

# Lesions to the subthalamic nucleus decrease impulsive choice but impair autoshaping in rats: the importance of the basal ganglia in Pavlovian conditioning and impulse control

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## Abstract

Although the subthalamic nucleus (STN) is involved in regulating motor function, and inactivation of this structure relieves the motor symptoms in Parkinsonian patients, recent data indicate that corticosubthalamic connections are involved in both the regulation of attention and the ability to withhold from responding. Considerable evidence suggests that the neural circuitry underlying such behavioural disinhibition or impulsive action can be at least partially dissociated from that implicated in impulsive decision-making and it has been suggested that the tendency to choose impulsively is related to the ability to form and use Pavlovian associations. To explore these hypotheses further, STN-lesioned rats were tested on the delay-discounting model of impulsive choice, where impulsivity is defined as the selection of a small immediate over a larger delayed reward, as well as in a rodent autoshaping paradigm. In contrast to previous reports of increased impulsive action, STN lesions decreased impulsive choice but dramatically impaired the acquisition of the autoshaping response. When the STN was lesioned after the establishment of autoshaping behaviour, lesioned subjects were more sensitive to the omission of reward, indicative of a reduction in the use of Pavlovian associations to control autoshaping performance. These results emphasize the importance of the STN in permitting conditioned stimulus–unconditioned stimulus associations to regulate goal-seeking, a function which may relate to the alterations in impulsive choice observed in the delay-discounting task. These data bear a striking similarity to those observed after lesions of the orbitofrontal cortex and are suggestive of an important role for corticosubthalamic connections in complex cognitive behaviour.

## Introduction

Basal ganglia dysfunction, including hyperactivity within the subthalamic nucleus (STN), is thought to be responsible for the motoric impairments seen in Parkinson's disease and both high-frequency stimulation of the STN in Parkinsonian patients and STN lesions in animal models of Parkinson's disease reduce motor symptoms associated with the disease (Bennazzouz *et al.*, 1993; Baunez *et al.*, 1995; Limousin *et al.*, 1995; Phillips *et al.*, 1998; Phillips & Brown, 1999). However, the STN is a key node within corticostriatal-thalamic loops connecting the basal ganglia to cortical and limbic regions involved in goal-directed behaviour, including the nucleus accumbens (NAC) and frontal cortices (Alexander *et al.*, 1986). Hence, STN lesions also affect attention, working memory and motivation, which may relate to the dysexecutive syndrome observed in Parkinson's disease and other disorders (Baunez & Robbins, 1997; Baunez *et al.*, 2001, 2002).

Subthalamic nucleus lesions also increase behavioural disinhibition in rats, suggestive of increased impulsivity. For example, STN lesions increase premature responding during anticipation of a visual target in the five-choice serial reaction time task (5CSRT) and other attentional

paradigms (Baunez & Robbins, 1997; Phillips & Brown, 2000) and also increase locomotor activity conditioned to food (Baunez *et al.*, 2002). However, the impulsivity construct encompasses numerous behaviours ranging from such motoric disinhibition ('impulsive action') to inappropriate or risky decision-making ('impulsive choice') (e.g. Evenden, 1999). Although structures within the limbic corticostriatal loop are implicated in impulsivity, dissociations have been observed between those regulating impulsive action vs. impulsive choice. For example, whereas damage to the orbitofrontal cortex (OFC) increases premature responding on the 5CSRT, similar lesions decrease impulsive decision-making in a delay-discounting procedure, where impulsive choice is defined as selecting a small immediate over a larger delayed reward (Chudasama *et al.*, 2003b; Winstanley *et al.*, 2004a). Furthermore, anterior cingulate cortex lesions do not alter delay-discounting, despite increasing impulsive responding on the 5CSRT (Muir *et al.*, 1996; Cardinal *et al.*, 2001). As yet, only damage to the NAC increases impulsivity in both paradigms (Cardinal *et al.*, 2001; Christakou *et al.*, 2004).

It has been suggested that impulsive decision-making is related to Pavlovian autoshaping behaviour (Tomie *et al.*, 1998 but see Winstanley *et al.*, 2004b), a procedure during which subjects learn to approach a conditioned stimulus (CS) associated with food reward (e.g. Bussey *et al.*, 1997). Such behaviour persists even when approaching the CS cancels the scheduled reward (Brown & Jenkins, 1968), potentially indicative of impulsive or perseverative behaviour (Tomie *et al.*, 1998; Chudasama & Robbins, 2003; Winstanley *et al.*, 2004b). Whether

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perseverative responding is associated with or independent of impulsivity is currently unclear (e.g. Hollander & Rosen, 2000). STN lesions increased perseveration during the 5CSRT (Baunez & Robbins, 1997) but STN high-frequency stimulation reduced compulsive behaviours in Parkinsonian patients with obsessive-compulsive disorder (Alegret *et al.*, 2001; Mallet *et al.*, 2002).

Hence, in this study, the effects of STN lesions were assessed on delay-discounting and autoshaping behaviour to investigate the role that this region plays in these aspects of impulse control and to further understand the processes controlling these behaviours.

## Materials and methods

### Subjects

Subjects were 41 male Lister Hooded rats (Charles River, UK) weighing 300–320 g at the start of the experiment and maintained on 14 g of rat chow (Alegret *et al.*, 2001; Mallet *et al.*, 2002) per day. Water was available *ad libitum*. Animals were housed in pairs under a reverse light cycle (lights on from 19:00 to 07:00 h) and testing took place between 09:00 and 13:00 h 6 days per week. All experiments were carried out in strict accordance with the UK Animals (Scientific Procedures) Act 1986.

### Surgery

Subjects were anaesthetized with ketamine (Ketaset, 100 mg/kg *i.m.*; Vet Drug, Bury St. Edmunds, UK) and xylazine (Rompun, 10 mg/kg *i.m.*; Vet Drug) and secured in a stereotaxic frame fitted with atraumatic earbars. Bilateral lesions were made using ibotenic acid (9.4 µg/µL, *i.e.* 53 mM) infused at a rate of 0.5 µL over 3 min at the following co-ordinates taken from a stereotaxic atlas (Paxinos & Watson, 1998): AP, –3.8 from bregma; L, ±2.4 from the midline; DV, –8.35 from the skull surface. Sham-operated animals received corresponding vehicle infusions at the same co-ordinates. After each infusion, the injector was left in place for 5 min to allow the infusate to diffuse. The incisor bar was set at –3.0 mm relative to the interaural line. Postoperatively, animals were returned to their home cages for 7 days and given free access to food before behavioural testing.

### Behavioural testing

All apparatus was controlled and monitored by software written in Arachnid, a real-time extension to BBC BASIC running on Acorn Archimedes Series computers (Cambridge, UK). All data were analysed using SPSS version 9.0 (SPSS Inc., Chicago, IL, USA).

#### Experiment 1: effects of subthalamic nucleus lesions on delay-discounting

##### Behavioural apparatus

The apparatus and testing procedure have been described elsewhere in detail (Winstanley *et al.*, 2003). In brief, the apparatus consisted of eight operant conditioning chambers (30 × 24 × 30 cm; Medical Associates Inc., USA), each enclosed within a sound-attenuating wooden box fitted with a fan for ventilation and masking of extraneous noise. Each chamber was fitted with two retractable levers located on either side of a centrally positioned food magazine into which an external pellet dispenser could deliver 45-mg sucrose pellets (Noyes dustless pellets; Sandown Scientific, UK). The food magazine was illuminated by a diffused green LED (RS Components Ltd, UK). Entry to the food magazine could be detected by the breaking of an

infrared photobeam located horizontally across the entrance. General illumination was provided by a 2.8-W houselight.

### Behavioural testing

**Pretraining.** Subjects were first trained under a fixed ratio (FR1) schedule to a criterion of 50 presses in 30 min for each lever. They were then trained to nosepoke in the central magazine in order to trigger presentation of the levers using a training programme similar in structure to the full task. Every 40 s, a trial began with illumination of the houselight and the traylight. The subject was required to make a nosepoke response within 10 s to trigger presentation of a single lever. Responding on the lever within 10 s led to illumination of the traylight and the delivery of a single food pellet. The left and right levers were presented an equal number of times in the session with not more than two consecutive presentations of the same lever. Rats were trained to a criterion of at least 60 successful trials in 1 h.

**Delay-discounting task.** Each session lasted 100 min and consisted of five blocks of 12 trials, each lasting 100 s. Each block began with a pair of forced choice trials which consisted of one presentation of the left lever and one of the right lever in a random order. Throughout the task, a response on one lever would produce a reward of one pellet (lever A) whereas a response on the other would produce a reward of four pellets (lever B). The position of these levers (left or right) was kept constant for each rat but was counterbalanced between rats. The delay between responding on lever A and the concomitant delivery of reward was always 0 s whereas the delay between responding on lever B and the delivery of reward increased within the session in a step-wise manner between blocks from 0 s in block 1 to 10 s in block 2, 20 s in block 3, 40 s in block 4 and 60 s in block 5.

Each trial began with the onset of the houselight and traylight. As in pretraining, there was a limited hold period of 10 s in which the rat had to nosepoke in the magazine to trigger presentation of the two levers, upon which a 10-s response interval was initiated. Failure to respond in either 10-s period resulted in the trial being recorded as an omission and a return to the Inter-trial interval (ITI) state until the next trial was scheduled to begin. Once the rat had responded on one of the levers, both levers were retracted and the houselight and traylight were turned off. Food delivery, signalled by the traylight, occurred either immediately or after a delay, after which the operant chamber returned to the ITI state.

Subsequent to acquiring stable baseline behaviour on the task, which occurred within approximately 35 sessions, subjects were divided into two groups matched for levels of impulsive choice and received either bilateral lesions of the STN ( $n = 12$ ) or corresponding sham surgery ( $n = 8$ ).

#### Experiment 2: effect of lesions of the subthalamic nucleus on acquisition of the autoshaping response

The effects of lesions of the STN on acquisition of autoshaping were investigated using the same subjects as in experiment 1.

##### Behavioural apparatus

The autoshaping apparatus and behavioural training have been described previously in detail (Dalley *et al.*, 2002). Behavioural testing took place in six test chambers measuring 45 × 32 × 30 cm (Cambridge Cognition, Cambridge, UK), each housed within a sound-

attenuating wooden box fitted with a fan for ventilation and masking of extraneous noise and illuminated by a centrally located 3-W houselight. The chambers were fitted with two food magazines and pellet dispensers which allowed the controlled delivery of 45-mg sucrose pellets (Noyes dustless pellets; Sandown Scientific). One food magazine was located at the rear of the box, directly above a pressure-sensitive floor panel. The chamber was fronted at one end with a video display unit (Intasolve Ltd, UK) upon which the stimuli were presented. A second food magazine was centrally located within a vertical chimney placed 6 cm in front of the video display unit. The stimuli, consisting of vertical white rectangles (10 × 28 cm), were presented on either side of the chimney. Approaches to these stimuli could be detected by the breaking of photocell beams located to the right and left of the central magazine.

#### *Pretraining*

Subjects were initially given two 30-min habituation sessions to acclimatize to the test apparatus. During this time the houselight was on and pellets were delivered to the central magazine on a random time 40-s schedule. Pellets were also delivered to the second magazine at the rear of the chamber but only after the depression of the pressure-sensitive floor panel, in order to shape animals to approach the back of the box. This food magazine was only used during pretraining.

#### *Acquisition*

On the day after pretraining, subjects were trained to associate a 10-s stimulus presented on the video display unit with the delivery of a food pellet into the central magazine. A trial consisted of the consecutive presentation of the CS+ and CS− in a randomized order. The CS+ differed from the CS− in its spatial location (i.e. whether it appeared on the left or right of the food magazine) which was counterbalanced across subjects. After a random time (interstimulus interval, 10–40 s), which occurred before the presentation of each stimulus, the rat was required to locate itself centrally on the floor panel at the rear of the chamber, thus eliminating chance approaches to the stimuli and ensuring equal stimulus sampling. Depression of the floor panel triggered the 10-s presentation of either the CS+ or CS−. The offset of the CS+ was contingent with the delivery of a food pellet in the central magazine, whereas the CS− was never associated with food reward. The delivery of food started another variable interstimulus interval, thereby separating the presentation of stimuli to minimize interference. The minimum time between presentations of the stimuli was 10 s and the maximum number of consecutive presentations of either the CS+ or CS− was two. Acquisition was recorded over three consecutive days, with animals completing 50 trials per day, thereby giving 150 trials in total. Approaches were scored as the breaking of either the left or right photocell beam and only the first approach was measured during the stimulus presentation, regardless of the direction of the approach. Approaches were only included in the analysis if they were made to the stimulus that was actually presented on that trial. The number of approaches to the CS+ and CS− per block of 10 trials, as well as the latencies to make these approaches, were determined for statistical analysis. The difference score (number of approaches to CS+/number of approaches made to CS+ and to CS−) was also calculated as an index of the level of discrimination learning established in each block.

#### *Omission*

The day after the final acquisition session, the task contingencies were altered such that approaches to the CS+ prevented the delivery of a

food pellet. The session consisted of 50 trials and again only the first approach was scored.

#### *Experiment 3: effect of lesions of the subthalamic nucleus on performance of the autoshaping response*

Subjects completed the acquisition phase of the autoshaping procedure as outlined in experiment 2, whereupon they were divided into two groups matched for level of acquisition of the autoshaping response using the difference score for the final block of trials. One group received bilateral lesions of the STN ( $n = 12$ ) and the other corresponding sham surgery ( $n = 9$ ). The performance of the autoshaping response in these subjects was then assessed over 150 trials. The day after the final performance session, subjects were tested during the omission phase.

#### *Locomotor activity*

As described previously (Winstanley *et al.*, 2003), locomotor activity was assessed in activity cages (25 × 40 × 18 cm) fitted with two photocell beams located at each end of the cage. The test session lasted for 2 h and the data were collated over 5-min bins. Subjects were initially habituated to the locomotor boxes for two sessions before determination of baseline levels of locomotor activity.

#### *Data analysis*

*Delay-discounting.* The number of choices of the large reward per delay per session was analysed to determine levels of impulsive choice. Preoperatively, stable baseline behaviour was established across daily sessions by subjecting data from seven consecutive sessions to repeated-measures ANOVA with DAY and DELAY as within-subjects factors. The effects of lesioning the STN were assessed through comparison of data collected over seven stable preoperative sessions and seven postoperative sessions using a repeated-measures ANOVA with DAY and DELAY as within-subjects factors and LESION (two levels, sham and lesion) and SURGERY (two levels, preoperative and postoperative) as between-subjects factors. Data from the postoperative session only were also analysed using a repeated-measures ANOVA with DAY and DELAY as within-subjects factors and LESION as a between-subjects factor. The number of omissions, latency to respond on either lever (response latency) and latency to collect reward (collection latency) were also analysed. A more detailed description of the data analysis for this behavioural task can be found in Winstanley *et al.* (2003).

*Autoshaping.* Data were subjected to repeated-measures ANOVA with BLOCK (15 levels) as a within-subjects factor and LESION (2 levels, lesion and sham) as a between-subjects factor. Any significant effects were analysed further using simple main effects analysis.

*Locomotor activity.* Any significant differences between the activity of lesioned animals and sham-operated controls were determined by a one-way ANOVA with LESION as the factor.

#### *Histology*

Once behavioural testing was complete, subjects were anaesthetized with a fatal dose of sodium pentobarbitone (Euthatal, 200 mg/mL; Genus Express, UK) and perfused transcardially with 0.01 M phosphate-buffered saline followed by 4% paraformaldehyde. The brains were removed and postfixed in paraformaldehyde. Before being cut, the brains were transferred to a solution of 20% sucrose in 0.2 M phosphate buffer and left for 24 h. Coronal sections were cut at 40 μm

on a freezing microtome and stained with Cresyl violet. The size and extent of the lesion were then verified by an observer who was blind to the experimental conditions using a stereotaxic atlas (Paxinos & Watson, 1998).

## Results

### Lesion analysis

Of the subjects which completed experiments 1 and 2, six animals in the lesion group were excluded from the analysis as the lesions were either unilateral or incomplete. A complete lesion of the medial part of the STN was required to satisfy inclusion criteria, although some neurones remained in the lateral part of the nucleus in the majority of subjects. From the subjects used in experiment 3, seven animals were excluded from the lesion group for similar reasons. A schematic indicating the size and extent of the lesion and a photomicrograph of tissue from a lesioned subject as compared with a sham-operated control are shown in Fig. 1.

### Experiment 1: the effect of subthalamic nucleus lesions on delay-discounting

A typical pattern of delay-dependent choice behaviour was observed before surgery, with subjects initially preferring the large reward when its delivery was immediate and shifting their preference to the small reward across the session as the delay to the large reward increased. However, even when there was no delay to the large reward, subjects still sometimes selected the smaller reward (Fig. 2A), a pattern of responding which is often observed during performance of this paradigm (e.g. Cardinal *et al.*, 2001; Winstanley *et al.*, 2003, 2004a). Although it is unclear as to why subjects fail to exclusively choose the large reward when its delivery is immediate, it may reflect a generalization of responding across the session, in that the delay associated with delivery of the large reward which occurs during the

majority of the behavioural test session may decrease the incentive value of responding on the large reward lever even when the delivery of the large reward is immediate. Previous studies have also found that, when the delay to the large reward is removed completely during the entire test session, animals already trained to perform the delay-discounting test will exclusively choose the large reward on every trial (Cardinal *et al.*, 2001; Winstanley *et al.*, 2004a).

Postoperatively, animals with STN lesions chose the larger reward to a greater extent than sham-operated controls, i.e. decreased impulsive choice (comparison of preoperative and postoperative sessions, SURGERY  $\times$  LESION,  $F_{2,17} = 4.376$ ,  $P < 0.029$ ; comparing data from postoperative sessions only, LESION,  $F_{1,12} = 6.557$ ,  $P < 0.025$ , Fig. 2). Postoperatively, the lesion did not affect the number of omissions made [mean  $\pm$  SEM sham,  $0.73 \pm 0.11$ ; lesion,  $1.48 \pm 0.61$ ; LESION,  $F_{1,12} = 0.338$ , not significant (NS)] or the latency to collect reward (mean  $\pm$  SEM sham,  $0.39 \pm 0.05$  s; lesion,  $0.44 \pm 0.07$  s; LESION,  $F_{1,12} = 2.613$ , NS) and nor did the lesion alter the latency to respond (mean  $\pm$  SEM sham,  $1.40 \pm 0.88$  s; lesion,  $1.70 \pm 0.37$  s; LESION,  $F_{1,12} = 0.538$ , NS).

### Experiment 2: the effect of subthalamic nucleus lesions on acquisition of the autoshaping response

Lesioning the STN profoundly impaired the acquisition of autoshaping behaviour (LESION,  $F_{1,12} = 5.248$ ,  $P < 0.041$ , Fig. 3A). Rats with STN lesions made fewer responses to the CS+ (STIMULUS  $\times$  LESION,  $F_{1,12} = 14.971$ ,  $P < 0.002$ ; approaches to CS+ only, LESION,  $F_{1,12} = 21.824$ ,  $P < 0.001$ ) but not to the CS- (LESION,  $F_{1,12} = 0.461$ , NS). Unlike sham-operated controls, STN-lesioned rats did not increase the number of approach responses which they made to the CS+ with increased exposure to the contingencies (STIMULUS  $\times$  BLOCK  $\times$  LESION,  $F_{14,168} = 5.805$ ,  $P < 0.0001$ ; approaches to CS+ only, LESION  $\times$  BLOCK,  $F_{14,68} = 3.165$ ,  $P < 0.019$ ; sham-operated subjects only, BLOCK,  $F_{14,98} = 6.406$ ,  $P < 0.0001$ ; lesion subjects only, BLOCK,  $F_{14,70} = 1.220$ , NS).

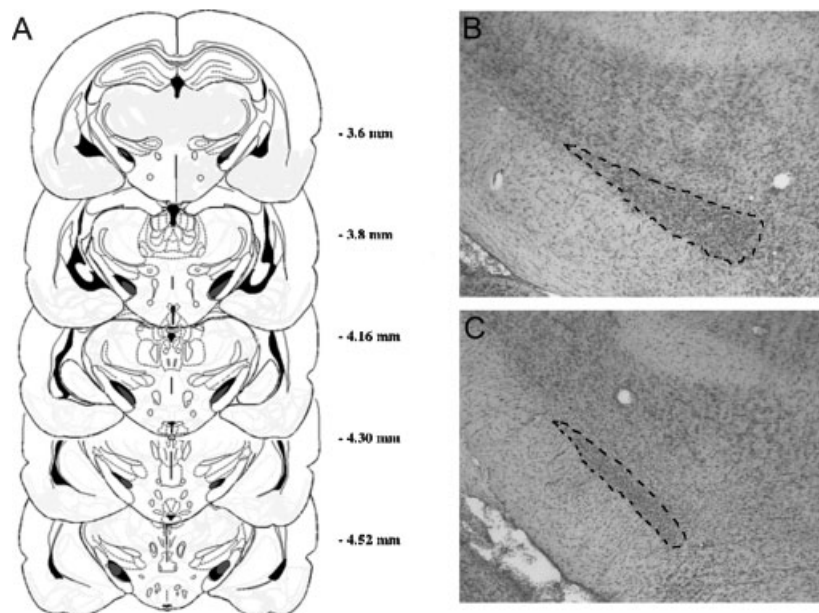


FIG. 1. Histological verification of the extent of the subthalamic nucleus (STN) lesion. (A) Schematic depicting the size and extent of the damage to the STN. The largest lesion is shown in black and the smallest in grey at each anteroposterior level. A photomicrograph of the STN taken from a representative animal in the sham-operated group (B) and in the lesion group (C) at approximately  $-3.6$  mm posterior to bregma is also shown. The extent of the STN is marked by dotted lines.

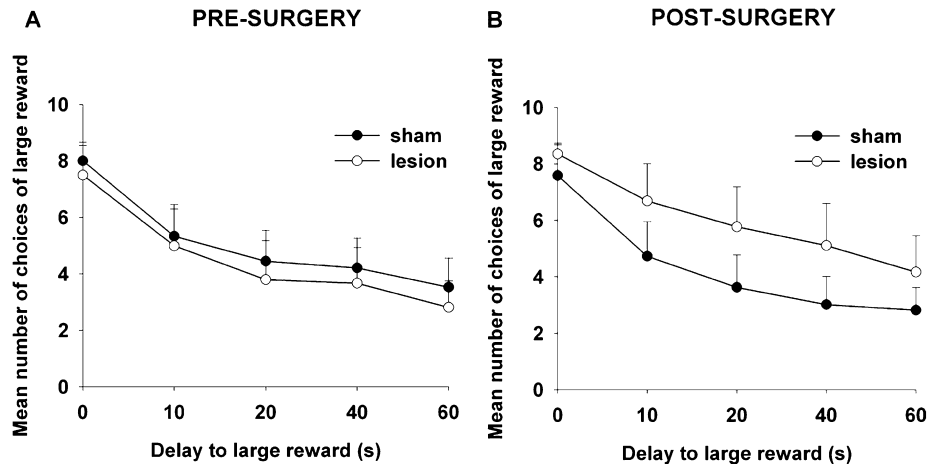


FIG. 2. Effect of subthalamic nucleus lesions on delay-discounting performance. Data shown are mean and SEM.

Analysis of the difference scores confirms that STN-lesioned rats showed no evidence of improved discrimination between the CS+ and CS- over time (LESION,  $F_{1,12} = 14.971$ ,  $P < 0.002$ ; BLOCK  $\times$  LESION,  $F_{14,168} = 5.805$ ,  $P < 0.0001$ , Fig. 3B). Similarly, during the omission phase of the experiment, sham-operated animals continued to approach the CS+, whereas STN-lesioned animals did not (STIMULUS,  $F_{1,12} = 10.427$ ,  $P < 0.007$ ; STIMULUS  $\times$  LESION,  $F_{1,12} = 19.104$ ,  $P < 0.001$ , Fig. 3C).

Due to the small number of approach responses made by rats with STN lesions, it was not statistically viable to analyse the latency to approach the CS+ or CS- in each successive training block. However, if the average time to approach the stimuli was calculated over all trials during acquisition, STN-lesioned animals were significantly slower to approach the CS+ but not the CS- (CS+,  $F_{1,13} = 14.007$ ,  $P < 0.003$ ; CS-,  $F_{1,13} = 0.282$ , NS, Fig. 3D). Similarly, rats with STN lesions took longer to respond to the CS+ but not the CS- during the omission phase as compared with sham-operated controls (CS+,  $F_{1,13} = 9.677$ ,  $P < 0.009$ ; CS-,  $F_{1,13} = 0.563$ , NS).

### Experiment 3: the effect of subthalamic nucleus lesions on performance of the autoshaping response

In contrast to the marked deficits caused by lesioning the STN before the acquisition of autoshaping behaviour, STN lesions made subsequent to training had very little effect on performance of the autoshaping response (Fig. 4). Both sham-operated and lesioned rats approached the CS+ more than the CS- after surgery (STIMULUS,  $F_{1,12} = 718.718$ ,  $P < 0.0001$ ; STIMULUS  $\times$  LESION,  $F_{1,12} = 0.922$ , NS; LESION,  $F_{1,12} = 0.491$ , NS, Fig. 4B) and there were no significant differences in the level of discrimination between the CS+ and CS- as indicated by analysis of the difference scores (LESION,  $F_{1,12} = 0.922$ , NS; LESION  $\times$  BLOCK,  $F_{14,168} = 0.540$ , NS, Fig. 4D). However, during the omission phase, STN-lesioned rats did not continue to approach the CS+ to the same extent as the sham-operated controls (STIMULUS  $\times$  LESION,  $F_{1,12} = 7.473$ ,  $P < 0.018$ ; CS+ approaches only, LESION,  $F_{1,12} = 6.160$ ,  $P < 0.029$ , Fig. 4E), although the number of approaches made to the CS- was similar between the two groups (CS- approaches only, LESION,  $F_{1,12} = 0.175$ , NS).

With regard to the speed of responding, lesioning the STN did not affect the latency to approach the CS+ (LESION,  $F_{1,12} = 2.156$ , NS; LESION  $\times$  BLOCK,  $F_{14,168} = 0.995$ , Fig. 4F) or the CS- (averaging

over block, mean  $\pm$  SEM, sham  $4.92 \pm 0.39$  s, lesion  $3.72 \pm 0.42$  s; LESION,  $F_{1,12} = 2.272$ , NS) during performance (Fig. 4F). Similarly, the latency to approach the CS+ was similar in lesioned animals and sham-operated controls in the omission phase of the paradigm (LESION,  $F_{1,12} = 1.957$ , NS), although there was a tendency for the STN-lesioned rats to be faster to approach the CS- (averaging over block, mean  $\pm$  SEM, sham  $5.16 \pm 0.49$  s, lesion  $3.77 \pm 0.39$  s,  $F_{1,13} = 4.088$ ,  $P < 0.066$ ). However, due to the low number of responses made to this stimulus, and the relatively small number of animals in each group, this analysis is based on very few data points.

### Locomotor activity

In keeping with a previous report (Baunez *et al.*, 2002), there were no significant differences in baseline levels of locomotor activity in STN-lesioned rats as compared with sham-operated controls, although there was a trend for the lesioned animals to be more active ( $F_{1,12} = 4.223$ ,  $P < 0.062$ , Fig. 5).

### Discussion

Subthalamic nucleus-lesioned rats showed an increase in preference for the larger delayed reward, i.e. were less impulsive in this delay-discounting task. In contrast, STN lesions severely impaired acquisition of the autoshaping response, suggestive of a fundamental deficit in the ability to form Pavlovian conditioned stimulus-unconditioned stimulus (CS-US) associations. However, if the STN was lesioned subsequent to the acquisition of autoshaping behaviour, subjects continued to approach the CS+ and to discriminate between the CS+ and CS- to the same level as sham-operated controls but they made fewer responses under the omission schedule. This complex pattern of data suggests dissociable roles for the STN in the acquisition and utilization of reward representations and increases our understanding of the role which this structure plays in cognitive behaviour.

The finding that STN-lesioned rats showed a reduction in impulsive choice in the delay-discounting task contrasts with previous reports of increased motoric impulsivity after STN lesions, as indicated by increases in the number of premature responses made on the 5CSRT and in other choice procedures (Baunez & Robbins, 1997; Baunez *et al.*, 2001). These data add to a growing body of evidence demonstrating that impulsive choice and impulsive action are subject

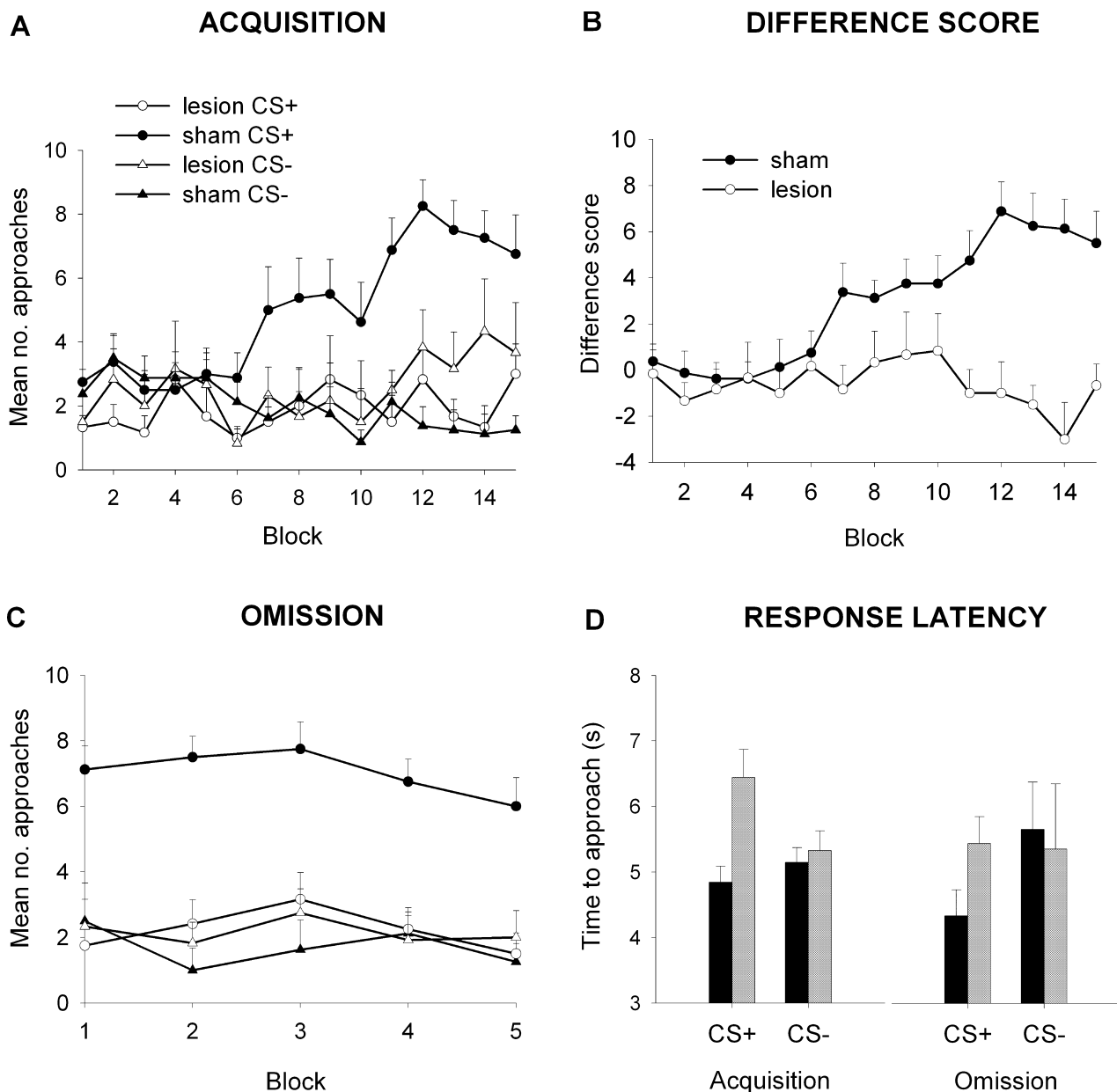


FIG. 3. Effect of subthalamic nucleus lesions on different behavioural measures during the acquisition of the autoshaping paradigm. (A) Responses to the conditioned stimulus (CS)+ and CS- during acquisition; (B) responses to the CS+ and CS- during the omission phase; (C) the difference scores calculated for each block during acquisition; (D) the latency to approach the CS+ and CS- during acquisition and omission (grey, lesioned animals; black, sham-operated controls). Data shown are mean and SEM.

to independent regulatory mechanisms which may nevertheless involve some similar neural structures, such as the NAC, STN and OFC, while others, such as the anterior cingulate and infralimbic cortex, have a more specific role in motoric inhibition (Muir *et al.*, 1996; Cardinal *et al.*, 2001; Chudasama *et al.*, 2003a,b; Christakou *et al.*, 2004; Winstanley *et al.*, 2004a). Although lesions of both the NAC and STN increase break-points during progressive ratio responding for food reward and increase conditioned locomotor activity (Baunez *et al.*, 2002; Bowman & Brown, 1998; Parkinson *et al.*, 1999), lesions to these two areas have differing effects not only on impulsive choice (Cardinal *et al.*, 2001; current study) but also on responding for reward-associated stimuli, i.e. conditioned reinforcers (Parkinson *et al.*, 1999, 2002; Baunez *et al.*, 2002). Furthermore, unlike lesions of the STN, lesioning the NAC core impairs both the

acquisition and performance of the conditioned approach response in the autoshaping paradigm (Cardinal *et al.*, 2002; Parkinson *et al.*, 2000) but NAC lesions do not produce such profound behavioural changes during 5CSRT performance when compared with STN lesions (Baunez & Robbins, 1997; Christakou *et al.*, 2004). Overall, these data suggest that, although the STN and NAC do participate in reward-related processes, the STN is not simply an output relay for the NAC but is significantly involved in processing information from additional structures within the limbic corticostriatal loops.

Although the STN is involved in motor function, the reduction in CS+ approaches observed in STN-lesioned animals during autoshaping is unlikely to be due to a decrease in ambulation as the lesion did not affect baseline levels of locomotor activity. Indeed, it has previously been reported that STN-lesioned rats are more active

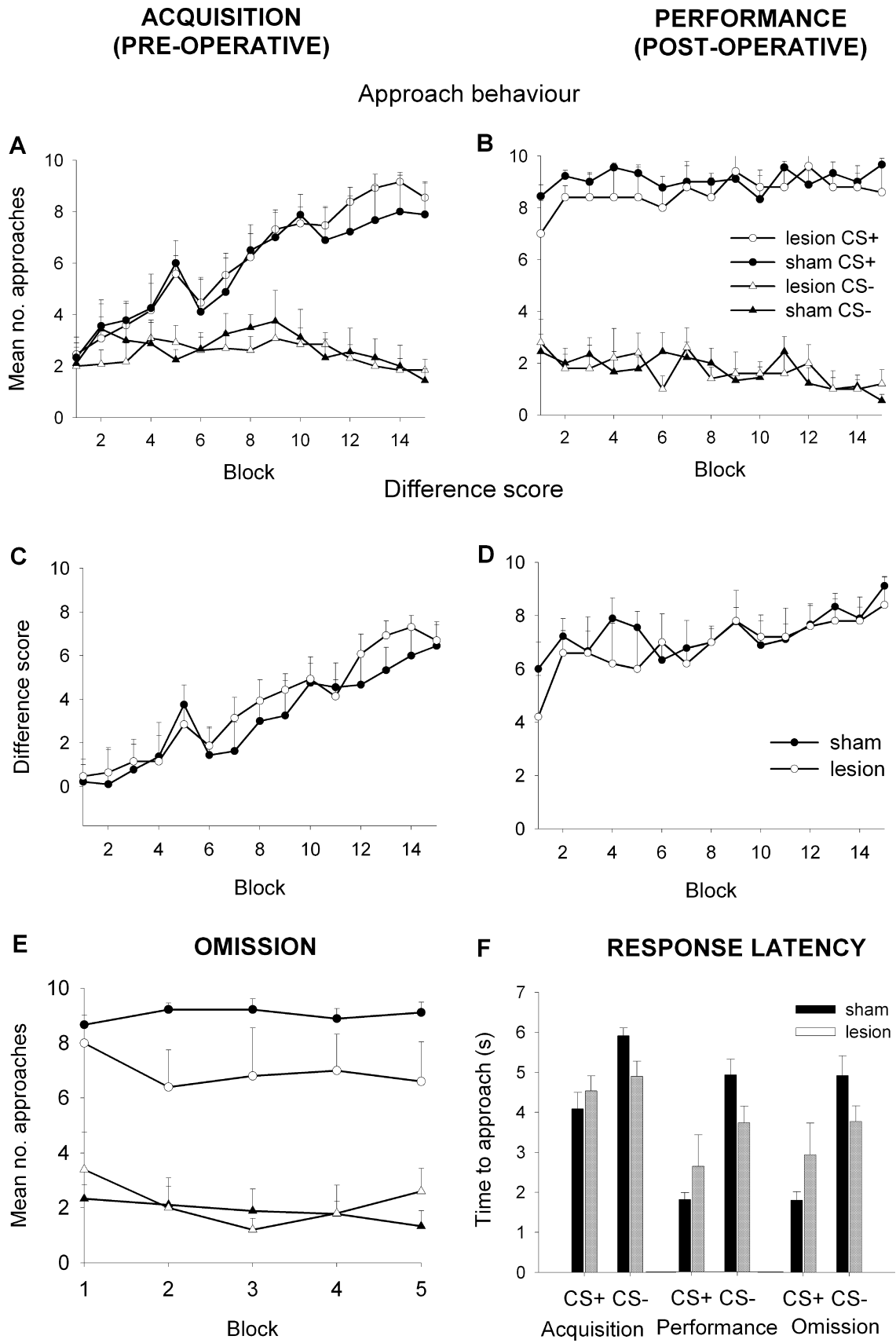


FIG. 4. Effect of subthalamic nucleus lesions on different behavioural measures during performance of the autoshaping paradigm; the number of approaches made to the conditioned stimulus (CS)+ preoperatively (A) and postoperatively (B); the difference score preoperatively (C) and postoperatively (D) and the latency to approach the stimuli preoperatively (E) and postoperatively (F). Data shown are mean and SEM.

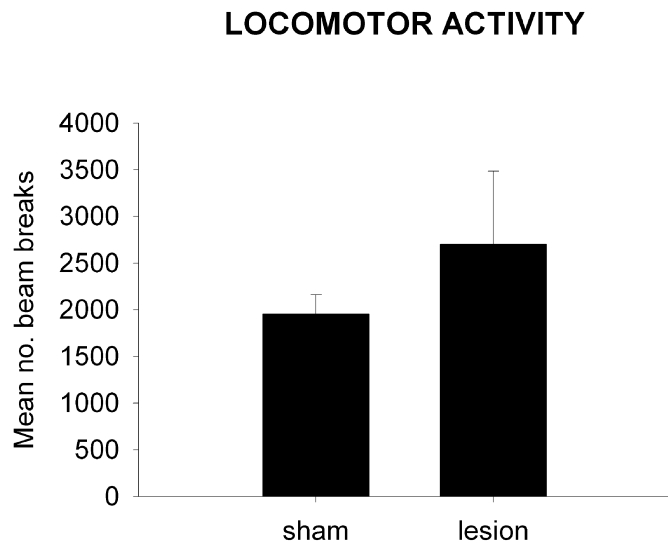


FIG. 5. Effect of subthalamic nucleus lesions on locomotor activity. Data shown are mean and SEM.

during the anticipation of food delivery (Baunez *et al.*, 2002). This increase in conditioned locomotor activity to food further suggests that lesions of the STN do not impair Pavlovian conditioning *per se*, as animals were capable of forming an association between the context and the delivery of food. However, data presented here indicate that STN lesions nevertheless prevent the acquisition of novel associations between a discrete CS and reward.

It has previously been reported that animals which demonstrate higher levels of impulsive choice during a delay-discounting paradigm also make more autoshaping responses to the CS+ (Tomie *et al.*, 1998). A previous investigation examining the effects of serotonergic lesions on these behaviours failed to observe such a relationship (Winstanley *et al.*, 2004b). However, the current data provide partial support for the hypothesis that the number of autoshaping responses that animals make may be related to their level of impulsive choice in that STN-lesioned, in comparison to OFC-lesioned, rats (Chudasama & Robbins, 2003) made many fewer approaches to the CS+ but showed decreased levels of impulsive choice. The fact that sham-operated controls continue to respond to the CS+ during the omission phase strongly argues that their behaviour is under the control of Pavlovian stimulus–reward associations (Brown & Jenkins, 1968). In contrast, in experiment 3 the STN-lesioned rats were sensitive to the cancellation of the food reward after approach responses, a result that also mimics that seen after OFC lesions (Chudasama & Robbins, 2003), indicating instead that their behaviour may result from an instrumental strategy.

It is possible that a reduction in the ability to utilize Pavlovian associations may impact upon delay-discounting performance. The making of the approach response in autoshaping is thought to be rewarding in itself due to its association with the appearance of food (Monterosso & Ainslie, 1999). Likewise, there is evidence to suggest that the making of an instrumental response associated with reward delivery can itself acquire appetitive value through Pavlovian mechanisms (Garrud *et al.*, 1981). Such conditioning processes would be stronger in the case of the response for the small reward as delivery of this reward is always immediate and temporal contiguity between events is a critical factor in the formation of such associations (see Mackintosh, 1974). However, the fact that the

delivery of the large reward is delayed at most points during the paradigm would probably decrease the effectiveness of any such conditioning. It is therefore possible that disrupting the use of basic CS–US associations would lead to an increase in choice for the large delayed reward through the potentiation of goal-directed, instrumental action due to the lack of conflict with Pavlovian, stimulus-driven responses. It has been demonstrated previously that STN-lesioned rats are more motivated to earn reward as demonstrated by increased breakpoints on progressive ratio schedules (Baunez *et al.*, 2002), which may also relate to their ability to inhibit their approach response to the CS+ during the omission phase of the autoshaping task in order to earn reinforcement. Increased motivation for reward may therefore potentially result in increased choice of the larger reward regardless of the aversive delay associated with such a response.

Alternatively, increased responding for the large reward when it is delayed may reflect perseveration in the response associated with reward. Lesions to the OFC, like damage to the STN, increase both premature and perseverative responding during 5CSRT performance and decrease impulsive choice in this delay-discounting paradigm (Baunez & Robbins, 1997; Chudasama *et al.*, 2003b; Winstanley *et al.*, 2004a). It was suggested in the Introduction that increased responding during autoshaping, particularly during the omission phase of the procedure, could reflect perseverative tendencies but lesions to both of these structures produce the opposite effect on this behaviour. Hence, autoshaping may be related to impulsive decision-making due to the fundamental role played by CS–US associations in regulating goal-seeking, rather than through a common element of perseverative-type responding present in both behaviours.

Such striking similarities between the pattern of behavioural effects observed after damage to the STN and OFC may suggest that they participate in common circuitry to regulate certain types of goal-directed and affective behaviour. Disconnection lesions of the STN and medial prefrontal cortex produce a similar pattern of behavioural effects on the 5CSRT compared with bilateral STN lesions, emphasizing the importance of cortico-subthalamic connections in the regulation of complex behaviours (Chudasama *et al.*, 2003a). Not only does the STN receive indirect connections from the OFC through its involvement in the limbic corticostriatal loop (Alexander *et al.*, 1986) and through its connections with the globus pallidus but a direct projection from the medial OFC to the STN has recently been demonstrated using neuroanatomical tracing techniques (Maurice *et al.*, 1998). Dysfunction within the OFC has been heavily implicated in obsessive-compulsive disorder (Stein, 2002) and such dysregulation of this frontal region doubtless affects activity within the corticostriatal loops in which it participates. For example, pathological correlations in brain activity between the OFC and the caudate nucleus have been observed in patients with obsessive-compulsive disorder and this aberrant activity is remedied by successful treatment (Schwartz *et al.*, 1996). High-frequency stimulation of the STN has been shown to reduce compulsive behaviour in Parkinsonian patients with obsessive-compulsive disorder (Aleget *et al.*, 2001; Mallet *et al.*, 2002) and also to increase the processing of emotional stimuli (Schneider *et al.*, 2003). Furthermore, in comparison to the effects of STN lesions in rats on cognitive tests, STN high-frequency stimulation in human patients with Parkinson's disease can impair tests sensitive to frontal damage, such as verbal fluency and the Stroop test, and other tests of working memory and recall (Trepanier *et al.*, 2000). Inactivation of the STN may therefore disinhibit nonmotor as well as motor limbic circuits passing through the basal ganglia and projecting to the frontal cortex, thus affecting cognitive performance.

In summary, the finding that lesions to the STN alter impulsive decision-making as well as affecting the utilization and formation of CS-US associations supports and extends the hypothesis that the STN plays an important role in the regulation of cognitive function. The finding that damage to this structure impairs the use of environmental stimuli to guide behaviour may relate to the lesion-induced changes in the performance of more complex tasks such as impulsive decision-making. Although the STN is involved in regulating motor output, its functions clearly extend beyond this behavioural dimension due to its interconnections with both limbic and cortical structures, including the OFC, which are associated with a range of neuropsychiatric disorders. The similarities observed between damage to this frontal region and lesions of the STN support the view that there may be a common pathophysiological principle in neuropsychiatric and movement disorders based on the interconnectivity of the basal ganglia and frontal cortices (Middleton & Strick, 2000). Further elucidation of the pattern of behavioural effects produced by lesions to cortical and subcortical areas will increase our understanding of which frontostriothalamic loops are implicated in specific behaviours and this knowledge can be used to improve our modelling and understanding of cognitive processes in both healthy and disease states.

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## Abbreviations

CS, conditioned stimulus; 5CSRT, five-choice serial reaction time task; NAC, nucleus accumbens; NS, not significant; OFC, orbitofrontal cortex; STN, subthalamic nucleus; US, unconditioned stimulus.

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