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Dissociation in Effects of Lesions of the Nucleus Accumbens Core and Shell on Appetitive Pavlovian Approach Behavior and the Potentiation of Conditioned Reinforcement and Locomotor Activity by D-Amphetamine

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Dopamine release within the nucleus accumbens (NAcc) has been associated with both the rewarding and locomotor-stimulant effects of abused drugs. The functions of the NAcc core and shell were investigated in mediating amphetamine-potentiated conditioned reinforcement and locomotion. Rats were initially trained to associate a neutral stimulus (Pavlovian CS) with food reinforcement (US). After excitotoxic lesions that selectively destroyed either the NAcc core or shell, animals underwent additional CS–US training sessions and then were tested for the acquisition of a new instrumental response that produced the CS acting as a conditioned reinforcer (CR). Animals were infused intra-NAcc with D-amphetamine (0, 1, 3, 10, or 20 μg) before each session. Shell lesions affected neither Pavlovian nor instrumental conditioning but completely abolished the potentiative effect of intra-NAcc amphetamine on responding with CR. Core-lesioned animals were impaired during the Pavlovian retraining sessions but showed no deficit in the acquisition of responding with CR. However, the selectivity in stimulant-induced potentiation of the CR lever was reduced, as intra-NAcc amphetamine infusions dose-dependently increased responding on both the CR lever and a nonreinforced (control) lever. Shell lesions produced hypoactivity and attenuated amphetamine-induced activity. In contrast, core lesions resulted in hyperactivity and enhanced the locomotor-stimulating effect of amphetamine. These results indicate a functional dissociation of subregions of the NAcc; the shell is a critical site for stimulant effects underlying the enhancement of responding with CR and locomotion after intra-NAcc injections of amphetamine, whereas the core is implicated in mechanisms underlying the expression of CS–US associations.

Key words: ventral striatum; reward; dopamine; psychomotor stimulant; associative learning; drugs of abuse

The mesolimbic dopamine (DA) system that projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) has been implicated in the rewarding properties of intracranial self-stimulation, drugs of abuse, and natural reinforcers (for review, see Wise and Bozarth, 1987; Robbins and Everitt, 1996). Although there is general agreement that the dopaminergic innervation of the NAcc contributes to reinforcement processes, the functions of this system remain unclear. It is unlikely that mesolimbic DA simply mediates primary reinforcement because lesions of this system or infusions of DA receptor antagonists into the NAcc do not affect consummatory responses to food, water, or sex (Koob et al., 1978a; Everitt, 1990; Robbins and Everitt, 1992).

Conversely, there is psychopharmacological, neurochemical, and electrophysiological evidence that DA release in the NAcc is associated with anticipatory responses to reinforcing stimuli (Everitt, 1990; Phillips et al., 1991; Schultz et al., 1992; Williams et al., 1993), indicating that DA modulates reinforcement signals at the level of the NAcc (Robbins and Everitt, 1992). In this way, DA release in the NAcc may enhance behavioral responses during both Pavlovian and instrumental conditioning by potentiating approach responses to conditioned stimuli and increasing the control over instrumental behavior of stimuli associated with reinforcement (conditioned reinforcers; Everitt and Robbins, 1992; Robbins and Everitt, 1992).

The acquisition of responding with conditioned reinforcement (CR) provides a powerful means of investigating the contribution of Pavlovian conditioning to reinforcement-related instrumental behavior. Conditioned reinforcement is the process whereby a previously conditioned stimulus (CS) acts as the reinforcer for an instrumental action (Mackintosh, 1974). Responding for a conditioned reinforcer is potentiated by systemic (Hill, 1970) or intra-NAcc (Taylor and Robbins, 1984) administration of D-amphetamine, having behavioral, anatomical, and neurochemical specificity (Cador et al., 1989; Taylor and Robbins, 1984, 1986). Of particular relevance for this study is that these effects have been shown to depend critically on DA receptor activation within the NAcc (Taylor and Robbins, 1986; Wolterink et al., 1993). Thus, use of this procedure allows the control over behavior by a conditioned reinforcer and how this control is modified by increasing dopamine transmission in the nucleus accumbens to be measured, rather than the ability of an appetitive conditioned stimulus to affect new learning per se.

In addition to its role in reinforcement processes, the NAcc has been implicated in spontaneous and psychomotor stimulant-potentiated locomotion. Systemic or intra-NAcc infusions of DA receptor agonists increase spontaneous activity (Kelly et al., 1975;

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Kelley et al., 1989; Swerdlow and Koob, 1989). This increased activity is blocked by DA-depleting lesions of the VTA or by previous intra-NAcc administration of DA receptor antagonists (Kelly et al., 1975; Wolterink et al., 1993). Dopamine depletion from the NAcc or VTA generally produces hypoactivity (Koob et al., 1978b), whereas cell body lesions of the entire NAcc produce a significant increase in spontaneous locomotor activity (Kelly and Roberts, 1983; Kafetzopoulos, 1986; Everitt et al., 1991).

The NAcc is a heterogeneous structure (Graybiel and Ragsdale, 1978; Jongen-Relo et al., 1993). It can be separated anatomically into core and shell subdivisions (Zaborszky et al., 1985; Voorn et al., 1989) situated in the dorsolateral and ventromedial regions of the nucleus, respectively, that can be dissociated immunocytochemically (Graybiel and Ragsdale, 1978; Voorn et al., 1994) and on the basis of their differential patterns of connectivity (Groenewegen et al., 1987; Berendse and Groenewegen, 1990; Zahm and Heimer, 1990, 1993; Hurley et al., 1991; Berendse et al., 1992; Wright et al., 1996).

The distinct pattern of core and shell output targets, with the core projecting to pallidal structures and the shell, in addition, projecting to more limbic domains, such as the lateral hypothalamus (Zahm and Heimer, 1990), suggests that the two regions may mediate different behavioral processes. Recently, functional dissociations of the NAcc shell and core have been provided using both excitotoxic lesions and excitatory amino acid modulation of the two NAcc subregions selectively (Maldonado-Irizarry and Kelley, 1995; Kelley et al., 1997). By using excitotoxic lesions that selectively destroy neurons in either the NAcc core or shell, we have investigated the functions of these two subregions in the processes underlying CR and its potentiation by stimulant drugs and also in mediating the locomotor stimulant effects of D-amphetamine.

MATERIALS AND METHODS

Subjects

Ninety-five male Lister hooded rats (Olac, Bicester, UK) were housed in pairs in a temperature-controlled (21°C) room on a 12 hr light/dark cycle. After recovery from surgery, animals were placed on a restricted feeding schedule and maintained at ~85% of their free-feeding weight. Water was available *ad libitum* in the home cages. All animals used in these studies were treated in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (project license PPL 80/00668).

Surgery

Animals in the locomotor activity study received bilateral lesions of the NAcc core or shell before any behavioral testing. Animals in the CR study received lesions after the Pavlovian CR training sessions. Before surgery, rats were anesthetized with Avertin [2,2,2-tribromoethanol, 2-methylbutan-2-ol, Dulbecco "A" PBS tablets, and ddH_20 in tertiary amyl alcohol (Sigma, Poole, UK); 10 ml/kg body weight] and secured in a Kopf stereotaxic instrument with the incisor bar set at −3.3 mm. Fifteen animals for the CR study and eighteen animals for the activity study received NAcc core lesions, induced by infusing 0.5 μl of quinolinic acid (0.09 M) over 3 min at the following site: anteroposterior (AP), +1.2 mm; lateral (L), ±1.8 mm; and dorsoventral (DV), −7.1 mm from dura. The pipette was removed 2 min after the infusion. Fourteen animals for the CR study and twelve animals for the activity study received NAcc shell lesions that were induced by infusing ibotenic acid (0.06 M) at three different sites. The coordinates, infusion volume, infusion time, and diffusion time for each injection were as follows: (1) AP, +1.6 mm; L, ± 1.1 mm; DV, −6.4 mm; 0.1 μl; 1 min; 2 min; (2) AP, +1.6 mm; L, ±1.1 mm; DV, −6.9 mm; 0.1 μl; 1 min; 1 min; (3) AP, +1.6 mm; L, ±1.1 mm; DV, −7.9 mm; 0.2 μl; 2 min; 1 min. Twelve rats (six core and six shell) for the CR study and twenty-four (12 core and 12 shell) for the activity study received NAcc sham lesions induced by infusing vehicle using the coordinates and infusion parameters described above.

All neurotoxin injections were made through a single burr hole using either a 1 or 5 μl SGE (Baton Rouge, LA) syringe (26 gauge code, 1BR-OC-7/0.47) with a custom-made glass micropipette attached to the end. Initially, pipettes (Intracel Ltd.) measured 1.2 mm external diameter and 0.69 mm internal diameter by 10 cm in length and were pulled using a Stoelting App-1 all-purpose Puller, model 52500, giving a final tip diameter of 50–100 μm and a length of 12 mm. Micropipettes were attached to the syringe using Araldite epoxy resin (CIBA) to ensure an airtight seal. All animals were given injections of glucose–saline (5–10 ml, i.p.) after surgery to aid recovery. There was no significant animal loss in the first week after surgery.

Animals in the CR study were also implanted bilaterally with stainless steel guide cannulae (22 gauge) 2 mm dorsal to the intended injection site in the NAcc (AP, +1.6 mm; ML, ±1.5 mm; DV, −4.1 mm) during the same surgery as that in which the lesions were made. Cannulae were fixed to the skull with dental cement, and stainless steel screws and were closed with stainless steel stylets. All animals were allowed to recover for 2 weeks before behavioral testing began.

Drugs and infusions

D-Amphetamine sulfate (Sigma) was dissolved in sterile PBS, pH 7.2, for intracerebral infusions and in sterile 0.9% saline for systemic injections. Intracerebral infusions were made through 29 gauge cannulae that extended 2 mm beyond the guide cannulae tips and were attached to an infusion pump (Harvard Apparatus) by polyethylene tubing. Infusions were in a volume of 0.5 μl per side over 60 sec with a 60 sec diffusion period. Before the first drug infusion, all animals were given a preliminary infusion of PBS and returned to their home cages so that any behavioral effects of tissue damage mechanically induced by the injection cannulae would occur before the test session.

Apparatus

Conditioned reinforcement training and testing took place in sound-attenuated operant chambers (26.5 × 22 × 20 cm) that were fitted with two retractable levers and a sucrose dipper situated between the levers (Med Associates). The operant chambers could be illuminated by a ceiling house light, and external noise was masked by ventilating fans mounted on the side of each box. Access to the dipper was allowed through a magazine (3.8 cm from the side and 5.5 cm from the grid floor) that could be illuminated with a tray light. The apparatus was controlled and data were collected by BBC or Acorn Archimedes microcomputers (Acorn Computers, Cambridge, UK) running the control languages Spider or Arachnid (Genes Cognition, Cambridge, UK).

Locomotor activity was tested in individual wire photocell cages (40 × 25 × 18 cm) that were transected by two parallel infrared photocell beams 6 cm from the cage ends and 1 cm from the cage floor. Beam breaks were registered in 10 min bins on-line by a BBC Master Series microcomputer equipped with a Spider extension (Genes Cognition). All testing was conducted in the dark phase of the light/dark cycle.

Behavioral procedures

Conditioned reinforcement. Forty-one animals underwent Pavlovian conditioning sessions in which the presentation of a CS (5 sec illumination of the tray light and house light off) preceded the US (5 sec elevation of the dipper filled with a 10% sucrose solution). The CS–US pairing was presented 30 times per session on a variable interval (VI), 60 sec schedule of reinforcement. All animals received 20 training sessions. The frequency and duration of magazine entries were detected by infrared beams that transected the entrance. The number of magazine entries during the VI, CS, and US periods was recorded, and a measure of discriminated approach was determined for each animal. Discriminated approach was calculated as the mean number of magazine entries during the CS period as a ratio of the mean number of magazine entries during the total trial period, excluding the duration of US presentation (CS + VI). Animals were given four retraining sessions when they had recovered from surgery to ensure a stable baseline of responding before the start of the test phase.

In Pavlovian to instrumental transfer test sessions, sucrose was not available, i.e., animals were tested in extinction. The two levers were introduced into the chambers, and depression of one lever (CR) resulted in the presentation of the CS (under a random ratio 2 schedule), whereas depression of the second lever had no programmed consequences (NCR). The ability of the CS to selectively increase responding on the CR lever provides a measure of the conditioned reinforcing properties of the CS (Mackintosh, 1974). The assignment of CR and NCR levers was counterbalanced within groups. Immediately before each of five tests,

animals were infused intra-NAcc with D-amphetamine (0, 1, 3, 10, or 20 μg). All drug doses were administered in a Latin square design. The number of responses on each lever, as well as the number of magazine entries, were recorded during each 30 min test. All test sessions were separated by 48 hr. For statistical analyses, the responses on the CR and NCR lever were square root-transformed to maintain homogeneity of variance (Winer, 1971). Furthermore, the homogeneity of variance across groups in repeated-measures design ANOVAs was assessed by the Mauchly Sphericity test. When data sets significantly violated this requirement for a repeated-measures design ANOVA, the Greenhouse Geisser Epsilon correction parameter for degrees of freedom (Geisser and Greenhouse, 1959; Winer, 1971) was used to calculate a more conservative p value for each F ratio. Finally, when appropriate, further analyses of three-way ANOVA interactions was undertaken using weighted mean-adjusted (pooled) Mean Square Error terms as described by Winer (1971).

Locomotor activity. Thirty-six animals were tested for locomotor responses to systemic injections of D-amphetamine. They were given four 2 hr sessions in the activity cages to measure spontaneous locomotor activity and habituation to the cages. After this, all animals received 0, 0.5, 1.5, and 5.0 mg/kg of D-amphetamine (administered in ascending order of concentration) systemically (intraperitoneally), on separate days, and their activity levels were measured for 2 hr.

Histological assessment of lesions

At the completion of behavioral testing, animals were killed under deep barbiturate anesthesia and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde (PFA). The brains were removed and post-fixed for 1 hr in PFA and then stored in a 20% w/v sucrose solution for 12–15 hr.

Coronal sections (40 μm) were cut through the forebrain on a freezing microtome. Every third section was stained for immunocytochemical analysis using antibodies raised against substance P and Calbindin 28 K. These sections were quenched for 5 min at room temperature in a solution of 10% methanol, 10% concentrated hydrogen peroxide, and 80% distilled water. After two 5 min washes in 0.05 M Tris-buffered saline (TBS; pH 7.4), the sections were subjected to blocking with 1% goat serum (NGS) in TBS containing 0.2% Triton X-100 (TTBS) for 1 hr before being transferred without washing into a solution containing the primary antibody in the following dilution: monoclonal anti-Calbindin-D (mouse) antibody (Sigma) 1:500 and 1% NGS in TTBS; anti-substance P antibody 1:1000 with 1% NGS in TTBS. Sections were left overnight at room temperature on a shaker and then washed thoroughly in TBS. Sections were then incubated in goat anti-mouse (biotinylated) at a dilution of 1:200, or goat anti-rabbit (biotinylated) at a dilution of 1:50, with 1% NGS in TBS for 3 hr, then given three 5 min washes in TBS before a further 1 hr incubation with the avidin–biotin–peroxidase complex (ABC; Vector Laboratories, Burlingame, CA) at a dilution of 1:200 with 1% NGS in TBS (this solution was mixed 30 min before use). The sections were then washed once in TBS and twice in Tris nonsaline (TNS) before treatment with the chromogen diaminobenzidine (DAB): 10 mg/ml DAB and 0.67 μl/ml 30% hydrogen peroxide in TNS. Sections were incubated for ~5 min until the required intensity of reaction was attained. After a final rinse in TBS, sections were mounted on gelatinized slides, allowed to dry overnight, and then dehydrated in ascending alcohols and coverslipped.

Alternate sections were mounted on gelatin-coated glass slides, then stained for Nissl substance using cresyl violet. The combined Nissl staining and immunocytochemistry allowed visualization of the NAcc core and shell regions. Calbindin is prevalent within the core subregion, whereas substance P immunoreactivity is relatively more intense in the shell (Voorn et al., 1989; Zahm and Brog, 1992). Thus, immunocytochemistry highlights the core and shell subregions during histological analysis, whereas staining with cresyl violet stain enables assessment of the extent and nature of excitotoxin-induced neuronal damage as well as gliosis-associated intracerebral infusions of quinolinic or ibotenic acids.

RESULTS

Histological assessment

Figures 1 and 2 show schematic representations of lesions of the NAcc core and shell, respectively, based on the stereotaxic atlas of the rat brain by Paxinos and Watson (1998). Delineation of the NAcc core and shell was also based on immunocytochemical and histological analyses of the striatum (Voorn et al., 1989; Zahm and Brog, 1992; Jongen-Relo et al., 1993, 1994; Heimer et al., 1995). NAcc shell lesions, resulting from infusions of ibotenic acid, destroyed all, or a great majority of, neurons in the caudal, mediodorsal shell (sometimes termed the septal pole), thus leaving the medial rostral pole and also the entire ventral and ventrolateral aspects of the shell intact (see Fig. 3 for photomicrograph of the lesion). Neuronal loss typically extended in an anteroposterior direction from +1.7 to +0.48 mm anterior to bregma and from the base of the lateral ventricle dorsally, to the ventral portions of the medial shell occasionally reaching the region of the olfactory tubercle. There was, in some cases, unilateral damage to the lateral septum or to the medial NAcc core.

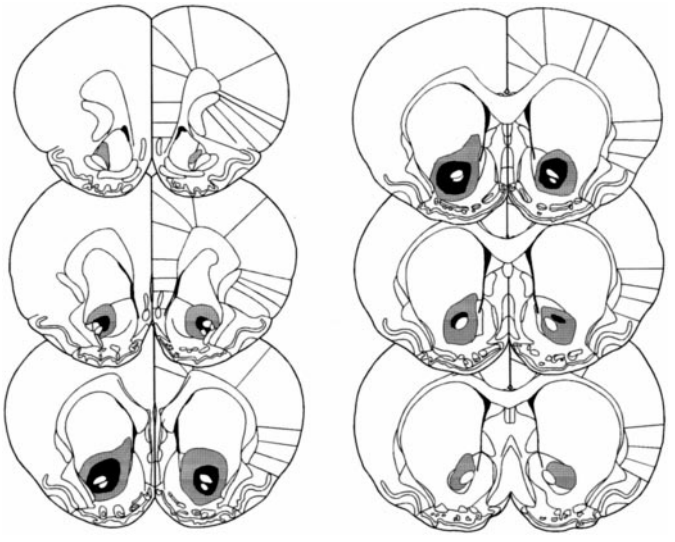

Figure 1. Schematic representation of excitotoxic lesions to the NAcc core. *Shaded areas* represent the smallest (*black*) and largest (*gray*) extent of neuronal damage in a single animal. Coronal sections are +2.7 mm anterior through +0.48 mm posterior to bregma (Paxinos and Watson, 1998).

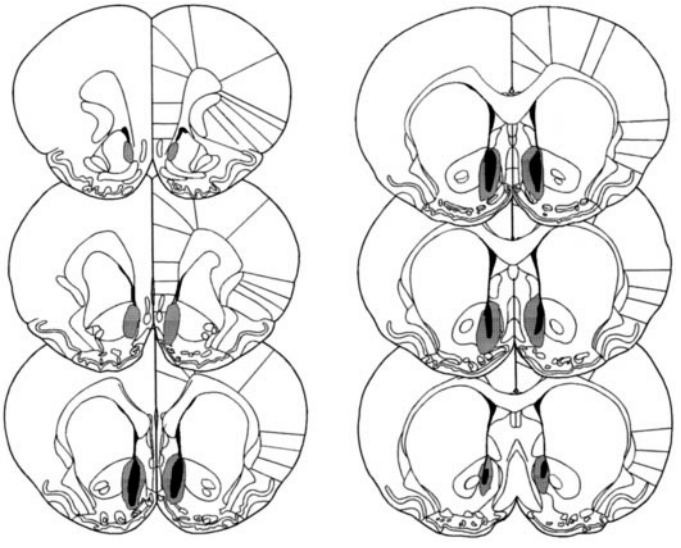

Figure 2. Schematic representation of excitotoxic lesions to the NAcc shell. *Shaded areas* represent the smallest (*black*) and largest (*gray*) extent of neuronal damage in a single animal. Coronal sections are +2.7 mm anterior through +0.48 mm posterior to bregma (Paxinos and Watson, 1998).

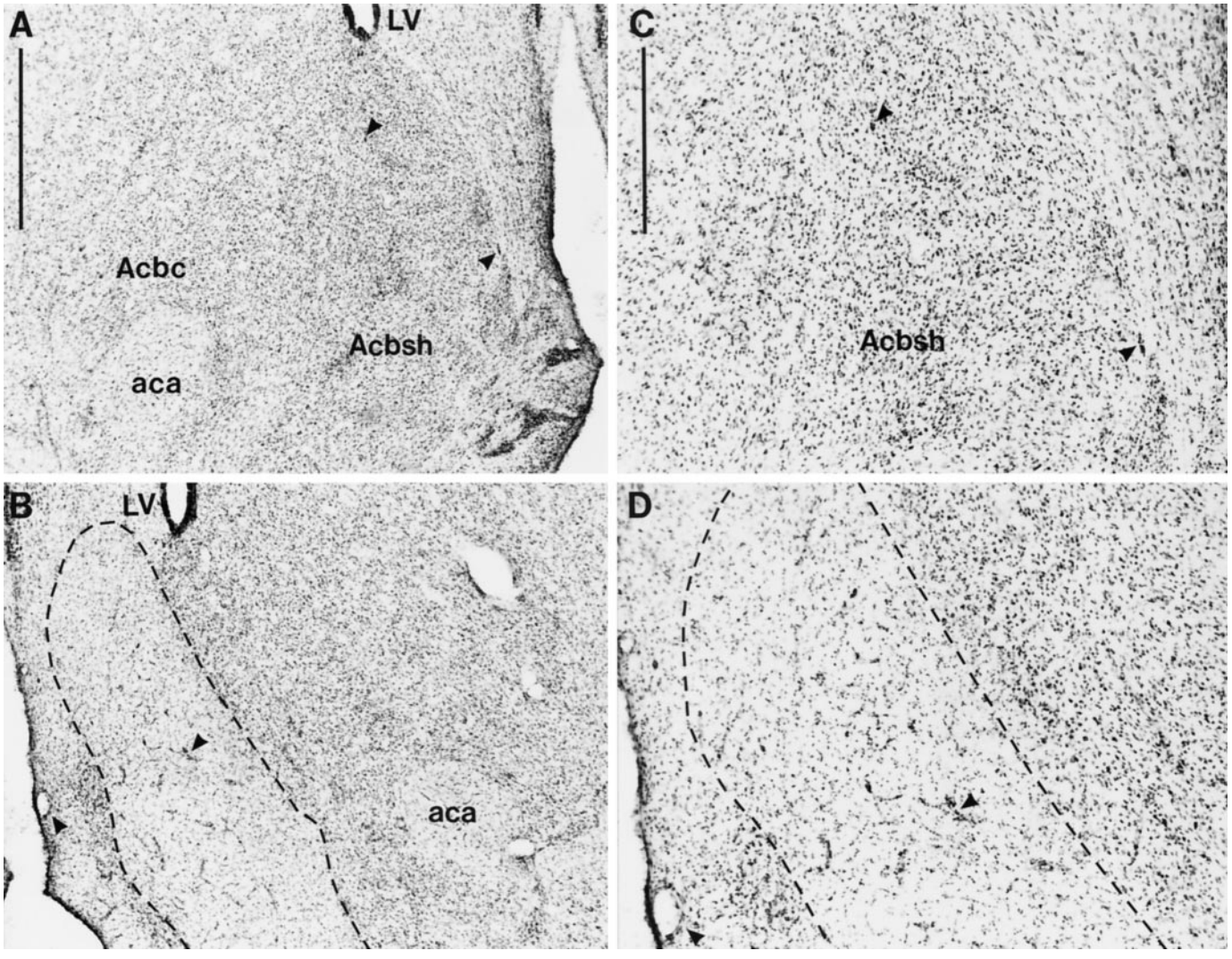

Figure 3. Photomicrographs showing cresyl violet-stained coronal sections through the nucleus accumbens (~+1.2 mm from bregma). *A*, Sham lesion; *B*, nucleus accumbens shell lesion. *C*, High magnification of the sham lesion section shown in *A*; *D*, high magnification of the shell lesion section shown in *B*. The lesioned area is indicated by the *dotted lines*. Scale bars: *A*, 1 mm; *C*, 500 μm. *Arrowheads* show identical landmarks in *A* and *B*, and *C* and *D*. *aca*, Anterior commissure; *Acbc*, nucleus accumbens core; *Acbsh*, nucleus accumbens shell; *LV*, lateral ventricle.

Animals with bilateral damage to any structures extraneous to the shell, including especially the medial core, were excluded from any further analysis. Finally, there was no evidence of damage to the ventral pallidum or the nucleus of the vertical limb of the diagonal band of Broca in any animals.

Lesions of the NAcc core resulting from infusions of quinolinic acid encompassed most of the core subregion. Figure 4 shows photomicrographs of a representative coronal section from an NAcc core lesion and a sham control stained with cresyl violet. Neuronal loss and associated gliosis extended, in an anteroposterior direction, rostrally from +2.5 to +0.5 mm anterior to bregma. Generally, the lesion did not extend ventrally or caudally into ventral pallidum or olfactory tubercle. Neuronal damage was often caused to ventral parts of the overlying caudate putamen, although this was usually unilateral in nature. Similarly, neuronal loss was occasionally seen in the lateral or ventrolateral shell. Animals with bilateral damage of this kind were excluded from further analysis of the behavioral data. Finally, animals with any damage to the medial and dorsomedial shell were excluded from the behavioral analysis.

During experimental testing, implanted cannulae became detached from the head mountings of some subjects. The data from these animals were not included in the subsequent statistical analyses (four core, two shell, and four sham animals). Similarly, the data from animals with lesions that were incomplete or extended beyond the target area, as revealed by histological examination, were also discarded (six core and 11 sham). Twenty-two animals remained in the CR study, seven shell-lesioned, seven core-lesioned, and eight sham-lesioned. Forty-six animals remained in the locomotor study, six shell-lesioned, 16 core-lesioned, and 24 sham-lesioned.

Conditioned reinforcement

Pavlovian conditioning

The effects of NAcc core and shell lesions on discriminated approach during CS–US training sessions are shown in Figure 5. Discriminated approach was calculated as the number of approaches during the CS period as a ratio of approaches during the total CS and VI period. An increase in this ratio across sessions indicates that responses during the CS period are increasing

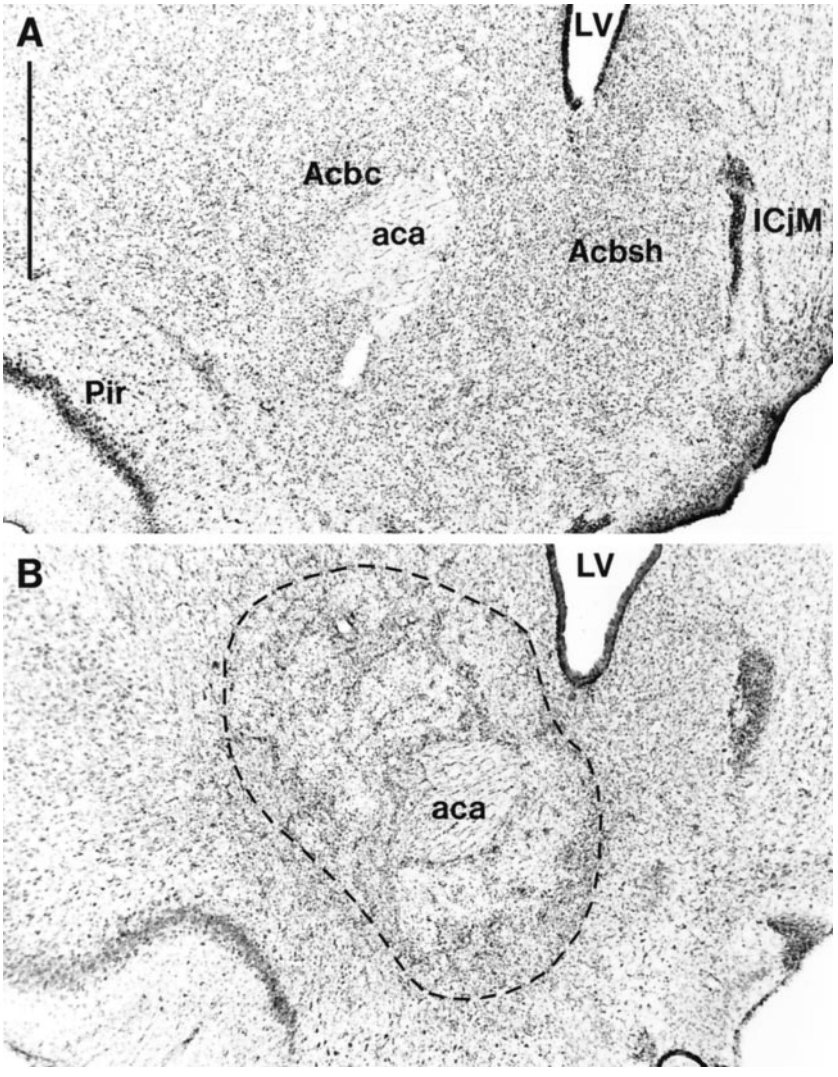

Figure 4. Photomicrographs showing cresyl violet-stained coronal sections through the nucleus accumbens (~+1.2 mm from bregma). *A*, Sham lesion; *B*, nucleus accumbens core lesion. The lesioned area is indicated by the *dotted lines*. Scale bar: *A*, 1 mm. *aca*, Anterior commissure; *Acbc*, nucleus accumbens core; *Acbsh*, nucleus accumbens shell; *ICjM*, major islands of calleja; *LV*, lateral ventricle; *Pir*, piriform cortex.

relative to responses during the VI period. ANOVA of the four presurgical Pavlovian sessions (comparing the three experimental groups before surgery) showed that there were no differences in this measure of discriminated approach between the three groups either through a main effect of lesion ($F_{(2,19)} = 0.52$; $p = 0.6$) or through a lesion × session interaction ($F_{(6,57)} = 0.33$; $p = 0.92$). However, all animals demonstrated Pavlovian learning as expressed as an increase in conditioned responding over sessions ($F_{(3,57)} = 22.28$; $p = 0.0001$).

ANOVA comparing postsurgery discriminated approach revealed a significant lesion × surgery interaction ($F_{(6,57)} = 2.9$; $p < 0.05$). *Post hoc* analysis of simple interactions demonstrated that animals with core lesions exhibited a reduced level of discriminated approach relative to shell- and sham-lesioned animals during the postsurgery conditioning sessions ($p < 0.05$). Furthermore, whereas shell- and sham-lesioned animals showed a significant increase in discriminated approach over the four trials ($p < 0.05$), core-lesioned animals showed no significant change in their approach behavior ($p = 0.13$).

Baseline activity (overall magazine entries during the session), as measured by the total frequency and duration of magazine entries, was not affected by either lesion (duration: lesion × session interaction, $F_{(2,22)} = 2.26$, $p = 0.13$; main effect of lesion, $F_{(2,22)} = 3.04$, $p = 0.09$; frequency: lesion × session interaction, $F_{(2,22)} = 0.13$, $p = 0.73$; main effect of lesion, $F_{(2,22)} = 1.49$; $p = 0.25$), demonstrating the specific discriminated nature of the core lesion deficit.

Acquisition of a new response with conditioned reinforcement

The effects of NAcc core and shell lesions on the acquisition of responding with CR are shown in Figures 6 and 7. The actual mean number of responses over the 30 min testing periods made by sham-lesioned animals varied from 56.6 and 10.5 (for CR and NCR levers, respectively) after saline to 208 and 19.9 at the 20 μg D-amphetamine dose. Responses made by core-lesioned animals similarly ranged from 52.1 and 19.6 (CR and NCR after saline infusion) to 121.2 and 30.2 (after 20 μg D-amphetamine), whereas those for shell-lesioned animals ranged from 36.9 and 12.2 (CR and NCR, respectively) to 43.8 and 13.9 (after 20 μg D-amphetamine).

The data were square root-transformed to maintain homogeneity of variance. ANOVA comparing CR and NCR responses for each group across D-amphetamine doses revealed statistical significance for all interactions, including lesion × lever × dose ($F_{(5,50)} = 4.14$; $p < 0.005$), lesion × lever ($F_{(2,19)} = 11.08$; $p < 0.005$), lesion × dose ($F_{(8,76)} = 3.85$; $p < 0.005$), and lever × dose ($F_{(3,50)} = 9.17$; $p < 0.0001$). All three main effects were also statistically significant: lesion ($F_{(2,19)} = 8.41$; $p < 0.005$), lever

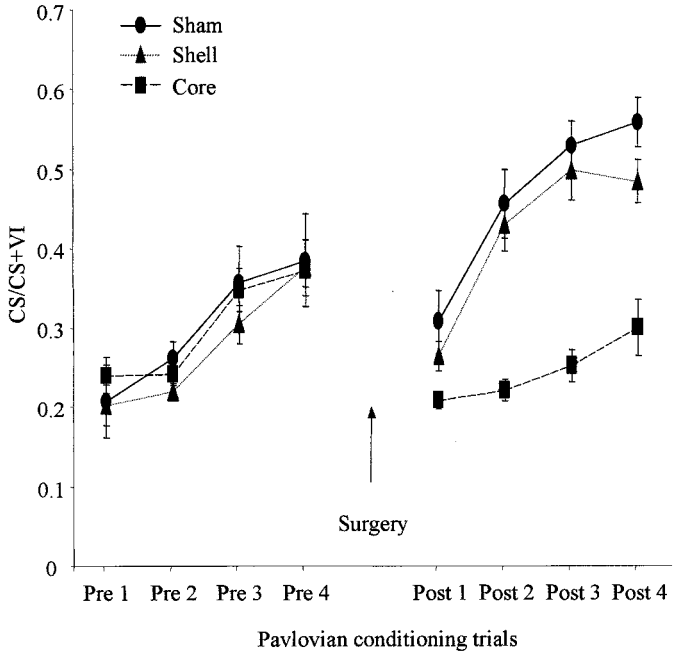

Figure 5. Effect of NAcc core and shell lesions on Pavlovian-discriminated approach. The mean ± SEM ratio of approach responses during the CS relative to the CS plus VI period is shown for four presurgical and four postsurgical sessions for core-, shell-, and sham-lesioned animals.

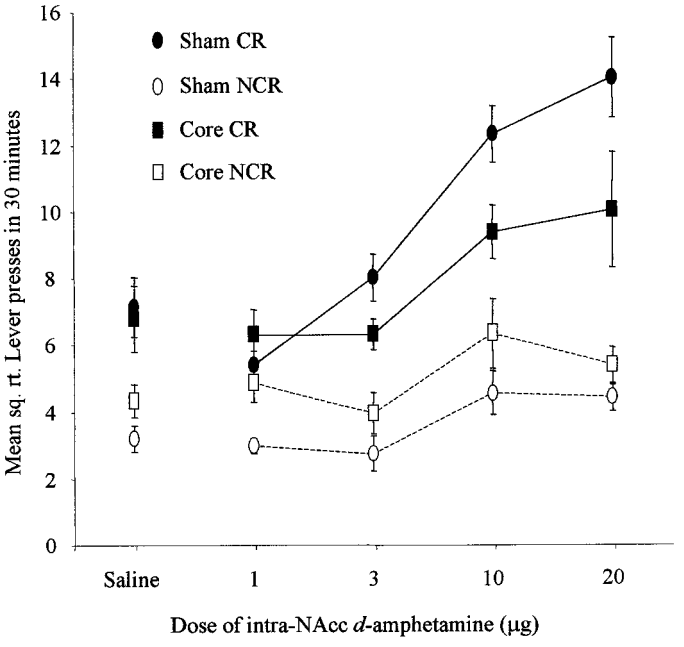

Figure 6. Effect of NAcc core lesions on the acquisition of responding with CR. Data points represent the mean square root ± SEM responses on the lever producing the conditioned reinforcer (*CR*) and the control lever (*NCR*) for sham- and core-lesioned animals after intra-NAcc injections of D-amphetamine (0, 1, 3, 10, and 20 μg).

($F_{(1,19)}$ = 137.55; $p < 0.0001$), and dose ($F_{(4,76)}$ = 9.85; $p < 0.0001$). Because of the three-way interaction, the nature of these effects was investigated further by *post hoc* analysis of simple interactions and simple main effects by studying the factors of lever and dose separately in each experimental group.

These *post hoc* analyses revealed that sham-lesioned animals made significantly more responses on the CR than the NCR lever and indicated that these animals further showed a dose-dependent increase in responding on the CR lever but not the NCR lever (lever × dose interaction, $F_{(4,76)}$ = 15.33; $p < 0.05$).

The analysis of core-lesioned animals produced significant main effects of lever ($F_{(1,19)}$ = 24.58; $p < 0.05$) and dose ($F_{(4,19)}$ = 3.72; $p < 0.05$), revealing that the stimulant effects of intra-NAcc D-amphetamine and the control over behavior by CR were intact. However, core-lesioned animals did not show a significant lever × dose interaction ($F_{(4,76)}$ = 2.06; p = ns), suggesting that the interaction of CR and its potentiation by amphetamine was absent. There was a dose-dependent increase in responding, but this was not selective for the CR lever.

Shell-lesioned animals were not impaired in responding with CR (main effect of lever, $F_{(1,19)}$ = 21.38; $p < 0.05$). However, the stimulant effects of intra-NAcc D-amphetamine were abolished after shell lesions (main effect of dose, $F_{(4,19)}$ = 0.39; p = ns; lever × dose interaction, $F_{(4,76)}$ = 0.84; p = ns).

In summary, sham-lesioned animals responded more on the CR lever at all doses of D-amphetamine, and this response was selectively potentiated in a dose-dependent manner. Core-lesioned animals were not significantly impaired in the acquisition of a new response with CR. Moreover, intra-NAcc infusions of D-amphetamine produced a significant stimulation of responding. However, the stimulus control over responding on the CR and NCR levers after intra-NAcc D-amphetamine was impaired in these animals. Shell-lesioned animals were completely unimpaired

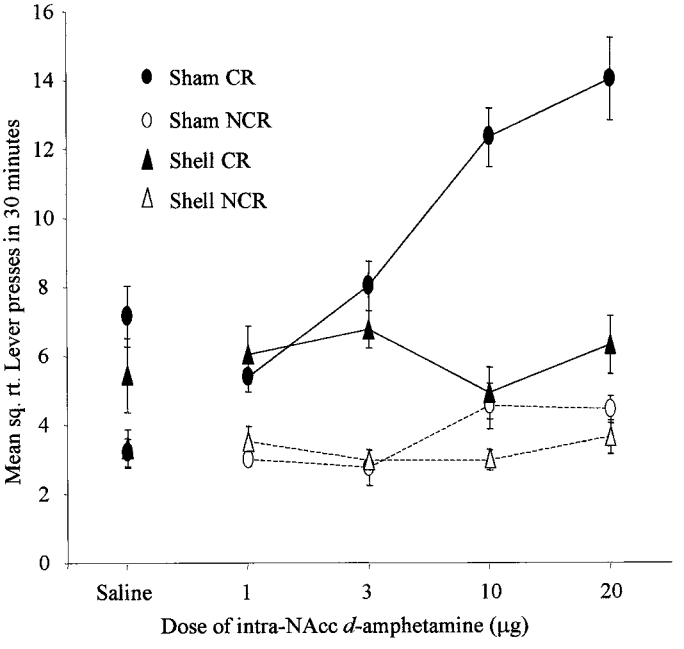

Figure 7. Effect of NAcc shell lesions on the acquisition of responding with CR. Data points represent the mean square root ± SEM responses on the lever producing the conditioned reinforcer (*CR*) and the control lever (*NCR*) for sham- and shell-lesioned animals after intra-NAcc injections of D-amphetamine (0, 1, 3, 10, and 20 μg).

in the acquisition of a new response with CR, but these lesions abolished the potentiative effect of intra-NAcc D-amphetamine. Thus, the CS successfully acted as a conditioned reinforcer for operant lever pressing in all groups. D-amphetamine selectively potentiated responding with CR in sham-lesioned animals only,

whereas there was less control over this behavior by the conditioned reinforcer after NAcc core lesions and a complete abolition of the potentiative effects of D-amphetamine in animals with NAcc shell lesions.

Discriminated approach to the magazine during CR testing

ANOVAs were used to compare the duration and frequency of magazine entries during CR testing. Under control vehicle treatment there was a significant main effect of lesion on duration of magazine entries ($F_{(2,21)} = 4.31$; $p < 0.05$) caused by the fact that core-lesioned animals spent significantly more time at the magazine than either sham- or shell-lesioned animals. Statistical analysis of the effect of D-amphetamine on the duration of magazine approaches revealed a significant lesion × dose interaction ($F_{(8,84)} = 8.15$; $p < 0.0001$) as the duration of approaches was reduced in both the core- and sham-lesioned animals equivalently and dose-dependently, whereas the shell-lesioned group showed a dose-dependent increase in approach duration.

Analysis of the frequency of magazine entries revealed no significant group differences after saline injections ($F_{(2,21)} = 2.73$; $p = 0.09$). Comparisons of the effect of D-amphetamine on the frequency of magazine approaches showed no significant lesion × dose interaction ($F_{(3,32)} = 1.95$; $p = 0.14$) or main effect of dose ($F_{(2,32)} = 0.8$; $p = 0.43$). The significant main effect of lesion ($F_{(2,21)} = 5.16$; $p < 0.05$) was caused by a reduced frequency of approaches made by shell-lesioned animals across doses of D-amphetamine relative to both sham- and core-lesioned animals.

In summary, core-lesioned animals showed an increase in the duration of magazine approach under saline treatment. Furthermore, compared with both sham- and core-lesioned animals, animals with NAcc shell lesions showed a general reduction in the frequency of magazine entries and a dose-dependent increase in the duration of magazine entry after D-amphetamine injections, perhaps reflecting an overall reduction in locomotor activity by these animals.

Locomotor activity

Figure 8 shows the activity levels of shell-, core-, and sham-lesioned animals during habituation sessions and after each drug injection. ANOVA comparing locomotor scores of the three groups across habituation sessions revealed no lesion × session interaction ($F_{(4,93)} = 0.59$; $p = 0.69$). Activity levels of all three groups declined over the four habituation sessions (main effect of session, $F_{(2,93)} = 39.03$; $p < 0.001$), but there was also a significant main effect of lesion ($F_{(2,42)} = 8.97$; $p = 0.001$). *Post hoc* tests revealed that this was caused by the increased activity of core-lesioned animals compared with both sham- and shell-lesioned animals.

A one-way ANOVA comparing activity levels after saline injections revealed a significant effect of lesion ($F_{(2,43)} = 6.81$; $p = 0.003$), and *post hoc* tests indicated that the core-lesioned group's activity was significantly higher than that of shell- and sham-lesioned groups (which were not different from one another). Because of this baseline difference, a multivariate analysis of covariance was undertaken to compare activity levels after the three systemic drug injections, using the activity levels after saline injections as the covariates. This analysis revealed that activity levels after injections of 0.5 and 1.5 mg/kg D-amphetamine differed significantly from saline ($F_{(1,41)} = 17.31$; $p < 0.001$ and $F_{(1,41)} = 14.20$; $p = 0.001$, respectively), but not after the 5.0 mg/kg D-amphetamine injection ($F_{(1,41)} = 0.62$; $p = 0.44$). Thus, activity was significantly elevated in all groups after 0.5 and 1.5 mg/kg injections but not after 5.0 mg/kg. There was also a lesion × drug interaction ($F_{(2,41)} = 7.11$; $p = 0.002$) at the 1.5 mg/kg dose. *Post hoc* analysis of this interaction revealed that the activity scores of the core-lesioned rats were significantly higher than both shell- and sham-lesioned groups, whereas the shell-lesioned group showed significantly lower locomotor activity scores than shams. A repeated-measures ANOVA using the percentage change from baseline activity levels corroborated the results from the multivariate analysis of covariance.

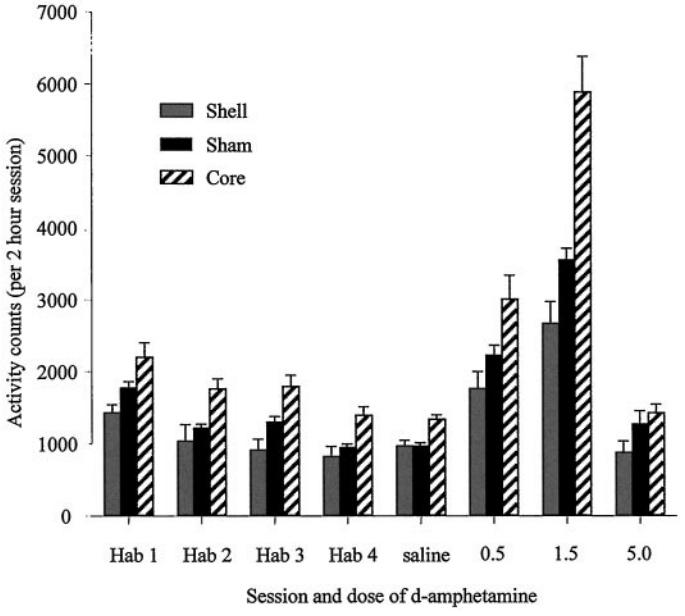

Figure 8. Effect of NAcc core and shell lesions on spontaneous and D-amphetamine-induced locomotion. *Vertical bars* represent the mean ± SEM photocell beam breaks for each group during habituation sessions (*Hab*) and after systemic injections of D-amphetamine (*saline*, 0.5, 1.5, 5.0 mg/kg).

Table 1. Effect of NAcc core and shell lesions on stereotypy

Group	30	60	90	120
Sham	4.33	4.66	4.29	3.33
Core	3.67	4.68	4.37	3.37
Shell	4.16	4.83	4.66	3.5

Mean stereotypy scores during each 30 min interval of a 2 hr test after a 5.0 mg/kg D-amphetamine injection.

Ratings of stereotypy were taken (ranging from 0 to 8; see Mittleman et al., 1991) at 30, 60, 90, and 120 min during the final (5.0 mg/kg) test session. For example, a score of 0 was given for inactivity (of behavior), 2 for continuous locomotor activity, 4 for continuous stereotypy over a wide area, and 6 for pronounced, continuous stereotypy in a restricted area. Average stereotypy scores for each group over the 2 hr session are shown in Table 1. Because of the nature of these data, nonparametric tests were used to analyze stereotypy ratings. Thus, a Kruskal–Wallis one-way ANOVA assessed group differences at each of the four time points. There were no significant group differences at any time point (critical value for χ^2 at $p < 0.05 = 5.99$; calculated values were 5.22 at 30 min, 0.19 at 60 min, 1.09 at 90 min, and 0.18 at 120 min). Thus, whereas all animals demonstrated stereotypy after a systemic injection of 5.0 mg/kg D-amphetamine, there were no differences between groups.

In summary, animals with lesions of the core were more active than their sham-lesioned controls during a test of spontaneous locomotor activity. Furthermore, their locomotor response to systemic D-amphetamine was significantly enhanced relative to sham controls. Thus, lesions of the core produced an enhancement of the locomotor potentiative effects of D-amphetamine. In contrast, rats with lesions of the shell were less active than sham-lesioned controls during the measurement of spontaneous locomotor activity, and the potentiative effects of systemic D-amphetamine were significantly attenuated in these animals (at the 1.5 mg/kg dose).

DISCUSSION

To investigate the possibly dissociable functions of the NAcc core and shell, we have developed excitotoxic amino acid-induced lesions that selectively destroy these two regions and investigated their effects on two fundamental effects of psychomotor stimulant drugs; the potentiation of conditioned reinforcement and locomotor hyperactivity, as well as appetitive Pavlovian behavior and the acquisition of instrumental responding with CR. Lesions of the NAcc shell completely abolished the potentiation of instrumental behavior with CR after intra-NAcc infusions of D-amphetamine. Shell lesions also produced locomotor hypoactivity and attenuated D-amphetamine-induced increases in locomotor activity. By contrast, in core-lesioned animals, intra-NAcc D-amphetamine infusions dose-dependently increased responding on CR and NCR (control) levers, thus demonstrating intact stimulant potentiation combined with a reduction of stimulus control. Core lesions also produced locomotor hyperactivity and enhanced the locomotor-stimulating effect of systemic D-amphetamine. NAcc shell lesions affected neither Pavlovian conditioning nor CR as assessed in the acquisition of a new response procedure. In contrast, NAcc core lesions impaired discriminated approach to a Pavlovian-conditioned stimulus but did not affect the acquisition of a new instrumental response with CR.

These findings indicate basic interactions between the dopamine-dependent effect of stimulants such as amphetamine and associative information that we have shown to be dependent on limbic afferents to the NAcc (Cador et al., 1989). They also indicate that different aspects of the learned control over appetitive behaviors are mediated by distinct regions within the NAcc, as revealed here by demonstrating a double dissociation between the effects of lesions of the NAcc shell and core on responses to amphetamine and associative learning mechanisms.

Effects of NAcc shell and core lesions on responses to amphetamine

NAcc shell lesions completely abolished the potentiative effects of intra-NAcc D-amphetamine on the control over behavior by a conditioned reinforcer. Shell lesions also resulted in hypoactivity during the habituation sessions to locomotor activity cages and attenuated the stimulant effect of systemic D-amphetamine. These results suggest that a major property of stimulant drugs to potentiate both the impact of motivationally relevant environmental cues on instrumental behavior and locomotor activity rely critically on the integrity of the NAcc shell.

These findings are consistent with reports of changes in DA transmission selectively within the NAcc shell (relative to the NAcc core or the dorsal striatum), in response to intravenous infusions of several drugs of abuse (Pontieri et al., 1995, 1996; Carlezon and Wise, 1996; Tanda et al., 1997) and selective increases in energy metabolism as measured by 2-deoxyglucose autoradiography in the NAcc shell produced by such drugs (Pontieri et al., 1994; Orzi et al., 1996). Highly palatable, preferred foods also increase DA selectively in the NAcc shell (Tanda et al., 1994) as do Pavlovian CSs paired with food (Phillips et al., 1993; Wilson et al., 1995). Such observations (Robbins and Everitt, 1992) have led authors to suggest a role for NAcc DA in incentive motivation (Phillips and Fibiger, 1987) and reward (Wise and Bozarth, 1987) and more specifically, a role for DA in the shell in the attribution of incentive properties to CSs (DiChiara, 1998), with drugs of abuse usurping this process, thereby producing abnormal "incentive learning". Although the NAcc shell is clearly implicated in aspects of responding to both drug-related and natural reinforcers, the present findings of intact Pavlovian and instrumental conditioning in NAcc shell-lesioned animals suggest that, rather than an associative or incentive motivational role, a key function of the dopaminergic innervation of the NAcc shell is to potentiate ongoing instrumental responding in the presence of motivationally significant stimuli.

Lesions of the central nucleus of the amygdala (CeA) also block the potentiative effects of intra-NAcc D-amphetamine on responding with CR (Burns et al., 1993; Robledo et al., 1996). Although the CeA does not project directly to the NAcc, it may influence striatal DA function via its projections to midbrain DA neurons (Simon et al., 1979; Wallace et al., 1992; Han et al., 1997). Alternatively, the NAcc shell and CeA may be functionally related as part of the continuum known as the extended amygdala (Heimer et al., 1991; Alheid et al., 1995). Dopamine-depleting lesions of the amygdala (Simon et al., 1988), as well as infusions of D_1 receptor antagonists (Hurd et al., 1997) both profoundly alter dopamine concentration or release in the NAcc, indicating the tight functional relationship between these components of the extended amygdala.

Lesions of the ventral subiculum block the potentiative effects of D-amphetamine on locomotor activity and abolish the effects of intra-NAcc D-amphetamine without affecting the impact of the CR on instrumental performance (Burns et al., 1993), much like the shell lesions in the present study. This similarity in the functional effects of NAcc shell and ventral subiculum lesions is significant in the context of the strong preferential glutamatergic projection from the ventral subiculum to that part of the NAcc shell (septal pole) that was lesioned here (Fig. 2). Thus, information reaching the NAcc concerned with the nature and direction of behavior, which depends on the integrity of the basolateral amygdala (BLA) (Cador et al., 1989; Burns et al., 1993), presumably via its projections to both the NAcc core and shell (Groenewegen et al., 1987) may be "gain-amplified" by dopamine transmission in the shell in a way that is critically dependent on the integrity of its glutamatergic inputs arising from the ventral subiculum (Burns et al., 1993; Blaha et al., 1997; Brudzynski and Gibson, 1997).

Although NAcc core lesions did not affect the acquisition of responding with CR under control (saline) conditions, the interaction between intra-NAcc D-amphetamine and responding with CR appears to depend on the integrity of the NAcc core. It is of interest, therefore, that animals with lesions of the BLA show similar, although greater, impairments in the same task, including a loss of control over responding for the CR under control conditions (Cador et al., 1989; Burns et al., 1993). Similarities in the effects of NAcc and BLA manipulations have been reported previously (Everitt et al., 1989, 1991; Everitt and Robbins, 1992) and have led us to suggest that the integrity of the BLA is critical for stimulus–reward information to gain influence over voluntary

behavior (Everitt et al., 1991; Burns et al., 1993; Robbins and Everitt, 1996). Thus, limbic corticostriatal circuits involving the BLA and NAcc core may be essential for the influence of associative stimulus–reward information on goal-directed action. The effects of NAcc shell lesions to abolish the potentiative effects of intra-NAcc D-amphetamine, whereas NAcc core lesions disrupt discriminative control after intra-NAcc infusions of D-amphetamine are reminiscent of models of striatal function based on separate striatal zones being responsible for behavioral "choice" and "vigour" (Kelly and Moore, 1976; Robbins and Everitt, 1982; Koshikawa et al., 1996). Such models can now be refined both behaviorally and neuroanatomically on the basis of the functional dissociations revealed in the present and related studies (Balleine and Killcross, 1994; Kelley et al., 1997) and the neuronal interactions known to occur in shell and core compartments of the striatum determined by the pattern of termination of discrete limbic cortical afferents (Pennartz et al., 1994).

NAcc core lesions also resulted in increased spontaneous locomotor activity and an enhanced response to systemic D-amphetamine. This may reflect motoric response disinhibition after core lesions resulting from reductions in inhibitory striatal GABAergic outflow to several striatal output target structures directly concerned with the control of locomotor activity (Alexander and Crutcher, 1990) that are also further susceptible to the effects of DA release within remaining parts (shell) of the NAcc. Similar mechanisms may underlie the significant increase in responding on the NCR control lever during the conditioned reinforcement procedure.

Effects of NAcc shell and core lesions on associative learning mechanisms

Animals with lesions of the NAcc shell were not impaired in Pavlovian conditioning as assessed by their discriminated approach to a Pavlovian CS+, or in the acquisition of responding with CR. Intra-NAcc infusions of D-amphetamine also dose-dependently increased magazine approach duration, relative to NAcc core- and sham-operated groups, supporting further the selective effects of D-amphetamine in the NAcc core on the mechanisms subserving discriminated approach. Thus, the NAcc shell does not appear to be significantly involved in associative learning mechanisms assessed in this study. Lesions of the NAcc core, by contrast, retarded the expression of the CS–US association such that animals in this group showed a decrease in levels of discriminated approach relative to prelesion performance. It may seem paradoxical that core-lesioned animals who showed a deficit in discriminated approach subsequently acquired responding with CR. However, Kelley et al. (1997) reported similar effects with blockade of NMDA receptors in the NAcc. Furthermore, we have demonstrated previously that disruptions in the formation of a CS–US association do not necessarily produce deficits in the acquisition of a new response for the Pavlovian-paired stimulus (Olmstead et al., 1998) and vice versa (Burns et al., 1993). Our results confirm, therefore, that the neural mechanisms through which conditioned reinforcers control instrumental behavior are dissociable neurally from processes that mediate the expression of a CS–US relationship through Pavlovian approach behavior (Cador et al., 1989; Burns et al., 1993).

Implications for the functions of the nucleus accumbens

There are two major findings in this study: (1) the NAcc shell mediates both the potentiation by D-amphetamine of the control over instrumental responding by conditioned reinforcers and of locomotor activity; (2) the NAcc core is involved in the conditioned preparatory aspects of Pavlovian associative learning and may also modulate the associative control over instrumental responding after intra-NAcc D-amphetamine. These results, therefore, indicate that there is functional specificity within the NAcc and its associated circuitries. The potentiative effects on behavior of D-amphetamine are critically dependent on the NAcc shell, whereas the expression or potentiation of Pavlovian conditioned responses generated by the presentation of incentive stimuli (Rescorla and Solomon, 1967; Dickinson and Balleine, 1994) depends on the integrity of the NAcc core. Furthermore, control over goal-directed actions and incentive motivation per se may depend on functional interactions between limbic cortical structures, including the BLA, cingulate, and prefrontal cortex, which may interface with different striatal zones (Cador et al., 1989; McAlonan et al., 1993; Bussey et al., 1997; Floresco et al., 1997). Thus, different limbic corticostriatal afferents passing through the NAcc within ventral striatopallidal circuits may be involved in qualitatively different functional processes but may undergo similar modulation at the level of the NAcc.

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