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Differential control over cocaine-seeking behavior by nucleus accumbens core and shell

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Nucleus accumbens (NAc) dopamine is widely implicated in mediating the reinforcing effects of drugs of abuse. However, the precise function of the NAc itself in drug self-administration has been difficult to establish. Here we show a neural double-dissociation of the behavioral processes that underlie cocaine self-administration in rats. Whereas selective excitotoxic lesions of the NAc core had only a minor effect on the acquisition of responding for cocaine under a standard schedule of continuous reinforcement, these lesions profoundly impaired the acquisition of drug-seeking behavior that was maintained by drug-associated conditioned reinforcers and assessed using a second-order schedule of cocaine reinforcement. In contrast, selective excitotoxic lesions of the NAc shell did not impair drug self-administration or the acquisition of cocaine-seeking, but they did attenuate the psychostimulant effects of cocaine. These results further our understanding of how the NAc controls drug-seeking and drug-taking behavior.

Much evidence supports the hypothesis that the dopamine innervation of the nucleus accumbens (NAc) is a key neural substrate mediating the primary reinforcing and psychomotor stimulant effects of drugs of abuse. Intravenous cocaine self-administration is reduced by 6-hydroxydopamine (6-OHDA)-induced dopamine depletion from the NAc[1–3]. Infusions of dopamine receptor agonists and antagonists directly into the NAc alter rates of intravenous drug self-administration, as if rats are compensating for changes in the reinforcing effects of the drug[4,5]. Furthermore, extracellular dopamine in the NAc is consistently increased in response to experimenter-delivered or self-administered cocaine, amphetamine, nicotine, opiates and ethanol in rats and in primates[6–10]. The glutamatergic innervation of the NAc has also been implicated in modulating drug self-administration behavior, though less clearly so[11,12].

Given the evidence that various neural systems innervating the NAc contribute to drug self-administration, it is perhaps surprising that excitotoxic lesions of the NAc itself, which destroy its medium spiny neuron output and other intrinsic neurons, have variable, and often no, effects on drug self-administration[13–16]. This leaves some measure of doubt about the NAc having an essential role in drug reinforcement.

In resolving this issue, it is important to bear in mind that drug self-administration probably depends on a complex interaction of several distinct behavioral processes. It involves conditioning as well as the unconditioned effects of the drug itself—these factors may underlie different aspects of drug-seeking and drug-taking behavior[17,18]. Thus, data from humans and animals indicate that environmental stimuli previously associated with self-administered drugs may potently affect subjective (*i.e.*, craving) as well as behavioral measures of drug-seeking behavior and relapse[19–25]. Moreover, these conditioned and unconditioned effects of drugs are neurally dissociable. For example, lesions of the basolateral amygdala do not affect drug self-administration, but they do prevent the acquisition of cocaine-seeking behavior under the control of drug-associated stimuli[26] and reinstatement of drug-seeking after extinction[27].

Distinguishing among these conditioned and unconditioned processes in self-administration protocols is especially important in light of evidence that the NAc itself is a heterogeneous structure with at least two distinct regions, shell and core[28,29], that may contribute in different ways to drug self-administration[30–33]. There is strong evidence that the NAc core is involved in the control of goal-directed behavior by associative processes, consistent with its central position within limbic cortical-ventral striatal circuitry[34]. For example, selective dopaminergic or excitotoxic lesions of the NAc core, but not the shell, disrupt learnt Pavlovian influences on appetitive behavior[35–37]. One of the important ways in which Pavlovian conditioned stimuli influence behavior is as conditioned reinforcers that can, by themselves, support instrumental behavior such as drug-seeking[23]. Excitotoxic lesions of the NAc core disrupt the capacity of food-associated conditioned reinforcers to control behavior, whereas lesions of the NAc shell specifically impair the mechanism by which drugs such as cocaine and d-amphetamine enhance the effects of such stimuli[38]. Moreover, intra-NAc shell infusions of amphetamine enhanced the motivational effects of Pavlovian conditioned stimuli on instrumental behavior (Pavlovian-to-instrumental transfer, a form of Pavlovian arousal[39]).

The aim of the present study was to investigate the extent to which the NAc core and shell contribute to conditioned, as well as unconditioned, influences that govern drug-seeking and drug-taking. To this end, we not only studied drug self-administration under standard

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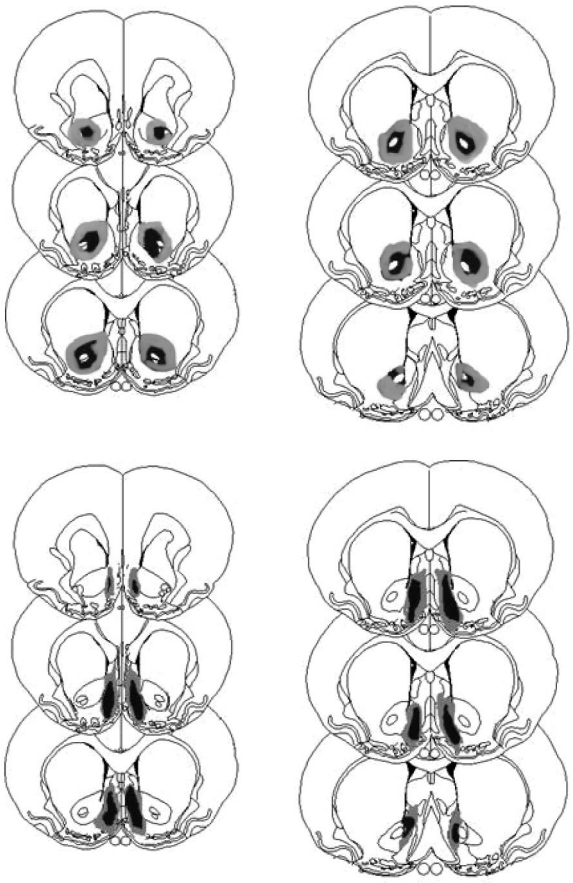

Figure 1 Excitotoxic lesions of the NAc core and shell. Schematic representation of quinolinic acid lesions of the NAc core (top) and ibotenic acid lesions of the NAc shell (bottom). Areas shaded in gray and black represent the largest and smallest extent of neuronal damage in a single animal, respectively. Coronal sections are +2.2 mm anterior through +0.48 mm posterior to bregma[49].

conditions of continuous reinforcement, where every instrumental response is followed by a contingent cocaine infusion, but we also used a procedure in which drug-seeking becomes increasingly under the control of drug-associated conditioned reinforcers (in a so-called second-order schedule of reinforcement[23]). We found that selective excitotoxic lesions of the NAc core profoundly disrupted the acquisition of cocaine-seeking behavior when this behavior was substantially under the control of drug-associated conditioned reinforcers. Although lesions of the NAc shell did not impair drug self-administration or the acquisition of cocaine-seeking, they did attenuate the response rate–enhancing (or psychostimulant) effects of cocaine. These findings imply a neural dissociation between the mechanisms underlying the associative control of drug-seeking and those underlying the psychomotor stimulant effects of cocaine. The present results enhance our understanding of how the NAc controls drug-seeking and drug-taking behavior.

RESULTS

Cocaine self-administration under continuous reinforcement

Intravenously catheterized rats with selective excitotoxic lesions of the NAc core or shell regions, and their sham-operated controls (**Figs. 1** and **2**), were initially trained daily for 2 h to acquire cocaine self-administration under a continuous reinforcement schedule. Response on one of two identical levers (active lever) led to a contingent infusion of cocaine (0.25 mg per infusion). They were deemed to have acquired the task when stable responding with less than 10% variance across three consecutive days was achieved.

All three treatment groups (core, shell and sham) reached criterion levels of responding within 10 d of beginning cocaine self-administration under a continuous reinforcement schedule (**Fig. 3a**). Three-way ANOVA of square-root transformed lever presses during acquisition revealed a significant group × lever × day interaction ($F_{18,315} = 1.62$, $P < 0.05$) and a significant main effect of lesion group ($F_{2,65} = 6.24$, $P < 0.005$). Separate analyses of the pattern of responding on the active and inactive levers using two-way ANOVA followed by Newman-Keuls multiple comparisons revealed that the core-lesioned rats responded at a significantly higher rate on the active lever on days 1 and 2 only ($P < 0.01$) compared to control rats (group × day interaction, $F_{9,261} = 2.61$, $P < 0.01$; **Fig. 3b**). There was no difference between sham and shell groups in responding on the active lever ($F_{1,20} = 0.09$, $P = 0.76$). Inactive lever responses in the core-lesioned group were slightly, but significantly, increased overall compared with those in the sham group (group effect, $F_{1,29} = 7.01$, $P < 0.01$; **Fig. 3a**), whereas there was no significant group effect on inactive lever responding between shell and sham groups ($F_{1,21} = 1.91$, $P < 0.18$). Nevertheless, significantly more responses were made on the active lever, as compared to the inactive lever, in all groups (lever, $F_{1,35} = 276.03$, $P < 0.0001$).

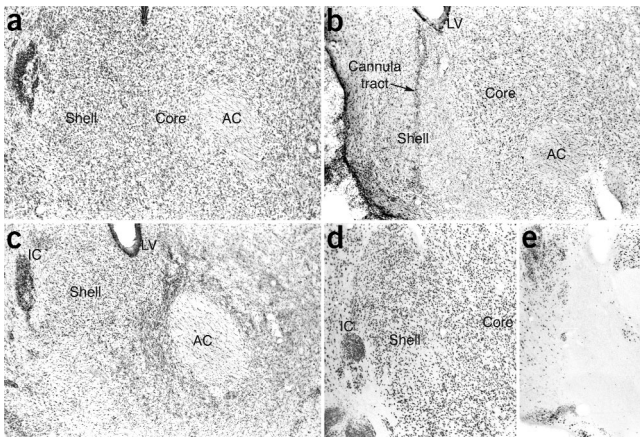

Figure 2 Representative photomicrographs showing Cresyl Violet-stained and NeuN-stained coronal sections through the NAc in rats with NAc shell or core lesions and sham-operated control subjects. (**a**) Nissl-stained section through the NAc of a control subject, showing the region of the shell and core and other markers at this antero-posterior level (island of Calleja, lateral ventricle and anterior commissure). (**b**) Nissl-stained section of a NAc shell lesion, showing the marked loss of staining in the shell region and preservation of neurons in the core region, as well as the infusion cannula tract. (**c**) Nissl-stained section of a NAc core lesion; marked gliosis can be seen around the medial surface of the anterior commissure. Note the apparent medial shift of the anterior commissure, which is the result of the loss of neurons in the core region. The integrity of the shell region is preserved. (**d**,**e**) NeuN-stained sections through the shell region of sham (**d**) and shell-lesioned subjects. Note the complete disappearance of NeuN-immunoreactivity in the shell region (compare **e** with **d**), indicating the loss of neurons in this region of the NAc following infusion of ibotenic acid. Abbreviations: AC, anterior commissure; Core, NAc core; Shell, NAc shell; LV, lateral ventricle; IC, Isle of Calleja.

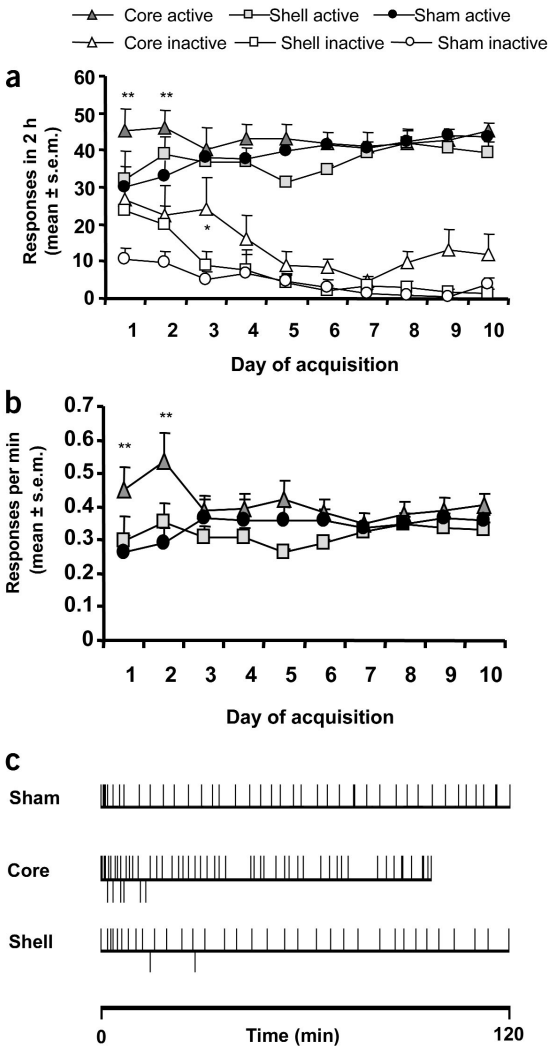

Figure 3 Acquisition of intravenous cocaine self-administration under a continuous reinforcement schedule. (**a**) Mean (±s.e.m.) number of responses on the active and inactive lever during each 2 hr session. $*P < 0.05$, $**P < 0.01$, compared to sham. (**b**) The rate of responding (per min) on the active (drug-paired) lever in each session, after sham or excitotoxic lesions of the NAc core and shell regions. $**P < 0.01$ compared to sham. (**c**) Representative response records of individual rats from NAc sham, core and shell lesion groups on the last day of acquisition of cocaine self-administration under a continuous reinforcement schedule. Each bar above the horizontal line represents an individual response on the active lever, whereas each bar below the line represents a response on the inactive lever.

Typical response records on the last day of acquisition (day 10) in sham, core and shell-lesioned rats (**Fig. 3c**) showed that response patterns in both sham and shell groups were characterized by an initial, rapidly occurring burst of cocaine self-administration ('loading' phase) followed by a period of stable 'titrating pattern,' evenly spaced responses on the active lever. Responding in core-lesioned rats, however, had the following characteristics: (i) higher response rates, such that core-lesioned rats gained the maximum number of cocaine infusions (50) significantly earlier in the session than the shell and sham lesioned groups across the 10 d of acquisition; (ii) for the post-reinforcement pause (PRP; the period after each cocaine infusion and before the first response made subsequently, which provides a measure of the impact of cocaine reinforcement), there were no significant differences, indicating unimpaired control over instrumental responding by cocaine (sham, 2.71 (mean PRP (min) on day 10) ± 0.19 (s.e.m.); core, 2.73 ± 0.26; shell, 3.07 ± 0.25; $F_{2,37} = 0.60$, nonsignificant, n.s.)

In summary, core-lesioned rats showed only minor changes in the acquisition of cocaine self-administration. They had somewhat higher rates of responding on the active lever, compared with the sham and shell groups, on the first two days of acquisition only. In addition, responding on the inactive lever by core-lesioned rats was inconsistently elevated, thereby reducing discrimination between the active and inactive levers on some days of testing. By contrast, there was no difference between the sham and shell groups in the pattern of acquisition of cocaine self-administration.

Cocaine dose-response function

In all three groups, variations in the dose of cocaine produced orderly, monotonic changes in the rates of responding on the active lever, with evidently more persistent responding in extinction (*i.e.*, under saline) in the core-lesioned rats (**Fig. 4**). ANOVA with repeated measures on response rates revealed significant main effects of dose ($F_{4,120} = 41.24$, $P < 0.0001$), lever ($F_{1,30} = 171.21$, $P < 0.0001$) and lesion ($F_{2,30} = 5.59$, $P < 0.01$), with significant group × lever × dose ($F_{8,120} = 2.14$, $P < 0.04$) and dose × lesion ($F_{8,120} = 5.36$, $P < 0.001$) interactions. Separate ANOVA on response rates on the active lever at each dose revealed significant main effects of lesion group in the saline ($F_{2,17} = 12.31$, $P < 0.001$), 0.25 mg/infusion ($F_{2,17} = 7.40$, $P < 0.005$) and 0.5 mg/infusion ($F_{2,17} = 9.05$, $P < 0.003$) conditions. Newman-Keuls pairwise comparisons revealed that core-lesioned rats responded at significantly higher rates than sham and shell group animals on the active lever in extinction (*i.e.*, on saline substitution) and at marginally, yet significantly, higher rates at doses of 0.25 and 0.5 mg/infusion. One-way ANOVAs on response rates on the inactive lever revealed no significant lesion group effect in any of the conditions.

Cocaine-seeking under a second-order schedule of reinforcement

Once cocaine self-administration under continuous reinforcement had been acquired, training began under the second-order schedules, where the response requirement for cocaine and the conditioned reinforcer was progressively increased (see Methods). It was determined that each rat would have to satisfy a certain criterion in order to move on to the next stage of training. This criterion was to obtain at least ten cocaine infusions per session at each schedule stage for three consecutive days. Significantly more core-lesioned animals failed to reach criterion at each stage beyond FR10(FR4:S), the core group size diminishing from 15 at FR10(FR1:S) to 8 at FR10(FR10:S) (**Fig. 5**). Responding on the active lever increased in all groups as the schedule requirement was progressively increased across days (day; $F_{1,35} = 713.41$, $P < 0.0001$). However, overall ANOVAs showed that the groups responded at significantly different rates (group, $F_{2,113} = 4.00$, $P < 0.0001$; group × day $F_{2,35} = 10.80$, $P < 0.0002$). Further analyses by lever and training stage separately revealed significantly lower responding on the active lever in the core-lesioned group compared to controls under FR10(FR2:S) ($F_{1,124} = 6.04$, $P < 0.02$), FR10(FR4:S) ($F_{1.116}$=7.28, $P < 0.02$), FR10(FR7:S) ($F_{1,104}$=11.69, $P < 0.002$) and FR10(FR10:S) ($F_{1,96} = 17.06$, $P < 0.0004$) schedules. Responding on the active lever in the shell-lesioned group was significantly lower than the controls under the FR10(FR10:S) schedule ($F_{1,88} = 10.40$, $P < 0.004$), but not at other stages. By contrast, responding on the inactive lever in the core group was significantly more elevated than that in the sham group ($F_{1,1056} = 7.48$, $P < 0.01$), at all stages of the

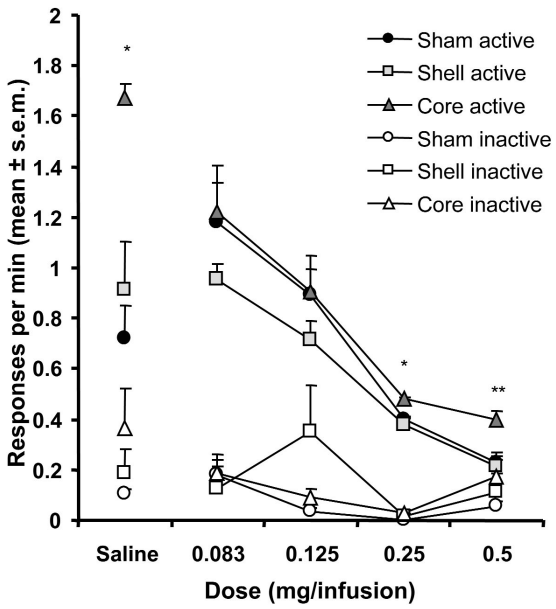

Figure 4 Cocaine dose-response function. Between-sessions dose-response function and effects of substituting saline for cocaine (responding in extinction) in sham-lesioned and NAc core- and shell-lesioned rats. Error bars, ±s.e.m. $^*P < 0.05$.

second-order schedules of reinforcement. Responding on the inactive lever in the shell group, however, was not significantly different from that of the sham group ($F_{1,786} = 1.24$, n.s.).

Pattern of responding under FR10(FR10:S)

The response patterns of the core-lesioned rats differed from those of the sham- and shell-lesioned rats in three main ways (**Fig. 6a**,**b**): (i) whereas sham- and shell-lesioned rats showed rapid burst-like patterns of responding on the active lever, core-lesioned rats exhibited temporally dispersed patterns of responding; (ii) core-lesioned rats took much longer to complete the FR10(FR10:S) response requirement before and even after the first cocaine infusion than did sham- and shell-lesioned rats; (iii) the sham- and shell-lesioned rats showed a regular 'titrating' pattern of responding, wherein a period of inactivity followed each cocaine infusion (post-reinforcement pause, PRP); no such regular pauses were observed in core-lesioned rats.

Post-reinforcement pause

The PRP is the period following the reinforcer delivery and before the start of the next ratio of responding, often taken to represent the rewarding impact of the drug (**Fig. 7a**). ANOVA revealed significant main effects of group ($F_{2,1836} = 569.39$, $P < 0.0001$) and training stage ($F_{4,1824} = 7.83$, $P < 0.0001$) as well as a significant group × training interaction ($F_{8,1824} = 41.64$, $P < 0.0001$) in the mean duration of the PRP within a 2-h self-administration session at each of the different second-order schedule requirements in sham, core- and shell-lesioned rats. In both sham- and shell-lesioned groups, the duration of the PRP increased as the response requirement rose at each stage of the second-order schedule. *Post-hoc* analysis showed the PRP duration in the core-lesioned rats to be significantly shorter than in the sham controls and shell groups at all second-order schedules of cocaine reinforcement tested ($P < 0.01$).

Post-conditioned reinforcement pause

The post-conditioned reinforcement pause (PCRP) is the period between the brief presentation of the conditioned reinforcer and the first response made subsequently. It provides a measure of the impact of the conditioned stimulus acting as a conditioned reinforcer (**Fig. 7b**). Two-way ANOVA with lesion as the between-group factor and cocaine state (pre-cocaine versus post-cocaine) as the repeated measure showed significant main effects of group ($F_{2,280} = 3.26$, $P < 0.04$) and state ($F_{1,277} = 15.36$, $P < 0.0001$) but no interaction between these factors ($F_{2,277} = 2.56$, $P < 0.08$, n.s.) in the mean PCRP duration of the first 200 responses under the FR10(FR10:S) schedule of cocaine reinforcement in the three groups. *Post hoc* analysis revealed that PCRP pauses of core-lesioned rats in the drug-free state were significantly shorter compared with the shell and sham groups ($P < 0.01$). In addition, the PCRP duration during responding for the first infusion was significantly higher compared to that during responding under the influence of cocaine for the second infusion, in shell and sham groups, but not in the core-lesioned group. Thus, core-lesioned rats showed significantly shorter PCRP pauses before a cocaine infusion, compared to the sham- and shell-lesioned rats, but the PCRP duration remained unchanged after a cocaine infusion.

Rate of responding on the active lever

The effects of NAc lesions on the mean rate of responding before and after the first cocaine infusion during three sessions under the FR10(FR10:S) schedule were also investigated (**Fig. 7c**). Two-way ANOVA showed significant main effects of group ($F_{2,28} = 10.3$, $P < 0.0001$) and cocaine state ($F_{1,28} = 103.2$, $P < 0.0001$), and significant interaction between these factors ($F_{2,28} = 7.6$, $P < 0.002$). Separate between-subject one-way ANOVAs revealed that the rate of responding during the pre-cocaine period was not significantly different between the lesion groups ($F_{2,29} = 2.42$, $P = 0.11$), whereas it was for the post-cocaine state ($F_{2,29} = 12.08$, $P < 0.0001$). Separate within-subject one-way ANOVAs showed the difference in the rate of responding pre- and post-cocaine to be significant only for the core and sham groups (core, $F_{1,5} = 14.85$, $P < 0.006$; sham, $F_{1,15} = 303$, $P < 0.0001$; shell, $F_{1,5} = 4.36$, $P = 0.10$, n.s.). Therefore, significant cocaine-induced increases in response rate were evident in sham and core-lesioned, but not in shell-lesioned rats.

In summary, core-lesioned rats were profoundly impaired in the acquisition of drug cue–controlled cocaine-seeking behavior. Only half of the core-lesioned group completed the training, and the overall number of responses made on the active lever as well as the rates of responding were significantly reduced at all stages of training under the second-order schedule in these rats, as compared to sham-lesioned rats. Moreover, the pattern of responding of core-lesioned rats showed a marked absence of PRPs and significantly shorter PCRP pauses compared to the sham rats. In contrast, lesions of the NAc shell did not significantly affect the acquisition of cocaine-seeking behavior. However, shell-lesioned rats showed significantly smaller increases in the rate of responding in the periods after cocaine intake, as compared to sham- and core-lesioned rats.

Cocaine-induced locomotor activity

We also recorded locomotor activity during cocaine self-administration sessions under the FR1 schedule on days 1–3 and 10 (**Fig. 8**). Two-way ANOVA revealed a significant main effect of group ($F_{2,17} = 4.75$, $P < 0.02$) but no significant interaction between the day of acquisition and group. A subsequent *post-hoc* repeated measures ANOVA showed that cocaine-induced activity measures of the

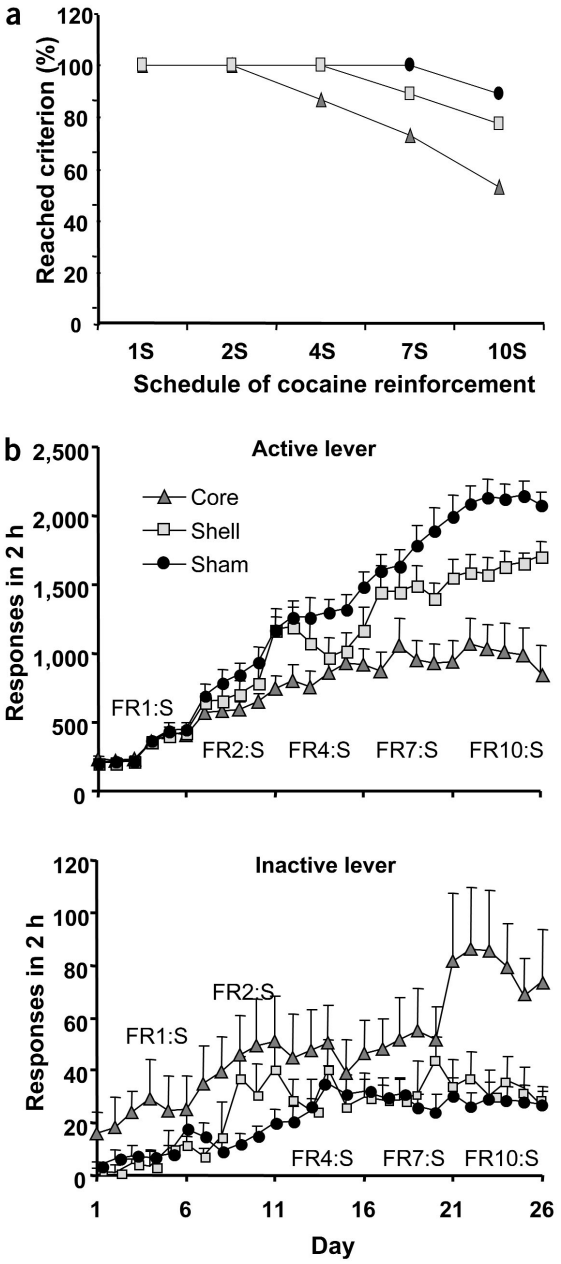

Figure 5 Acquisition of cocaine self-administration under the second-order schedule. (**a**) The proportion of sham, core and shell-lesioned rats attaining criterion at each successive stage of acquisition of the second-order schedule of cocaine reinforcement. Abbreviations: *y*S = FR10(FR*y*:S). The criterion was set as obtaining at least ten cocaine infusions within a 2-h self-administration session for three consecutive days. (**b**) Mean (±s.e.m.) responses on the active and inactive lever at each stage of the second-order schedule of intravenous cocaine reinforcement in rats with lesions of the NAc core and shell.

core-lesioned rats ($P < 0.023$), but not shell-lesioned rats, were significantly higher than those of the shams.

DISCUSSION

NAc core and cocaine-seeking

The present data provide evidence for an important role of the core region of the nucleus accumbens in cocaine-seeking behavior and help to resolve previous data showing only variable effects of lesions of this structure on drug self-administration behavior. NAc core-lesioned rats were impaired in the conditioned control over cocaine-seeking, similar to the disruptive effects of NAc core lesions on Pavlovian approach behavior[35], Pavlovian-to-instrumental transfer[36] and conditioned reinforcement[38]. The results are also consistent with our previous finding that lesions of the NAc core impair the capacity of a food-related conditioned reinforcer to acquire discriminative control over instrumental behavior[38]. Taken together with the evidence that lesions of the basolateral amygdala (BLA) also impair the ability of a food-associated conditioned reinforcer to support the acquisition of a new instrumental response[40,41], the NAc core may also be a component of the neural circuitry involved in behavioral selection based on reward-related information derived from conditioned reinforcers, mediated via amygdaloid or other limbic cortical afferents to the NAc[34]. Indeed, selective lesions of the BLA produce a similar pattern of relative sparing of continuously reinforced cocaine self-administration, but a failure to acquire drug cue–controlled cocaine-seeking behavior[26].

The deficits observed in cocaine-seeking by core-lesioned rats were unlikely to have been due to an inability to form effective stimulus-reward associations, as post-training NAc core lesions also impair performance under a second-order schedule of cocaine reinforcement (data not shown), indicating that the deficits observed in the present study are not specific to the acquisition of this behavior.

It is important to emphasize that core-lesioned rats in the present study were able to acquire instrumental responding for cocaine under a continuous reinforcement schedule, and indeed they responded more than controls under certain conditions. Similarly, NAc core-lesioned rats are also able to learn to respond for food and intravenous heroin[15,16,36], as well as to adapt their responding to changes in the dose of cocaine or heroin[15] (and present results). These findings indicate intact learning of instrumental action-outcome contingencies and a largely intact efficacy of primary reinforcement, whether drug or food. The lack of a rightward shift in the dose-response curve is also strong evidence against the simple hypothesis that acquisition impairments in cocaine-seeking are primarily due to an attenuation in drug reinforcement. Nevertheless, there was some evidence of elevated response rates on both the active and inactive lever under continuous reinforcement, which could be taken to indicate a mild attenuation of cocaine reinforcement. However, this generally enhanced operant output could also be attributed to the locomotor hyperactivity known to result from core lesions[38,42]. The lesion-induced hyperactivity may also contribute to the persistence of responding in extinction frequently seen in core-lesioned rats[15,43,44]. However, it is important to note that the hyperactive model of behavior produced by core lesions could not in itself account for the pattern of effects seen in the drug-taking (where responding increased) and drug-seeking (where responding decreased) paradigms, nor could it account for the differential responding on the active and inactive levers in both situations.

NAc shell and cocaine-seeking

Compared to NAc core lesions, shell lesions had no major effects on the acquisition of cocaine-seeking behavior or self-administration under continuous reinforcement. Only at the most stringent stage of the second-order schedule was there an indication of significantly reduced responding on the active lever. However, there were no differences in the rate of responding in the period prior to cocaine infusion. Therefore, this effect can be entirely attributed to an atten-

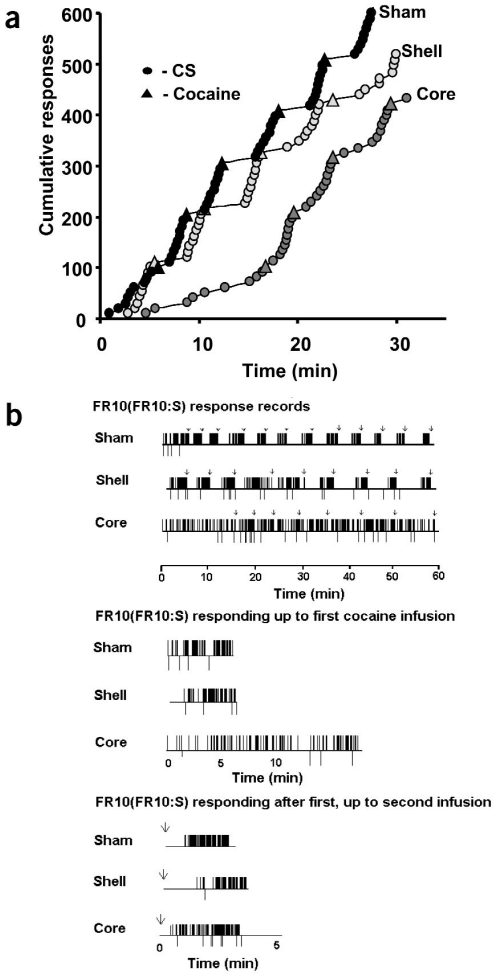

Figure 6 Effects of NAc lesions on qualitative measures of cocaine self-administration under the second-order schedule. Representative individual records of (**a**) cumulative responses and (**b**) responses in sham, core and shell-lesioned rats under the second-order FR10(FR10:S) schedule of cocaine reinforcement. (**a**) The triangles represent the points at which the rat obtained a cocaine infusion (0.25 mg/infusion) and the circles represent the presentation of a light CS that was contingent on every tenth lever press. (**b**) The top panel shows individual records of the first 60 min of a 2-h session, with the arrows depicting the point of cocaine infusion. This overall response record is then truncated into response records for (i) the period up to the first cocaine infusion (middle panel; a period of responding completely unaffected by any self-administered cocaine) and (ii) the period between the first and second infusion (bottom panel), for each lesion group.

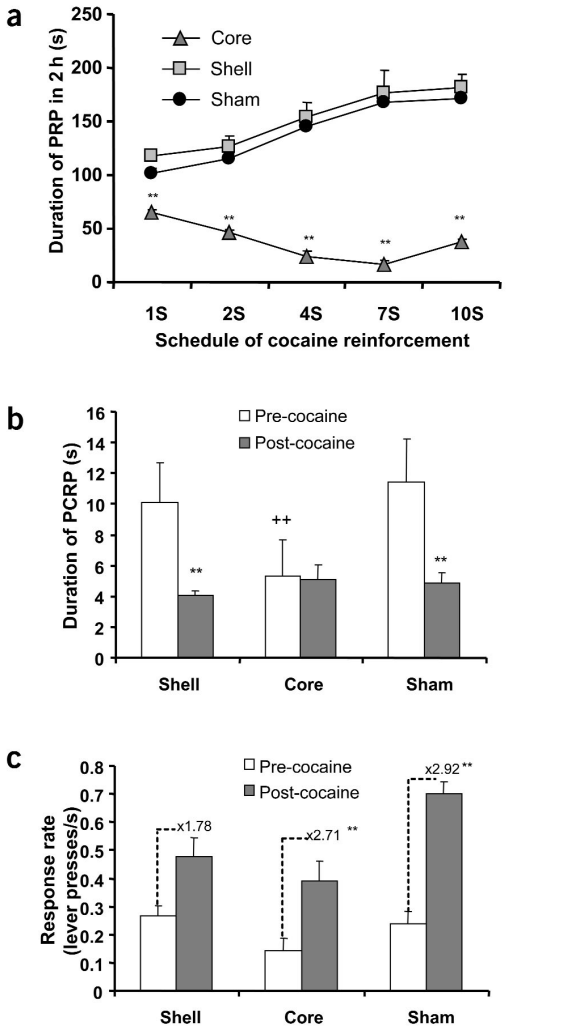

Figure 7 Effects of NAc lesions on quantitative measures of cocaine self-administration under the second-order schedule. (**a**) Mean duration of PRPs within a 2-h session, during each stage of second-order schedules of cocaine reinforcement. $^{**}P < 0.01$. Abbreviations: *y*S = FR10(FR*y*:S). (**b**) Mean duration of post-conditioned reinforcement pause (PCRP) during the first 100 responses in a drug-free state (pre-cocaine) and the next 100 responses following the first cocaine infusion (post-cocaine), under the FR10(FR10:S) schedule of reinforcement in sham, core and shell-lesioned rats. $^{**}P < 0.01$, compared to pre-cocaine value, $^{++}P < 0.01$, compared to sham. (**c**) Mean rate of responding before (pre-cocaine) and after (post-cocaine) the first cocaine infusion of self-administration under the FR10(FR10:S) schedule of reinforcement in core, shell and sham-lesioned rats. $^{**}P < 0.01$ compared to pre-cocaine rate of responding.

uation of the effect of cocaine itself to increase rates of responding under second-order schedules[18,22]. A similar, though quantitatively larger, effect of similar NAc shell lesions on the potentiation of responding with conditioned reinforcement by amphetamine as well as other psychomotor stimulant manifestations of the drug, such as locomotor hyperactivity, has previously been reported[38]. Furthermore, infusions of amphetamine into the NAc shell enhance the ability of non-contingently presented Pavlovian cues to potentiate instrumental lever pressing[39]. Thus, these data, together with those from the present study, show that the caudomedial NAc shell is not critical for the primary reinforcing effects of cocaine, but it is essential for the invigorating effect of stimulant drugs on conditioned and unconditioned behavioral responses—that is, for their psychomotor stimulant actions.

Theoretical implications

The deficits observed in core-lesioned rats most likely reflect a loss of the impact of conditioned reinforcement on cocaine-seeking behavior. Highly relevant to such an interpretation is the observation that lesions of the NAc core induce persistent impulsive choice behavior as evidenced by an inability to tolerate delays of food reinforcement[45]. It should be noted that a second-order schedule of reinforcement necessarily introduces a delay to primary reinforcement, but conditioned reinforcers normally help to mediate this delay by maintaining

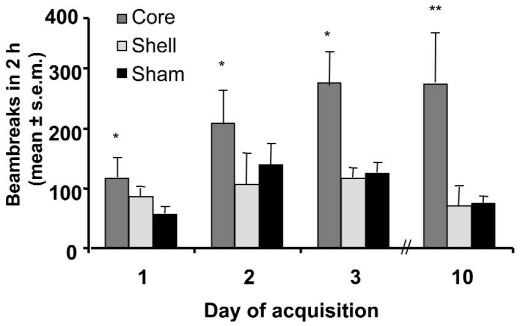

Figure 8 Effects of NAc lesions on cocaine-induced locomotor activity during self-administration sessions. Vertical bars represent the mean ± s.e.m. photocell beam breaks for each group during a 2-h self-administration session on the first three and last days of acquisition. $^{**}P < 0.01$, $^{*}P < 0.05$ compared to the sham.

instrumental responding[34]. As core-lesioned rats showed no deficits when responding for cocaine under a continuous reinforcement schedule, the impaired acquisition of cocaine-seeking behavior reported here may depend on an inability of core-lesioned rats to tolerate a delay in cocaine reinforcement. We infer that this reflects a loss of impact of cocaine-associated conditioned reinforcers. The similar effects of BLA lesions[26] may implicate both of these structures in a neural system by which conditioned associations help to mediate delays between responses and outcomes.

The integrity of the NAc core, unlike that of the caudomedial NAc shell, is not required for the response rate–increasing effects of psychostimulant drugs[38]. These data thus re-open the issue of the relationship between the stimulant and reinforcing effects of psychomotor stimulants such as cocaine, which have been suggested to be isomorphic[46]. However, the doubly dissociable effects reported here indicate that the NAc core and shell mediate distinct processes associated with cocaine self-administration behavior. The core seems to mediate control by conditioned reinforcers, whereas the shell seems to mediate the potentiation of that control by cocaine, perhaps reflecting stimulant or motivational effects of the drug.

The additional importance of this double dissociation is in demonstrating a selective functional effect produced by the relatively discrete shell lesion (restricted to its caudomedial domain), that contrasts with the qualitatively distinct nature of the deficits produced by the more complete lesion of the nucleus accumbens core. It is significant that this region of the NAc shell is most often implicated in mediating both the reinforcing and response-invigorating effects of psychomotor stimulant drugs[30–33,38].

How the NAc core and shell interact remains unclear, but recent anatomical evidence suggests that the striatum is organized in a hierarchical fashion, with the shell and its limbic connections capable of influencing the behavioral output of the core, via 'spiralling' connections with the midbrain dopamine neurons[47]. The NAc shell can thus serve to amplify the expression in behavior of information flowing through the NAc core[17]. Such anatomical relationships may also underlie different factors affecting intravenous drug self-administration behavior. These data emphasize the complex nature of cocaine reinforcement mechanisms, while specifying a particular role for the NAc core subregion that has hitherto not been apparent.

METHODS

Animals. Male Lister Hooded rats (Charles River Ltd., UK) were housed in pairs and then individually after surgery, in a room held at a temperature of 21 °C under a reversed 12-h light/dark cycle (lights off at 09:00). Food (laboratory chow, Purina) and water were available *ad libitum* but, after recovery from surgery, food was restricted to 20 g of lab chow per day, sufficient to maintain pre-operative body weight and growth. All experiments were carried out during the dark phase, between 09:00 and 18:00 and in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act Project License No. 80/1324.

Surgery. In all surgical procedures, animals were anesthetized with Avertin (10 g of 99% 2,2,2-tribromoethanol (Sigma-Aldrich) in 5 mg tertiary amyl alcohol and 4.5 ml phosphate buffered saline (Dulbecco "A", Unipath Ltd.) in 40 ml absolute alcohol; 1 ml/100 g body weight, intraperitoneally (i.p.)).

Excitotoxic lesions. We used different excitotoxins to damage the core or shell sub-regions of the NAc (quinolinic acid for core; ibotenic acid for shell). This produced selective lesions of these structures with little, if any, overlap between them[38].

A 1-μl SGE syringe (SGE) was lowered stereotaxically into either the NAc core or shell, and the neurotoxin was infused bilaterally. For NAc core lesions, 0.3 μl of 0.09 M quinolinic acid (Sigma-Aldrich) buffered to pH 7.3–7.4 in 0.1 M sterile phosphate buffer (sterile PB), was infused for 1 min in each hemisphere, using the following coordinates (in mm from bregma); AP: +1.2, L: ±1.8, DV: –7.1 from the skull surface (SS). For NAc shell lesions, three separate infusions of 0.06 M ibotenic acid (Sigma-Aldrich) buffered to pH 7.4 using 0.1 M sterile PB were made at different points along the DV axis in each hemisphere: (i) 0.2 μl at AP = +1.6, L = ±1.1, DV = –7.9 (SS); (ii) 0.1 μl at AP = +1.6, L = ±1.1, DV = –6,9; (iii) 0.1 μl at AP = +1.6, L = ±1.1, DV = –6.4. Sham and lesion groups were treated identically, except that sham controls received injections of sterile PB instead of the toxin.

Intravenous catheterization. After a recovery period of at least 5 d with food available *ad libitum*, rats were then implanted with chronic intravenous jugular catheters as previously described[48]. Antibiotic treatment (daily subcutaneous administration of 0.1 ml Baytrill; Bayer) was given for 5 d after surgery. Thereafter, before each self-administration session, the animals were flushed with 0.1 ml sterile 0.9% saline and at the end of the session with 0.1 ml heparinised saline (CP Pharmaceuticals Ltd.; 30 units/ml 0.9% sterile saline) to maintain catheter patency.

Apparatus. Twelve operant chambers (24 cm wide × 20 cm high × 22 cm deep; Med Associates) contained within a sound-attenuating box with a ventilating fan were used in the experiment. Each chamber was equipped with two retractable levers, a stimulus light above each lever, a house light and three infrared beams (See **Supplementary Methods** online). Intravenous infusions of cocaine were delivered by a software-operated infusion pump (Semat Technical Ltd.) placed outside the sound-attenuating box, through a counterbalanced single-channel liquid swivel. Animals were tethered to the counterbalanced arm by a metal spring and a skull-mounted plastic-post. The apparatus was controlled by an Acorn Archimedes microcomputer (Acorn Computers Ltd.) running a program written in the BASIC control language, Arachnid (Paul Fray Ltd.).

Drugs. Cocaine hydrochloride (McFarlan-Smith) was dissolved in sterile 0.9% saline. The dose of cocaine was calculated as the salt.

Behavioral procedures. Cocaine self-administration under continuous reinforcement: Animals were trained to acquire cocaine self-administration under a continuous reinforcement schedule (fixed ratio1) during daily 2-h sessions until stable baseline responding was achieved (defined as 2–3 consecutive d of stable responding with as much as ±10% variation). For each rat, one of the two levers was designated the active or drug lever, and the other was the inactive lever on which responding had no programmed consequence. No drug priming was given at any stage of training. The beginning of the session was marked by illumination of the house light. Subsequent depression of the active lever resulted in the retraction of both levers, extinction of the house light and simultaneous illumination of the drug stimulus light for 20 s, as well as the activation of an infusion pump,

delivering 0.1 ml intravenous infusion of cocaine solution (0.25 mg/infusion). On completion of the 20-s CS presentation per time out period, the levers were re-extended, the house light illuminated and the stimulus light extinguished. Throughout training, the maximum number of infusions per session was fixed at 50 to prevent overdosing. Once this number of cocaine infusions had been reached, the session terminated.

Second-order schedule of cocaine reinforcement: Once self-administration under a continuous reinforcement schedule had been attained, a second-order FRx(FRy:S) schedule of cocaine reinforcement was introduced. Under this schedule, rats were required to make *y* responses to obtain a single presentation of a 2 s light CS (or conditioned reinforcer) while completion of *x* of these response units resulted in the delivery of cocaine, the illumination of the light CS for 20 s, the retraction of both levers and extinction of the house light during a 20 s time out period. In the initial stage of training, *x* was set at 5 and *y* was 1. The value for *x* was then increased from 5 to 10 and remained at this value throughout the training. The value for *y* was progressively increased from 1 to 10 until stable responding was established at FR10(FR10:S). Animals were allowed to move from one stage to the next when at least ten cocaine infusions within a 2-h session were made over three consecutive days at each stage.

Cocaine dose-response function. A separate group of 18 rats (4 or 5 per treatment group) was subjected to a between-sessions cocaine dose-response function once stable acquisition of cocaine self-administration under a CRF schedule at the training dose of 0.25 mg/infusion had been attained. The training dose was substituted by 0.083, 0.125 or 0.50 mg per infusion of cocaine or saline in a 2-h self-administration session on five consecutive days, and the order in which the rats received each dose was counterbalanced.

Histological assessment of lesions. Within a week after completion of the testing, all rats were anesthetized with sodium pentobarbitone (1.5 ml/animal, 200 mg/ml Euthatal, Rhone Merieux) and perfused intracardially via the ascending aorta with 0.01 M phosphate-buffered saline (PBS) for 4 min, followed by 4% paraformaldehyde (PFA) in PBS for 6 min. Brains were then removed, stored in PFA and transferred to a 20% sucrose cryoprotectant solution on the day before sectioning (See **Supplementary Methods** online). For the verification of lesions, coronal sections (60 μm) of the brain were cut using a freezing microtome.

Statistical analysis. All behavioral data were analyzed using SPSS version 9. Responses during the acquisition of self-administration under CRF and second-order schedules were square-root transformed to preserve homogeneity of variance and analyzed using a three-factor analysis of variance (ANOVA) with group (core, shell, sham) as the between-group factor and training day and lever (active vs. inactive) as repeated, within-subjects factors. Data collected from the seven core-lesioned animals that failed to complete the second-order schedule training were included in the statistical analyses, as their exclusion did not alter statistical significance of the overall data.

Two-way ANOVAs (lesion group as between-subjects factor and training schedule or cocaine state as repeated measures) were conducted for all quantitative data extracted from the response patterns for the second-order schedule contingencies.

For all analyses, upon confirmation of significant main effects, differences among individual means were analyzed using the Newman-Keuls *post-hoc* test. Significant interactions were further analyzed as appropriate using two-way or one-way ANOVAs, with appropriate α adjustments using Sidak's method ($\alpha' = 1 - (1 - \alpha)^{1/C}$, where C is the number of within-experiment analyses).

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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