Published in final edited form as:

Behav Pharmacol. 2009 September ; 20(5-6): 437–446. doi:10.1097/FBP.0b013e3283305e4d.

QUANTITATIVE ANALYSIS OF THE EFFECT OF LESIONS OF THE SUBTHALAMIC NUCLEUS ON INTER-TEMPORAL CHOICE: FURTHER EVIDENCE FOR ENHANCEMENT OF THE INCENTIVE VALUE OF FOOD REINFORCERS

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Abstract

Recent evidence suggests that the subthalamic nucleus (STN) is involved in regulating the incentive value of food reinforcers. The objective of this study was to examine the effect of lesions of the STN on inter-temporal choice (choice between reinforcers differing in size and delay). Rats with bilateral quinolinic acid-induced lesions of the STN ($n=15$) or sham lesions ($n=14$) were trained in a discrete-trials progressive delay schedule to press levers A and B for a sucrose solution. Responses on A delivered 50 μ l of the solution after a delay d_A ; responses on B delivered 100 μ l after a delay d_B . d_B increased across blocks of trials; d_A was manipulated across phases of the experiment. Indifference delay, $d_{B(50)}$ (value of d_B corresponding to 50% choice of B), was estimated for each rat in each phase, and linear indifference functions ($d_{B(50)}$ vs. d_A) were derived. The STN-lesioned group showed a flatter slope of the indifference function (implying higher instantaneous reinforcer values) than the sham-lesioned group; the intercepts did not differ between the groups. The results agree with recent evidence for a role of the STN in incentive value. Unlike some previous studies, these results do not indicate a role of the STN in delay discounting.

Keywords

Subthalamic nucleus; Lesion; Quinolinic acid; Inter-temporal choice; Incentive value; Rat

Introduction

The subthalamic nucleus (STN) is an important relay in the indirect striatofugal pathways (Alexander et al. 1990; Gerfen 2004). Its role in extrapyramidal motor control has been recognized for many years (see Parent and Hazrati 1995a, 1995b; Gerfen 2004). More recently it has transpired that the STN makes an important contribution to voluntary response inhibition and attention. Thus, destruction of the STN has been found to promote

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premature responding in serial reaction time tasks (Baunez and Robbins 1997, Baunez et al. 2001; Aron and Poldrack 2006; Eagle et al. 2008) and differential-reinforcement-of-low-response-rate (DRL) schedules (Uslaner and Robinson 2006), and to facilitate unreinforced responding in the fixed-interval peak procedure (Wiener et al. 2008). These effects have been attributed to the release of positively reinforced operant behaviour from learned inhibitory control (Baunez and Robbins 1997; Phillips and Brown 2000 Wiener et al. 2008; for reviews, see Temel et al. 2005; Tan et al. 2006).

In addition, there is a growing body of evidence that the STN exerts some limiting control over the incentive value of reinforcers. For example, destruction of the STN has been found to increase the locomotor activation induced by conditioned stimuli that have been associated with food reward (Baunez et al. 2002), to prolong responding on progressive-ratio schedules of food (Baunez et al. 2005) and cocaine (Uslaner et al. 2005) reinforcement, and to facilitate sign-tracking behaviour conditioned with food or cocaine reinforcement (Uslaner et al. 2008). In a recent experiment employing a quantitative approach based on Killeen's (1994) mathematical model of schedule-controlled behaviour, it was found that lesions of the STN enhanced a quantitative index of the incentive value of food reinforcement (Bezzina et al. 2008c).

Another aspect of operant behaviour in which the STN may play a significant role is inter-temporal choice – that is, choice between reinforcers delivered after different delays. In a typical inter-temporal choice situation, the subject is presented with two operanda, A and B; a response on A produces a smaller reinforcer of size q_A after a delay d_A , whereas a response on B produces a larger reinforcer of size q_B after a longer delay d_B . Preference for the larger reinforcer, expressed as percentage choice of that reinforcer, %B, declines as a function of increasing values of d_B . Lesions of the STN have been found to suppress preference for the smaller, earlier reinforcer in inter-temporal choice schedules (Winstanley et al. 2005; Uslaner and Robinson 2006).

It is generally agreed that one important determinant of inter-temporal choice is 'delay discounting', i.e. degradation of the incentive value of an outcome as a function of the delay interposed between the operant response and delivery of the primary reinforcer. A considerable body of evidence indicates that the delay discounting function is hyperbolic in form (Ainslie 1975; Mazur 1987; 1997):

$$V = 1 / [1 + Kd] \quad [1]$$

where V is the incentive value of the outcome, d is delay, and K is a free parameter expressing the rate of delay discounting (Mazur 1987). However, inter-temporal choice behaviour depends not only on delay discounting, but also on the absolute and relative sizes of the two reinforcing outcomes (Ho et al. 1997, 1999). According to one model of inter-temporal choice (the multiplicative hyperbolic model [MHM]: Ho et al. 1999), the relationship between reinforcer size and instantaneous incentive value is an increasing hyperbolic function, and the overall incentive value of a delayed outcome is determined by multiplicative combination of the two hyperbolic expressions:

$$V = \frac{1}{1 + Kd} \cdot \frac{1}{1 + Q/q} \quad [2]$$

where q is reinforcer size and Q is a free parameter expressing the size corresponding to the half-maximal value (Ho et al. 1999).

Equation 2 implies that preference in an inter-temporal choice situation is necessarily influenced by the reinforcer sizes and delays associated with each outcome, as well as by the

individual organism's sensitivity to reinforcer size and delay (represented by Q and K , respectively). This may complicate the interpretation of experiments in which biological interventions are found to affect inter-temporal choice behaviour, since it is often unclear whether an alteration of preference is brought about by a change in Q , a change in K , or both. However, according to MHM, the separate influences of delay- and size sensitivity may be teased apart by measuring a series of indifference delays to the larger of the two reinforcers ($d_{B(50)}$), when the values of the two outcomes may be assumed to be equal (i.e. $V_A = V_B$). Expanding this equality using Equation 2 and solving for $d_{B(50)}$ yields the following linear relation:

$$d_{B(50)} = \frac{1}{K} \cdot \left[\frac{1+Q/q_A}{1+Q/q_B} - 1 \right] + d_A \cdot \left[\frac{1+Q/q_A}{1+Q/q_B} \right] \quad [3]$$

(Ho et al. 1999). The slope of this relation is determined only by the sizes of the reinforcers (q_A and q_B) and the size-sensitivity parameter Q , whereas the intercept of the function is jointly determined by the sizes of the reinforcers, the size-sensitivity parameter Q and the delay-discounting parameter K . It follows that, according to MHM, a single preference function ($\%B$ vs. d_B) corresponding to any given delay to the smaller reinforcer (e.g. $d_A=0$) does not necessarily convey any information about the rate of delay discounting. However, if several preference functions are obtained, using a range of values of d_A , the linear relation defined by Equation 3 may be constructed, from which valid inferences may be drawn about both the rate of delay discounting (K) and the organism's sensitivity to reinforcer size (Q). Since Q is the only free parameter expressed in the slope of Equation 3, a change in slope induced by a biological intervention necessarily implies a change in Q , whereas a change in the rate of delay discounting, K , may be inferred from a change in the intercept, with or without a concomitant change in slope (Ho et al. 1999). Using this approach it was found that lesions of the orbital prefrontal cortex (OPFC) altered both K and Q (Kheramin et al. 2002), whereas lesions of the nucleus accumbens core (AcbC) (Bezzina et al. 2007) or disconnecting the OPFC from the AcbC (Bezzina et al. 2008b) increased K without altering Q .

The present experiment examined the effect of lesions of the STN on the linear indifference function defined by Equation 3. Based on the evidence, discussed above, that destruction of the STN enhances the incentive value of food reinforcers (Baunez et al. 2002; Uslaner et al. 2005; Uslaner and Robinson 2008; Bezzina et al. 2008c), it was expected that the lesion would result in a reduction of Q , which would be reflected in flattening of the linear indifference function. The logic underlying this prediction is as follows. Q represents the reinforcer size (q) corresponding to the half-maximal incentive value (Equation 2). Therefore, a reduction of Q implies an increase in the incentive value corresponding to any particular reinforcer size. Moreover, given the negatively accelerated (hyperbolic) relationship between value and size postulated by MHM (Ho et al. 1999), a reduction of Q entails a reduction of the ratio of the incentive values of any two reinforcer sizes. Since the slope of the linear indifference function reflects the ratio of the incentive values of two reinforcing outcomes (Equation 3), it is evident that a reduction of Q implies a reduction of the slope of the indifference function. In summary, therefore, the evidence favouring an enhancement of (instantaneous) reinforcer value following lesions of the STN suggests that this lesion reduces the size-sensitivity parameter Q . This in turn implies a reduction of the *relative* incentive value of the larger of two reinforcers, which should be reflected in a reduction of the slope of the linear indifference function (see Kheramin et al., 2005, for discussion and graphical illustration of this principle). In addition, the proposal that lesions of the STN reduce the rate of delay discounting (Winstanley et al. 2005) suggested that the intercept of the function would be elevated by the lesion.

Methods

The experiment was carried out in accordance with UK Home Office regulations governing experiments on living animals.

Subjects

Thirty experimentally naive female Wistar rats approximately 4 months old and weighing 250-300 g at the start of the experiment were used. They were housed under a constant cycle of 12 h light and 12 h darkness (light on 0600-1800 hours), and were maintained at 80% of their initial free-feeding body weights throughout the experiment by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cages.

Surgery

The rats received either lesions of the STN (n=16) or sham lesions (n=14). Anaesthesia was induced with isoflurane (4% in oxygen), and the rat was positioned in a stereotaxic apparatus (David Kopf), with the upper incisor bar set 3.3 mm below the inter-aural line. Anaesthesia was maintained with 2% isoflurane in oxygen during surgery. A hole was drilled in the skull over each hemisphere for microinjection of quinolinic acid into the STN. The following coordinates were used to locate the STN: AP -3.6, L $\pm$ 2.6, V -8.0 (mm, measured from bregma: Paxinos and Watson 1998). Injections were given via a 0.3-mm diameter cannula connected by a polyethylene tube to a 10- μ l Hamilton syringe. In the case of the lesioned group, the cannula tip was slowly lowered to the position of each site and 0.3 μ l of a 0.1-M solution of quinolinic acid (2,3-pyridinedicarboxylic acid) in phosphate-buffered 0.9% NaCl (PBS: pH 7.0) was injected at a rate of 0.1 μ l per 15 s. The cannula was left in its position for three minutes after completion of the injection in each site. In the case of the sham-lesioned group, the procedure was identical, except that the vehicle alone was injected. The rats were given diazepam 5 mg kg⁻¹ intraperitoneally in order to suppress seizures during the immediate post-operative period.

Apparatus

The rats were trained in standard operant conditioning chambers (CeNeS Ltd, Cambridge, UK) of internal dimensions 25 × 25 × 22 cm. One wall of the chamber contained a recess into which a peristaltic pump could deliver a 0.6 M sucrose solution. Two apertures situated 5 cm above and 2.5 cm to either side of the recess, through which motor-operated retractable levers could be inserted into the chamber. The levers could be depressed by a force of approximately 0.2 N. A 2.8-W lamp was mounted 2.5 cm above each lever; a third lamp was mounted 10 cm above the central recess. Six red light-emitting diodes were mounted in a row, 4 cm apart, 5 cm above the levers. The operant chamber was enclosed in a sound-attenuating chest; masking noise was generated by a rotary fan. An Acorn microcomputer programmed in Arachnid BASIC (CeNeS Ltd, Cambridge, UK), located in an adjoining room, controlled the schedules and recorded the behavioural data.

Behavioural training

Two weeks after surgery, the food-deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press two levers (A and B) for sucrose reinforcement, and were exposed to a discrete-trials continuous reinforcement schedule in which the two levers were presented in random sequence for three sessions. After this initial training, they underwent daily training sessions under the discrete-trials delayed reinforcement schedule for the remainder of the experiment. Each experimental session consisted of six blocks of six trials, except in phases 4 and 5

when sessions consisted of five blocks. The trials were 90 s in duration, with the exception of phase 5, in which the duration was increased to 120 s in order to accommodate the long delay to reinforcement (see below). The six blocks were signalled by illumination of the six light-emitting diodes: in block 1 the first (left-most) diode was illuminated, in block 2 the first and second diodes were illuminated, and so on. The first two trials of each block were forced-choice trials in which each lever was presented alone in random sequence. The other four trials were free-choice trials in which both levers were presented. The beginning of each trial was signalled by illumination of the central light above the reinforcer recess. After 2.5 s the lever or levers (depending on the type of trial) were inserted into the chamber. When a lever-press occurred, the lever(s) were withdrawn, the central light was extinguished, and the light located above the lever that had been depressed was illuminated. This light remained illuminated until the delivery of the reinforcer, and was then extinguished. The chamber remained in darkness until the start of the following trial. If no lever-press occurred within 5 s of the lever(s) being inserted, the lever(s) were retracted and the central light extinguished. A response on lever A initiated a fixed delay d_A , following which 50 μ l of the 0.6 M sucrose solution was delivered. A response on lever B initiated a variable delay d_B , after which 100 μ l of the same sucrose solution was delivered. The positions of levers A and B (left vs. right) were counterbalanced across subjects.

The experiment consisted of six phases, in which the value of d_A was set at 1, 2, 4, 8, 12 and 0.5 s, respectively. In each phase, the value of d_A was held constant. In each session the value of d_B was set equal to d_A in the first block of trials. In subsequent blocks d_B was increased in increments of 75%. In phases 4 and 5, when d_A was 8 s and 12 s, respectively, computing five increments of 75% would have generated a value of d_B that was longer than the duration of a trial in the sixth block of trials; therefore the number of blocks was limited to five in these phases. The first phase continued for 100 sessions and the remaining phases for 40 sessions.

Experimental sessions were carried out 7 days a week, at the same time each day, during the light phase of the daily cycle (between 0800 and 1400 hours).

Histology

At the end of the experiment, the rats were deeply anaesthetised with sodium pentobarbitone and perfused transcardially with 0.9% sodium chloride, followed by 10% formol saline. The brains were removed from the skull and fixed in formol saline for one week. 40- μ m coronal sections were taken through the region of the STN using a freezing microtome.

Cresyl violet staining—The procedure was similar to that described previously (Kheramin et al. 2005). Alternate sections were mounted on chrome-gelatine-coated slides and air dried, hydrated by successive immersion in 95%, 70% and 50% ethanol, stained in 0.25% cresyl violet for 2 min at room temperature, dehydrated by successive immersion in 50%, 70%, 95%, 100% ethanol and xylene, and mounted with DPX.

Immunocytochemistry—In the other sections neurone-specific nuclear protein (NeuN) was labelled as described by Jongen-Relo and Feldon (2002). Our protocol has been described elsewhere (Bezzina et al. 2007). Briefly, freshly sliced sections were rinsed in 0.1 M PBS and placed in 0.5% H_2O_2 in PBS for 30 min. After twice rinsing in PBS, they were placed for 60 min in a blocking solution (10% normal horse serum [Vector Laboratories, Peterborough, UK], 1% bovine serum albumin [BSA, Sigma-Aldrich, Gillingham, UK] and 0.3% Triton X-100 [Sigma-Aldrich] in PBS). They were incubated for 48 h at 4°C with the primary antibody (monoclonal mouse anti-NeuN serum [1:5000, Chemicon, Chandlers Ford, UK] in 1% normal horse serum, 1% BSA and 0.3% Triton X-100 in PBS), washed twice in

PBS, and incubated for 2 h at room temperature in biotinylated horse antimouse serum (Vector Laboratories) (1:1000 in 1% BSA and 0.3% Triton X-100 in PBS). After further rinsing in PBS, they were placed for 2 h in avidin-biotin-horseradish peroxidase complex (1:200, ABC-Elite, Vector Laboratories) in PBS. After two further rinses in PBS, they were placed in a chromagen solution (0.05% diaminobenzidine [Sigma-Aldrich] and 0.01% H₂O₂ [Sigma-Aldrich]) for 5 min. The reaction was observed visually and stopped by rinsing in PBS. The sections were floated on to chrome-gelatine-coated slides and mounted with DPX.

An investigator who was blind to the behavioural results performed the microscopic examination. Drawings of the area of the lesions were superimposed on the appropriate coronal sections in the stereotaxic atlas of Paxinos and Watson (1998).

Data analysis

Data from one rat in the STN-lesioned group were discarded because the lesions were found to be misplaced, leaving 15 rats in the STN-lesioned group and 14 in the sham-lesioned group.

Preference functions and linear indifference functions—For each rat, the percentage choice of lever B in the free-choice trials ($%B$) was computed for each block of trials from the pooled data from the last 10 sessions of each phase of the experiment. Plots of $%B$ vs. d_B were derived for each rat, and the indifference delay ($d_{B(50)}$; the value of d_B corresponding to $%B=50\%$) was estimated by linear interpolation between the two delays which fell on either side of $%B=50\%$ (i and j) using the formula: $d_{B(50)} = d_{B(i)} + [(d_{B(j)} - d_{B(i)}) \cdot (%B_i - 50) / (%B_i - %B_j)]$ (Snedecor and Cochran 1989). Indifference delays were subjected to a two-factor analysis of variance (group $\times$ phase, with repeated measures on the latter factor) followed by multiple comparisons between groups at each phase using the least significance difference test. Plots of $d_{B(50)}$ vs. d_A were obtained for each rat, and linear functions were fitted by the method of least squares; goodness of fit was expressed as r^2 , the proportion of the data variance accounted for by the fitted function. The slope and intercept of the linear indifference functions were compared between the two groups using Student's t -test. (According the Equation 3, the slope reflects the ratio of the instantaneous values of the two reinforcers, and is influenced only by the sizes of the reinforcers and the size-sensitivity parameter Q , whereas the intercept is also influenced by the rate of delay discounting, K ; see Introduction.)

Psychophysical analysis of preference functions—Logistic functions were fitted to the group mean $%B$ data and the $%B$ data from each rat in each phase of the experiment: $%B = 100 / (1 + [d_B / d_{B(50)}]^\epsilon)$. This function defines a descending sigmoid curve which is symmetrical in semi-logarithmic co-ordinates; $d_{B(50)}$ and ϵ are parameters, $d_{B(50)}$ being the point of intersection of the logistic curve with the indifference line, and ϵ being the slope of the function. These parameters were used to derive the limen $([d_{B(25)} - d_{B(75)}] / 2)$, where $d_{B(25)}$ and $d_{B(75)}$ are the estimated values of d_B corresponding to $%B=25$ and $%B=75$, respectively), and the index of relative precision, the Weber fraction, was defined as limen / $d_{B(50)}$. The Weber fraction was subjected to repeated-measures analysis of variance (phase); as no significant effect of phase was revealed, the Weber fractions were averaged across phases compared between groups using Student's t -test.

A significance criterion of $P < 0.05$ was adopted in all statistical analyses.

Results

Behavioural Data

Preference functions and linear indifference functions—Preference functions (%B vs d_B) derived for each group in all six phases of the experiment are shown in Fig. 1 (left-hand graphs). In each group, preference for lever B declined as a function of the delay to reinforcer B (d_B). The horizontal lines in the graphs show the indifference level (i.e., %B = 50); the value of d_B at which the preference function crossed this level (i.e. $d_{B(50)}$) increased as a function of increasing values of d_A , reflecting a progressive rightward displacement of the curve.

Analysis of variance of the $d_{B(50)}$ data revealed a significant main effect of phase [$F(5,135)=52.0$, $P<0.001$]; the main effect of group was not significant [$F(1,27)=2.5$, N.S.], but there was a significant group $\times$ phase interaction [$F(5,135)=2.4$, $P<0.05$], reflecting an increasing between-group difference in $d_{B(50)}$ as a function of increasing delays to the smaller reinforcer (d_A). Multiple comparisons between groups at each value of d_A (LSD test) indicated that the value of $d_{B(50)}$ was significantly lower in the STN-lesioned group than in the sham-lesioned group at $d_A = 8$ s and $d_A = 12$ s. Fig. 2 shows the indifference functions ($d_{B(50)}$ vs d_A) for the group mean data. In each group, the linear function accounted for more than 99% of the variance of the group mean data ($r^2>0.99$ in each case). Linear indifference functions were also fitted to the data from the individual rats. The group mean values (+SEM) of the slope and intercept of the function are shown in Fig. 3. The STN-lesioned group had a significantly flatter slope than the sham-lesioned group [$t(27)=2.1$, $P<0.05$], indicating a smaller ratio of the instantaneous values of the two reinforcers (Equation 3). There was no significant difference between the intercepts derived for the two groups [$t(27)=0.2$, N.S.] The goodness of fit of the linear function did not differ significantly between the groups [$t(27)=0.8$, N.S.]; the function accounted for >85% of the data variance for individual rats in both groups [sham-lesioned group: $r^2=0.927\pm0.015$; STN-lesioned group: $r^2=0.881\pm0.050$].

In order to facilitate comparison with previous results in the literature, an additional analysis was carried out on the data from the final phase of the experiment, in which d_A was 0.5 s. Fig 2 (left-hand panel) shows the mean $\pm$ SEM preference data from the two groups (percent choice of the larger reinforcer plotted against delay to the larger reinforcer). Analysis of variance showed that there was a significant main effect of delay [$F(5,135)=98.1$, $P<0.001$], but no significant main effect of group [$F(1,27)=2.4$, N.S.] and no significant interaction [$F<1$]. Fig. 2 (right-hand panel) shows the mean $\pm$ SEM indifference delays from the two groups. There was no significant difference between the groups [$t(27)=0.6$, N.S.].

Psychophysical analysis of preference functions—The logistic psychometric functions derived for the group mean data (Fig. 1, right-hand panels) accounted for 97% of the data variance ($r>0.97$) in all cases. The logistic function could be fitted to 164 of the 174 preference functions obtained for the individual rats in the six phases of the experiment (94.3%); functions could be fitted to the data from all six phases in 25 of the 29 rats. The goodness of fit did not differ significantly between the two groups [sham-lesioned group: $r^2=0.969\pm0.009$; STN-lesioned group: $r^2=0.948\pm0.011$; $t(27)=0.8$, N.S.].

The group mean values of the slope of the logistic functions (ϵ) (+SEM) are shown in Fig. 4 (left-hand histogram). There was no significant difference between the values of the slope derived for the two groups [$t(27)=0.8$, N.S.].

The Weber fraction derived from the logistic function was not systematically related to the value of d_A . Single-factor analysis of variance with repeated measures (phase),

incorporating the data from the rats that generated Weber fractions from all six phases showed no significant effect of phase in either group [sham-lesioned group: $F(5,55)=2.1$, N.S.; STN-lesioned group: $F(5,60)=1.1$, N.S.]. The Weber fraction was therefore averaged across phases for each rat; the group mean values (+SEM) are shown in Fig. 4 (right-hand histogram). The values of the Weber fraction did not differ significantly between the two groups [$t(27)=1.1$, N.S.]

Histology

Bilateral lesions were found to be accurately placed in 15 of the 16 rats that had received injections of quinolinic acid into the STN (the behavioural data from the remaining rat were excluded from all analyses: see above). There was marked neuronal loss in the STN compared to the sham-lesioned rats. Neuronal loss was mainly restricted to the STN, although some minor loss was seen in the zona incerta and lateral hypothalamus immediately adjacent to the STN in some animals. Examples of NeuN-labelled sections are shown in the left-hand panels of Fig. 5; the approximate extent of the lesion is shown in the right-hand diagrams.

Discussion

Injection of quinolinic acid produced a substantial lesion of the STN, of approximately the same extent as those seen in previous experiments using similar surgical protocols with the excitotoxin ibotenic acid (e.g. Baunez et al. 2005; Uslaner et al. 2005; Uslaner and Robinson 2006; Wiener et al. 2008). The STN was almost completely destroyed in most of the lesioned rats.

The discrete-trials progressive delay schedule used in this experiment was an adaptation of the schedule developed by Evenden and Ryan (1996). In agreement with many previous experiments employing this method (Evenden and Ryan 1996, 1999; Cardinal et al. 2001; Kheramin et al. 2002, 2004; Winstanley et al. 2004, 2005; Bezzina et al. 2007, 2008b), the rats in both groups in this experiment shifted their preference from the larger to the smaller reinforcer as the delay to the larger reinforcer (d_B) was progressively increased.

As in our previous experiments (Kheramin et al. 2002, 2004; Bezzina et al. 2007, 2008b), we used a geometric progression to establish the values of d_B in successive blocks of trials in each session. This allowed the range of values of d_B to be adapted to the value of d_A in the six phases of the experiment. The preference functions ($\%B$ vs. d_B , see Fig. 1) were used to compute indifference delays ($d_{B(50)}$) in each phase, which were then used to construct linear indifference functions ($d_{B(50)}$ vs. d_A ; Equation 3). According to MHM (see Introduction) the slope of this function expresses the ratio of the instantaneous values of the larger and smaller reinforcer, which is determined by the physical sizes of the reinforcers (q_B and q_A) and the size-sensitivity parameter, Q . The flatter slope of the function in the STN-lesioned group than the sham-lesioned group implies a smaller value of Q in the STN-lesioned group, which in turn implies that the absolute instantaneous values of the reinforcers were higher in the STN-lesioned group than in the sham-lesioned group (Ho et al. 1999; Kheramin et al. 2005). Thus the present results are consistent with a growing body of evidence which indicates that destruction of the STN results in enhancement of the incentive values of outcomes associated with food reinforcement (Baunez et al. 2002; 2005; Uslaner and Robinson 2006; Uslaner et al. 2008; Bezzina et al. 2008c).

The present results do not, however, confirm previous reports of enhanced preference for the larger, more delayed reinforcer following STN lesions (Winstanley et al. 2005; Uslaner and Robinson 2006). Using the methodology of the present experiment, enhanced preference for the delayed reinforcer should have been reflected in higher values of $d_{B(50)}$ in the STN-

lesioned group than in the sham-lesioned group; no such trend was seen in this experiment. The reason for this discrepancy is not entirely clear. In the previously cited studies (Winstanley et al. 2005; Uslaner and Robinson. 2006), the smaller reinforcer was delivered immediately following a response (i.e. $d_A=0$). This condition was not included in the present experiment. However, as the shortest value of d_A used in the present experiment (0.5 s) was not associated with any between-group difference in $d_{B(50)}$, it seems unlikely that inclusion of a $d_A=0$ condition would have revealed a significantly higher value of $d_{B(50)}$ in the STN-lesioned group. Moreover, the intercepts of the linear functions, which represent the extrapolated indifference delays corresponding to $d_A=0$ did not differ significantly between the two groups.

Apart from the application of Equation 3 to derive quantitative indices of inter-temporal choice, there are other methodological differences between the present experiment and the previous studies of Winstanley et al. (2005) and Uslaner and Robinson (2006). One conspicuous difference, which might account for the differing results, is the timing of the lesion. In the present experiment the lesion was inflicted before behavioural training, whereas Winstanley et al. (2005) and Uslaner and Robinson (2006) trained their subjects under the inter-temporal schedule task before inflicting the lesion. Infliction of the lesion before training was essential in the present experiment, which comprised multiple phases associated with different values of d_A , in order to ensure that the subjects were trained under each condition in the same (post-lesion) state. It is possible that reduction of the rate of delay discounting is a transient effect of STN lesions, which may have dissipated during the extended periods of training used in the present experiment. Consistent with this suggestion, Uslaner and Robinson (2006) noted that enhanced preference for the larger delayed reinforcer was only apparent for a brief period after destruction of the STN, although it remained possible to induce the effect by systemic treatment with *d*-amphetamine over a longer period. In this context it is of interest to note that a transient effect on delay discounting may be particular to STN lesions, as previous studies have found that the effects of AcbC and OPFC lesions on delay discounting are still evident many months after the lesion has been inflicted (Kheramin et al. 2002; Bezzina et al. 2007, 2008b).

As in previous experiments using this protocol (Bezzina et al. 2007, 2008b), the preference functions ($\%B$ vs. d_B) could be described by a two-parameter logistic function. The slope of this function (ϵ) and the Weber fraction derived from the function did not differ significantly between the two groups. This suggests that the STN lesion did not impair the rats' ability to discriminate the incentive values of the outcomes associated with the two levers (see Bezzina et al. 2007). In this respect, the effect of the STN lesion differed from the effect of ablating the AcbC, which resulted in a significant increase in the Weber fraction (Bezzina et al. 2007).

The present results do not directly address the mechanism whereby destruction of the STN produces an increase of incentive value. One possibility is that the efficacy of the primary reinforcer itself (food) is enhanced, as suggested by Winstanley et al. (2005) and Bezzina et al. (2008c). An alternative, or additional, mechanism has been proposed by Uslaner et al. (2008). These authors found that destruction of the STN facilitated approach to a stimulus previously paired with food ('sign tracking'), and proposed that the STN normally helps to regulate the incentive salience that is assigned to conditioned reinforcers. The present results are compatible with this suggestion if, as proposed by Mazur (1995), inter-temporal choice is regarded as choice between outcomes based on the incentive values of the conditioned reinforcing stimuli that are present at the moment of choice. A conceptual advantage of Mazur's (1995) position is that it accounts for delay discounting without assuming delay-dependent devaluation of the primary reinforcer itself (see Mazur 2001).

In conclusion, this experiment employed a quantitative analysis of inter-temporal choice behaviour. The results provided further evidence that destruction of the STN enhances incentive reinforcer value, suggesting that the STN may exert some limiting or 'dampening' influence over reinforcer value.

Acknowledgments

We are grateful to Mrs Victoria Bak and Mr R.W. Langley for skilled technical help.

Financial support: This work was supported by the Wellcome Trust

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Fig. 1.

Group mean data from the sham-lesioned group (*upper panels*) and STN-lesioned group (*lower panels*). *Left-hand panels* show preference functions (percent responding on B, %B, vs. delay to the larger of the two reinforcers after a response on B [d_B , s]). Each set of points shows the data collected from one phase of the experiment, in which the delay to the smaller reinforcer (d_A) was set at the value indicated (see inset). The horizontal reference lines denote indifference (%B=50). The intersection between each preference function and the indifference level denotes the indifference delay ($d_{B(50)}$) for that phase. *Right-hand panels* show transformations of the preference functions with d_B on a logarithmic scale and fitted logistic psychophysical functions for each value of d_A .

Fig. 2.

Left-hand panel. Preference functions (percent responding on B, %B, vs. delay to the larger of the two reinforcers after a response on B [d_B , s]) for the sham-lesioned group (open symbols) and STN-lesioned group (filled symbols) in the final phase of the experiment in which the delay to the smaller reinforcer (d_A) was 0.5 s. Points are group mean data; vertical bars indicate SEMs. *Right-hand panel.* Mean (+SEM) indifference delays ($d_{B(50)}$, s) for the two groups corresponding to the preference functions shown in the left-hand graph. There was no significant difference between the groups (see text for statistical analysis).

Fig. 3. Linear indifference functions obtained from the sham-lesioned (*empty circles*) and STN-lesioned (*filled circles*) groups. *Ordinate*: indifference delay to the larger reinforcer ($d_{B(50)}$); *abscissa*: imposed delay to the smaller reinforcer (d_A , s). Points show group mean data; vertical bars indicate SEMs; lines are best-fit linear functions. Significant difference between groups: * $P < 0.05$.

PARAMETERS OF INDIFFERENCE FUNCTIONS

Fig. 4.

Parameters of linear indifference functions from individual rats in the sham-lesioned group (*empty columns*) and STN-lesioned group (*filled columns*). Columns show group mean values; vertical bars indicate SEMs. *Left-hand panel*, slope of linear function; *right-hand panel*, intercept of linear function. Significant difference between groups: * $P < 0.05$.

Fig. 5. Weber fraction (*left-hand panel*) and slope of the logistic function (*right-hand panel*) derived from psychophysical analysis of preference functions (see logistic functions in right-hand panels of Fig. 1). See text for derivation of parameters. Columns show group mean values; vertical bars indicate SEMs.

Fig. 6. Sample photomicrographs from NeuN-stained coronal sections of the brains of a sham-lesioned rat (upper panel) and an STN-lesioned rat (lower panel). CP: cerebral peduncle. The dotted outline shows the extent of the STN in the sham-lesioned rat. Note the cell loss in the STN in the lesioned rat. *Right-hand diagrams.* Representation of the approximate area of destruction of the STN in the lesioned group. Drawings were made from the microscopic sections, and were superimposed on the relevant pages from Paxinos and Watson's (1998) stereotaxic atlas. The black area in the region of the STN represents the smallest, and the surrounding grey area the largest extent of the lesion. LV: lateral ventricle; IIIrd V: third ventricle.