European Journal of Neuroscience, Vol. 22, pp. 2605–2616, 2005

Double dissociation of the effects of selective nucleus accumbens core and shell lesions on impulsive-choice behaviour and salience learning in rats

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Keywords: behavioural inhibition, delayed reinforcement, DRL, latent inhibition, ventral striatum

Abstract

The nucleus accumbens can be subdivided into at least two anatomically distinct subregions: a dorsolateral 'core' and a ventromedial 'shell', and this distinction may extend to a functional dissociation. Here, we contrasted the effects of selective excitotoxic core and medial shell lesions on impulsive-choice behaviour using a delayed reward choice paradigm and a differential reward for low rates of responding (DRL) test, against a form of salience learning known as latent inhibition (LI). Core lesions led to enhanced impulsive choices as evidenced by a more pronounced shift from choosing a continuously reinforced lever to a partially reinforced lever, when a delay between lever press and reward delivery was imposed selectively on the former. The core lesions also impaired performance on a DRL task that required withholding the response for a fixed period of time in order to earn a reward. Medial shell lesions had no effect on these two tasks, but abolished the LI effect, as revealed by the failure of stimulus pre-exposure to retard subsequent conditioning to that stimulus in an active avoidance procedure in the lesioned animals. As expected, selective core lesions spared LI. The double dissociations demonstrated here support a functional segregation between nucleus accumbens core and shell, and add weight to the hypothesis that the core, but not the shell, subregion of the nucleus accumbens is preferentially involved in the control of choice behaviour under delayed reinforcement conditions and in the inhibitory control of goal-directed behaviour.

Introduction

On anatomical, neurochemical as well as electrophysiological grounds (e.g. Zahm & Brog, 1992; Brog *et al.*, 1993; O'Donnell & Grace, 1993; Jongen-Rêlo *et al.*, 1994; Pennartz *et al.*, 1994; Heimer *et al.*, 1997), the nucleus accumbens (NAC) can be partitioned into at least two subregions: a dorsolateral 'core' and a ventromedial 'shell' (Záborszky *et al.*, 1985). Rodent studies employing lesion techniques and intracerebral pharmacological manipulations have revealed that the shell–core distinction is also valid across a wide range of behavioural functions (e.g. Pennartz *et al.*, 1994; Maldonado-Irizarry & Kelley, 1995; Weiner *et al.*, 1996; Heimer *et al.*, 1997; Parkinson *et al.*, 1999; Setlow & McGaugh, 1999; Murphy *et al.*, 2000; Alderson *et al.*, 2001; Corbit *et al.*, 2001; Di Chiara, 2002; Sellings & Clarke, 2003; Fuchs *et al.*, 2004; Kelley, 2004). Direct comparisons of focal lesions or reversible transient inactivation targeted at NAC shell or core in our laboratory have revealed that the two subregions can be distinguished in the control of locomotor and explorative behaviour, latent inhibition, spatial working memory, and prepulse inhibition of the acoustic startle reflex (Jongen-Rêlo *et al.*, 2002, 2003; Pothuizen *et al.*, 2005). It remains to be elucidated whether the distinction between core- and shell-dependent behaviours corresponds to any systematic psychological demarcation or not.

Recent evidence has shown that the NAC is also involved in the control of choice behaviour, and that excitotoxic lesions restricted to the core lead to persistent impulsive choice, as exemplified by choosing a small reward that is available immediately, in preference to a larger but delayed reward (Cardinal *et al.*, 2001). The contribution of the shell to this behavioural control, however, remains unexplored. Here, we directly compared selective shell and core lesions on a similar delayed reward choice paradigm (Newman *et al.*, 1983), and on a differential reinforcement for low rates of responding (DRL) operant task that requires withholding response for a fixed period of time in order to obtain a reward. DRL represents another procedure for the assessment of impulsive-like behaviour, although it may be sensitive to different elements of impulsivity (see Evenden, 1999; Monterosso & Ainslie, 1999; also see Discussion later). Both impulsive paradigms are sensitive to damage of the hippocampal system (Johnson *et al.*, 1977; Rawlins *et al.*, 1985; Bannerman *et al.*, 1999), which projects directly to the NAC. Nonrestrictive NAC lesions are known to impair efficient DRL performance (Reading & Dunnett, 1995), but the relative contributions of the NAC core and shell subregions have hitherto not been examined. To confirm the efficacy of the selective medial shell lesions employed, we initially evaluated latent inhibition (LI, a form of salience learning in which repeated nonreinforced pre-exposures to a neutral stimulus reduces the stimulus' subsequent associability; Lubow & Moore, 1959) using a two-way active avoidance test. LI was abolished by shell lesions

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Received 17 March 2005, revised 17 August 2005, accepted 19 August 2005

doi:10.1111/j.1460-9568.2005.04388.x

specifically. The present results also confirm that the selective core lesions did not affect the functional integrity of the shell, because they spared the LI effect (see also Weiner *et al.*, 1999; Jongen-Rêlo *et al.*, 2002).

Materials and methods

Subjects

Naive male Wistar rats ($n = 58$, bred in the animal facilities of the Behavioural Neurobiology Laboratory, Schwerzenbach, Switzerland) weighing 280–300 g at the time of surgery, were housed under controlled conditions of temperature (21 ± 1 °C) and humidity (55 ± 5%), and maintained under a reversed 12 h light : 12 h dark cycle (lights on 1900–0700 h). All behavioural tests were conducted during the dark phase. The animals had free access to food chow (Kliba 3430, Klibamuhlen, Kaiseraugst, Switzerland) and water throughout the experimental period unless otherwise specified. The rats were caged in groups of four in Makrolon IV cages.

The animals were handled daily for at least 1 week before surgery, when they were randomly assigned to one of the four surgical groups: receiving selective core lesions ($n = 15$), selective medial shell lesions ($n = 15$), sham operation ($n = 13$) or no operation ($n = 14$).

The Swiss Federal Veterinary Office approved all procedures used in this study, and every effort was made to minimize the number of animals used.

Surgery

The rats were first sedated with sodium pentobarbital (50 mg/kg, i.p., Nembutal, Abbott Laboratories, IL). General anaesthesia was subsequently achieved by two intramuscular injections (0.045 mL into each hind leg per rat) containing a 3 : 8 mixture of Dormicum® (Midazolamum, 5 mg/mL, Roche Pharma, Reinach, Switzerland) and Domitor® (Medetomidin, 1 mg/mL, Orion, Espoo, Finland). When the animal was fully unconscious, the head was secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with bregma and lambda positioned in the same horizontal plane. An incision was made along the midline of the scalp and the skin deflected. A craniotomy was performed to expose the neocortex overlying the injection sites. Selective bilateral lesions were made by multiple injections of *N*-methyl-D-aspartate (NMDA, Sigma, St. Louis, MN) dissolved in 0.1 M phosphate buffered saline (PBS, pH 7.4, Fluka, Buchs, Switzerland) at a concentration of 14 mg/mL.

The anterior–posterior (AP) coordinates were measured (in mm) with respect to bregma, the medio-lateral (ML) coordinates with respect to the sagittal sinus, and the dorso-ventral (DV) coordinates with respect to the surface of the dura. The two injections per hemisphere for the core lesions were: 0.10 μL at +1.4 (AP), ±2.0 (ML), −6.5 (DV); and 0.075 μL at ±2.4 (AP), ±1.8 (ML), −6.5 (DV). The three injections per hemisphere for the lesions targeted at the medial shell were: 0.075 μL at +1.2 (AP), ±0.8 (ML), −6.9 (DV); 0.10 μL at +1.2 (AP), ±0.8 (ML), −6.4 (DV); and 0.025 μL at +1.2 (AP), ±0.8 (ML), −6.2 (DV).

Injections were made via a 34 G stainless steel needle attached to a 5-μL syringe (SGE-Germany GmbH, Weiterstadt, Germany) at an infusion rate of 0.5 μL/min. The needle was left in place for 1 min after each infusion to allow for diffusion and to minimize backtracking of the NMDA toxin. The rats assigned to the sham group were infused with the appropriate amount of PBS at either the core or the medial shell coordinates. At the end of the surgery, the wound was sutured and the rat was given a subcutaneous injection of atropine sulphate (0.5 mg/mL, Sintetica, Mendrisio, Switzerland) to aid recovery. The animals were monitored for at least 3 h postsurgery.

Experimental design

The rats were allowed 4 weeks to recover before the start of behavioural testing. Prior to the present study, the animals had been tested on three behavioural tests examining open field activity, startle response and conditioned taste aversion (not presented here). Approximately 11 weeks had elapsed after surgery when the behavioural tests reported here commenced.

The present study comprised three experiments. First, all animals were employed in Experiment 1 designed to assess latent inhibition. The animals were then subdivided into two balanced cohorts: one destined for the delayed reward choice experiment (Experiment 2), and the other for the DRL experiment (Experiment 3).

Experiment 1. Latent inhibition in the acquisition of conditioned two-way active avoidance

Apparatus

The apparatus consisted of four identical shuttle boxes (Coulbourn Instruments, Allentown, PA, model E10–16TC), each set in a ventilated, sound- and light-attenuating cubicle (model E10-20). They were located in a testing room illuminated by red lights. The internal dimensions of each box were 35 × 17 × 21.5 cm as measured from the raised grid floor. An aluminium hurdle (17 cm wide, 4 cm high) served as a barrier separating the box into two identical compartments. It was low enough to allow the rats to shuttle freely between the two compartments, and sufficiently thin to prevent the rats from avoiding the shock by balancing themselves on it. The box was constantly illuminated by two house lights (1.8 W) mounted on the walls at each end of the shuttle box, at a level of 19 cm above the grid floor, providing an ambient light level of 4 lux throughout the test sessions. The grid floor (model E10–16RF) consisted of 24 parallel stainless steel rods with a diameter of 0.48 cm, spaced 1.5 cm apart centre to centre, through which scrambled foot-shocks could be delivered by a constant direct current shock generator (model E13-14) and a scanner (model E13-13) set at 0.5 mA. The conditioned stimulus was an 85-dB[A] tone produced by a 2.9-kHz tone module (model E12-02) placed behind the shuttle box on the floor of the cubicle. A personal computer (Compaq) together with a set of four universal environment interfaces (model E91-12) controlled the sessions and recorded the behavioural data.

Procedure

The rats in each of the four surgical groups were subdivided into two conditions: pre-exposed (PE) and nonpre-exposed (NPE). The allocation of subjects to the four boxes was counterbalanced across surgical treatments and pre-exposure conditions. The test comprised two sessions (pre-exposure and conditioning) separated by 24 h.

Pre-exposure

On the first day, the PE rats were placed in the shuttle box for 50 min and were exposed to 50 presentations of a 10-s tone stimulus at variable intervals of 50 ± 40 s. The NPE rats were placed in the shuttle box for an equivalent period of time without receiving any tone exposure. The total number of spontaneous crossings during pre-exposure was taken as a measure of basal locomotor activity.

Conditioning

On the second day, the rats were placed in the shuttle box and received 100 trials of conditioned avoidance according to a variable interval schedule of 50 ± 40 s. Each trial began with a 10-s presentation of the tone, followed immediately by a 2-s 0.5-mA electric foot-shock, with the tone remaining on until termination of the foot-shock. If the rat crossed the barrier during the first 10 s after the tone onset, the tone was terminated and no shock was delivered, and an avoidance response was recorded. Crossing the barrier during the shock terminated both the shock and tone, and an escape response was recorded. An escape failure was recorded if the animal received the entire 2-s shock.

The number of avoidance responses over successive 10-trial blocks provided a measure of conditioned avoidance learning. The LI effect refers to retarded avoidance responding in the PE relative to the NPE subjects.

Experiment 2. Delayed reward choice paradigm

Apparatus

The test apparatus comprised eight identical operant chambers (Coulbourn Instruments, model E10–10TC), each set in a ventilated, sound- and light-attenuating cubicle (model E10-20). They were located in a testing room illuminated by red lights. Each chamber measured 30 × 25 × 21.5 cm from the stainless steel grid floor. Two light sources (1.8 W) mounted on the manipuladum-panel wall, 19 cm above the grid floor, provided a constant illumination in the chamber (4 lux) throughout all test sessions. A magazine tray (model E14-24) was positioned in the centre of the panel wall, 2 cm above the grid floor; 3 cm on either side of the magazine tray was a retractable lever (model E23-07) positioned 3 cm above the grid floor. An infrared beam detected magazine entry, and a pellet dispenser delivered 45-mg reward food pellets (Bioserv Frenchtown, NJ) into the magazine, which could be illuminated by a light (1.8 W) to signal reward delivery. Control of the chambers and collection of the data were accomplished using the Graphic State Notation 1.013–00 software (Coulbourn Instruments) implemented on a personal computer running the Windows 98 operating system, which was connected via a set of eight universal environment interfaces (model E91-12) to the operant chambers.

Procedure

Testing commenced 4 weeks after Experiment 1. The subjects consisted of approximately half of the animals in Experiment 1 ($n = 7$ selective core lesions, $n = 8$ selective medial shell lesions, $n = 6$ sham operations, $n = 7$ with no operation) with, as far as possible, an equal number of subjects with PE and NPE experience from Experiment 1. Food deprivation was introduced gradually 3 weeks before the experiment commenced, such that each rat was finally maintained on ∼13 g of laboratory food chow per day. The weight of the animals was monitored weekly and was not allowed to fall below 85% of their free feeding weight. For the 3 days before the start of Experiment 2, rats were also familiarized with the reward pellets in their home cage.

The rats were allocated to one of the eight operant chambers, counterbalanced across surgical treatment. The delayed reward choice task was based on that described in Newman *et al.* (1983) and in Rawlins *et al.* (1985) and adapted here for the operant boxes using food reward.

Pre-training

On day 1, the animals were trained to consume food from the magazine: 20 pellets were delivered according to a random time interval (mean 25 s, range 20–30 s), with the levers retracted. Every pellet delivery coincided with a 1-s illumination of the magazine light.

On day 2, the animals were trained to press a lever for food: there were 20 discrete trials in which either the left or the right lever (10 trials per lever), was inserted into the chamber. One press of the inserted lever (i.e. fixed ratio 1, FR1) led to the immediate retraction of the lever and the delivery of 1 pellet accompanied by a 1-s magazine light. The ITI was 30 s.

On day 3, the first 6 trials were as described before (i.e. FR1), the following 6 trials required two presses to earn a reward (FR2), and the final 8 trials required 5 presses (FR5).

On day 4, the rats received 10 FR5 trials, and thereafter proceeded to the delayed reward choice paradigm.

Delayed reward choice paradigm

The right lever was designated as the continuously reinforced (CRF) lever, and the left the partially reinforced (PRF) lever. The test was conducted in separate phases, to allow the evaluation of impulsive choice behaviour under different delayed reward conditions, as imposed via the CRF lever. Each phase comprised 3–9 days of 'forced-trial' training, followed by 2–10 days of 'choice-trial' testing. Prior to the assessment of choice behaviour under a specific delay condition (> 0 s), the animals were always evaluated under the 'no-delay' (0 s delay) condition in order to ascertain and to (re-)establish a stable preference for the CRF lever.

Forced-trial training. This served to familiarize the animals to the differential outcomes associated with the two levers. On each day, the rats were given 12 forced CRF trials followed by 12 forced PRF trials, in which only the CRF or the PRF lever, respectively, was presented. The ITI was 30 s. For all trials, FR5 response to the given lever was followed by the retraction of the lever and the illumination of the magazine. For CRF forced-trials, continuous reinforcement with 1 pellet was associated with 0, 20, 0, 10, 0, 15, 0, and 20 s delay (from the time of magazine entry) in phases 1–8, respectively. The magazine light remained on during the delay and was terminated 1 s after food delivery. In PRF forced-trials, the probability of reinforcement was 25% (3 out of 12 trials), and the delivery of the reward pellet (if any) was always immediate upon the nose-poke entry, followed by the termination of the magazine light 1 s later. In phases 1 and 2, there were 9 days of forced-trial training. In all subsequent phases (3–8), the forced-trial training lasted 3 days.

Choice-trial testing

A daily session of choice-trial testing consisted of 20 discrete trials in which both levers were presented, with an ITI of 30 s. A trial began with the simultaneous presentation of the two levers. At the first press of one of the two levers, the nonselected lever was immediately retracted. Following the fifth press, the selected lever was also retracted, and the magazine light switched on. If the CRF lever had been selected, a nose-poke magazine entry initiated the programmed delay period, followed by the delivery of 1 food pellet. The magazine light remained on during the delay period and was terminated 1 s after reward delivery. If the PRF lever was selected, a magazine entry led immediately either to a reward or not, and the termination of the magazine light 1 s later. In every 5-trial block, the one (or maximally two) PRF trial(s) to be rewarded was assigned randomly.

The programmed event associated with the PRF lever during choice-trial testing remained identical across phases 1–8. In contrast, the delay of reward delivery associated with the CRF lever varied across the 8 phases, matching that of the preceding forced-trial training. Across phases 1–8 the delays were 0, 20, 0, 10, 0, 15, 0 and 20 s, respectively, and the respective numbers of choice-trial testing days were 2, 5, 5, 5, 2, 5, 2, and 10 days.

Finally, one forced trial was interspersed after every 5th choice trial. There were thus four such forced trials per session, in which the lever not selected in the preceding choice trial was presented, and the animals were required to press this lever to proceed. These interspersed forced trials ensured that the rats remained familiar with the programmed consequences of the two levers within a given session (Newman *et al.*, 1983; Rawlins *et al.*, 1985).

Experiment 3. Differential reinforcement for low rates of response (DRL) task

Apparatus

The same set of eight operant chambers described in Experiment 2 was employed here.

Procedure

The subjects from Experiment 1 that were not allocated to Experiment 2 participated in this experiment. There were 29 rats in total ($n = 8$ selective core lesions, $n = 7$ selective medial shell lesions, $n = 7$ sham operations, $n = 7$ with no operation). They were gradually introduced to a food deprivation schedule 3 weeks before commencement of the DRL experiment, and on the last 3 days they were also familiarized with the reward pellets in their home cage. The allocation of the rats to the eight operant chambers was counterbalanced across surgical conditions. The chamber house light was always on.

Pretraining

On the first day, the rat was familiarized with consumption of reward pellets in the operant chamber with the levers retracted. Forty reward pellets were delivered in the magazine tray according to a variable time schedule (mean 16 s, range 12–20 s). Each pellet delivery also coincided with a 1-s illumination of the magazine tray.

Next, subjects were trained to lever press as described by Bannerman *et al.* (1999). On each day, the left lever was present for the entire session, which ended after 40 rewards had been earned. First, they were trained to lever press on a VI schedule, in which every lever press after a variable interval (mean 16 s, range 12–20 s) earned a pellet, the delivery of which coincided with 1-s illumination of the magazine tray light. Afterwards, the VI schedule was no longer enforced. Over the next 2 days, a reward was delivered after every two (FR2) and four (FR4) lever presses, respectively. The FR pretraining provided the basis for the effective assessment of behavioural inhibition under the subsequent DRL schedule(s).

DRL schedule

The demand of the DRL requirement was such that only responses (except for the very first lever press) made after a specific time period had elapsed after the preceding response were reinforced with one food pellet. Premature responses were not rewarded and reset the expired time to zero. Each daily session lasted for a fixed duration of 40 min.

On the first 6 days, the animals were tested on a DRL-4 s schedule, followed by another block of 6 days on DRL-8 s, and then onto 6 days of DRL-12 s, and finally for 18 days on a DRL-18 s schedule. The total number of rewards obtained and the total number of responses made per session were recorded. A ratio denoting the mean lever-presses per reward earned was used to index DRL performance on each day – thus the lower the ratio, the better was the DRL performance. All three dependent measures were analysed separately.

Statistical analysis

Preliminary analyses in all experiments indicated that the sham-operated and unoperated control subjects did not differ significantly from each other. They were therefore combined into one single control group ($n = 27$ two-way active avoidance, $n = 13$ delayed reward choice paradigm, and $n = 14$ DRL) in all the reported statistical analyses.

All data were subjected to parametric ANOVA performed using the statistical software StatView 5.01 (SAS Institute Inc., Cary, NC) implemented on a PC running the Microsoft Windows XP operating system. Statistical significance was set at a probability level of $P < 0.05$ for all tests. Significant main effects or interaction terms were further investigated by post hoc comparisons using Fischer's protected least significance tests.

Experiment 1. Two-way active avoidance

Pre-exposure. The total number of spontaneous crossings was subjected to a 3×2 split-plot ANOVA with the between-subjects factor of treatment (core, medial shell and control group) and pre-exposure (PE, NPE).

Conditioning. The number of avoidance responses in successive blocks of 10 trials, expressed in percentage, was submitted to a $3 \times 2 \times 10$ split-plot ANOVA with the between-subjects factor of treatment and pre-exposure, and the repeated measurements factor of blocks.

Experiment 2. Delayed reward choice paradigm

Choice behaviour was indexed by percentage preference for the CRF lever on each day of choice-trial testing. The 8 phases were analysed separately by a split-plot ANOVA with the between-subjects factor of treatment and the repeated measurements factor of days.

Experiment 3. Differential reinforcement for low rates of response (DRL) task

Data on the four DRL schedules (DRL-4 s, DRL-8 s, DRL-12 s and DRL-18 s) were subjected to separate split-plot ANOVA with the between-subjects factor of treatment and the repeated measurements factor of 2-day blocks. Three sets of analyses were conducted, one with each of the three dependent measures: total number of rewards obtained, the total number of responses made per session, and the mean lever presses per reward earned.

Histology

At the end of behavioural testing, the rats were deeply anaesthetized with sodium pentobarbital (60 mg/kg, i.p) and then perfused with 0.9% NaCl solution (Fluka) at room temperature for 2 min (flow rate

35 mL/min), followed by 4% solution of freshly depolymerized paraformaldehyde in 0.1 M phosphate buffer at 4 °C (PB, pH 7.2, Fluka) for 15 min at a flow rate of 35 mL/min. The brains were removed and post-fixed for 24 h in the same fixative, and then transferred to 30% sucrose solution (in 0.1 M PBS, pH 7.4, Fluka) for 2 days at 4 °C under gentle agitation, for the purpose of cryoprotection.

Five adjacent series of coronal sections (40 μm) were cut on a freezing sliding microtome. Four series were collected in a cryoprotectant solution (30% ethylene glycol, 25% glycerol in 50 mM PB, pH 7.4, Fluka), and stored at −20 °C until used. The remaining series of sections were mounted directly onto gelatine-coated slides and stained with Cresyl violet. After staining, the sections were dehydrated through an alcohol series, cleared with xylene, and cover-slipped with the mounting medium Eukitt (Kindler, Freiburg, Germany).

NeuN immunostaining

One of the remaining series was processed for the immunohistochemical demonstration of the neuronal nuclei (NeuN) protein to enable a more accurate evaluation of the extent of excitotoxic lesions in the NAC (Jongen-Rêlo & Feldon, 2002).

Free-floating sections were rinsed in 0.1 M PBS and treated with 0.5% H_2O_2 (Fluka) in 0.1 M PBS for 30 min to suppress endogenous peroxidase activity. Sections were then incubated for 1 h with blocking solution consisting of 10% normal horse serum, 1% bovine serum albumin (BSA, Sigma), and 0.3% Triton X-100 (Sigma) in PBS. They were subsequently incubated for 48 h at 4 °C in the monoclonal anti-NeuN serum (1 : 5000, Chemicon, Temecula, CA) diluted in 1% normal horse serum, 1% BSA, and 0.3% Triton X-100 in PBS. After rinsing in 0.1 M PBS, the sections were incubated for 2 h at room temperature in biotinylated horse antimouse serum (1 : 1000, Vector Laboratories, Burlingame, CA) in secondary diluent consisting of 1% BSA and 0.3% Triton X-100 in 0.1 M PBS. After three rinses in 0.1 M PBS, they were incubated for 2 h in an avidin-biotin-horseradish peroxidase complex (1 : 200, ABC-Elite, Vector Laboratories) in secondary diluent. After rinses in 0.1 M PB, the sections were placed for 10 min in a chromagen solution consisting of 0.05% diaminobenzidine (Sigma) and 0.01% H_2O_2. The reaction was visually monitored and stopped in rinses of 0.1 M PB. The sections were mounted and dried on gelatin-coated slides, and then prepared for cover-slipping as described before.

Histological evaluation of the lesions

Core lesions

One rat in this group was excluded because the lesion extended into the rostral part of the NAC. A coronal section of a representative core lesion from the remaining animals is depicted in Fig. 1A. The lesions were characterized by marked cell loss and intense gliosis in a discrete area around the injection site. The cell loss induced by the NMDA injection resulted in shrinkage of the core region (see Fig. 1A and B) and occasionally of the anterior commissure. Examination of NeuN immunostained sections clearly revealed an area devoid of immunoreactivity (Fig. 1A). In the Nissl stained materials, glial cell accumulation was evident in the region surrounding the anterior commissure (Fig. 1B). As a general feature of the core lesions, the lateral ventricles were slightly enlarged in comparison with that of animals in the medial shell lesion group. However, such ventricular enlargement did not result in damage to either the caudate putamen laterally or the septal nuclei medially. In three cases, minor gliosis was observed in the bed nucleus of the stria terminalis and in the subcommissural part of the ventral pallidum. According to the rat brain atlas of Paxinos & Watson (1997), the largest lesion extended 2.20–0.48 mm, and the smallest lesion 1.70–0.70 mm anterior to bregma (see Fig. 2A).

The final numbers of subjects in the core lesion group were: $n = 14$ (Experiment 1), $n = 7$ (for Experiments 2 and 3).

Medial shell lesions

Two rats in the medial shell lesion group were excluded because the lesions were either too small (sparing of most of the medial shell) or extra damage was observed extending into the core in one hemisphere. A coronal section of a representative medial shell lesion from the remaining animals is depicted in Fig. 1C. The medial shell lesions were invariably characterized by extensive gliosis and cell loss in the ventromedial part of the shell (Fig. 1C and D). In five cases, the most dorsomedial part of the shell was spared. No damage was evident in the ventrolateral aspect of the shell. Marked atrophy along the medial wall of the brain was observed in three cases. In two cases, damage extended into neighbouring areas such as the olfactory tubercle and the vertical limb of the diagonal band of Broca. As a general feature, restricted damage was evident in the medial and ventrolateral septum. Similar to the animals in the core lesion group, restricted gliosis was observed in the bed nucleus of the stria terminalis and the adjoining ventral pallidum. As can be seen in Fig. 2B, the largest medial shell lesion extended 2.20–0.48 mm, and the smallest 1.60–0.70 mm anterior to bregma (Paxinos & Watson, 1997).

The final numbers of subjects in the shell lesion group were: $n = 13$ (Experiment 1), $n = 8$ (Experiments 2), $n = 5$ (Experiment 3).

Sham operated controls

In the sham operated controls no sign of cell damage could be detected, except for the injection tracks (Fig. 1E), which were comparable to those seen in subjects from the core and shell lesion groups. In three of the sham operated animals, the lateral ventricles were slightly enlarged. No sham subjects were excluded on histological grounds.

Results

Experiment 1. Latent inhibition in the acquisition of conditioned two-way active avoidance

Pre-exposure

No significant difference in the total number of crossings was observed among the treatment groups ($F_{2,48} = 0.032$) indicating that neither core nor shell lesions affected spontaneous locomotor activity. Comparison of the two exposure conditions revealed that PE subjects exhibited fewer crossings (mean ± SEM, 31.11 ± 2.87) than the NPE animals (49.51 ± 6.68); this effect of pre-exposure ($F_{1,48} = 4.52$, $P < 0.05$) was similar across groups.

Conditioning

There was a clear increase in the number of avoidance responses in all groups across the 10 blocks of avoidance training, yielding a highly significant main effect of blocks ($F_{9,432} = 48.45$, $P < 0.0001$). There was a general presence of LI as evident by the retarded avoidance learning in the PE (36.5% ± 0.9) relative to the NPE subjects (57.4% ± 1.0), and supported by the significant main effect of pre-exposure ($F_{1,48} = 10.852$, $P < 0.005$) (see Fig. 3).

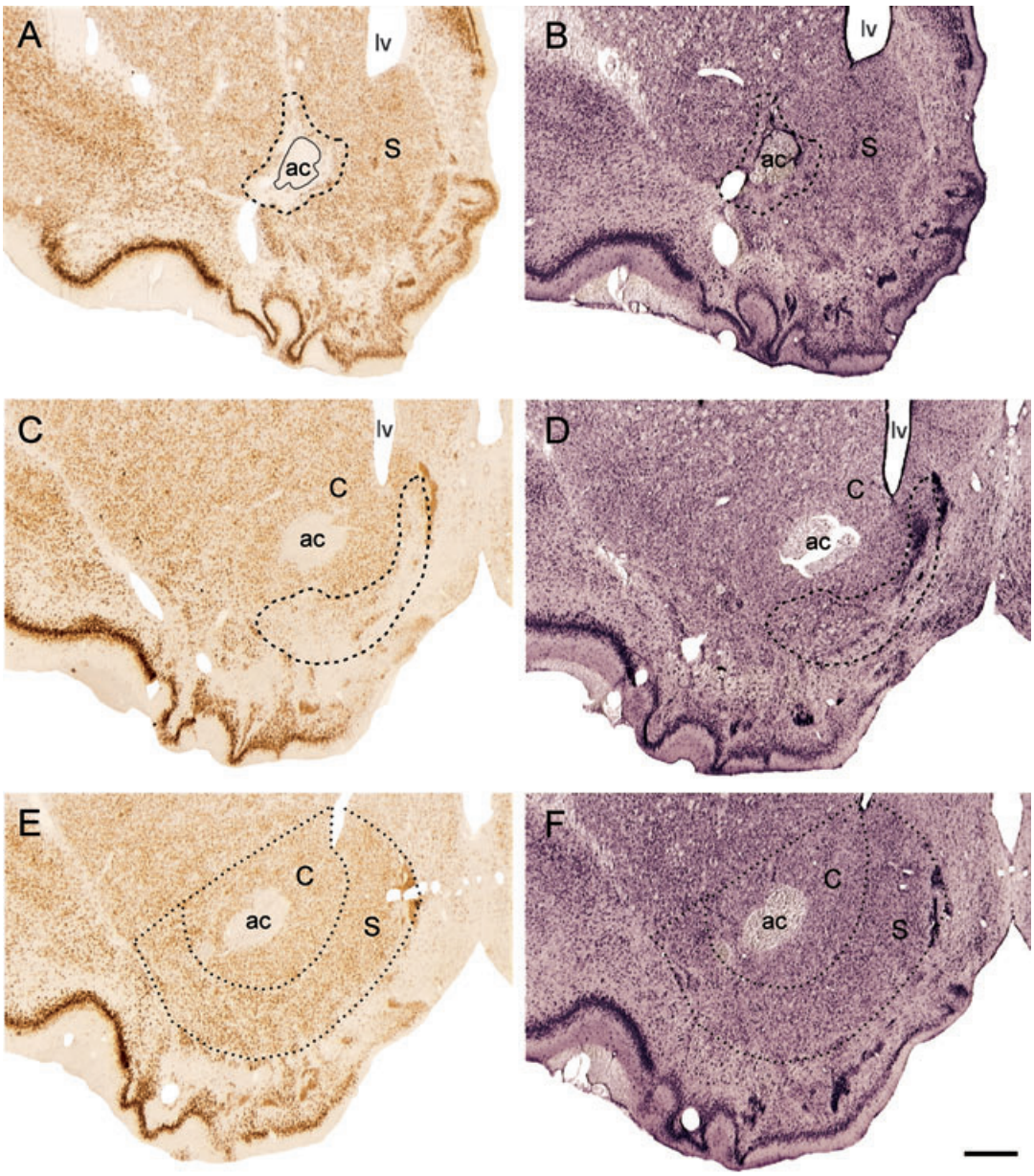

FIG. 1. Examples of selective nucleus accumbens shell and core cytotoxic lesions. Photomicrographs of adjacent coronal sections through the nucleus accumbens immunostained for NeuN (left column) and stained for cresyl violet (right column) from a subject taken from the core-lesioned group (A and B), a subject from the medial shell-lesioned group (C and D) and a sham-operated control (E and F). Since the NeuN immunostaining selectively labels neurons, the area of lesion can be readily discerned by the clear absence of immunoreactivity (as delineated by the black dashed lines in A and C). The solid line in A outlines the boundary of the anterior commissure to facilitate its distinction from the area of lesion. The black dotted line in E and F delineates the core ('C') from the shell ('S') subregion of the nucleus accumbens. Scale bar in F applies to all photomicrographs and represents 500 μm. Abbreviations: ac, anterior commissure; lv, lateral ventricle.

Inspection of Fig. 3 indicates that LI was reduced in the shell-lesioned group in comparison to the sham and core-lesioned groups. However, neither the interaction between treatment and pre-exposure, nor the three-way interaction attained statistical significance. Given the a priori purpose of the experiment to demonstrate LI, and the overall presence of LI in the overall ANOVA, we in addition conducted restricted two-way ANOVAs to examine if LI was consistently present in each of the three groups. Significant presence of LI was revealed in the core-lesioned ($F_{1,12} = 6.16$, $P = 0.03$) and sham operated group ($F_{1,25} = 14.63$, $P < 0.001$), but it was distinctly absent in the medial shell-lesioned group ($F_{1,11} = 0.01$). Hence, there was sufficient reason to conclude that the expression of LI was relatively weak, if not entirely absent, in the shell group in comparison to the others.

Experiment 2. Delayed reward choice paradigm

Immediate-CRF vs. immediate-PRF choice

On each of the four occasions (phases 1, 3, 5, 7) when the animals were assessed on this particular choice, they demonstrated a preference for the immediate-CRF lever (see Fig. 4). No significant difference was detected between groups. The overall mean of CRF-lever preference was 83.5 ± 1.7% (mean ± SEM).

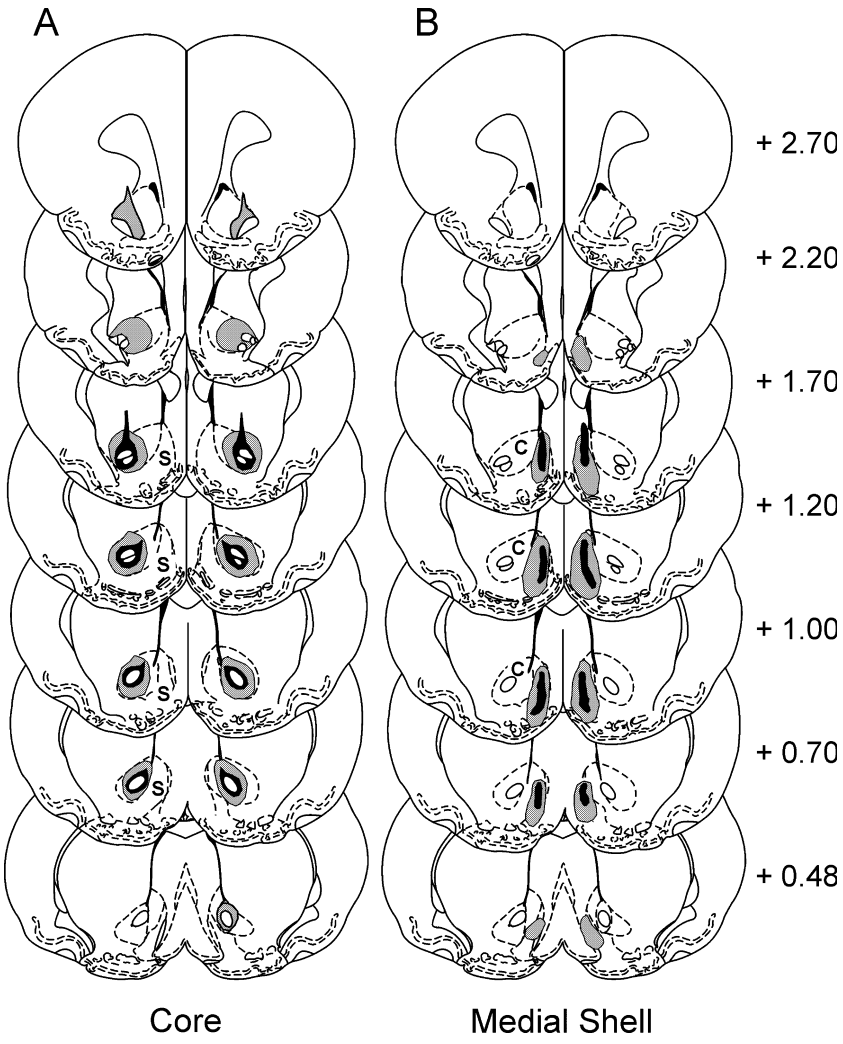

FIG. 2. Reconstructions of the maximum (grey plus black shading) and minimal (black shading) extent of the core ('C', column A) and medial shell lesions ('S', column B) in the coronal plane of subjects included in the final analysis. The numbers (in mm relative to bregma) denotes the anterior-posterior level of the illustrated sections in correspondence to the stereotaxic atlas by Paxinos & Watson (1997).

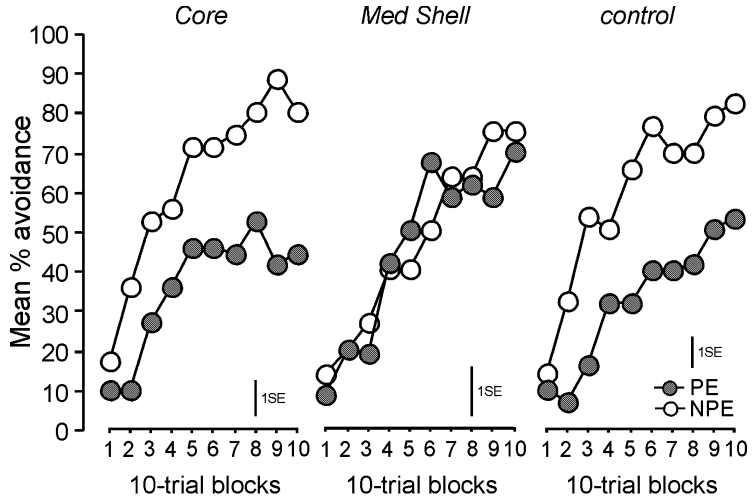

FIG. 3. Effects of excitotoxic lesions restricted to either the core or the medial shell subregions on two-way active avoidance learning. The line graphs depict the mean percentage avoidance of the NPE and the PE animals as a function of 10-trial blocks in each of the three treatment groups. The error bars indicate one standard error (1SE) derived from the appropriate error terms in the restricted 2-way ANOVAs. Core-PE, $n = 7$; core-NPE, $n = 7$; medial shell-PE, $n = 7$; medial shell-NPE, $n = 6$; control-PE, $n = 13$; and control-NPE, $n = 14$.

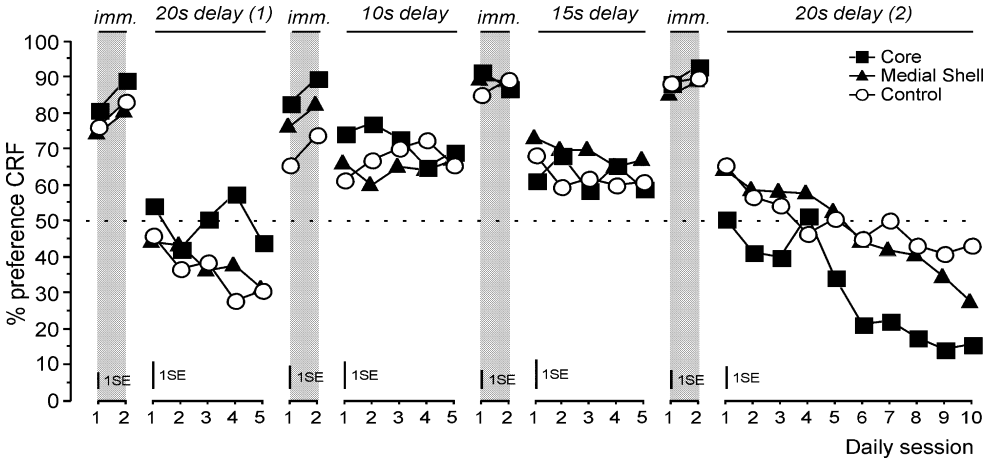

FIG. 4. Effects of excitotoxic lesions restricted to either the core or the medial shell subregions on performance in the delayed reward choice paradigm. Mean percentage choice preference for the CRF lever of each treatment is expressed as a function of daily sessions (with free choice trials – see Methods for details). The animals were subjected to choice tests with various delays being imposed on the CRF lever. The 'imm' (0 s delay for CRF lever) sessions served as baseline prior to the assessment of each specific delay intervals (20 s, 10 s, 15 s, and then 20 s again). The dashed line represents chance level of preference (i.e. 50%). The error bars indicate one standard error (SE1) derived from the appropriate error terms taken from the ANOVA of the corresponding phase. Core, $n = 7$; medial shell, $n = 8$; and control, $n = 13$.

20 s delayed-CRF vs. immediate-PRF choice

With the first introduction of a 20-s delay to the CRF lever (phase 2), preference for the CRF lever dropped to near chance level. The overall mean of CRF-lever preference in this phase was 40.2 ± 3.2%. This shift away from the CRF lever and towards the PRF lever was exacerbated over the 5 days of testing, giving rise to a main effect of days ($F_{4,100} = 3.04$, $P < 0.05$). No significant group difference was detected. Nonetheless, there was a nonsignificant tendency that while the medial shell lesions and sham groups were showing an overall preference away from the CRF lever (i.e. < 50%), the core lesion group chose at a level close to chance level performance (∼50%). No other main effects or interaction attained statistical significance.

10 s delayed-CRF vs. immediate-PRF choice

Introduction of the 10 s delay on the CRF lever again resulted in a shift away from choosing the CRF lever (phase 4), but there remained an overall bias for the CRF lever that was observable in all groups. The overall mean of preference for the CRF lever in this phase was 67.6 ± 4.0%. Moreover, the level of preference for the CRF level remained stable over days. No main effects or interactions attained statistical significance.

15 s delayed-CRF vs. immediate-PRF choice

Subsequent introduction of the 15 s-delay on the CRF lever (phase 6) yielded behaviour comparable to that observed previously under the 10-s delay, with a slightly lower overall level of preference for the CRF lever (64.3 ± 5.1%). Again, no main effects or interactions attained statistical significance.

20 s delayed-CRF vs. immediate-PRF choice

The re-introduction of a 20-s delay to the CRF lever resulted again in a shift of preference away from the CRF lever (phase 8). However, unlike the first introduction of the same delay condition (i.e. phase 2), the initial shift was maintained at a level above 50% (preference for CRF lever), this was at least the case for the medial shell lesion and control groups (Fig. 4). Over the extended 10 days of testing, subjects increasingly preferred the PRF lever, and this effect was more pronounced in the core lesion group. These impressions were supported by the main effect of days ($F_{9,225} = 20.88$, $P < 0.0001$), and the significant interaction between treatment and days ($F_{18,225} = 1.76$, $P < 0.05$). The latter was solely attributable to the anomalous core lesion group, because the interaction term was no longer significant in an additional analysis restricted to the other two groups.

Experiment 3. Differential reinforcement for low rates of response (DRL)

DRL-4 s

Performance improved over the three 2-day blocks of DRL-4 s as evident by a reduction in the mean number of lever presses required per reward ($F_{2,4} = 19.50$, $P < 0.0001$) and an increment in the total number of reinforcement earned ($F_{2,4} = 34.35$, $P < 0.0001$) (see Fig. 5A). No other main effects or interactions attained statistical significance.

DRL-8 s

Increasing the DRL requirement to 8 s led to an initial reduction in performance compared to the previous stage, but performance improved as training progressed over the three blocks of training (Fig. 5A). The latter was evident in terms of the number of responses per reward ($F_{2,4} = 6.36$, $P < 0.005$) and total number of rewards ($F_{2,4} = 9.28$, $P < 0.0005$). There was a tendency for the core lesion group to perform less well, but no other main effects or interactions reached statistical significance in the analyses.

DRL-12 s

Further extension of the DRL requirement to 12 s had the predictable effect in first reducing performance, followed by an improvement as training progressed (Fig. 5A). Analysis of the number of responses per reward and total reinforcements earned yielded a significant main effect of 2-day blocks ($F_{2,4} = 7.25$, $P < 0.005$, and $F_{2,4} = 18.67$, $P < 0.0001$, respectively). Despite the continual trend indicative of a relatively poor performance in the core lesion group compared with the other two groups, no other main effects or interactions attained statistical significance.

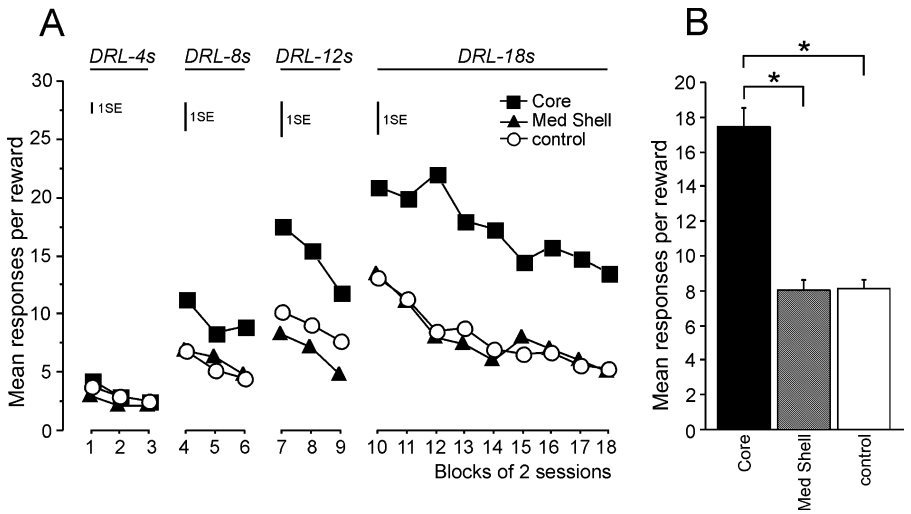

FIG. 5. Effects of excitotoxic lesions restricted to either the core or the medial shell subregions on DRL performance. Mean response per reward earned over every 2-day block is expressed as a function of blocks for each treatment group across the various DRL requirements: DRL-4 s, DRL-8 s, DRL-12 s and then DRL-18 s. The histogram in B depicts the mean (+ SEM) number of responses emitted per earned reward across the 9 blocks of DRL-18 s. The asterisks in B indicate a significant difference ($P < 0.05$) of the Core group compared to the other groups as revealed by the post hoc pair-wise comparison. The error bars in A indicate one standard error (1SE) as derived from the appropriate error terms taken from the corresponding ANOVA for each of the four DRL requirements. Core, $n = 7$; medial shell, $n = 5$; control, $n = 14$.

DRL-18 s

Finally, the animals were challenged with DRL-18 s and this lasted over a more extended period of testing (18 days). Throughout, the medial shell lesions and control groups performed at a highly comparable level. By contrast, the core lesion group showed a clear and persistent deficit, despite evidence of improvement as testing continued (Fig. 5A). This impression is consistent with the nonsignificant trend observed previously under lower DRL demands. This pattern of results gave rise to a main effect of blocks ($F_{8,16} = 9.53$, $P < 0.0001$) and of treatment ($F_{2,23} = 3.98$, $P < 0.05$). Post hoc pair-wise comparisons confirmed that the treatment effect was solely attributed to an inferior performance in the core lesion group relative to the other two groups (Core vs. controls $P = 0.01$, and core vs. medial shell $P = 0.04$), which did not differ from each other. The interaction between treatment and blocks was far from statistical significance ($F < 1$), implying that improvement seen across blocks did not differ among groups.

Discussion

The present study extends the original finding of Cardinal *et al.* (2001) that lesions of NAC core can lead to impulsive-like behaviour in a delayed reward choice paradigm, utilizing a contrast between reward probability instead of reward size. This effect was not seen after selective NAC medial shell lesions. Similarly, DRL performance was impaired by core but not shell lesions. The emergence of impulsive-like behaviour was selectively promoted by core damage, and this contrasts with the disruption of LI that was only seen after shell (but not core) damage. The present study thus provides evidence for two double dissociations, depending on whether Experiment 1 is contrasted with Experiment 2 or 3, lending further support for a functional segregation between NAC core and shell.

The finding that LI is selectively disrupted by shell lesions is in line with previous studies employing either an active avoidance paradigm as here (Weiner *et al.*, 1999; Jongen-Rêlo *et al.*, 2002) or the conditioned lick-suppression paradigm (Tai *et al.*, 1995; Weiner *et al.*, 1996). The differential involvement in LI between shell and core has been linked to the shell being the preferential target for the limbic cortical inputs (originating from the ventral hippocampus/subiculum and entorhinal cortex) that are destined for the NAC (Totterdell & Smith, 1989; Sesack & Pickel, 1990; Totterdell & Meredith, 1997). These excitatory cortical afferents converge with the ascending dopaminergic projection arising from the ventral tegmental area (Totterdell & Smith, 1989; Sesack & Pickel, 1990); and their interactive modulation over NAC neuronal activity (Grace, 2000; Floresco *et al.*, 2001) is of critical importance to the control of LI expression according to a number of theories (e.g. Weiner, 1990; Gray *et al.*, 1991; Gray *et al.*, 1995; Gray *et al.*, 1997; Weiner & Feldon, 1997). One theory that has explicitly predicted this pattern of shell–core differentiation in LI is the 'switching' hypothesis of Weiner (1990, 2003).

Weiner (1990) characterizes an attenuation of LI as enhanced 'switching' from responding according to the 'CS → nothing' contingency that prevails during pre-exposure, to responding according to the 'CS → US' contingency subsequently acquired during conditioning. The relevance of response switching to behavioural control in general is lucidly captured by Weiner's own description: 'switching to respond according to the reinforcement contingency when environmental contingencies demand so' (p.18, Weiner, 2003). Although the 'switching' hypothesis primarily focuses on LI, the description of response switching clearly captures some essential elements of the delayed reward choice paradigm that are central to its use as a test of impulsivity. Thus, animals switch their preference for the CRF to the PRF lever as the delay in reward delivery associated with the CRF lever is lengthened. It follows that LI attenuation and enhanced CRF-to-PRF shifting can both be understood as an increased propensity to switch. Contrary to this view, however, our results suggested that impaired LI is not associated with enhanced impulsive choice behaviour. Furthermore, the fact that core lesions facilitate the emergence of impulsive choice behaviour further contradicts the proposal of Weiner (2003) that core lesions can blunt switching – based on the observation that core lesions can eliminate the impact of a context change between CS pre-exposure and conditioning upon LI expression (see Gal *et al.*, 2005).

Taken together, it is apparent that the double dissociation between shell and core lesions revealed by the LI and delayed reward choice experiments cannot be accommodated within a singular concept of 'switching'. It suggests the existence of multiple expressions of behavioural switching, which may be distinguished by their relative sensitivity to selective shell and core damage. Alternatively, the effect of selective core lesions on the delayed reward choice paradigm may not represent enhanced response switching of any form.

Cardinal *et al.* (2001) first reported that core lesions led to reduced tolerance to delayed reinforcement: rats with core lesions showed enhanced preference for the smaller reward when increasing delays were imposed on the delivery of the larger reward. This forgoing of the larger reward was interpreted as impulsivity (Ho *et al.*, 1999; Rachlin, 2000). Here, the extension of their finding to a choice between high and low probabilities of reward excludes the possibility that the observation of Cardinal *et al.* (2001) was specific to the interaction between differentiation of reward size and delayed reinforcement.

The procedure of the delay-reinforcement (Ainslie, 1975) also incurs an additional mnemonic demand as the response and reward become increasingly discontiguous (see Garrud *et al.*, 1981). This confounding factor is unlikely to offer an alternative explanation of the effects of core lesion because working memory function is unaffected by similar lesions (Jongen-Rêlo *et al.*, 2003). Yet, from a more general perspective, this current emphasis on delayed-discounting ought to be further evaluated against other means of reinforcement discounting procedures. For example, the larger reward can be discounted by a lower reinforcement probability, or an increase in operant effort (Ishiwari *et al.*, 2004; also see Salamone *et al.*, 2003), thus allowing an extension of impulsivity research in animals beyond delayed reinforcement.

Even though both Cardinal *et al.* (2001) and the present study used a delay to discount the reinforcement associated with the more favourable reward, certain procedural differences deserve further discussion, as they may explain why the effect of core lesions emerged only on the second (but not the first) introduction of a 20-s delay here. Whilst the delay imposed on the larger reward lengthened progressively within a test session in the experiment of Cardinal *et al.* (2001), a specific delay was maintained across test days here. We observed that the first imposition of a 20-s delay led to an immediate loss of preference for the CRF lever and a rapid development of an overall bias towards the PRF lever. Intriguingly, the core lesioned animals did not show any sign of enhanced impulsivity at this stage. Instead, they showed if anything a reduced sensitivity to the delay (albeit nonsignificantly so) compared to the other groups, because only their performance appeared to remain around chance level. The latter may point to a failure in distinguishing between the two levers and their respective consequences, possibly reflecting a generalization between delayed-reward and nonreward. This interpretation is already suggestive of one possible consequence of an increase in sensitivity to delayed reinforcement in the core group.

Next, when shorter delays (10 s and 15 s) were employed, their impact was less severe: controls subjects were maintaining an above-chance level of preference for the CRF lever. Yet, there was still no evidence for enhanced sensitivity to the delayed reinforcement in the core lesioned group. Notably, at these two delays, performance remained remarkably stable across test days. In comparison, when a clear effect of core lesions finally emerged in the second introduction of the 20-s delay (i) the control animals maintained an initial above-chance preference for the CRF lever, and (ii) they developed a clear shift of choice across test days. However, this does not necessarily imply that core lesions enhanced the rate of the shift. The rate of preference change across days was not particularly faster in the core group apart from day 4 (see Fig. 4). This is remarkably similar to the results of Cardinal *et al.* (2001), when the preference for the larger reward was depicted as a function of increasing delay (see their Fig. 2D, p. 2500). The changes observed between the two tests with a 20-s delay must be attributable to the intervening tests with delays of 10 s and 15 s, when the subjects had learned to tolerate the delay (at least as reflected by the reduction in the initial impact of the introduction of a 20-s delay) and to maintain a distinction between the two levers. It can be envisaged that the paradigm adopted by Cardinal *et al.* (2001) is particularly conducive for such an ongoing shift from choosing preferentially the high to the low reward. This could be an important element, if not a prerequisite, for a successful demonstration of the effect of core lesions on impulsive choice. This is consistent with the notion that the NAC plays a critical role in translating motivation into action in the guidance of goal-directed behaviour (Mogenson *et al.*, 1980), and such guidance would be particularly active when a continuous shift of choice is taking place.

The shell–core dissociation demonstrated in the delayed reward choice paradigm is paralleled by a similar core-specific effect on DRL, which confirmed an earlier report using nonrestrictive NAC lesions (Reading & Dunnett, 1995) and further specified that this effect stemmed solely from damage to the core. Although we did not evaluate both DRL and the delayed reward choice test in the same subjects (to avoid potential confounding transfer effect between tasks), an intriguing question arises as to whether these two observations could be attributed to a unitary psychological dysfunction. According to Cardinal *et al.* (2001) core lesions impaired the tolerance to delay reinforcement. Could this have predicted the outcome in the DRL experiment, in which reinforcement is provided only when responses are spaced out in time? We suspect, however, that such an extension of argument is far from straightforward.

It has been proposed that although reduced preference for the more favourable outcome in the delayed reward choice task and poor performance in the DRL schedule can both be interpreted as a sign of impulsive behaviour, the two appear to reflect distinct expressions of impulsivity (Evenden, 1999; Ho *et al.*, 1999; Monterosso & Ainslie, 1999). First, DRL does not entail an explicit choice between responding to two alternative discriminanda or operanda. Instead, the subjects need to choose between responding and not responding, which arguably may only be considered as a 'go/no-go' choice.

Second, the 'impulsive behaviour' in DRL (viz., a premature response) leads to the omission of an expected reward. In contrast, the selection of the known (and therefore not unexpected) lesser reward constitutes an impulsive choice in the delayed reward choice paradigm. Omission of an expected reward is an aversive event; it inhibits responding (and its avoidance reinforces nonresponding), leading to more efficient DRL performance. DRL does not have a trial discrete design and is therefore more susceptible to a global assessment of reinforcement rate – withholding response increases the global probability of reinforcement, to a maximum that every response earns a reward when response rate is below that of the specified DRL period. DRL is thus highly effective in taxing the ability to inhibit or withhold learned purposeful behaviour (Gray, 1982a, 1982b; Ho *et al.*, 1999; Monterosso & Ainslie, 1999). Interestingly, ad hoc reasoning may equate poor DRL performance to impaired switching from responding to nonresponding, and thus lends some support to Weiner's (2003) behavioural characterization of selective core lesion. However, our observation that the DRL impairment after core lesions was weakest at the beginning when the impact of the DRL requirement is expected to be strongest (from the preceding FR4 schedule to DRL-4 s), and the progressive

emergence of a clear effect of the lesions over days, and across increasing DRL demand, are contrary to the expectations of Weiner's (2003) switching hypothesis.

Thirdly, DRL does not involve any delayed reinforcement, and the hypothesis that core lesions induce intolerance to delayed reinforcement would not have predicted the present outcome. A given response in DRL is either immediately rewarded or not. Maintenance of responding to the higher reward against the discounting effect produced by delay has been central to the delayed reward choice paradigm as a test of impulsive behaviour, but inhibition against choosing the lesser reward is certainly equally important in maintaining nonimpulsive choice. Likewise, nonimpulsive behaviour in DRL entails learning to inhibit responding. Disinhibition may represent a parsimonious account for the emergence of two forms of impulsive-like behaviour after selective core lesion, which is not in conflict with its lack of an effect in LI (see Clark *et al.*, 1992). However, this hypothesis does not necessarily contradict the view that NAC core lesions can induce intolerance to delayed reinforcement (Cardinal *et al.*, 2001).

In conclusion, the present study provides important and novel qualifications to the shell–core functional dissociation across tests previously known to be sensitive to NAC lesions. Its primary contribution is incremental: confirming previous tentative conclusions, and establishing two double dissociations in a more explicit within-subjects manner. Our results support the hypothesis that dysfunction of the NAC core (but not the shell) may be associated with the appearance of impulsive-like behaviour, but this certainly still warrants investigation and characterization using additional paradigms. On the other hand, additional specifications and modifications to the 'switching' hypothesis of Weiner (2003) are needed in light of the present data. Further integration with theories relating NAC to behavioural inhibition and reward prediction (Gray, 1982a,b; Gray *et al.*, 1991; Schultz, 2002) would be useful for a comprehensive and accurate psychological description of NAC functions.

Acknowledgements

The present study was supported by grants awarded by the Swiss Federal Institute of Technology and the Swiss National Science Foundation. We are also indebted to the additional support from the National Centre of Competence in Research (NCCR): Neural Plasticity and Repair. The authors would like to express their gratitude to Ms. Liz Weber for preparing the histology, Mr Peter Schmid for his technical expertise, Dr Daniela Rüedi-Bettschen and Mr Francis Bambico for their assistance in running parts of the experiments, Mrs Misa Kuper-Yamanaka for her editorial assistance, and the animal facility team for taking care of the animals.

Abbreviations

ANOVA, analysis of variance; CRF, continuous reinforced; CS, conditioned stimulus; DRL, differential reward for low rates of responding; FR, fixed ratio; LI, latent inhibition; NAC, nucleus accumbens; NeuN, neuronal nuclei (protein); NMDA, *N*-methyl-D-aspartic acid; NPE, nonpre-exposed; PB, phosphate buffer; PBS, phosphate buffered saline; PE, pre-exposed; PRF, partially reinforced; SEM, standard error of the mean; US, unconditioned stimulus.

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