Disconnection of the Anterior Cingulate Cortex and Nucleus Accumbens Core Impairs Pavlovian Approach Behavior: Further Evidence for Limbic Cortical–Ventral Striatopallidal Systems

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The nucleus accumbens (NAcc) has been implicated in a variety of forms of reward-related learning, reflecting its anatomical connections with limbic cortical structures. After confirming that excitotoxic lesions of the anterior cingulate cortex (Ant Cing) impaired the acquisition of appetitive Pavlovian conditioning in an autoshaping procedure, the effects of excitotoxic lesions to the NAcc core or shell on autoshaping were also assessed. Only selective core lesions impaired Pavlovian approach. A subsequent experiment studied the effects of a disconnection of the Ant Cing and NAcc core, using an asymmetric lesion procedure, to determine whether these structures interact sequentially as part of a limbic corticostriatal system. Such lesioned rats were also significantly impaired relative to controls at autoshaping. These results demonstrate that the NAcc core and Ant Cing are “nodes” of a corticostriatal circuit involved in stimulus-reward learning.

Lesions of the central nucleus of the amygdala and of the anterior cingulate cortex (Ant Cing) impair discriminated Pavlovian approach behavior, as measured in an autoshaping procedure (Bussey, Everitt, & Robbins, 1997; Parkinson, Robbins, & Everitt, 1996; Parkinson, Robbins and Everitt, in press). Such deficits may be interpreted in terms of a more general involvement of these structures in stimulus–reward learning (Bussey, Everitt, & Robbins, 1997; Bussey, Muir, Everitt, & Robbins, 1996; Everitt & Robbins, 1992). These critical components of the limbic forebrain have converging projections onto the ventral striatum and may process informational and affective aspects of emotion and motivation that are in turn integrated at the level of the nucleus accumbens (NAcc), where relevant behavioral responses are gated. Indeed, Pennartz, da Silva, and Groenewegen (1994) have suggested that “distinct neuronal ensembles” operate within and through the NAcc and are functionally related to the nature of their limbic–cortical afferents.

This neuronal ensemble hypothesis gains further support from observations of NAcc neurons during reward-related learning and behavior. For example, conditioned stimuli (CSs) that have, through association, become predictive of primary rewards (unconditioned stimuli [USs]) produce significant changes in extracellular dopamine (DA) concentrations in the NAcc, as measured by voltammetry (DiCiano, Blaha, & Phillips, 1998a, 1998b). Similarly, DA neurons that project to the NAcc (and NAcc neurons themselves) also produce increases in single-unit neuronal firing in response to a reward-related CS, as measured by electrophysiology (Schultz, Apicella, Scarnati, & Ljungberg, 1992; Schultz, Dayan, & Montague, 1997). Further, lesions or dopaminergic manipulations of the NAcc have significant effects on Pavlovian conditioning (Balleine & Killcross, 1994; Parkinson, Otmstane, Burns, Robbins, & Everitt, 1999) and on tests of conditioned place preference (Carr & White, 1983; Everitt, Morris, O’Brien, & Robbins, 1991; White, Packard, & Hiroi, 1991). Although the acquisition and performance of conditioned place preferences (CPP) are probably dependent on other learning mechanisms rather than simply Pavlovian conditioning (e.g., involving an instrumental locomotor response), Everitt et al. (1991) found that excitotoxic lesions of the NAcc impaired the acquisition of CPP when animals were given access to sucrose in a distinct environment. Further, lesions of the basolateral amygdala (BLA) also impaired such a place preference and, more importantly, a disconnection lesion of the BLA and NAcc, produced by making unilateral lesions of the two structures, contralateral to each other also abolished a CPP to sucrose presentation. Such evidence strongly implies a serial and functional connection between the BLA and NAcc in reward-related processes.

In fact, there is evidence of a role for the NAcc in spatial learning (Annett, McGregor, & Robbins, 1989; Maldonado-Irizarry & Kelley, 1995; Ploeger, Spruijt, & Cools, 1994; Riedel, Harrington, Hall, & MacPhail, 1997), Pavlovian conditioning (Balleine & Killcross, 1994; Kelley, Smith-
These diverse functions attributed to the NAcc may reflect several factors. First, different behavioral procedures use different measures of learning. Thus, it is difficult to compare or be precise about the nature of the learning or the response being assessed. Second, different manipulations of the NAcc often yield contradictory findings. Important assumptions apply to different neural manipulations that in some cases preclude simple comparisons between techniques. Finally, there are potentially different contributions to behavior from subregions within the NAcc, most notably the core and shell. Notwithstanding, the NAcc may be involved in more fundamental processes that influence, to some extent, all of the above forms of learning, such as Pavlovian associative mechanisms (Rescorla & Solomon, 1967).

An important modification of earlier conceptions of striatal connectivity was made by questioning the view that the striatum operated as a funnel to integrate diverse cortical projections onto a focused set of output targets (Alexander & Crutcher, 1990; Alexander, Delong, & Strick, 1986) and arguing instead that there are segregated circuits through the striatum that operate in parallel. These circuits originate in specific areas of the cortex and project back to a restricted set of cortical areas via the striatum, pallidum, and thalamus (but see Joel & Weiner, 1994). In this anatomical system, each structure within a “loop” would perform a distinct function, and structures at the same anatomical level of separate loops (e.g., the putamen in the “motor” loop and the NAcc in the “anterior cingulate” loop) would perform qualitatively similar functions, though for potentially different purposes.

The ventral striatum, and in particular the NAcc, conforms to this corticostriatal circuitry in that there are topographic differences between afferent and efferent projections between the ventral and dorsal striatum and, more importantly, between the core and shell of the NAcc (Zahn & Brog, 1992). Further, the examination of a circuit of neural structures is likely to provide a more precise understanding of the functional interactions between areas within the brain. Thus, not only by studying the effects of selective bilateral lesions to several areas on a well-defined behavioral task, but also by using disconnection techniques (between structures), it is possible to provide evidence for functionally segregated circuits (Everitt et al., 1991; Floresco, Seamans, & Phillips, 1997; Gaffan & Harrison, 1987).

The present experiments therefore examined the effects of anatomically connected structures on an autoshaping task that measured appetitive Pavlovian conditioning, unconfounded by instrumental learning mechanisms, in an attempt to define neural circuits subserving appetitive Pavlovian learning. The apparatus, procedure and theory have been discussed in more detail elsewhere (Bussey, Everitt, & Robbins, 1997). Briefly, autoshaping was first described when pigeons came to approach and peck a key light, which had been presented in temporal contiguity with a food reward, before approaching and eating the food (P. L. Brown & Jenkins, 1968). Regardless of whether there was a necessary contingency between the subjects’ behavior and presentation of reward, the pigeons reliably approached and pecked the key light stimulus. This approach behavior has subsequently been interpreted as a Pavlovian sign-tracking response (Hearst & Jenkins, 1974) because it lacks the flexibility and goal-directed nature of instrumental actions (Williams & Williams, 1969), is produced by the association of the stimulus and reward, and has been observed in rats, monkeys, and humans, both children and adults (Boakes, 1977; Sidman & Fletcher, 1968; Wilcove & Miller, 1974; Zeiler, 1972).

Previously it has been shown that autoshaping is sensitive to Ant Cing lesions (Bussey, Everitt, & Robbins, 1997). The present study therefore attempted to replicate and extend these findings by also studying the effects of selective excitotoxic lesions to a major limbic efferent target, namely the NAcc, on the autoshaping task in an attempt to define the neural structures that are involved in preparatory Pavlovian mechanisms and may underlie emotional learning.

**EXPERIMENT 1: LESIONS OF THE ANT CING AND THE NACC CORE AND SHELL**

Initially, the effects of lesions to the Ant Cing were studied on the autoshaping task to replicate the previous findings of Bussey, Everitt, and Robbins (1997). Further, the effects of selective lesions of the NAcc core or shell were studied on acquisition of the autoshaping task, as the ventral striatum is a major target of cingulate projections. Any dissociable effects found after NAcc lesions could then be compared with those seen after lesions of limbic–cortical structures that are major sources of afferents to the NAcc, such as the cingulate cortex, amygdala, and hippocampal formation (Bussey, Everitt, & Robbins, 1997; Parkinson et al., 1996; Parkinson et al., in press).

**Method**

**Subjects**

Subjects were 60 male Lister hooded rats (Olac, Bicester, UK) weighing between 310 and 410 g at the time of surgery. They were housed in pairs in a temperature-controlled room (minimum 22 °C) under a 12-hr reversed light–dark cycle. All testing took place during the dark phase. The rats were food deprived to 85% of their free-feeding body weight for the duration of the experiment. Subject groups and numbers were as follows (final group numbers in parentheses): Ant Cing-lesioned n = 10 (10); Ant Cing sham n = 7 (7); NAcc core-lesioned n = 12 (9); NAcc core sham n = 11 (11); NAcc shell-lesioned n = 12 (6); and NAcc shell sham n = 8 (8). All rats used in this study were treated in accordance with the UK 1986 Animals (Scientific Procedures) Act (Project License PPL 80/00668).

**Surgical Procedure**

Surgery was performed under Avertin anesthesia. Infusions were made with a 1-μl syringe (26 gauge, code 1BR-OC-7/0.47, SGE, Baton Rouge, LA) with a custom-made glass micropipette attached...
to the end. The pipettes (Intracel, Royston, UK) initially measured 1.2 mm in external diameter, 0.69 mm in internal diameter, and 10 cm in length, and were pulled with a Stoelting App-1 All Purpose Puller (Model 52500, Stoelting, IL), giving a final tip diameter of 50–100 μm (outer diameter) and a length of 12 mm. Micropipettes were attached to the syringe with Araldit epoxy resin (Ciba, UK) to ensure an airtight seal. Infusion coordinates (taken from Paxinos & Watson, 1998) and excitotoxin volumes are shown in Table 1.

All subjects were given injections of glucose–saline (5–10 ml ip) after surgery to aid recovery. Behavioral testing began 7–10 days after surgery.

**Behavioral Procedure**

The autoshaping paradigm examines the acquisition of Pavlovian approach behavior (P. L. Brown & Jenkins, 1968; Williams & Williams, 1969) by presenting a visual stimulus followed by the delivery of food. Over training, animals develop the conditioned response (CR) of approaching the food-predicting CS before returning to the food hopper to retrieve the primary reward. This preparatory approach behavior is deemed to be under the control of Pavlovian mechanisms because it lacks the bidirectionality of conditioning of which operant responses are capable (Williams & Williams) and has been described as a form of sign tracking (Tomie, Brooks, & Zito, 1989). The procedure and apparatus have been described in detail elsewhere (Bussey, Everitt, & Robbins, 1997) but will be outlined below.

**Apparatus**

The apparatus consisted of a testing chamber attached to a video display unit (VDU) within a sound-attenuating box (fitted with an extractor fan) and is an adaptation of the apparatus described by Bussey, Muir, and Robbins (1994). The inner chamber (48 cm long × 30 cm high × 30 cm wide) consisted of a metal frame and Perspex walls, with an aluminum floor. A 3-W houselight was attached to the center of the ceiling. A food magazine hopper was attached to a pellet dispenser (Campden Instruments, Loughborough, UK) outside the sound-attenuating box and allowed the controlled delivery of sucrose pellets (Noyes, Lancaster, NH). Pressure-sensitive floor pads (14 × 10 cm) were attached to microswitches, enabling the measurement of approaches to stimuli (10 × 28-cm white triangles presented on the far left or far right sides of the VDU). The floor pad at the rear of the chamber triggered successive trials by detecting when a subject was equidistant from both stimulus locations. The apparatus was controlled and monitored by a BBC Master series microcomputer that used programs written in BASIC (Bussey, Everitt, & Robbins, 1997) and allowed the presentation of computer graphic stimuli on the VDU monitor while measuring rats' motor responses through the use of a touch-sensitive screen and the pressure-sensitive floor pads.

**Procedure**

**Pretraining.** On the 1st day, rats were given one 15-min habituation session in the chamber. The houselight was switched on, and rats were allowed access to food pellets (dustless sucrose pellets; 45 mg sucrose, Noyes), which were delivered into the magazine under a variable time (VT) 40-s schedule. Subjects were observed during this session to ensure that they were successfully retrieving and consuming pellets.

**Acquisition.** On the 2nd day of testing, rats were trained to associate stimuli with the sucrose pellet reward. Stimuli were presented on the VDU for 10 s, followed by the delivery of a sucrose pellet into the magazine. One stimulus was designated the CS+ and was always followed immediately by reward. Another was designated the CS− and was never followed by reward. The two stimuli differed only in the side of the screen on which they were presented. Half the rats received a right-sided CS+ and the other half received a left-sided CS+. Stimuli were presented under a VT 40-s schedule, and training consisted of a total of 100 trials (2 consecutive days of 50 trials per day), each consisting of one CS+ stimulus presentation and one CS− stimulus presentation. An approach was recorded if a rat stepped onto the floor panel directly in front of a stimulus; no other approaches were recorded for that stimulus presentation.

Stimuli were presented only when the subject was centrally located at the back of the chamber, eliminating chance approaches and allowing the reliable calculation of approach latencies. There was a minimum time of 10 s between CS+ and CS− presentation to reduce interference across trials. There was a maximum of two consecutive presentations of either the CS+ or CS−. Several performance measures were taken, including the number of approaches to both the CS+ and CS− per each block of 10 trials and the mean latency to approach both the CS+ and CS− over the course of acquisition training.

**Omission training.** On the 4th testing day, subjects were given an additional block of 50 trials with the same parameters used in the acquisition sessions, except that an approach to the CS+ now

**Table 1**

**Infusion Coordinates and Excitotoxin Volumes for Lesions of the Anterior Cingulate Cortex (Ant Cing) and Nucleus Accumbens (NAcc)**

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Excitotoxin</th>
<th>Infusion volume</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant Cing</td>
<td>0.09 M quinolinic acid</td>
<td>0.5 μl</td>
<td>AP +0.8 ±0.5 −2, −3*</td>
</tr>
<tr>
<td>Ant Cing</td>
<td>0.09 M quinolinic acid</td>
<td>0.5 μl</td>
<td>L +0.2 ±0.5 −2, −2.5</td>
</tr>
<tr>
<td>Ant Cing</td>
<td>0.09 M quinolinic acid</td>
<td>0.5 μl</td>
<td>−0.4 ±1.5 ±0.5 −1.5, −2</td>
</tr>
<tr>
<td>NAcc core</td>
<td>0.09 M quinolinic acid</td>
<td>0.5 μl</td>
<td>+1.2 ±1.8 −7.1b</td>
</tr>
<tr>
<td>NAcc shell</td>
<td>0.06 M ibotenic acid</td>
<td>0.2 μl</td>
<td>+1.6 ±1.1 −7.9b</td>
</tr>
<tr>
<td>NAcc shell</td>
<td>0.06 M ibotenic acid</td>
<td>0.1 μl</td>
<td>+1.1 ±1.1 −6.9b</td>
</tr>
<tr>
<td>NAcc shell</td>
<td>0.06 M ibotenic acid</td>
<td>0.1 μl</td>
<td>+1.6 ±1.1 −6.4b</td>
</tr>
</tbody>
</table>

*Note. Coordinates are in millimeters and are from Paxinos and Watson (1998). The incisor bar was set at 3 mm below the interaural line.

*Indicates distance from dura. *Indicates distance from skull surface.
prevented the delivery of a sucrose pellet. This type of omission training has previously been used as a corroborative measure of whether animals approach the stimuli by using Pavlovian conditioning mechanisms (Williams & Williams, 1969).

Statistical Analysis

Behavioral results were subjected to a two- or three-factor repeated-measures analysis of variance (ANOVA) with one between-subjects factor (lesion) and one or two within-subject factors (CS, block) depending on the analysis, using the statistical package SPSS for Windows (Version 6.1.3, 1998) including the post hoc Newman–Keuls test. Where necessary, a post hoc analysis of simple main effects and simple interactions was undertaken with CLR ANOVA for Macintosh (Version 2.0, 1995). Statistical analyses of acquisition data were primarily used to determine the development of discriminated autoshaping behavior and to observe experimental group differences. However, analysis of the omission procedure was undertaken primarily to assess continued discriminated approach within groups; therefore Student’s t test was used to analyze omission approach difference scores (number of approaches to the CS+ minus number of approaches to the CS−, per block). The homogeneity of variance across groups in repeated-measures ANOVAs was assessed by the Mauchly sphericity test. When data sets significantly violated Mauchly test requirements, the Greenhouse–Geisser epsilon correction parameter for degrees of freedom (Geisser & Greenhouse, 1959; Winer, 1971) was used to calculate a more conservative p value for each F ratio. Further, for presentation of descriptive statistics, the standard error of the differences of the means (SED) was used, as it provides a better estimate of the population variance for between-group comparisons. The SED is calculated by using the formula provided in Cochran and Cox (1957).

Histological Procedure and Lesion Analysis

Rats were overdosed with sodium pentobarbitone (1.5 ml ip, Euthatal, Rhône Mérieux, UK) and perfused via the ascending aorta with cold phosphate-buffered saline (PBS; 0.01 M, pH 7.4) over 4 min, followed by 4% (wt/vol) paraformaldehyde with 0.2% (wt/vol) saturated picric acid in 0.2 M phosphate buffer for a further 4 min. The brains were then removed and postfixed for 2 hr before being transferred into 20% (wt/vol) sucrose in 0.01 M PBS overnight. Coronal sections (60 μm) were cut on a freezing microtome throughout the full extent of the lesioned area. Every third section was mounted on gelatin-coated glass slides, then stained for Nissl substance with cresyl violet. Mounted sections were immersed for 3 min in descending alcohol concentrations (absolute, 95%, 70%), then rinsed in distilled water before being stained for 2–5 min in cresyl violet (Cresyl Fast Violet, Raymond Lamb, Eastbourne, UK). Once the required intensity of staining was reached, sections were rinsed in distilled water and 70% alcohol, then differentiated in 95% alcohol before a final rinse in absolute alcohol and immersion in Histoclear for 3 min. After this treatment, slides were coverslipped and dried.

Sections stained with cresyl violet were then examined to assess the extent and nature of excitotoxic-induced neuronal damage, including gliosis associated with intracerebral infusions of quinolinic or ibotenic acids. Further, this analysis was used to prepare schematic representations and photomicrographs of the lesions used in this study.

Results

Histological Assessment

Lesions of the Ant Cing were similar (though slightly more anterior) to those obtained previously, using the same infusion parameters (Bussey et al., 1996; Bussey, Everitt, & Robbins, 1997; Bussey, Muir, Everitt, & Robbins, 1997). Neuronal loss and gliosis extended from approximately 3 mm anterior to 1 mm posterior to bregma and encompassed both areas Cg1 and Cg2. Figure 1a shows a schematic representation of the lesion, and Figure 1b and 1c show photomicrographs of rostral sections from lesioned rats and sham-lesioned controls. In some cases, there was bilateral damage to the rostromedial prelenticular cortex (these subjects were included in the behavioral analysis) and in others, damage to the overlying shoulder region. There was no visible evidence of damage to the cingulum or neuronal loss extending below the corpus callosum into the septum or hippocampal formation. Ultimately, all rats in the Ant Cing lesion group were retained for behavioral analysis.

Lesions of the NAcc core selectively destroyed neurons within the core subregion (Groenewegen, Wright, & Beijer, 1996; Zahm & Brog, 1992). In all cases, the rostral pole was spared, as was the mediodorsal NAcc shell. In some cases, gliosis was found in the ventrolateral NAcc shell; these data were discarded for rats with bilateral damage in this area. The data from 3 subjects were removed from further analysis due to damage extending beyond the proposed target area (1 rat with bilateral ventral shell damage and 2 with bilateral damage in the dorsal striatum). Schematic representations and a representative photomicrograph of NAcc core lesions are shown in Figure 2 (a–e).

Lesions of the NAcc shell were targeted at the mediodorsal aspect of the shell subregion for both anatomical and theoretical reasons. Data from 5 rats were discarded due to bilateral damage to the septum or globus pallidus or damage to the core subregion. Thus, only rats with very selective lesions of the mediodorsal shell (encompassing the septal pole of the shell) were included. One NAcc shell-lesioned subject died after surgery. Schematic representations based on the atlas of Paxinos and Watson (1998) and a representative photomicrograph of NAcc shell lesions can be seen in Figure 2 (f–h).

Behavioral Results

All subjects in the experiment reliably collected and consumed sucrose pellets from the magazine. Final group numbers used for statistical analysis were: Ant Cing lesion, n = 10; Ant Cing sham, n = 7; NAcc core lesion, n = 9; NAcc core sham, n = 11; NAcc shell lesion, n = 6; NAcc shell sham, n = 8.

Effects of Ant Cing Lesions

Discriminated Approach

Approach scores for acquisition (approaches to the CS+) and approaches to the CS− across 10 blocks of 10 stimulus
Figure 1. a: Schematic representation of lesions to the anterior cingulate cortex. Shaded areas represent the smallest (black) and largest (gray) extent of neuronal damage in a single rat. Coronal sections are from +2.7 to +0.48 mm relative to bregma (Paxinos & Watson, 1998). b–c: Photomicrographs showing cresyl violet-stained coronal sections through the anterior cingulate cortex (approximately +1.7 mm from bregma). b: Sham lesion, c: Anterior cingulate cortex lesion. The lesioned area is indicated by the dotted lines. Cg1 = cingulate cortex area 1; Cg2 = cingulate cortex area 2; M2 = secondary motor cortex.
Figure 2. Schematic representations and photomicrographs of excitotoxic lesions to the nucleus accumbens. Shaded areas represent the smallest (black) and largest (gray) extent of neuronal damage in a single rat. Lesions are represented by dotted lines in photomicrographs. Coronal sections are from +2.7 to +0.48 mm relative to bregma (Paxinos & Watson, 1998). a: Nucleus accumbens shell (Acbsh) lesion; b–c: Photomicrographs showing cresyl violet-stained coronal sections through the nucleus accumbens (approximately +1.2 mm from bregma); b: Sham lesion; c: Acbsh lesion; d: High magnification of the sham lesion section shown in b; e: High magnification of the Acbsh lesion section shown in c; f: Schematic representation of excitotoxic lesions to the nucleus accumbens core (Acbc); g–h: Photomicrographs showing cresyl violet-stained coronal sections through the nucleus accumbens (approximately +1.2 mm from bregma); g: Sham lesion; h: Acbc lesion. ac = anterior commissure; icjm = major islands of Calleja; LV = lateral ventricle.

Presentations were analyzed for each sham and lesion group and are shown in Figure 3a. Analysis of the number of approaches during the acquisition of autoshaping revealed a main effect of lesion, $F(1,15) = 4.95, p < .05$, characterized by an increased number of approaches by the Ant Cing-lesioned group relative to sham controls. There was also a main effect of CS, $F(1,15) = 20.16, p < .01$, due to a greater number of approaches toward the CS+ relative to the CS−. More importantly, there was a Lesion $\times$ CS interaction, $F(1,15) = 8.53, p < .05$, and a CS $\times$ Block interaction, $F(9,135) = 7.12, p < .01$. Post hoc analysis revealed that the former interaction was due to discriminated approach demonstrated by the sham control group ($p < .05$) but not by the lesioned group. Further, there was no significant difference between the Ant Cing sham CS+ approaches and the Ant Cing CS+ approaches, but there was a significant difference between the CS− approaches of the two groups, with the Ant Cing group showing a higher
Approach Latencies

Whereas the number of absolute approaches gives a good indication of learning the association between the CS+ and food reward, measuring the latency to make an approach can offer not only a corroborative indication of the extent of learning, but also an indication of lesion effects on response times and, therefore, an index of locomotor capacity. Thus, the mean latency to approach a stimulus was calculated for all the instances that a rat actually approached (a maximum of 100 approaches per stimulus) during acquisition. There was a significant effect of lesion, $F(1, 15) = 5.01, p < .01$, on approach latencies (see Figure 4). Specifically, lesioned rats were significantly faster to approach than were sham controls ($p < .05$). There was also a significant main effect of CS, $F(1, 15) = 15.11, p < .01$, characterized as faster approaches to the CS+ relative to the CS−. There was no Lesion × CS interaction, $F(1, 15) = 0.88, p = .36$.

Omission Session

Analyzing the omission data not only reveals any between-group differences, but also can offer insight as to whether learning during the acquisition of autoshaping is due predominantly to Pavlovian mechanisms or other forms of learning (Bussey, Everitt, & Robbins, 1997; Williams & Williams, 1969). Thus, the data were subjected to both a repeated-measures ANOVA as well as individual Student’s $t$ tests on the difference scores of the lesion and sham groups separately. The difference score measure indicated whether there was discriminated approach behavior (toward the CS+) over the entire omission session. Analysis of omission data revealed a main effect of CS, $F(1, 15) = 24.90, p < .001$.
and a main effect of block, $F(4, 60) = 5.10, p < .01$, produced by an overall discriminated approach toward the CS+ during omission, but with a general reduction in approaches over blocks. There were no significant effects of lesion, $F(1, 15) = 2.17, p = .16$, Lesion × CS, $F(1, 15) = 3.62, p = .07$; Lesion × Block, $F(4, 60) = 0.12, p = .91$; or Lesion × CS × Block, $F(4, 60) = 0.27, p = .78$.

Difference scores were obtained, and individual Student's $t$ tests were carried out on each block of approach data to determine exactly when a significant level of discriminated approach behavior was attained by each experimental group. The sham group showed a significant level of discriminated approach throughout the omission session ($p < .05$), whereas the Ant Cing group showed a significant level of discriminated approach on Blocks 2 and 3 only ($p < .05$).

In summary, whereas sham controls developed a significant level of discriminated approach toward the CS+ relative to the CS−, rats with Ant Cing lesions were impaired in this behavior. The level of approach to the CS+ demonstrated by lesioned rats was no different from that of sham, but the level of CS− approach was significantly higher than that of sham controls. However, toward the end of training, Ant Cing rats did begin to show a greater level of approach toward the CS+ versus the CS−, but this did not reach significance.

Both groups approached the CS+ with lower latencies than those for the CS−. Further, the lesioned group responded more quickly in general when approaching either stimuli.

Finally, sham controls showed a preserved discriminated level of approach during the entire omission session. Rats with Ant Cing lesions actually exhibited discriminated approach on two of the five blocks even after showing no significant degree of discrimination during the acquisition of autoshaped responding. Thus, although Ant Cing-lesioned rats were significantly impaired at autoshaping, there was evidence to suggest that they were beginning to learn toward the end of the experiment.

**Effects of NAcc Core Lesions**

**Discriminated Approach**

As shown in Figure 5a, repeated-measures ANOVAs carried out for NAcc core lesioned rats and sham-lesioned controls revealed a significant main effect of lesion, $F(1, 18) = 6.12, p < .05$, and CS, $F(1, 18) = 47.07, p < .001$; a Lesion × CS interaction $F(1, 18) = 15.03, p < .001$; a CS × Block interaction, $F(9, 162) = 14.66, p < .001$; and a Lesion × CS × Block interaction, $F(9, 162) = 5.96, p < .001$. There was no main effect of block $F(5, 87) = 1.43, p = .18$, or a Lesion × Block interaction, $F(5, 87) = 1.66, p = .12$. Newman–Keuls post hoc analysis of simple main effects and interactions revealed that the main effect of lesion was due to significantly lower approach scores in the NAcc core-lesioned group ($p < .05$), and the main effect of CS revealed lower approach scores to the CS− ($p < .05$) relative to the CS+. The Lesion × CS interaction revealed a significant difference between CS+ and CS− in the NAcc core shams ($p < .05$) but not the NAcc core-lesioned group.

There was also a significant difference between the CS+ scores for the NAcc core shams and NAcc core-lesioned groups ($p < .05$); but this was not true for the CS− ($p > .05$). Thus, sham subjects showed discriminated approach toward the CS+, relative to the CS−, but core-lesioned subjects did not. Post hoc analysis of the CS × Block interaction revealed an increase in discriminated approach over blocks between CS+ and CS− from Block 4 to Block 10, as shown in Figure 5a.

**Figure 5.** Acquisition of autoshaping behavior (a) after lesions of the nucleus accumbens core (and sham control). The performance of these rats was also assessed during an omission session (b). Data points represent the mean number of approach responses made toward the conditioned (CS+) and control (CS−) stimuli per block of 10 trials. SED bar represents 1 standard error of the difference of the means.
onward ($p < .05$). Because there was a Lesion $\times$ CS $\times$ Block interaction, separate ANOVAs were calculated for the NAcc core sham and NAcc core-lesioned groups to further analyze their autoshaping behavior. Analysis of the NAcc core sham group alone revealed significant main effects of CS, $F(1, 10) = 56.17$, $p < .001$, characterized by a higher level of approach to the CS+, a main effect of block, $F(9, 90) = 2.19$, $p < .05$, demonstrating an increase in approaches over blocks, and a CS $\times$ Block interaction, $F(9, 90) = 24.14$, $p < .0001$, revealing a significant discriminated approach toward the CS+ from Block 4 onward ($p < .05$) and a significant change in approaches to both the CS+ (increased) and CS− (decreased) over blocks. Analysis of the NAcc core-lesioned group alone revealed no significant effects. However, there was a trend toward a significant main effect of CS ($p = .068$), characterized by an increased approach toward the CS+.

**Approach Latencies**

An ANOVA revealed a significant main effect of lesion, $F(1, 18) = 12.31$, $p < .005$, and a Lesion $\times$ CS interaction, $F(1, 18) = 13.72$, $p < .005$, but no main effect of CS (see Figure 4). NAcc core shams were significantly faster to approach both the CS+ and CS−. Examination of the Lesion $\times$ CS interaction showed that there was a significant difference between approach latencies for the CS+ and CS− in the NAcc core sham group ($p < .005$) but not in the NAcc core-lesioned group (ns) and that the CS+ approach latencies were significantly faster for the NAcc core sham group relative to the NAcc core-lesioned group ($p < .005$) but with no difference in the CS− approach latencies ($p > .05$). Thus, NAcc core shams approached the CS+ more quickly than the CS− and with shorter latencies than either approach in the NAcc core-lesioned group. There was no significant difference between CS+ and CS− scores in the NAcc core-lesioned group ($p > .05$). Although rats with sham lesions demonstrated faster approach latencies toward the predictive CS+ than toward the CS−, core-lesioned rats did not show any differences between their CS+ and CS− approach latencies.

**Omission Session**

Analysis of approach scores revealed a main effect of CS, $F(1, 18) = 35.32$, $p < .001$, and of block, $F(3, 46) = 8.81$, $p < .05$, and a Lesion $\times$ CS interaction, $F(1, 18) = 7.98$, $p < .05$, but no main effect of lesion, $F(1, 18) = 2.92$, $p = .1$, or the following interactions: Lesion $\times$ Block, $F(3, 46) = 0.11$, $p = .98$; CS $\times$ Block, $F(2, 42) = 1.05$, $p = .39$; or Lesion $\times$ CS $\times$ Block, $F(2, 42) = 1.12$, $p = .35$ (see Figure 5b).

Newman–Keuls post hoc analysis revealed that the main effect of CS was due to a higher number of approaches to the CS+ ($p < .01$) relative to the CS−. The main effect of block was due to a general reduction in the number of approaches over blocks, and the Lesion $\times$ CS interaction was due to a significant difference between the NAcc core shams’ CS+ approaches compared with the NAcc core-lesioned CS+ approaches ($p < .05$). Although there was a significant difference between the CS+ and CS− approaches in the NAcc core sham group ($p < .01$), there was no such difference in the behavior of the NAcc core-lesioned group. Student’s $t$ tests of the difference between CS+ and CS− approaches were performed on individual blocks to determine whether NAcc core shams and NAcc core-lesioned rats (separately) continued to show a discriminated approach during the omission training. Difference scores were significantly different from zero for all five blocks ($ps < .05$) for the NAcc core sham group. None of the NAcc core-lesioned groups’ scores were significantly different from zero ($ps > .05$). In summary, NAcc core sham-operated rats continued to show discriminated approach behavior during the omission procedure, although their approach scores did decrease over blocks. NAcc core-lesioned rats, in contrast, did not reach a significant level of discrimination.

**Food Consumption Test After NAcc Core Lesions**

Because of the severe impairment in autoshaping after NAcc core lesions, a food consumption test was carried out to determine whether there was a deficit in primary motivation, rather than in Pavlovian approach (see Figure 6). Core-lesioned rats and sham controls were tested under four conditions: They were first given 50 Noyes food pellets (the amount presented in a typical 2-hr autoshaping session), and the time to consume them was recorded. Second, the two groups were given an amount of Rat Chow equivalent to the weight of 50 Noyes pellets (i.e., 2.3 g), and again the time to consume was measured. The third and fourth conditions involved giving rats’ free access to either pellets or chow for a 30-min period, and the amount consumed was measured in grams.

A one-way ANOVA comparing the time to consume 50 Noyes food pellets by the core-lesioned and sham control groups revealed no significant main effect of lesion, $F(1, 18) = 2.05$, $p = .18$. Analysis of the time to consume 2.3 g of laboratory chow revealed slightly (though not significantly) longer times for NAcc core-lesioned rats relative to sham controls, as revealed by the lack of a main effect of lesion, $F(1, 18) = 3.16$, $p = .1$. Thus, there was no significant difference in the time it took NAcc core-lesioned subjects to consume the amount of food available in an autoshaping session, relative to sham controls.

Analysis of the amount of pellets consumed over a 30-min period revealed no significant effect of lesion, $F(1, 18) = 0.02$, $p = .9$. However, core-lesioned rats ate relatively less laboratory chow over a 30-min period, $F(1, 18) = 43.36$, $p < .001$. Thus, core-lesioned subjects appeared to have a selective impairment in the consumption, overall, of laboratory chow but showed no significant deficit when consuming Noyes food pellets.

**Effects of NAcc Shell Lesions Discriminated Approach**

As shown Figure 7a, there were no significant effects of lesion on autoshaping performance, $F(1, 23) = 1.07$, $p =
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Figure 6. A test of food consumption after nucleus accumbens core lesions (core) and sham controls (sham), in a food-deprived state. a: Mean latency to consume 50 reinforcer pellets. Fifty pellets is the total number of reinforcers given in a standard 2-hr autoshaping session (50 trials). b: Mean latency to consume 2.3 g of laboratory chow. This amount of chow is equivalent in weight to 50 pellets. c: Mean weight of reinforcer pellets consumed in 30 min. d: Mean weight of laboratory chow consumed in 30 min. Asterisk indicates a significant main effect of lesion (p < .05). SED bar represents 1 standard error of the difference of the means.

.31, nor any interactions between Lesion × CS, F(1, 23) = 2.06, p = .17; Lesion × Block, F(9, 207) = 1.04, p = .41; or Lesion × CS × Block, F(9, 207) = 0.87, p = .56. There was a main effect of CS, F(1, 23) = 76.66, p < .0001, manifest by increased approach toward the CS+ relative to the CS− (p < .01). There was also a significant CS × Block interaction, F(9, 207) = 27.89, p < .001, but no main effect of block, F(9, 23) = 1.64, p = .12, showing that, although the overall number of approaches made did not significantly change, the nature of those approaches did. Thus, approaches to the CS+ increased over blocks and became significantly higher than approaches to the CS− (which reduced over blocks) from Block 4 onward (p < .001). In summary, NAcc shell shams and NAcc shell-lesioned groups did not significantly differ in their acquisition of autoshaping; both groups developed a significant discriminated approach to the CS+ relative to the CS−.

Approach Latencies

Analysis of the approach latencies revealed a main effect of CS, F(1, 23) = 19.87, p < .0005, demonstrating that approaches to the CS+ were made with shorter latencies than approaches to the CS− (p < .01; see Figure 4). There were no effects of lesion on approach latency, F(1, 23) = 1.15, p = .29, or Lesion × CS interaction, F(1, 23) = 0.40, p = .53. Thus, both NAcc shell and sham-lesioned rats approached the CS+ with shorter latencies than those for the CS−.

Omission Session

Figure 7b shows that, consistent with the lack of any effects of the shell lesion in the acquisition of autoshaping, there were no significant effects of lesion for the omission
session, $F(1, 23) = 0.32, p = .58$. There also were no significant interactions of Lesion × CS, $F(1, 23) = 0.1, p = .99$; Lesion × Block, $F(4, 92) = 0.28, p = .89$; or Lesion × CS × Block, $F(4, 92) = 0.39, p = .82$. However, there was a main effect of CS, $F(1, 23) = 35.33, p < .0001$, showing the continuing discriminated approach to the CS+ over the omission session. There was also a main effect of block, $F(4, 92) = 6.09, p < .001$, revealed as a reduction in overall approaches over blocks. There was no significant interaction between CS × Block, $F(4, 92) = 1.15, p = .34$. In summary, both the NAcc shell sham and NAcc shell-lesioned groups continued to show a discriminated approach toward the CS+, and there were no significant differences between these groups. This was further emphasised by the individual t tests on the difference scores, which were significantly different from zero for the NAcc shell shams over all blocks ($p < .05$) whereas those for the NAcc shell-lesioned group were significant on all but Block 4 ($p < .01$ for Blocks 1–3, $p < .05$ for Block 5).

**EXPERIMENT 2: DISCONNECTION OF ANT CING AND NACC CORE**

Experiment 1 provided evidence that lesions to the NAcc core, but not the shell, impair the acquisition of discriminated approach behavior. Rats with lesions of the Ant Cing, which provides a major source of afferents to the NAcc core (Brog, Salyapongse, Deutch, & Zahn, 1993) were also significantly impaired at autoshaping (see also Bussey, Everitt, & Robbins, 1997). Ant Cing-lesioned rats showed an increased level of approaches to the CS− (to a comparable level of CS+ approaches), but no significant effect on CS+ approaches relative to sham controls. This effect has been interpreted by Bussey et al. as being due to a disruption in stimulus–reward learning, though whether it was a deficit in acquiring the association (possibly an attentional or mnemonic deficit) or in expressing it (due to possible confounds of impulsive responding by Ant Cing-lesioned rats; Muir, Everitt, & Robbins, 1996) was unclear.

There are important implications for the finding that both Ant Cing lesions and NACC core lesions produced impairments in autoshaping. Although it is possible that these two effects are independent and that the lesion-induced impairments on the same task reflect quite different mechanisms, there are strong reasons to hypothesize that the Ant Cing and NACC core may operate in a functionally integrated way. As Alexander et al. (1986) have argued, the corticostriatal loop involving the NACC is functionally related to processing in the Ant Cing (the “affective” loop in their scheme), and the fact that lesions of the core and of the Ant Cing produce different, but functionally related, impairments also supports the view that distinct processes might contribute to learning at different levels of a corticostriatal circuit (Alexander & Crutcher, 1990; Alexander et al., 1986).

Experiment 2 used the autoshaping procedure to investigate whether the Ant Cing and NACC core interact serially as part of a larger cortico-striato-pallido-thalamic circuit involved in Pavlovian stimulus–reward learning. To test the hypothesis, a disconnection procedure was adopted to prevent direct neuronal communication between these two structures, but without destroying either structure bilaterally. Thus, a unilateral lesion of the Ant Cing was made on one side of the brain, and a unilateral lesion of the NACC core was made on the opposite side. Due to the predominantly ipsilateral connections between these structures, this procedure prevented any direct communication between the NACC core and Ant Cing (see Figure 8). If there is indeed a serial and functional connection between these nodes, then such a disconnection should produce deficits in Pavlovian autoshaping that resemble those produced by bilateral lesions of either structure alone. Such a disconnection procedure has
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Figure 8. Schematic representation of the disconnection hypothesis. a: Pavlovian stimulus–reward processes involve both the anterior cingulate cortex (Ant Cing) and the nucleus accumbens core in a serial manner. b: Bilateral lesions of the Ant Cing, in rats, impair performance of the autoshaping task (Bussey et al., 1997; Experiment 1). c: Bilateral lesions of the NAcc core also impair task performance (Experiment 1). d: If the two structures operate serially, a unilateral lesion of one combined with a contralateral lesion of the other should also impair performance on the autoshaping task.

previously been used successfully in both rats and monkeys (Everitt et al., 1991; Floresco et al., 1997; E. A. Gaffan, Gaffan, & Harrison, 1988; D. Gaffan & Harrison, 1987) to provide evidence for serial functional connections between distinct brain structures.

Method

Subjects

Subjects were 40 male Lister hooded rats (Olac, Bicester, UK) weighing between 290 and 385 g at the time of surgery. They were housed in pairs in a temperature-controlled room (minimum 22 °C) under a 12-hr reversed light–dark cycle and were tested during the dark phase. The rats were food deprived to 85% of their free-feeding body weight for the duration of the experiment. Subject groups and numbers were as follows (final group numbers in parentheses): disconnection lesion n = 14 (8); disconnection shams n = 12 (12); unilateral NAcc core lesions n = 7 (6); and unilateral Ant Cing lesions n = 7 (6).

Procedures

The surgical procedure was derived from that of Experiment 1. Lesions were produced by using identical coordinates and procedures to those in Experiment 1. Behavioral procedures, statistical analysis, histological procedure, and lesion analysis were identical to those in Experiment 1.

Results

Histological Assessment

One subject in the unilateral NAcc core group showed neuronal loss and gliosis in the dorsal, rather than ventral, striatum. Data from that rat were excluded. Lesions of the Ant Cing were almost identical, though unilateral, to those produced in Experiment 1 (See Figure 1). One rat in the unilateral Ant Cing lesion group sustained only minor damage to the Ant Cing, and the data from this subject were excluded from further analysis. Six subjects in the disconnected cingulate and core group showed neuronal loss that was either incorrectly placed or too minor, and their data were also discarded. Schematic representations of the unilateral Ant Cing lesions, unilateral NAcc lesions, and the disconnection lesion are shown in Figure 9.

Behavioral Results

All subjects completed the experiment and reliably collected and consumed sucrose pellets from the magazine. Final group numbers for statistical analysis were: disconnection lesion n = 8; disconnection sham n = 12; unilateral NAcc core n = 6; unilateral Ant Cing n = 6.
**Figure 9.** Schematic representations of disconnection and unilateral excitotoxic lesions of the anterior cingulate cortex and the nucleus accumbens core. Shaded areas represent the smallest (black) and largest (gray) extent of neuronal damage in a single rat. Coronal sections are from +2.7 to +0.48 mm relative to bregma (Paxinos & Watson, 1998). a: Disconnection lesions of the anterior cingulate cortex and nucleus accumbens core. b: Schematic representation of unilateral excitotoxic lesions to the anterior cingulate cortex. c: Schematic representation of unilateral excitotoxic lesions to the nucleus accumbens core.

The Effects of Disconnection Lesions (and Unilateral Controls)

**Discriminated Approach**

A repeated-measures ANOVA comparing all four experimental groups was performed for the acquisition of autoshaping. The analysis revealed a trend toward a main effect of lesion, $F(3, 28) = 2.64, p = .069$, a main effect of CS, $F(1, 28) = 47.78, p < .001$, and a main effect of block $F(9, 252) = 7.59, p < .001$. The effect of CS was due to an increased number of approaches to the CS+ overall, and the effect of block was due to a general increase in approaches.
over training (see Figure 10, a and c). There was a significant CS × Block interaction, $F(9, 252) = 14.47, p < .001$, and a Lesion × CS × Block interaction, $F(27, 252) = 1.60, p < .05$. There were no other significant effects: Lesion × CS, $F(3, 28) = 1.70, p = .42$; Lesion × Block, $F(27, 252) = 0.87, p = .71$. Due to the three-way interaction between lesion, CS, and block, the experimental groups were analyzed separately to determine the nature of the interaction.

The analysis of the disconnection sham group revealed a main effect of CS, $F(1, 11) = 35.32, p < .001$, characterized by an increase in CS+ approaches and a CS × Block interaction, $F(9, 99) = 9.92, p < .001$, revealing the increase in discriminated approaches over training. There was no main effect of block, $F(9, 99) = 1.67, p = .26$.

Both of the unilateral lesion groups showed the same pattern of results as the disconnection shams (greater number of approaches to the CS+ and increasing discrimination of the CS+ and CS− over training) in terms of a main effect of CS and a CS × Block interaction, unilateral NAcc core: CS, $F(1, 5) = 14.15, p < .05$, CS × Block, $F(9, 45) = 8.32, p < .01$; unilateral Ant Cing: CS, $F(1, 5) = 15.82, p < .05$, CS × Block, $F(9, 45) = 3.30, p < .01$. However, both unilateral NAcc core and unilateral Ant Cing groups also showed a main effect of block, unilateral NAcc core: $F(9, 45) = 2.66, p < .05$; unilateral Ant Cing: $F(9, 45) = 2.15, p < .05$, which revealed a general increase in the number of approaches over training.

Analysis of the disconnection lesion group revealed a

Figure 10. a: Acquisition of autoshaping behavior after disconnection lesions of the anterior cingulate cortex (Ant Cing) and nucleus accumbens (NAcc) core (and sham control). b: Autoshaping performance by these groups during an omission session. c: Acquisition after unilateral lesions of the Ant Cing and NAcc core. The performance of these rats was also assessed during an omission session. d: Mean number of approach responses made toward the conditioned (CS+) and control (CS−) stimuli per block of 10 trials. SED bar represents 1 standard error of the difference of the means.
main effect of block, $F(9, 63) = 4.02$, $p < .001$, characterized by a general increase in approaches over training. However, there was no main effect of CS, $F(1, 7) = 2.38$, $p = .13$, and no CS × Block interaction, $F(9, 63) = 0.99$, $p = .31$, demonstrating that there was no significant degree of discriminated approach during the autoshaping session.

In summary, rats with unilateral lesions of the NAcc core, unilateral lesions of the Ant Cing, and sham controls developed significant discriminated approach behavior toward the CS+ relative to the CS− over the duration of the autoshaping session. In contrast, subjects with asymmetric unilateral lesions of the NAcc and Ant Cing (and therefore functional disconnection of the two areas) were significantly impaired at autoshaping. Although the overall number of approaches increased during autoshaping, these subjects never reached a significant level of discriminated approach directed toward the CS+.

**Approach Latencies**

A repeated-measures ANOVA of approach latencies over the entire autoshaping training (100 trials) revealed a significant main effect of CS, $F(1, 28) = 14.00$, $p < .001$, but no effect of lesion, $F(3, 28) = 2.06$, $p = .3$, and no Lesion × CS interaction, $F(3, 28) = 1.04$, $p = .47$ (see Figure 11). In all groups, approaches to the CS+ were made more quickly than approaches to the CS−. Thus, there was no effect of any lesion manipulation on approach latency.

![Figure 11. Mean approach latencies during the acquisition of autoshaping behavior (calculated over the 100 acquisition trials) for rats with disconnection lesions (Disc) and their controls (Disc SHAM, unilateral core, and unilateral anterior cingulate cortex (Ant Cing)). Open bars represent latencies to approach the conditioned stimulus; filled bars represent latencies to approach the control stimulus. SED bar represents 1 standard error of the difference of the means.](image)

**Omission Session**

As shown in Figure 10 (b and d), analysis of approach scores during omission revealed a main effect of CS, $F(1, 28) = 23.65$, $p < .001$, and of block, $F(4, 112) = 3.44$, $p < .05$, and a Lesion × CS interaction, $F(3, 28) = 5.03$, $p < .01$, but no main effect of lesion, $F(3, 28) = 0.69$, $p = .55$, or the following interactions: Lesion × Block, $F(12, 112) = 0.29$, $p = .86$; CS × Block, $F(4, 112) = 2.18$, $p = .18$; or Lesion × CS × Block, $F(12, 112) = 1.74$, $p = .2$. Whereas the main effect of CS revealed a maintained discriminated approach toward the CS+, the effect of block showed a general reduction in approaches over the omission session. To determine whether each experimental group maintained a significant level of discriminated approach during omission (the Lesion × CS interaction suggested there should be group differences), individual $t$ tests of the difference scores were performed on each block for each group. The disconnection sham group showed a significant level of discriminated approach over all five omission blocks ($p < .05$), but the unilateral NAcc core group showed a difference only on Blocks 1, 2, and 3 ($p < .05$), and the unilateral Ant Cing group showed significant differences on Blocks 1, 3, 4, and 5 ($p < .05$). Thus, the control groups maintained discriminated autoshaping to different degrees over the omission session. In contrast to this, the disconnection lesion group did not show a significant level of discriminated approach at any time during the omission session and was the only group to show a significant reduction, selectively, in CS+ approaches over the session ($p < .05$).

In summary, the disconnection sham group persisted in approaching the CS+ in a discriminated manner over the entire omission session. The two unilateral control groups showed a lower level of discriminated approach relative to the disconnection sham group, but did maintain a discriminated approach behavior. The disconnection lesion group showed no discriminated autoshaping behavior at all during the omission session.

**DISCUSSION**

This study replicated the findings of Bussey, Everitt, and Robbins (1997), which demonstrated that lesions of the Ant Cing impair appetitive Pavlovian conditioning. Further, the two subregions of the NAcc examined in the present study can be dissociated in terms of their roles in appetitive Pavlovian conditioning: Lesions of the NAcc core significantly attenuated autoshaping behavior; lesions of the NAcc shell did not. A disconnection of the Ant Cing and NAcc core also impaired autoshaping, providing strong evidence for a serial functional connection between these two structures in terms of Pavlovian conditioning.

**Effects of Lesions to the Ant Cing**

The selective increase in the number of approaches to the CS− seen after lesions of the Ant Cing replicates the results of Bussey, Everitt, and Robbins (1997). However, though approaches to the CS+ and CS− were similarly affected in
Bussey et al. and in the present study, one difference was that in the present study, the lesions hastened approach latencies relative to shams, perhaps reflecting a form of impulsivity produced by the lesion in other contexts (Muir et al., 1996). In contrast, Bussey, Everitt, and Robbins (1997) found longer approach latencies after Ant Cing lesions. These differences are, at first sight, difficult to reconcile. They may reflect variability in performance of subjects that were failing to learn the stimulus associations within the experiment. Alternatively, they may result from subtle differences in the location and extent of the lesions in the two studies. In fact, the dorsal and ventral aspects of the Ant Cing can be dissociated anatomically, and potentially also functionally, in terms of motor output and emotional processing, respectively (Dum & Strick, 1993; Neafsey, Terreberry, Hurley, Ruit, & Frysztak, 1993). Thus, greater damage in the dorsal Ant Cing found in the present study could produce a combination of reward-based learning deficits (ventral) and increased impulsivity (dorsal). Importantly, the major finding that Ant Cing lesions impair appetitive Pavlovian conditioning by selectively increasing approaches to a stimulus that predicts nonreward is clearly a consistent and robust result (Bussey, Everitt, and Robbins, 1997).

The Ant Cing, a key component of the limbic system (MacLean, 1949; Papez, 1937), has been suggested to underlie processes of attention (Janer & Pardo, 1991), memory (Valenstein et al., 1987), emotion (Damasio, 1994b), and spatial learning (Kesner, Farnsworth, & DiMattia, 1989). More specifically, Bussey, Everitt, and Robbins (1997) argued that the Ant Cing forms part of a neural system underlying stimulus–reward learning. Further support for this hypothesis comes from the fact that Ant Cing lesions actually enhance the acquisition of a conditional visual discrimination (Bussey et al., 1996). Such a discrimination can be more effectively learned by means of a stimulus–response habit that might develop (presumably in a separate neural system) in parallel to (and potentially in conflict with) stimulus–reward mechanisms. Damage to the latter learning system would therefore allow the habit system to predominate, thereby resulting in enhanced learning, as was found after Ant Cing lesions by Bussey et al. (1996).

Others have suggested similar notions of multiple learning systems both experimentally (McDonald & White, 1993; Robbins & Everitt, 1996) and theoretically (Adams & Dickinson, 1981; Dickinson, Balleine, Watt, Gonzalez, & Boakes, 1995). In fact Gabriel and colleagues (Freeman, Cuppernell, Flannery, & Gabriel, 1996; Gabriel, Kubota, Sparenborg, Straube, & Vogt, 1991; Gabriel, Vogt, Kubota, Poremba, & Kang, 1991; and see Bussey et al., 1996) have also suggested, on the basis of both lesion studies and single-unit recordings, that the Ant Cing operates early in learning and the posterior cingulate cortex operates later in learning. Gabriel et al. suggested that the Ant Cing may therefore subserve a mnemonic function such as retrieving internal representations of CSs. The fact that lesions of the Ant Cing increased activity in reciprocally connected areas such as the mediodorsal nucleus of the thalamus and the striatum was argued to indicate its suppressive influence on its efferent targets (Gabriel, Kubota, et al., 1991). Such a suggestion is consistent with the increased approaches to the CS− seen after bilateral Ant Cing lesions in the present study.

Effects of Lesions to the NAcc Core and Shell on the Autoshaping Task

Rats with lesions of the NAcc core were severely impaired during the acquisition of autoshaping. Approaches to the CS+ were both significantly lower than sham CS+ approaches and not significantly different from CS− approaches. Thus, no significant discriminated approach was seen during autoshaping. Not surprisingly, these subjects did not show a significant level of discrimination during the omission session. However, it should be noted that though these rats were impaired, the actual pattern (tending toward a discrimination of the CS+ and CS−) of approach behavior toward the end of the acquisition period and during the omission test suggests that any conclusions regarding the precise role of the NAcc in appetitive Pavlovian approach should be made with caution.

Approach latency data corroborate the impairment in autoshaping. Although sham controls approached the CS+ with lower latencies than those for the CS−, core-lesioned rats did not show such a discrimination in approach latency and in fact were significantly slower to approach the stimuli in general relative to sham controls.

In contrast to the pattern of behavior after core lesions, rats with lesions of the NAcc shell were not significantly impaired in their autoshaping behavior. These subjects developed a significant level of discriminated approach toward the CS+ but away from the CS−. This pattern of approach persisted during the omission session, and the approach latencies for shell-lesioned rats were significantly lower for the CS+ relative to the CS−.

It is unlikely that the effects of NAcc core lesions are due to a simple motor deficit or reduction in motivation. First, NAcc core-lesioned rats are hyperactive (Parkinson et al., 1999). Thus, a motor deficit might be expected to interfere with autoshaping by producing an increase in the overall number of approaches, which is in fact the opposite of what was observed in subjects after core lesions. Second, the food-consumption test demonstrated that NAcc core-lesioned rats were not slower to collect food, nor significantly different in their consumption of the reinforcer used in the autoshaping procedure (see Figure 6). Finally, all core-lesioned rats consumed the entire 50 pellets during each autoshaping session.

The deficit seen after core lesions could have several explanations: (a) The effects of core lesions may be secondary to disrupted discrimination between the CS+ and CS−, and therefore the NAcc is involved in spatial attention and learning; (b) The NAcc core may be the site for the association between the CS and US; (c) The NAcc may be directly involved in the expression of conditioned responses; or (d) The NAcc may not directly produce a CR but instead influence the direction and vigor of such responses through motivational mechanisms (Balleine & Killcross, 1994; Dick-
It would be informative to explore further the ability of NAcc core-lesioned rats to develop autoshaped behavior over a more prolonged period of training or, alternatively, to test effects of lesions subsequent to the attainment of asymptotic performance on the autoshaping task.

Although there are few studies examining the effects of separate core and shell lesions on learning and memory, excitotoxic lesions of the entire NAcc have been shown to produce impairments in spatial learning (Annett et al., 1989). In the present study, though the CS+ and CS− were discriminable in terms of their spatial location, they were in fact identical stimuli. The ability to navigate egocentric space is necessary for accurate performance, therefore animals with an impairment in this ability would not be able to discriminate predictive from nonpredictive stimuli. Anatomically, projections from the dorsal hippocampal formation, which are implicated in spatial learning (Moser, Moser, & Andersen, 1993), target the ventrolateral region of the NAcc bordering the ventral aspects of the core and ventrolateral aspects of the shell, via the subiculum (Brog et al., 1993). Damage to these incoming projections could disrupt spatial performance. However, it is unlikely that the present results are due to such damage. Damage to either the dorsal or ventral subiculum does not result in significant impairments on the autoshaping task (Parkinson et al., 1996; Parkinson et al., in press). Further, the area that receives the dorsal subiculum projection was not significantly damaged in either of the lesions used in this study. Finally, a spatial deficit would not impair conditioning to the reward, so although animals might form an association between an autoshaping stimulus and the delivery of a food pellet, they would not discriminate between the CS+ and CS−. In this case, approaches to both stimuli would show a general increase, rather than the selective reduction in CS+ approaches seen in the core-lesioned group.

Thus, although there is limited evidence implicating the NAcc in spatial learning (Annett et al., 1989; Riedel et al., 1997; Westbrook, Good, & Kimura, 1997), other investigators (Floresco, Seamans, & Phillips, 1996a), using reversible lidocaine “lesions” of the NAcc, have demonstrated that such manipulations of the NAcc do not in fact lead to disruptions in spatial learning (using a radial arm maze). Further investigation from the same laboratory (Floresco, Seamans, & Phillips, 1996b; Floresco et al., 1997) suggested that any involvement of the NAcc in spatial behavior is specific to foraging or novelty-dependent exploration and not learning-related (conditioned) spatial navigation. Similarly, Reading and Dunnett (1991), found that, though NAcc lesions affected a matching-to-position task (involving the egocentric discrimination of two levers), the impairment was delay-dependent (NAcc lesioned animals being unimpaired in the zero delay condition); thus the basic ability to discriminate levers was unaffected.

It is likely, therefore, that the deficit in CS+ approach seen in rats with core lesions reflects a disruption of Pavlovian conditioning mechanisms. Evidence produced by in vivo recording techniques supports this suggestion. For example, Wilkinson et al. (1998), using in vivo microdialysis, found that NAcc DA release was related to the ability of a stimulus to elicit a CR (using a mild footshock as the US). Further, Schultz and colleagues, using in vivo electrophysiological recording techniques, observed that DA neurons projecting to the NAcc respond to stimuli that have become predictive of reward through conditioning (Schultz et al., 1992) and have argued that these neurons may operate as teaching signals for reward-related learning processes (Schultz, 1997; Schultz et al., 1997; but see Redgrave, Prescott, & Gurney, 1999 for an alternative, response-based hypothesis). Evidence based on both electrophysiology and microdialysis also suggests that DA within the NAcc is more involved in (or at least is a clear correlate of) incentive or preparatory aspects of conditioning (Bassareo & DiChiara, 1997; Richardson & Gratton, 1996).

Finally, there is a growing body of evidence implicating mesolimbic DA systems in preparatory aspects of behavioral responses to primary reward (Blackburn, Phillips, & Fibiger, 1987; Everitt, 1990; Robbins & Everitt, 1992, 1996; Simon, Sky, Bourbonais, & Smith, 1985), though there is some evidence for a role in certain consummatory aspects of conditioning (Pfaus, Mendelson, & Phillips, 1990; Plein, Matochik, Barfield, & Auerbach, 1990; Radhakishun, Van Ree, & Westerink, 1988). Comparisons between manipulations of mesolimbic DA and excitotoxic lesions of the NAcc cannot always be expected to yield similar results and should be made with caution. Impairments in preparatory aspects of Pavlovian conditioning have been documented after both procedures and may help guide understanding of the specific functions of the NAcc.

Evidence based on chemical manipulation and lesions of the NAcc provides further support for a role of the NAcc in Pavlovian conditioning. Lesions of the NAcc impair a CPP with sucrose reward (Everitt et al., 1991). Furthermore, dopaminergic receptor agonists appear to produce rewarding effects specifically within the NAcc, as intra-NAcc, but not intradorsal striatal, infusions of amphetamine support a CPP (Carr & White, 1983). Importantly, Balleine and Killcross (1994) demonstrated that lesions of the entire NAcc did not appear to disrupt instrumental, action–outcome contingencies, as animals with lesions were still sensitive to changes in the contingency between actions and reward and to changes in the value of the reward (see also V. J. Brown & Bowman, 1995). However, these same animals showed deficits in the influence of Pavlovian stimuli on instrumental actions (Dickinson & Dawson, 1987), thus the effects of NAcc lesions were interpreted as disrupting a process by which Pavlovian stimuli can influence responding through appetitive arousal (for a description of these associative mechanisms see Dickinson & Balleine, 1994). Kelley et al. (1997) have also demonstrated the disruptive effects of infusion of the N-methyl-D-aspartate antagonist AP5 into the NAcc core on lever pressing and on Pavlovian discriminated magazine approach. Given the sensitivity to changes in reward value and contingency seen to follow entire NAcc lesions (Balleine & Killcross, 1994), it is likely that the effects of NAcc manipulations on instrumental performance and magazine approach are secondary to Pavlovian associative mechanisms, including what we have previously termed...
the “activation” or gain-amplification of response output and behavior (Everitt & Robbins, 1992; Robbins & Everitt, 1992).

Although it is plausible that the NAcc core stores stimulus–reward associative information, it is perhaps unlikely (Balleine & Killcross, 1994; V. J. Brown & Bowman, 1995). It is more plausible that it mediates prepotent responses, in this case one relating to a CR based on stimulus–reward knowledge. In fact, Parkinson et al. (1999) found that NAcc core lesions still produced a severe disruption in Pavlovian discriminated approach when the lesions were performed after acquisition. The NAcc shell, on the other hand, does not appear to be involved in Pavlovian approach or, indeed, in any aspect of Pavlovian conditioning measured in this study, though it does appear to mediate the potentiative effects on behavior of stimulant drugs such as amphetamine (Parkinson et al., 1999). If the NAcc is involved in mediating the expression of motivated behavior, then it is likely that the associative knowledge underlying this influence is held in afferent structures, thus justifying their examination in an attempt to understand further the functional nature and interactions in limbic corticostriatal circuitry (Everitt et al., 1991).

Effects of Disconnection Lesions of the Ant Cing and NAcc Core

Rats with asymmetric lesions of the NAcc core and Ant Cing, which functionally disconnected the two structures, showed significant deficits on the autoshaping task and therefore in appetitive Pavlovian approach behavior. The deficit comprised a loss of discriminated approach, which appeared to consist of both an increase in the number of CS− approaches and a decrease in the number of CS+ approaches. Therefore, subjects with disconnection lesions showed an impairment, elements of which resembled that of both bilateral Ant Cing and bilateral NAcc core-lesioned subjects.

Control rats with unilateral lesions of either the NAcc core or the Ant Cing were not significantly different from sham-lesioned rats in their acquisition of autoshaping behavior. However, subjects with unilateral core lesions showed reduced maintenance of autoshaping during the omission session, relative to shams (see below). This result, following disconnection, offers strong support for the idea that the Ant Cing and NAcc core operate functionally and serially in Pavlovian conditioning. It is also consistent with the hypothesis that these two structures are part of a larger limbic corticostriatal circuit involved in stimulus–reward learning. The question of which particular processes are undertaken at different levels of such a circuit will be discussed below.

Effects of Unilateral Lesions of the NAcc Core or Ant Cing

Bilateral lesions of either the NAcc core or the Ant Cing (Bussey, Everitt, & Robbins, 1997; Experiment 1 of present study) produce marked impairments on the autoshaping task. Before any conclusions can be drawn regarding the significance of the effects of the disconnection lesion, it is important to ensure that unilateral lesions of either of the two areas being studied do not themselves cause deficits or other nonspecific effects on the autoshaping task. During acquisition, there was no significant effect of either unilateral lesion; both groups showed discriminated approach toward the CS+ over the session. Further, the sham and unilateral Ant Cing groups also maintained their discriminated approach over the omission session, whereas the unilateral NAcc core group did not. Rats with unilateral core lesions did show a greater number of approaches toward the CS+ than toward the CS− over all blocks during omission, but the difference was only significant for the first three blocks. It could therefore be argued that the unilateral lesion of the NAcc core did impair autoshaping performance, an impairment seen only during the omission session when the precise stimulus–reward contingencies were changing over trials. In fact, there is literature on such disruptive effects of striatal lesions, not only on locomotion, but also on sensorimotor processing, after unilateral lesions of the dorsal striatum (Brasted, Humby, Dunnett, & Robbins, 1997; V. J. Brown & Robbins, 1988; Taylor, Reavill, Jenner, & Marsden, 1981). Although the unilateral lesions of the core in the present study produced a small, but significant, disruption of behavior during the omission session, they did not affect the final level of discriminated approach in the acquisition of autoshaped behavior. This suggests that, though these lesions may produce some effects in themselves, they were not large enough to explain any significant effect of the disconnection procedure on the acquisition of autoshaping. Consistent with this, Everitt et al. (1991), studying a disconnection of the NAcc and BLA, found no significant effects of unilateral NAcc lesions on a test of CPP or other nonspecific measures of locomotor activity and sucrose consumption after 23 hr of food deprivation.

Functions of an Ant Cing–NAcc Circuit

The Ant Cing has been suggested to subserve aspects of emotional processing (Damasio, 1994a) or stimulus–reward associative learning (Bussey, Everitt, & Robbins, 1997). Interestingly, in a study of stimulus–response learning (Bussey et al., 1996), animals with Ant Cing lesions actually demonstrated enhanced acquisition but also showed a resistance to extinction after the habit had been learned to asymptote. Such inflexibility in responding, particularly during extinction, has also been seen after NAcc lesions (Annett et al., 1989; Reading, Dunnett, & Robbins, 1991). This persistence in behavior after NAcc lesions also applied to performance on habit-based tasks and was similarly interpreted by the authors as the loss of flexibility to change behavior in response to changing environmental contingencies.

In the context of the present study, it is important to consider the nature of the processes being undertaken by the Ant Cing and NAcc. Both structures appear to be involved in preparatory aspects of Pavlovian stimulus–reward learning. The Ant Cing may also underlie instrumental stimulus–reward information (Bussey et al., 1996, 1997), whereas the
NAcc may be more critical for the activation of such actions, dependent on glutamate–DA interactions (Balleine & Killcross, 1994; Burns, Everitt, Kelley, & Robbins, 1994; Taylor & Robbins, 1986). It is possible that the role of the NAcc core in the autoshaping task is to provide direction and activation for the learned prepotent response of approach through similar dopaminergic mechanisms, as has been demonstrated for instrumental actions (Taylor & Robbins).

The Ant Cing has been implicated in declarative memory in humans (Bachevalier & Mishkin, 1986; Devinsky, Morrell, & Vogt, 1995; Palmer et al., 1993) and in effortless attentional processes (Corbetta, Miezin, Dobmeyer, Shulman, & Petersen, 1994; Raichle et al., 1994). Thus, the Ant Cing may be involved in processing aspects of environmental stimuli either by directing attention toward, or retrieving internal representations of, such stimuli. During autoshaping, bilateral lesions of the Ant Cing caused a deficit in autoshaping characterized by an increase in approaches to the CS –. Although the precise nature of this impairment is unclear, attentional, impulsive, or memory-based explanations cannot be ruled out. For example, the Ant Cing might be involved in the decremental processes in attaching negative affect to stimuli (e.g., a CS – that is predictive of nonreward) and thus plays a role in associative mechanisms. Similarly, the Ant Cing may be involved in reducing processing directed toward nonsignificant stimuli and therefore plays a role in attentional mechanisms and stimulus salience. It is likely that the anatomical heterogeneity of the Ant Cing underlies some of the contrasting findings seen after circulate manipulations (Dum & Strick, 1993; Neafsey et al., 1993).

Bilateral lesions of the NAcc core decreased approaches to the CS + during autoshaping. In the context of the role of the NAcc core in Pavlovian mechanisms, this reduction in behavior directed toward CSs could plausibly be due to loss of the associative connection between either the CS and US or CS and CR. Alternatively, this effect could be due a loss of the energization produced by a motivatedly salient stimulus. Thus, although the associative knowledge relating to the CS and US would remain intact in core-lesioned animals, their approach behavior would not be directed or potentiated by motivational mechanisms subsequent to the lesion. In this sense, the role of the NAcc core would be to energize or gain-amplify prepotent responses.

Implications for Corticostratial Circuitry

The suggestion of separable learning systems, including those for stimulus–reward and stimulus–response habit learning, is consistent with the concept of segregated corticostratial circuits. The evidence presented in this article supports the existence of such a corticostratial circuit, originating from within the Ant Cing and projecting through the NAcc core. Bilateral damage to either structure or a disconnection of the two disrupts the circuit and causes impairments in Pavlovian approach behavior. The nature of those impairments is different and appears to depend on the location of the damage within the circuit.

A similar functional mode of organization may apply to other corticostratial circuit subsystems. For example, Phillips and colleagues (Floresco et al., 1996a, 1996b; Seams & Phillips, 1994) have demonstrated a serial functional interaction between the ventral subiculum and NAcc shell in foraging behavior. We have also provided evidence for a functional convergence of the BLA and ventral subiculum at the level of the NAcc in conditioned reinforcement (Burns, Robbins, & Everitt, 1993; Cador, Robbins, & Everitt, 1989; Parkinison et al., 1999). The integrity of the BLA is critical for the conditioned reinforcement effect itself, whereas the ventral subiculum mediates the potentiating effect of intra-NAcc d-amphetamine on responding with conditioned reinforcement. This is also consistent with electrophysiological evidence for the convergence and gating of glutamatergic and dopaminergic inputs within the NAcc (Blaha, Floresco, Phillips, & Yang, 1997; Cools, van den Bos, Bloeger, & Ellenbroek, 1991; Mulder, Hodenpijl, & da Silva, 1998).

Finally, parallels in the selective effects of manipulations of areas within the prefrontal cortex and of their striatal projection target fields have provided further evidence for parallel and distinct corticostratial circuits for the dorsal striatum (Divac, 1972; Divac, Rosvold, & Szwarcbart, 1967; Dunnett & Iversen, 1980, 1981, 1982; Rosvold, 1972). The present study provides further evidence for such interactions with subdomains of the ventral striatum.

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