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Research report

Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function

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Abstract

The involvement of mesoaccumbens dopamine in adaptive learning and behaviour is unclear. For example, dopamine may act as a teaching signal to enable learning, or more generally modulate the behavioural expression, or selection, of an already-learned response. The present study investigated the involvement of the mesoaccumbens dopamine system in a fundamental form of learning: Pavlovian conditioning. In this case, the temporal association of a previously neutral visual stimulus and a biologically significant unconditioned stimulus (US), subsequently led to the production of the conditioned response (CR) of discriminated approach behaviour directed toward the conditioned stimulus (CS+), relative to a control (CS−) stimulus. 6-hydroxydopamine lesions of the nucleus accumbens (NAcc), leading to approximately 80% reductions in tissue dopamine, were made at varying time points in four experimental groups of rats, either before or subsequent to the acquisition of the CR. NAcc dopamine depletion produced long-term neuroadaptations in dopamine function 2 months after surgery, and profoundly impaired discriminated Pavlovian approach regardless of when the lesion was made. Thus, NAcc dopamine not only plays a role in conditioned behavioural activation, but also in making the appropriate discriminated response i.e. the direction of response. Further, acquisition lesions produced a far greater impact on discriminated approach than performance lesions. This difference in lesion-induced impairment implies that mesoaccumbens dopamine may play differential roles in the learning and performance of preparatory Pavlovian conditioning.

Keywords: Autoshaping; Reward; Striatum; Core; Shell; Learning; Motivation

1. Introduction

Much research into nucleus accumbens (NAcc) function has focused on the role of the mesolimbic dopamine (DA) system in reward and reinforcement. Thus, the DA-ergic innervation of the NAcc has been argued to subserve a critical component of the reward process [63] or to act as a teaching signal for associative learning [49,50]. However, NAcc DA may alternatively be specifically involved in response selection and in the 'gain-amplification' of responses learned and selected via neural networks converging on the NAcc [43,44,48].

Neurochemical evidence demonstrates correlations between environmental events, or stimuli which predict those events, and changes in DA transmission within the NAcc, including stimulant and opiate drugs, copulation, novelty and general arousal [13,14,18,25,42]. More specifically, some evidence suggests that changes in extracellular DA release in the NAcc shell appear to reflect responses to primary reinforcers (including stimulant drugs such as cocaine), whilst increases in

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DA in the NAcc core appear to reflect associative learning processes i.e. conditioned increases [13,15,27]. From another perspective, Schultz and colleagues (for example [50]; and see [51]) have argued that DA neurons projecting to the striatum, including the NAcc operate to predict reward according to contemporary associative learning rules. The rapid and temporally precise manner in which DA neurons fire to unpredicted USs or predictive CSs parallels the rapid approach/preparatory response that is made toward biologically significant stimuli and may result in DA neurons firing synchronously to the transfer of information relating to the to-be-reinforced behaviour to the striatum by limbic-cortical afferents, thus allowing consolidation, or 'stamping in' of responses by reinforcement [50].

Alternatively, NAcc DA may be selectively involved in the response process, specifically the arousing and preparatory effects of reinforcers, though not in the learning process itself [32,44,54]. Thus, low doses of systemic DA receptor antagonists reduce preparatory behaviour, such as hoarding and anticipatory responses, without affecting consummatory behaviour such as eating [5]. Further, 6-hydroxydopamine (6-OHDA) lesions of the NAcc impair the potentiative effect of intra-NAcc amphetamine on instrumental responding for a conditioned reinforcer, but do not impair the ability of conditioned reinforcers to sustain new instrumental learning [55]. NAcc DA also appears to contribute to asymptotic response rates, or effort, as DA depletion leads to the re-allocation of response selection based on the relative kinetic requirements to attain alternative goals [1,11,48].

The present study investigated whether NAcc DA is necessary for Pavlovian associative learning, for the performance of an already established Pavlovian response, or both. Rats were tested using a Pavlovian autoshaping procedure which has previously been shown to be sensitive to excitotoxic NAcc lesions [39]. 'Autoshaping' describes the way in which an animal will track a stimulus that is predictive of reward. This has obvious adaptive value in helping to guide an animal towards objects in the environment that will provide food and warmth, and away from dangerous objects and places. In essence, it embodies that way in which biologically significant environmental stimuli can capture the attention and behavioural control of an animal. This approach response lacks the flexibility of instrumental learning [23,60] and is therefore deemed to be predominantly under Pavlovian control. This form of stimulus-elicited approach has been observed in pigeons [8], rats [9,39], monkeys [52] and humans [59] and a successful session of autoshaping has been shown to increase tissue levels of DA specifically within the NAcc of rats [56]. In the present procedure, the 10 s presentation of a white rectangle on a computer screen (this stimulus is designated the CS+) was immediately followed by the presentation of a food pellet in a spatially separate location. A second stimulus, identical visually, but presented on the opposite side of the screen to the CS+ was never followed by reward and operated as a control (designated the CS−). During training, control animals demonstrate progressive discrimination by approaching the CS+, but not the CS−.

In this study, DA depleting lesions of the entire NAcc were made before or after learning, in different groups of rats. In order to relate behavioural changes to the disruption of NAcc DA following 6-OHDA lesions, in vivo microdialysis and ex vivo neurochemistry were used to assess DA function at various time points after surgery. The neurochemical specificity of the lesion was also determined through post-mortem analysis of tissue monoamine levels. In the first group of animals, the relationship between DA depletion and learning was established. Rats were given 6-OHDA lesions of the NAcc and then after 10 days the levels of extracellular monoamines were measured in the NAcc using in vivo microdialysis. Animals were then sacrificed and brain tissue samples taken for analysis. A second group of animals was lesioned, and tested on the autoshaping procedure 10 days later. Thus, this group of animals operated as a time-matched control for the first group. A third group of animals was lesioned and left for 2 months before autoshaping. This group underwent in vivo microdialysis and tissue sampling shortly after they had been autoshaped. The main purpose of studying the effects of DA depletion across two different time points was to assess the neurochemical and behavioural nature of functional recovery which is known to occur following 6-OHDA striatal DA depletion [62,66]. In a fourth group, animals were first trained to autoshape before being given 6-OHDA lesions of the NAcc in order to assess whether NAcc DA is critical for the performance of Pavlovian approach behaviour.

2. Methods

2.1. Subjects

The subjects were 65 male hooded Lister rats (Olac, Bicester, UK) weighing between 289 and 366 g at the time of surgery. Animals were housed in pairs in a colony room maintained at 21 °C on a reverse 12 h light to 12 h dark cycle and were tested during the dark phase. For the preceding week and for the duration of the autoshaping task and locomotor activity study, animals were given restricted access to food (1 h access

at the end of the testing day). At all other times, animals had free access to both water and laboratory chow. All animals used in this study were treated in accordance with the UK 1986 Animals (Scientific Procedures) Act (Project Licence PPL 80/1324).

2.2. Surgery

Rats were assigned randomly to experimental groups. Sham control groups were treated identically to the rats in lesion groups except that they received injections of sterile phosphate-buffer (sterile PB) vehicle alone. Surgery was performed under Avertin anaesthesia (2,2,2-tribromoethanol, 2-methylbutan-2-ol, Dulbecco 'A' PBS tablets, and H_2O in tertiary amyl alcohol (Sigma, UK); 10 ml/kg body weight), using standard stereotaxic procedures. Injections were made through a single burr hole using a 5 μl SGE (SGE, Baton Rouge, USA) syringe (26 gauge: code 1BR-OC-7/0.47). Injections of 6-OHDA (Sigma, UK) into the NAcc were made using the following co-ordinates: AP+3.4, ML±1.7, DV, 7.2 (from dura) based on the atlas from Pellegrino et al. [40], with the incisor bar set at +5 mm. 6-OHDA (2.0 μl) were infused over a period of 4 min. All animals were given injections of glucose/saline (5–10 ml, i.p.) after surgery to aid recovery. Behavioural testing began 10 days after surgery.

2.3. In vivo microdialysis

2.3.1. Dialysis probe construction and implantation

Concentric-design microdialysis probes were constructed using 2 mm lengths of Hospal (Fitral 12) dialysis membrane (20 000 Da nominal molecular cut-off), deactivated fused silica tubing (140 μm o.d., SGE, UK) (outlet) and 24 g thin-wall stainless steel tubing (0.38 mm i.d., 2.21 mm o.d., Elkay Laboratory Products, UK) as described previously [12]. The dialysis probe was vertically implanted in the NAcc (stereotaxic co-ordinates relative to bregma and the dural surface: AP+1.5 mm; L±1.2 mm; Dura −8.5 mm with the incisor bar at −3.3 mm) under ethyl carbamate anaesthesia (1.2 g/kg i.p. urethane, Sigma, UK). The core body temperature was maintained at approximately 36.5–37 °C.

2.3.2. Dialysis procedures

The dialysis probe was perfused at a flow rate of 1.0 μl/min with artificial cerebrospinal fluid (aCSF) of the following composition: NaCl 146 mM; KCl 3.0 mM; $MgCl_2$ 1.0 mM; $CaCl_2$ 1.3 mM; sodium phosphate buffer 1.5 mM; pH 7.4. Dialysates were collected over 3 h as 15 min fractions into 4 μl 0.2 M perchloric acid commencing 3 h after surgery. DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxy-3-indoleacetic acid (5-HIAA) were determined in brain dialysates by high performance liquid chromatography and electrochemical detection (HPLC-ECD), as described previously [27].

2.4. Post-mortem assessment of lesion

Following behavioural testing and in vivo microdialysis, the extent of DA depletion in the medial prefrontal cortex, and ventral and dorsal striatum was assessed in post-mortem tissue of CO_2 euthanised animals, by HPLC-ECD, as described in detail previously [34].

2.5. Experimental design

2.5.1. Neurochemical sequelae of 6-OHDA lesions of the NAcc 10 days post-operatively

Animals received DA depleting lesions (or sham lesions; $n=8$ for both groups) of the ventral striatum, and then, 10 days following surgery, in vivo microdialysis was used to measure extracellular levels of DA in the NAcc of urethane-anaesthetised animals. Subsequently, the extent of the DA depletion in selected brain regions was assessed. This group served to provide an index of DA function in the NAcc at a time congruent to sham and lesioned animals autoshaped 10 days following surgery.

2.5.2. Effects of 6-OHDA lesions of the NAcc 10 days post-operatively (acquisition group)

Animals received intra-NAcc 6-OHDA ($n=8$) or sham ($n=8$) infusions and were autoshaped 10 days later. Spontaneous locomotor activity was assessed in sham and lesioned animals over 4 consecutive days following the autoshaping procedure (commencing approximately 15 days post-operatively).

2.5.3. Effects of 6-OHDA lesions of the NAcc 2 months post-operatively (acquisition group)

Animals were treated identically to those in the previous group ($n=8$ for both experimental groups) except that the behavioural testing commenced 2 months after surgery. In vivo microdialysis and tissue analysis was used to assess NAcc DA function, as before.

2.5.4. Effects of 6-OHDA lesions of the NAcc 10 days post-operatively (performance group)

Initially, 17 animals were autoshaped. Only those which demonstrated a significant level of discriminated approach (70% approach to CS+, during its presentation, over the last 30 trials of acquisition) were then randomly assigned to sham ($n=7$) and lesion ($n=7$)

groups and prepared for surgery as previously described (three animals failed to approach the CS+ to criterion and were dropped from the study). Following recovery from surgery they were tested on the autoshaping procedure. As for previous behavioural groups, spontaneous locomotor activity was assessed, and post-mortem tissue analysis was performed following the termination of behavioural testing.

2.6. Autoshaping

2.6.1. Apparatus

The apparatus consisted of a testing chamber attached to a video display unit (VDU) within a sound attenuating box (fitted with an extractor fan) and is an adaptation of the apparatus described by Bussey et al. [9]. The inner chamber measured $48 \times 30 \times 30$ cm consisting of a metal frame and Perspex walls, with an aluminium floor incorporating pressure sensitive pads. A 3 W houselight was attached to the centre of the ceiling. A food magazine hopper was attached to a pellet dispenser (Campden Instruments, Loughborough, UK) outside the sound attenuating box and allowed the controlled delivery of sucrose pellets (Noyes, UK). Pressure sensitive floor pads (14×10 cm) were attached to microswitches enabling the measurement of approaches to stimuli presented on the left or right of the VDU. Individual trials were triggered when the subject depressed a floor pad at the rear of the testing chamber, equidistant from both stimulus locations. Stimuli were white rectangles (10×28 cm) presented on either the far right or far left sides of the VDU. The apparatus was controlled and monitored by a BBC Master series microcomputer, using programs written in BASIC [9] allowing the presentation of computer graphic stimuli on a VDU monitor to rats whilst measuring their motor responses through the use of a touch-sensitive screen and pressure-sensitive floor pads.

2.6.2. Acquisition

Animals were initially given one 20-min habituation session to the chamber. The houselight was switched on and animals were allowed access to food pellets (dustless sucrose pellets; 45 mg sucrose, Bioserve Inc., NJ), which were delivered into the magazine on a VT 40 s schedule. Animals were observed during this session to ensure that they were successfully retrieving and consuming pellets. On the second day of testing, rats were trained to associate stimuli with reward. Stimuli were presented on the VDU for 10 s and followed by the delivery of a sucrose pellet into the magazine. One stimulus was designated the CS+ and was always followed immediately by reward. Another was designated the CS− and was never followed by reward. The two stimuli differed only in the side of the screen on which they were presented. Half the animals were given a right side CS+ and the other half a left side CS+. Stimuli were presented on a VT 40 s schedule, training consisting of a total of 100 trials (2 consecutive days of 50 trials per day), each trial consisting of one CS+ stimulus presentation and one CS− stimulus presentation. An approach was recorded if an animal stepped onto the floor panel directly in front of a stimulus, no other approaches being recorded for that stimulus presentation.

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

2.6.3. Discrimination probe

On the day following acquisition, animals were again put into the testing boxes though this time they received 20 trials of simultaneous presentation of the CS+ and CS−. The animals' choice of which stimulus to approach were measured to give a further assay of associative learning and an indication as to whether animals could discriminate between the CS+ and CS−.

2.7. Sontaneous locomotor activity

The spontaneous locomotor activity of rats was assessed in individual activity cages for 2 h a day, over 4 consecutive days (at the same time each day). In all cases locomotor activity tests began the day after completion of autoshaping. Photocell beam activity cages were used to measure spontaneous locomotor activity. There were 16 such cages ($25 \times 40 \times 18$ cm) with two photocell beams spaced along the length of the cage, 1 cm above the floor. The position of subjects within the array of racks was counterbalanced across experimental groups but remained the same for each individual animal over testing. A BBC microcomputer was used to measure activity during the test sessions.

2.8. Statistics

Data were subjected to repeated measures analyses of variance (ANOVA). These analyses were performed using SPSS for Windows release 6.1.3. The homogeneity

of variance across groups in repeated measures design ANOVAs was assessed by the Mauchly Sphericity Test. Where data sets significantly violated this requirement for a repeated measures design ANOVA, the Greenhouse-Geisser Epsilon correction parameter for degrees of freedom (Geisser and Greenhouse, 1959) was used to calculate a more conservative P value for each F ratio. Following ANOVA, further exploration of the data was conducted by determination of simple effects or interactions (Winer, 1971). Where appropriate, post hoc comparisons between individual data points were made with the Newman-Keuls test. Post hoc tests were carried out using CLR ANOVA for Macintosh, version 2 (Clear Lake Research, USA). The criterion for statistical significance was a probability level of $P < 0.05$. The between subjects factor was LESION (6-OHDA lesion and sham). Within subject factors included SAMPLE (extracellular DA, DOPAC, 5-HIAA, and tissue levels of DA, noradrenaline and 5-HT), PROBE (CS+ and CS−), LATENCY (CS+ and CS−), CS (CS+ and CS−) and BLOCK (1–10) for autoshaping, and day (1–4) for spontaneous locomotor activity.

3. Results

3.1. Lesion assessment

A lesion criterion was implemented to ensure that the behavioural results reflected the effects of significant DA depletion from the NAcc. Previously, Jones and Robbins [28] demonstrated differential functional effects of 'partial' versus 'total' DA-depletions. To ensure that the present results reflected 'total' DA depletions, any animals which demonstrated only a 'partial' lesion (67% or less mean tissue DA in the NAcc, relative to mean sham control levels) were removed from all subsequent analyses. Following surgery and lesion analysis, final group numbers were: Group 1: sham = 6, lesion = 4; Group 2: sham = 7, lesion = 4; Group 3: sham = 6, lesion = 6; Group 4: sham = 7, lesion = 6. The reduction in group numbers, excluding those removed due to an insufficient lesion, was due to the following reasons: in Group 1, two sham and three lesion animals died during surgery. In Group 2, one sham animal was also lost in this way, and one sham in Group 3. A further sham and one lesion animal died post-operatively (Group 3). Subjects maintained relatively stable body weights over the course of the experiment and, importantly, there were no significant effects of the lesion on body weights for any of the experimental groups (all $F < 2$).

3.1.1. Neurochemica sequelae of 6-OHDA lesions of the NAcc 10 days post-operatively

Table 1 shows the effects of 6-OHDA lesions of the NAcc on tissue levels of DA, NA and 5-HT in the ventral striatum, dorsal striatum and prefrontal cortex. DA levels were significantly decreased in the prefrontal cortex [$F(1,9) = 9.87$, $P < 0.05$] and ventral striatum [$F(1,9) = 26.01$, $P < 0.001$] relative to sham controls. In addition, levels of noradrenaline were reduced in the prefrontal cortex [$F(1,9) = 12.96$, $P < 0.005$] and ventral striatum [$F(1,9) = 25$, $P < 0.001$].

3.1.2. Effects of 6-OHDA lesions of the NAcc 10 days post-operatively (acquisition group)

Lesioned animals showed a significant reduction in tissue levels of DA in the ventral striatum [$F(1,10) = 78.42$, $P < 0.001$] and a trend for a reduction in DA in the prefrontal cortex [$F(1,10) = 4.18$, $P = 0.06$]. There was also a significant reduction in NA in the prefrontal cortex [$F(1,10) = 156.02$, $P < 0.001$] and ventral striatum [$F(1,10) = 12.32$, $P < 0.005$]. There were no significant effects of the lesion on tissue levels of 5-HT. (all Fs < 2 except 5-HT in the prefrontal cortex [$F(1,10) = 2,89$, $P = 0.102$] and NA in the dorsal striatum [$F(1,10) = 2.01$, $P = 0.18$]).

3.1.3. Effects of 6-OHDA lesions of the NAcc 2 months post-operatively (acquisition group)

Tissue levels of DA remained significantly depleted relative to sham controls 2 months after intra-NAcc 6-OHDA administration (ventral striatum: [$F(1,11) = 8.7$, $P < 0.05$]; prefrontal cortex: [$F(1,11) = 6.47$, $P < 0.05$]) In addition, prefrontal cortical NA was significantly reduced in lesioned animals [$F(1,11) = 21.32$, $P < 0.001$].

3.1.4. Effects of 6-OHDA lesions of the NAcc 10 days post-operatively (performance group)

ANOVA revealed significant reductions in levels of NAcc NA [$F(1,12) = 6.22$, $P < 0.05$] and DA [$F(1,12) = 73.44$, $P < 0.001$] and a trend towards a reduction in 5-HT [$F(1,12) = 4.06$, $P = 0.06$]. In addition, tissue levels of PFC NA were significantly depleted [$F(1,12) = 28.72$, $P < 0.001$].

In summary, intra-NAcc 6-OHDA produced a similar pattern of results across all four experimental groups. Tissue levels of NAcc DA were significantly reduced in all lesion groups, relative to sham control levels. NA content was also reduced in the NAcc of lesioned animals though not significantly in the group which was behaviourally tested 2 months after the lesion was produced. Both DA and NA were also generally reduced in the prefrontal cortex of DA-depleted rats. Serotonin was not affected by the lesion in any brain region nor were any monoamines in the dorsal striatum.

Table 1
Effect of NAcc 6-OHDA infusions on brain tissue concentrations of monoamines across all four experimental groups

Group		Brain region								
		Nacc			Dorsal Stri.			PFC		
		DA	NA	5-HT	DA	NA	5-HT	DA	NA	5-HT
1	Sham	46.8	3.56	4.36	96.9	ND	1.21	0.63	2.88	2.96
	Lesion	7.48	0.67	5.38	72.85	ND	1.38	0.24	0.72	3.02
	% dep.	**84***	**81.2***	**−23.4**	**24.8**	**–**	**−14.1**	**61.9***	**75***	**−2**
2	Sham	31.6	4.78	5	84.9	ND	1.55	0.46	2.68	2.78
	Lesion	6.48	1.45	4.5	70.3	ND	1.82	0.27	0.51	2.39
	% dep.	**79.5***	**69.7***	**10**	**17.2**	**–**	**−17.4**	**41.3**	**81***	**14**
3	Sham	70.89	8.49	6.4	112.92	ND	3.69	1.2	3.53	3.53
	Lesion	18.21	4.57	6.73	104.31	ND	3.5	0.47	1.28	3.5
	% dep.	**74.3***	**46.2**	**−5.2**	**7.6**	**–**	**5.1**	**60.1***	**63.7***	**0.85**
4	Sham	38.04	1.82	3.2	75.67	ND	2.19	1.5	2.74	2.57
	Lesion	6.03	0.74	2.28	38.32	ND	1.72	0.31	0.72	2.01
	% dep.	**84.1***	**59.3***	**28.8**	**49.4**	**–**	**21.5**	**79.3***	**73.7***	**21.8**

Data are expressed as mean concentrations (pmol/mg tissue) collapsed across both hemispheres. Percentage depletions for lesions relative to shams are shown in bold. Negative values indicate a higher concentration of monoamine in lesioned animals. *, $P < 0.05$. Group 1, neurochemical 10 day control; Group 2, behavioural 10 day acquisition; Group 3, behavioural and neurochemical 2 months acquisition; Group 4, behavioural 10 day performance; Nacc, nucleus accumbens; Dorsal Stri., dorsal striatum; Pfc, medial prefrontal cortex; % dep., percentage depletion, lesion relative to sham tissue levels of monoamines, ND, not detectable.

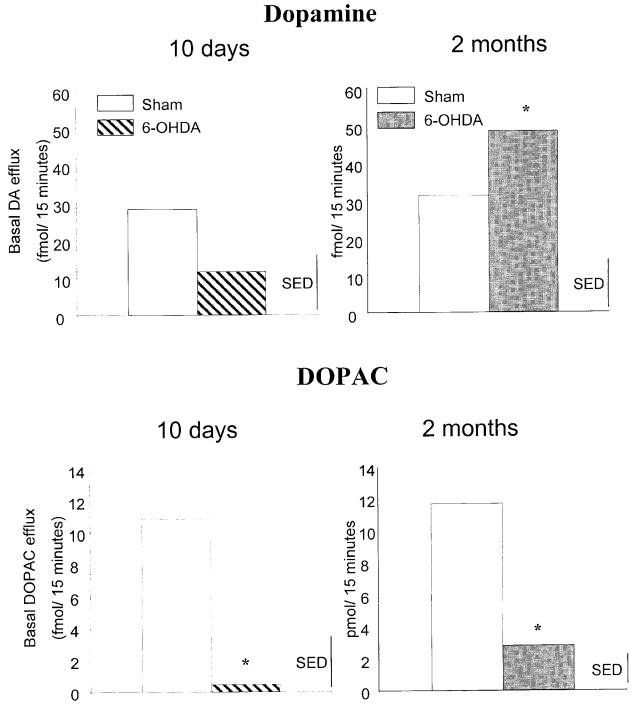

Fig. 1. Mean extracellular levels of DA and DOPAC, calculated over 12 × 15 min sampling bins from dialysis probes located in the NAcc of anaesthetised rats. Ten days, data are from animals in Group 1-Neurochemical 10 day control, in which in vivo dialysis measurements were made in animals 10 days after DA depletion. 2 months, data are from animals in Group 3, behavioural and neurochemical 2 months acquisition, in which in vivo dialysis measurements were made immediately following behavioural testing (vs. 2 months and 10 days after DA depletion). Significant between group differences are indicated by an * ($P < 0.05$).

3.2. In vivo microdialysis

3.2.1. Neurochemical sequelae of 6-OHDA lesions of the Nacc 10 days post operatively

Levels of extracellular DA (averaged over the 3 h sampling session) showed a large 59.3% reduction, which failed to reach significance [$F(1,9) = 2.34$, $P = 0.16$] in 6-OHDA lesioned animals, relative to controls. Further, there was a significant 95.5% reduction in the level of extracellular DOPAC [$F(1,9) = 22.33$, $P < 0.005$] in lesioned animals (see Fig. 1). Levels of extracellular 5-HIAA were not significantly affected by the lesion ($F < 2$).

3.2.2. Effects of 6-OHDA lesions of the NAcc 2 months post-operatively (acquisition group)

In this group, there was a significant 55.8% increase in DA in lesioned animals, relative to sham controls [$F(1,11) = 4.7$, $P < 0.05$] and a significant 76% reduction in the levels of DOPAC [$F(1,11) = 115.42$, $P < 0.001$]. There was no difference in 5-HIAA levels between groups ($F < 2$).

3.3. Behavioural results

3.3.1. The effects of 6-OHDA lesions of the NAcc produced 10 days before behavioural testing

Fig. 2A shows that whilst sham control animals showed a block dependent increase in discriminated approach towards the CS+ (i.e. autoshaped behaviour), the lesion group did not. A repeated measures analysis

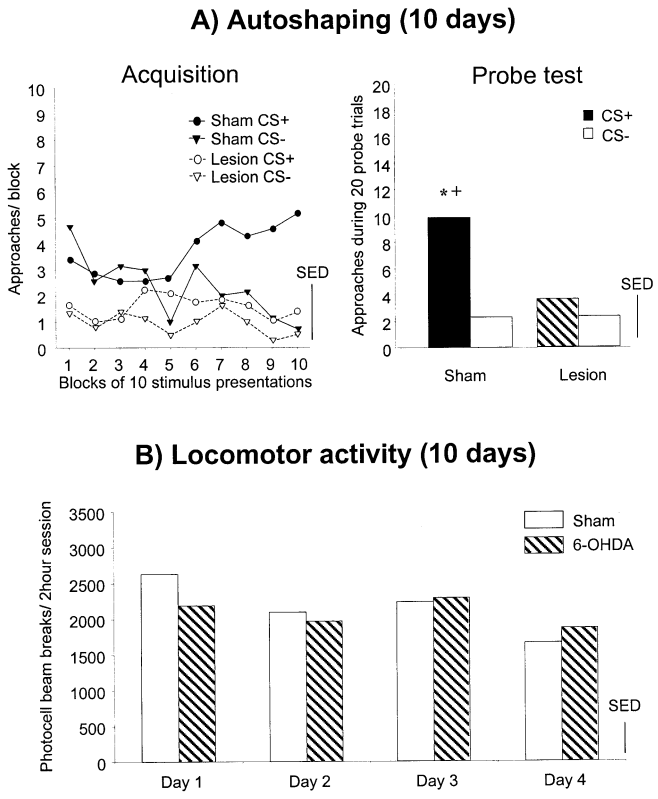

Fig. 2. (A) The acquisition of autoshaping in animals with DA depletions (and their sham controls). Lesions were produced 10 days before behavioural testing commenced. Mean approach scores are presented in blocks of ten trials, across the entire 100 autoshaping trials (a trial consisting of the presentation of both the CS+ and CS−, in a counterbalanced order). Thus the 'approaches/block' score gives the mean number of approaches to each stimulus out of a maximum of ten. Sham animals showed a block dependent development of discriminated approach behaviour towards the CS+ during autoshaping. They also showed a significantly higher number of approaches to the CS+ during the probe test, which consisted of the presentation of 20 trials in which both the CS+ and CS− were presented simultaneously. Lesioned animals did not develop discriminated approach during autoshaping or on the probe test. Significant difference between approaches to CS+ and CS−, during probe test *, $P < 0.05$; significant difference between sham CS+ and lesion CS+ approaches, during probe test +$P < 0.05$. For presentation of descriptive statistics the standard error of the differences of the means (SED) was used, as it provides a better estimate of the population variance for between-group comparisons. The SED is calculated using the formula provided in [61]. (B) Spontaneous locomotor activity was assessed in animals for 2 h/day for 4 days, commencing 1 day after the end of the autoshaping task. Animals were food deprived during this time. A main effect of day revealed a general reduction in activity scores over the 4 days. There were no significant effects of the lesion.

of variance, comparing lesion and sham groups during autoshaping, revealed a significant main effect of the Lesion [$F(1,13) = 11.37$, $P < 0.005$], with lesioned animals showing a reduction in approaches overall, but no other main effects of CS [$F(1,13) = 2.42$, $P = 0.14$] or Block ($F < 2$). There was a significant interaction of CS × Block [$F(9,117) = 5.53$, $P < 0.001$] revealed as a reduction, over blocks, of approaches to the CS− ($P < 0.001$), but no increase in approaches to the CS+. There were no other two way interactions: Lesion × CS or Lesion × Block (both $F < 2$) but there was an interaction of Lesion × CS × Block [$F(9,117) = 3.9$, $P < 0.001$]. Because of this, the sham and lesioned groups were analysed separately to determine the nature of the interaction.

Analysis of the sham group alone revealed no main effects of CS or Block (both $F < 2$) but a significant CS × Block interaction [$F(9,54) = 5.8$, $P < 0.0001$]. Analysis of simple interactions revealed a significant reduction in CS− approaches over blocks ($P < 0.001$) and a significant level of discriminated approach towards the CS+ (relative to the CS−) on blocks 9 and 10 ($P < 0.05$). Analysis of the lesioned group revealed an impairment in autoshaping behaviour with no significant effects of CS, Block or CS × Block (all $F < 2$).

Analysis of approach latencies during autoshaping revealed no significant effects: Lesion [$F(1,13) = 2.2$, $P = 0.16$], CS ($F < 2$), Lesion × CS ($F < 2$). Neither sham nor lesioned animals showed differences in their approach latencies to the CS+ and CS− (see Table 2).

Analysis of approaches towards stimuli during the probe test (Fig. 2A) revealed a main effect of Lesion [$F(1,13) = 8.42$, $P < 0.05$] and CS [$F(1,13) = 8.69$, $P < 0.05$] and a Lesion × CS interaction [$F(1,13) = 4.77$, $P < 0.05$]. These effects were revealed as (1) a higher number of approaches overall by the sham group ($P < 0.05$), (2) a greater number of approaches towards the CS+ relative to the CS− ($P < 0.05$) and (3) a significant level of discriminated approach by the sham group ($P < 0.005$) but not by the lesion group.

Table 2
Mean approach latencies calculated for the CS+ and CS− separately over approaches made during the 100 acquisition trails or 50 performance trials, as appropriate

Group		Approach latency (s)	
		CS+	CS−
Ten days acquisition	Sham	4.63	5.01
	Lesion	5.71	6.10
Two months acquisition	Sham	4.87	5.83
	Lesion+	3.84	4.40
Performance	Sham	1.82**	2.94
	Lesion	2.01**	3.49

All groups showed shorter approach latencies to the CS+ relative to CS−, though this difference was not significant in either of the acquisition autoshaping groups. **, $P < 0.005$ CS+ latencies quicker than CS− latencies; +, $P < 0.05$ Lesion group quicker than controls. The much shorter values for the performance group reflect the more extensive training that these animals received.

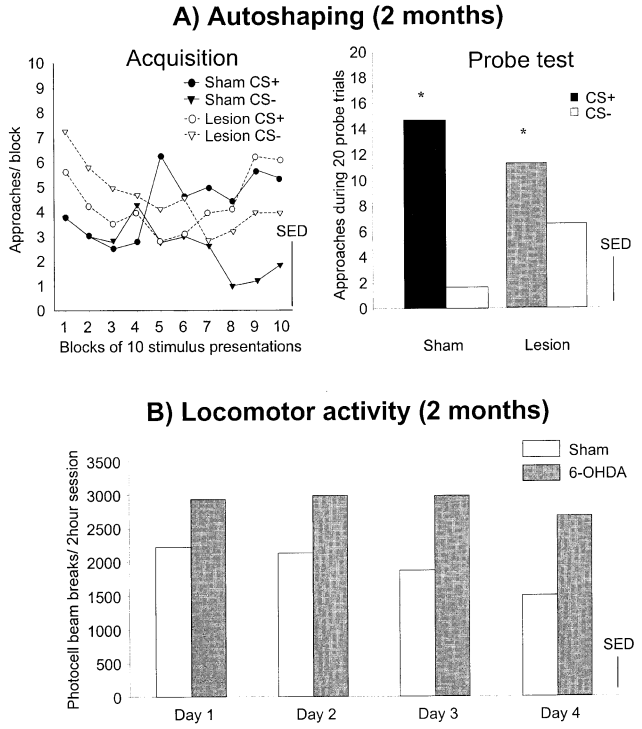

Fig. 3. Behavioural data presented in an identical manner to Fig. 2 (A) autoshaping and (B) activity. (A) In this case, behavioural testing commenced 2 months after DA depletions had been performed. Lesioned animals did not develop discriminated autoshaping behaviour. However, they did demonstrate a significant level of discriminated approach to the CS+, during the probe test. Significant difference between CS+ and CS− approaches *, $P < 0.05$. (B) Lesioned animals were hyperactive, relative to sham controls, across all 4 days of locomotor activity tests.

Fig. 2B shows that there was no significant difference between lesioned and sham control animals in their spontaneous locomotor activity levels. A repeated measures analysis of variance comparing lesion and sham groups over 4 days of locomotor activity tests revealed a significant main effect of Day [$F(3,39) = 9.34$, $P < 0.001$] but no main effect of Lesion ($F < 2$). There was a trend towards a Lesion × Day interaction [$F(3,39) = 2.29$, $P = 0.09$]. The main effect of Day was due to a reduction in overall activity across days (activity on day 1 was higher than on day 2 and activity on days 1, 2 and 3 were all higher than on day 4, $P < 0.05$ all comparisons).

3.3.2. The effects of 6-OHDA lesions of the NAcc produced 2 months before behavioural testing

Fig. 3A shows that lesioned animals showed a general increase in the number of approaches during autoshaping, and showed significant fluctuations in their overall approach behaviour. Sham animals showed a block-dependent development of discriminated approach. Whilst the overall number of approaches remained constant, between groups, these animals increased approaches to the CS+ and reduced approaches to the CS−. Analysis of approaches during autoshaping revealed a main effect of Lesion [$F(1,11) = 4.97$, $P < 0.05$] and a main effect of Block [$F(9,99) = 2.3$, $P < 0.05$], but no main effect of CS ($F < 2$). The effect of Lesion was due to a greater number of approaches overall by the lesion group ($P < 0.05$), whilst the Block effect was due to a greater number of approaches on block 1 relative to block 8 ($P < 0.05$). Furthermore, there was a significant CS × Block interaction [$F(9,99) = 4.11$, $P < 0.001$] characterised by the development of discriminated approach from block 5 onwards ($P < 0.05$, all comparisons). There was no Lesion × CS interaction [$F(1,11) = 2.55$, $P = 0.14$] or Lesion × CS x Block interaction ($F < 2$), though there was a Lesion × Block interaction [$F(9,99) = 2.1$, $P < 0.05$] due to the lesioned animals showing a significant change in the overall number of approaches being made during the autoshaping session ($P < 0.05$) whilst the sham animals did not. Thus there were significant differences between the lesion and sham groups (lesioned animals showing a greater number of approaches) during blocks 1, 2, 3 and 9 ($P < 0.05$).

Analysis of approach latencies during autoshaping revealed a main effect of Lesion [$F(1,11) = 6.61$, $P < 0.05$] but no effect of CS [$F(1,11) = 2.66$, $P = 0.13$] or a Lesion × CS interaction ($F < 2$). The Lesion effect was due to overall shorter latencies in the lesion group, relative to shams ($P < 0.05$), though there was no discriminated approach (in terms of a shorter latency) overall in either group towards the CS+ (see Table 2).

Analysis of the autoshaping probe test scores revealed a main effect of CS [$F(1,11) = 19.25$, $P < 0.001$] and a trend towards a Lesion × CS interaction [$F(1,11) = 4.211$, $P = 0.065$] but no effect of Lesion ($F < 2$). The CS effect was due to a greater number of approaches towards the CS+ overall. Thus there was no significant effect of the lesion on discriminated approach to the CS+ (relative to the CS−).

Fig. 3B shows that there was a significantly higher level of activity in the lesion group as shown by the main effect of Lesion [$F(1,11) = 7.88$, $P < 0.05$]. There was also a general reduction in activity over days [$F(3,33) = 6.51$, $P < 0.001$], day 4 activity being significantly lower than days 1, 2 and 3 ($P < 0.05$).

3.3.3. The effects of 6-OHDA lesions of the nucleus accumbens on behavioural performance

Pre-surgical acquisition: as can be seen in Fig. 4A, prior to surgery, animals acquired the autoshaping response. A comparison of CS+ and CS− approaches for the sham- and to-be-lesioned groups over the last three blocks of acquisition demonstrated that there were no pre-surgical group differences. A repeated measures ANOVA revealed no significant main effect of Lesion

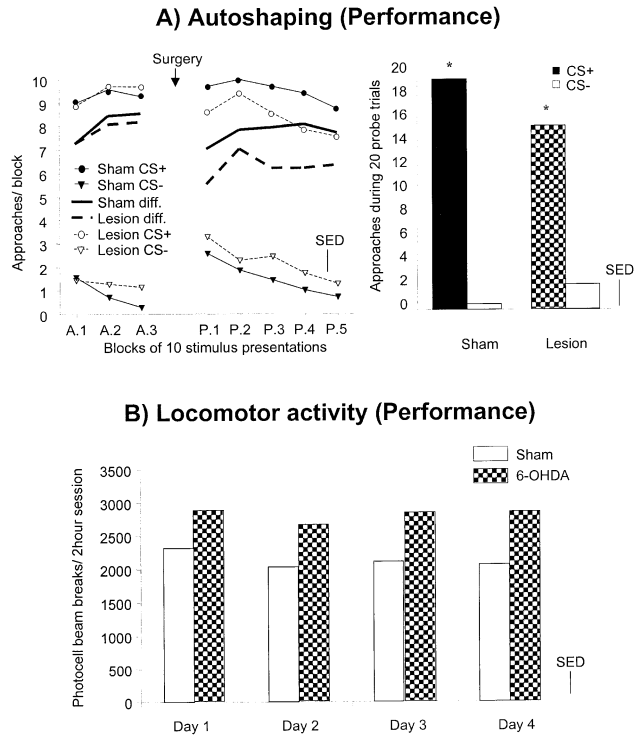

Fig. 4. Behavioural data presented in a similar manner to Figs. 2 and 3(A) autoshaping and (B) locomotor activity. (A) In this case data are from animals who had acquired autoshaping prior to DA depletion (performance). The difference between the number of approaches to the CS+ and to the CS− were also calculated for each block (difference score, Sham diff. and Lesion diff.) and are depicted in the autoshaping figure. Both experimental groups showed preserved discriminated approach following surgery, though the lesioned group showed a reduction in CS+ approaches relative to shams. Both groups showed a significant level of discriminated approach during the probe test *, $P < 0.05$. A 1–3, the last three blocks of autoshaping acquisition prior to surgery. P.1–P.5, blocks of 10 performance autoshaping trials. (B) Mean spontaneous locomotor activity during 2 h sessions over 4 days. DA depleted animals were hyperactive relative to controls across all days.

($F < 2$), Lesion × Block interaction [$F(4,48) = 2.13$, $P = 0.123$] nor Lesion × CS interaction ($F < 2$). A significant effect of Block [$F(4,48) = 3.22$, $P < 0.05$] and CS × Block interaction [$F(4,48) = 11.37$, $P < 0.001$] demonstrated the block-dependent increase in discriminated approach between the CS+ and CS−.

Repeated measures ANOVA of the post-surgical autoshaping test data revealed a deficit in the discriminated approach in the 6-OHDA lesioned group. Thus whilst there was no overall main effect of Lesion ($F < 2$), there was a significant Lesion × CS interaction[$F(1,12) = 8.06$, $P < 0.05$]. Separate analyses of approaches to the CS+ and CS− revealed that whilst there was no difference between the groups in their approaches to the CS− ($F < 2$), there was a main effect of Lesion [$F(1,12) = 5.58$, $P < 0.05$] for approaches to the CS+ demonstrating an attenuated level of CS+ approach in the 6-OHDA lesioned animals.

The lesion did not affect latencies to approach the stimuli. Mean latencies were calculated across all post-operative trials and analysed using a one-way ANOVA which revealed no effect of the Lesion ($F < 2$) but a significant effect of CS [$F(1,12) = 15.47$, $P < 0.005$] reflecting a shorter latency to approach the CS+ than the CS− in both groups (see Table 2).

Whilst the degree of discrimination during the performance of autoshaping was impaired in the lesion group, analysis of the probe test scores demonstrated that there were no group differences in the ability to select the approach stimulus to approach when both were presented simultaneously. Thus a one-way ANOVA comparing a discrimination ratio (the number of approaches to the CS+ divided by the total number of approaches during the probe session) revealed no main effect of Lesion [$F(1,12) = 2.71$, $P = 0.126$].

Fig. 4B shows that there was a significant overall reduction in activity over the 4 days of testing [$F(3,36) = 6.51$, $P < 0.005$] with significant hyperactivity in the 6-OHDA group [$F(1,12) = 4.91$, $P < 0.05$]. There was no significant Day × Lesion interaction ($F < 2$).

4. Discussion

The present study investigated the effects of DA depleting lesions of the NAcc on discriminated approach, measured in a Pavlovian autoshaping procedure. Animals were autoshaped either at 10 days or 2 months following intra-NAcc infusion of 6-OHDA, or were first trained to criterion on the approach task before receiving infusions of 6-OHDA. DA-depleted rats showed significant impairments in appetitive Pavlovian conditioned approach behaviour (autoshaping) regardless of when the lesion was produced and independent of its effects on unconditioned locomotor activity. In relative terms, DA depletion from the NAcc produced a greater impact on learning than on the performance of Pavlovian approach behaviour, suggesting that the effects on learning were not simply due to a gross disruption of sensory, motor or motivational processes. Further, 6-OHDA lesions were associated with reduced levels of extracellular DA in the NAcc 10 days after surgery but with increased DA levels following longer-term recovery.

DA was depleted in the ventral striatum in all lesion groups (average depletion 80%), though there were also reductions in DA in the prefrontal cortex (61%) and in NA from both the NAcc (64%) and prefrontal cortex (73%). Importantly, levels of DA measured in tissue post mortem showed no evidence of recovery over the 2-month experimental period. It should be noted that absolute control levels of monoamines in several brain

regions were consistently higher in animals in the 2 month autoshaping acquisition sham group (see Table 1, group 3). However, because analyses were conducted within each group of sham and lesioned subjects and not across different studies, this discrepancy is unlikely to affect the extent of monoamine depletion in percentage terms or the overall extent or significance of the results.

The general pattern of neurochemical results seen in this experiment has been observed previously after 6-OHDA lesions of the NAcc. For example, the percentage depletion of DA from the NAcc after similar lesions generally have been reported to be in the region of 75–85% [28,30,32,45,55]. Further, significant depletions of DA and NA in the frontal cortex has also been observed, particularly when a noradrenaline reuptake inhibitor was not administered pre-surgically [45], as in the present case. It is unlikely that such effects on frontal monoamine levels are responsible for the autoshaping results as it has been previously shown that excitotoxic lesions of the medial prefrontal cortex do not impair the acquisition of autoshaping [9]. It should also be noted that the 6-OHDA lesions in the present study produced significant reductions in NA in the striatum, as well as DA. Evidence suggests however, that NAcc NA does not play a major role in the learning- or reward-related processes studied here. For example, the potentiative effects of intra-NAcc infusions of stimulants on stimulus-reward learning are known to be mediated by DA, and not NA, in the NAcc (see [10]).

Further, it is not clear how levels of monoamines measured by in vivo microdialysis in anaesthetised subjects, as was the case in the present experiments, correspond to those measured in freely moving animals. Thus caution should be exercised in the interpretation of these data obtained from anaesthetised subjects. However, this approach was chosen as it allows DA function in the NAcc to be measured in a controlled setting free from confounding variables such as reinforcement density and motivational arousal. In addition, it was anticipated that 6-OHDA lesions would produce large and persistent changes in DA release which would be readily detected by this method. Finally, further systematic examination will be necessary to assess the possible interactive effects of such factors as depletion level, the anatomical specificity of the depletion (including core/shell differences) and recovery time, on appetitive Pavlovian learning.

4.1. The effects of nucleus accumbens dopamine depletion on the acquisition of autoshaping commencing 10 days post-surgery

Extracellular levels (measured by in vivo microdialysis) of both DA and DOPAC were reduced (though the reduction in DA showed only a trend towards significance) in 6-OHDA lesioned animals relative to controls. The remaining DA neurons innervating the NAcc presumably showed a compensatory increase in their firing rate following the lesion (see [68]). The behavioural results showed that, in a matched group of animals, spontaneous locomotor activity was not significantly affected by the lesion whilst appetitive Pavlovian autoshaping behaviour was severely impaired. This impairment was characterised by a global reduction in approach behaviour to both conditioned stimuli (CS+ and CS−) for the entire 100 autoshaping trials, including reduced exploration and approach to the novel visual stimuli during the initial autoshaping blocks. This general reduction in approach behaviour persisted through the probe test with lesioned animals making very few approaches to either stimulus. Thus, the 6-OHDA lesion produced a marked and profound impairment in Pavlovian approach. By contrast, sham control animals showed a significant level of discriminative approach during autoshaping and a preference for approaching the CS+ during the 20 probe trials. One simple explanation of the results, that the DA depletion produced a general and global reduction in behavioural output, is unlikely due to the comparable levels of spontaneous locomotor activity in the lesion and sham groups. However, these data do not conclusively demonstrate that NAcc DA depletion disrupted the acquisition of Pavlovian discriminated approach, because lesioned animals made very few approaches to either stimulus and hence a reduced level of conditioned responses (CRs). In sham animals, at a point in acquisition (in terms of responses) when they had made the same number of approach responses as the mean number of approaches made by lesioned animals (over the entire autoshaping task), there was only a trend towards a significant level of discriminated approach (around block 5). Thus, intact discrimination learning in lesioned animals may have been masked by the reduced level of approach responses, relative to controls. Nevertheless, lesioned animals did reliably collect all reinforcers and were thus exposed to the same density of Pavlovian pairings of CS and US.

It is unlikely that NAcc DA simply acts as a substrate of reward [64], the loss of which would have abolished autoshaped behaviour in the present study, not least because DA lesions of the NAcc do not impair the ability of natural unconditioned or conditioned stimuli to support instrumental behaviour [48,55]. However, DA may act as a teaching signal for error prediction in learning [50] or as a mechanism for Pavlovian incentive processes [16]. Thus a plausible account of the deficit observed during the acquisition of autoshaping, commencing 10 days after DA depletion, is that lesioned animals were unable to associate incoming representations of stimuli with appropriate Pavlovian responses or to reinforce those responses (see recent review [51]).

4.2. The effects of nucleus accumbens dopamine depletion on the acquisition of autoshaping commencing 2 months post-surgery

Two months after surgery there was a significantly lower level of extracellular DOPAC in the NAcc of 6-OHDA lesioned animals, but a relative increase in extracellular levels of DA, compared with sham control levels. This pattern of results contrasts with those seen 10 days after surgery. Thus, whilst extracellular DOPAC, like tissue levels of DA, appeared to be reduced permanently (or at least over the 2 month period), extracellular DA levels showed an initial trend towards a significant reduction followed by a significant increase in levels relative to sham control levels. The mechanism for this recovery of function leading to an increase in DA is unclear, but it may depend upon a number of factors. For example, the remaining DA neurons innervating the NAcc may show a compensatory increase in their firing rate or their synthesis and release of DA in the NAcc. In addition, the lower levels of DA due to the initial destruction of DA-ergic neurons may have resulted in an increase in the number of post-synaptic DA receptors and hence a supersensitive response to the transmitter [28,30,57].

Whatever the mechanism of compensation, the lesion resulted in significant effects on the behavioural performance of animals 2 months after surgery. Lesioned animals were spontaneously hyperactive relative to sham controls (and see [28]) and this may have been a direct result of the significantly raised basal levels of extracellular DA. Although hyperactive, the lesioned animals were nevertheless significantly impaired during autoshaping. The impairment was characterised by a general increase in approaches during the autoshaping session and stand in contrast to the decreased responding to the CS+ and CS− observed in animals 10 days after NAcc DA depletion. Thus, whilst animals both 10 days and 2 months following 6-OHDA lesions were significantly impaired in their discriminated approach to the CS+, the nature of the deficits in these two experiments was quite distinct.

Further, animals with 6-OHDA lesions produced 2 months before behavioural testing, had significantly shorter approach latencies during autoshaping relative to shams, contrasting with lesioned animals assessed 10 days after the lesion, who showed a trend towards significantly longer approach latencies. Finally, whilst there was a trend towards an impairment in the probe test (in lesioned animals), this did not reach significance, indicating that both 6-OHDA- and sham-lesioned animals showed a significantly higher level of approach towards the CS+ relative to the CS−. Whilst extracellular DA and general activity were somewhat independent shortly after surgery, the increase in extracellular DA seen 2 months after 6-OHDA lesions was matched by an increase in both spontaneous locomotor activity and autoshaped approaches. Thus, excess synaptic DA may influence general behavioural expression, perhaps characterised in this case by an inability of these animals to inhibit inappropriate behavioural output, including CS− approaches.

An apparent paradox demonstrated by these data is that, 10 days after DA depletion, lesioned animals were impaired at autoshaping, but not on a measure of general motoric output (spontaneous locomotor activity). In contrast, 2 months after DA depletion, lesioned animals showed signs of intact learning (significant probe test discrimination) but had abnormal spontaneous and conditioned locomotor responses. It would seem likely then that the NAcc DA may mediate or influence both conditioned (discriminated approach) and unconditioned (spontaneous locomotor activity) behaviour and thus its involvement in incentive motivated behaviour may supersede this dichotomy.

4.3. Post-acquisition dopamine depletion and the performance of discriminated approach

When animals received DA depleting lesions of the NAcc after reaching criterion levels of discriminated approach on the autoshaping task, their performance was also impaired, relative to controls. This was manifest as a reduced behavioural discrimination between the CS+ and CS−. However, although impaired, these animals were clearly capable of autoshaped behaviour, showing reduced latencies to approach the CS+, relative to the CS− and discriminated approach to the CS+ during the probe test.

The performance deficit in autoshaping was less severe than that seen when lesions were produced before conditioning (contrast the lesion effects shown in Fig. 4 with those in Figs. 2 and 3). This strongly suggests that NAcc DA is critical for learning a Pavlovian approach response. Alternatively, the NAcc may instead be required for selecting appropriate responses based on environmental conditions. Therefore, during the acquisition of conditioning, a deficit in the ability to produce the appropriate response may significantly retard learning. Such a deficit would manifest itself during both acquisition and performance, as was seen. The relatively greater impact of NAcc DA depletion during acquisition may have been due to the fact that the expression of autoshaped behaviour has been argued to become progressively and predominantly controlled by instrumental mechanisms, possibly stimulus-response habits (automaintenance; [26]) and thus may become less susceptible to the lesion-induced deficits in Pavlovian conditioning per se.

4.4. Implications for dopamine function in the nucleus accumbens

The data from these experiments imply that NAcc DA may function differentially during learning and performance. Grace and colleagues [19,36] have suggested that striatal DA is under the control of mechanisms having at least two components: (i) a brief phasic release which is dependent on VTA cell firing and is consistent with the properties of DA neurons observed by Schultz and colleagues (for example [50]), and suggested to be a teaching signal for stimulus-response learning; (ii) tonic DA release from terminals in the striatum that is more dependent upon glutamatergic-DA interactions at synapses in the striatum (see [19] for review). Tonic DA release provides the background level of extracellular DA and can modulate the level and impact of phasic DA cell firing. Tonic DA release is somewhat independent of DA cell firing and could contribute to the maintenance of cortico-striatal networks through its interaction with glutamatergic inputs [7,41]. 6-OHDA lesions of the striatum, at depletion levels comparable to those seen in the present study, are known to have relatively little effect on tonic levels of NAcc DA [47,67]. Indeed, the present study suggests that extracellular DA (in an anaesthetised preparation) may actually be increased in the longer-term following an 80% DA depletion.

It is suggested that the mild deficit in autoshaping expression following lesions of the NAcc DA system made after acquisition may be due to the reliance of this performance on the DA-ergic modulation of cortico-striatal networks [37] and hence tonic DA levels. In contrast, the acquisition of the autoshaped response may depend to a greater extent on phasic levels of NAcc DA, modulated by VTA cell firing, perhaps providing a teaching signal for the initial development of activity within cortico-striatal networks and, in this instance, appropriate stimulus-elicited behavioural responses.

According to this view, phasic activations of DA in the striatum may be required for learning to occur [31,50,51] i.e. association of a stimulus configuration (white rectangle on left) with an appropriate response (approach). However, once response 'sets' have been created, the performance and potentiation of a prepotent response may simply require a more tonic influence of DA at the neuronal population level [33,43,44]. Hence, an increase in DA (e.g. through systemic or intra-NAcc injection of amphetamine) will have the general effect of increasing prepotent response 'sets' [24,54]. At a cellular level, within the striatum, DA probably focuses activity by increasing output from the most active medium spiny neurons (which are in the minority) and decreasing output from the less active cells (gain-amplification; [58]). This is mirrored at a behavioural level; increasing doses of DA agonists produce higher rates of activity in more and more limited categories of response [33] until stereotypy ensues.

More generally, Salamone and colleagues (for example [48]) have demonstrated the importance of NAcc DA in mediating response effort and choice. Further, Ito et al. [27], have recently observed that extracellular DA release in the NAcc, in freely moving rats, is specifically correlated with the influence of Pavlovian stimuli on ongoing behaviour rather than in instrumental responding per se. Indeed, Berridge [4] and Dickinson [16], have demonstrated that Pavlovian and instrumental incentive processes can de dissociated according to their dependence on DA function, only the Pavlovian incentive salience process being critically dependent on the DA system. Consistent with this view, Ahn and Philips [2] observed that NAcc DA release appears to correspond to Pavlovian incentive processes, and several studies have now provided supporting evidence for the view that NAcc DA contributes to the way in which motivationally significant Pavlovian stimuli can direct and invigorate behaviour, including Pavlovian approach (as in the present study) and also the way in which Pavlovian cues can potentiate instrumental responses [16,21,22,65]. This mechanism may have a profound influence on more complex forms of learning, such as in goal-directed behaviour, and is consistent with the effects of NAcc manipulations that have been observed in instrumental effort, choice and responding [29,48].

4.5. Implications for dopamine mediated cortico-striatal function

Jones and Robbins [28] have demonstrated the sensitivity of the ventral striatal DA to the incentive activational effects of conditioned cues. The present study demonstrates a further role for ventral striatal DA in discriminated approach, that is, not simply in activation but also in response selection. Further, previous work in this laboratory [38,39] has demonstrated that the NAcc core and shell can be functionally dissociated on measures of both spontaneous locomotor activity and on discriminated approach; the shell appears to be the critical substrate for the potentiative effect of stimulants on locomotor activity, whilst the core is critical for Pavlovian discriminated approach during autoshaping [39]. It is possible, therefore, that the disrupted behaviour seen after 6-OHDA lesions of the entire NAcc may be further dissociated into separate core and shell effects. The general changes in behavioural output, seen in both the locomotor cages and during autoshaping might be due to fluctuating levels of extracellular DA selectively within the NAcc shell. Further, the lack of discriminated approach may be due to a disruption in the way that particular CRs are mediated by DA transmission in the NAcc core.

Consistent with this scheme, DA release in the NAcc shell has been correlated with the presentation of novel and with unconditioned stimuli (non-associative effects), whilst DA release in the core is observed predominantly during presentation of conditioned cues [3,15,27]. Through selective 6-OHDA lesions of the core and shell, Sokolowski and Salamone [53] found that depletions in the core produced a reduction in asymptotic levels of instrumental responding, consistent with a role for NAcc core DA in conditioned influences on behaviour.

The NAcc is unlikely to be directly involved in all forms of Pavlovian CR and appears to be particularly critical for preparatory aspects of conditioning [6,11,17,35,39,44,46]. Thus the NAcc may be specifically involved in the influence that motivationally significant stimuli can have on non-reflexive, goal-directed behaviour [21,37]. The ventral striatum is proposed to provide a DA-dependent modulatory influence on the functions of the rest of the striatum [20]. Thus within the context of the striatum as a whole, the NAcc may influence the motivational 'set' in which appropriate cognitive and sensorimotor behaviour is organised, based on both unconditioned and conditioned stimuli present in the environment [37,43]. The present study demonstrates the involvement of NAcc DA in both learning and the performance of Pavlovian approach behaviour. As such, DA may provide a differential contribution to the acquisition and performance of conditioned behaviour through its multifaceted mechanism of action at the level of the striatum.

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