative to bregma. (b) Photomicrograph of a section with a representative ACbC lesion. (c) Photomicrograph of a section with a representative ACbC sham lesion. Dotted areas represent the lesioned region in (b) and the target region in (c). Diagrams from Paxinos and Watson (1998); used by permission.

Fig. 2.

Histological results (a) Schematic representation of bilateral nucleus accumbens shell (ACbS) lesions showing the minimum (grey) and maximum (white) amount of neuronal damage. Coronal sections are 1.70 to 1.00 mm relative to bregma. (b) Photomicrograph of a section with a representative ACbS lesion. (c) Photomicrograph of a section with a representative ACbS sham lesion. Dotted areas represent the lesioned region in (b) and the target region in (c). Diagrams from Paxinos and Watson (1998); used by permission.

Fig. 3. Effects of nucleus accumbens core (ACbC) and shell (ACbS) lesions on sign-tracking in terms of (a) lever presses/min, (b) percentage of trials with a lever press, and (c) percentage of time spent in the food cup. Error bars represent $\pm$ SEM.

Fig. 4. Effects of nucleus accumbens core (CbC) and shell (ACbS) lesions on sign-tracking in terms of (a) approach behaviors (orienting and sniffing without lever contact), (b) consummatory lever contacts (biting/nibbling and grasping), (c) non-consummatory contacts, and (d) total number of lever contacts. The maximum number of behaviors that could be scored for each rat in each session was 25. Error bars represent $\pm$ SEM.

Table 1

Temporal distribution of lever press responding

Measure	ACbC	ACbS	Sham
Rate CS+ 1 st 5 s	13.6 ± 2.3	17.8 ± 5.8	11.3 ± 2.7
Rate CS+ 2 nd 5 s	18.5 ± 3.1	22.6 ± 6.5	15.9 ± 3.2
Rate CS− 1 st 5 s	2.9 ± 0.7	2.3 ± 0.7	1.6 ± 0.5
Rate CS− 2 nd 5 s	3.8 ± 1.0	3.7 ± 1.2	2.9 ± 0.8
% trials CS+ 1 st 5 s	40.8 ± 6.1	45.8 ± 9.8	41.8 ± 7.4
% trials CS+ 2 nd 5 s	51.0 ± 6.9	56.4 ± 10.3	50.9 ± 7.5
% trials CS− 1 st 5 s	14 ± 3.2	11.4 ± 3.9	7.3 ± 2.0
% trials CS− 2 nd 5 s	16.2 ± 3.7	14.8 ± 4.4	12.6 ± 2.9

Note: Rate = lever presses/min over all trials; % trials = percentage of trials on which at least one lever press response occurred; ACbC = nucleus accumbens core; ACbS = nucleus accumbens shell. Entries are mean ± SEM, averaged over all acquisition sessions.