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The role of the subthalamic nucleus in 'compulsive' behavior in rats

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Abstract

Different lines of evidence point to dysfunction of basal ganglia-thalamocortical circuits in obsessive-compulsive disorder (OCD). It has been hypothesized that the circuits' dysfunction in OCD may be characterized by a relative under-activity of the indirect compared with the direct pathway within these circuits. The present study tested whether lesions of the subthalamic nucleus (STN), a major node of the indirect pathway, would affect compulsive behavior, using the signal attenuation rat model of OCD. In this model, compulsive lever-pressing is induced by the attenuation of an external signal of reward delivery; an attenuation that is hypothesized to simulate the deficient response feedback suggested to underlie obsessions and compulsions in patients with OCD. Rats sustaining lesions to the STN showed a selective increase in compulsive lever-pressing compared with sham-operated rats. A *post mortem* biochemical analysis revealed a decrease in serotonin content in the prelimbic and infralimbic cortices, caudate-putamen (but not nucleus accumbens), globus pallidus and substantia nigra-ventral tegmental area, as well as a decrease in dopamine content in the caudate-putamen in STN-lesioned compared with sham rats. A comparison to recent findings that lesions to the orbitofrontal cortex, which also result in a selective increase in compulsive lever-pressing, lead to a decrease in serotonin and dopamine content in the caudate-putamen suggests that there may be a final common pathway by which different brain pathologies may lead to a pro-compulsive state.

Introduction

Obsessive-compulsive disorder (OCD) is a psychiatric affliction with a lifetime prevalence of 1–3% (Rasmussen & Eisen, 1992; Sasson *et al*., 1997), and is one of the most disabling of all psychiatric disorders (Murray & Lopez, 1996). The defining symptoms of OCD are recurrent, intrusive and unwanted thoughts (obsessions) and/or repetitive ritualistic behaviors (compulsions; American Psychiatric Association, 1994). The results of neuroimaging studies in patients with OCD have consistently pointed to a dysfunction of the orbitofrontal cortex and the striatum (for review, see Saxena *et al*., 1998; Friedlander & Desrocher, 2006), and have led to the prevailing view that obsessions and compulsions reflect malfunctioning of basal ganglia-thalamocortical circuits (e.g. Rapoport & Wise, 1988; Stahl, 1988; Insel, 1992; Saxena *et al*., 1998; Graybiel & Rauch, 2000). An important characteristic of the functioning of these circuits is that it depends on the balance between activity in the direct and indirect pathways that connect the striatum to the output nuclei of the basal ganglia (Alexander *et al*., 1986; Albin *et al*., 1989; Joel & Weiner, 1997; Bolam *et al*., 2000). It has further been speculated that the specific dysfunction of basal ganglia-thalamocortical circuits in OCD is characterized by a relative under-activity of the indirect pathway compared with the direct pathway (Baxter *et al*., 1996; Saxena *et al*., 1998).

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The present study tested whether lesions of the subthalamic nucleus (STN), a major node of the indirect pathway, would affect compulsive behavior, using the signal attenuation rat model of OCD (for a recent review of the model, see Joel, 2006). This model was developed on the basis of the theoretical proposition that compulsive behaviors result from a deficit in the feedback associated with the performance of goal-directed responses (e.g. Reed, 1977; Gray, 1982; Malloy, 1987; Pitman, 1987; Pitman, 1991; Baxter, 1999; Szechtman & Woody, 2004; for review, see Otto, 1992). In the signal attenuation model, attenuation of a signal indicating that a lever-press response was effective in producing a food reward, leads (in a subsequent extinction test) to excessive lever-pressing that is not accompanied by an attempt to collect a reward. This behavior, which has been named 'compulsive' lever-pressing because it may be analogous to the excessive and unreasonable behavior seen in OCD, is abolished by the selective serotonin reuptake inhibitors fluoxetine, paroxetine and fluvoxamine, but not by the anxiolytic drug, diazepam, the antipsychotic, haloperidol, or the tricyclic antidepressant, desipramine (Joel & Avisar, 2001; Joel & Doljansky, 2003; Joel *et al*., 2004), in accordance with the differential efficacy of these drugs in alleviating obsessions and compulsions in patients with OCD (e.g. Zohar *et al*., 1992; Piccinelli *et al*., 1995; Dolberg *et al*., 1996). Compulsive lever-pressing is also sensitive to manipulations of the orbitofrontal cortex (Joel *et al*., 2005a,b; Joel & Klavir, 2006) and the dopaminergic system (Joel *et al*., 2001; Joel & Doljansky, 2003), in line with different lines of evidence implicating these systems in the pathophysiology of OCD (for review, see Rauch *et al*., 2001; Denys *et al*.,

2004; Evans *et al*., 2004; Friedlander & Desrocher, 2006). In addition to the assessment of the behavioral effects of the lesion, the effects of the lesion on the levels of dopamine, serotonin, glutamate, γ-aminobutyric acid (GABA) and taurine in the striatum and frontal cortex were assessed.

Materials and methods

Subjects

Sprague–Dawley (Tel Aviv University, Israel) rats approximately 3–4 months old were housed four to a cage under a reversed 12 h light : dark cycle (lights on 19.00–07.00 h). Rats were maintained on a 22 h food restriction schedule (see below), with water freely available. They were weighed twice a week to ensure that their body weight was not reduced to below 90%. All experimental protocols were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University, and approved by it.

Surgery

Rats received 3 mg diazepam, and 20 min later were anesthetized with an i.p. injection of Avertin (10 mL/kg). They were placed in a stereotaxic frame (David Kopf Instruments, Tujungy, CA, USA) and an incision was made into the scalp to expose the skull. One microliter of ibotanic acid (Sigma Chemicals, Israel) dissolved in 0.9% saline at a concentration of 5 μg/μL was bilaterally infused into the STN at the following coordinates (Paxinos & Watson, 1998): 3.6 mm posterior to bregma, 2.4 mm lateral to the midline and 7.65 mm ventral to the dura at a flow rate of 1 μL/min. Infusions were made through a 25-μm-gage stainless steel cannula attached via a fine polyethylene tubing to a 20-μL Harvard microsyringe. After toxin injection the cannulae were left in place for another 5 min before being slowly retracted. Another group of rats (sham-operated) underwent the same surgical procedure as STN-lesioned rats; the cannulae were lowered to the injection site and remained there for the same amount of time as in lesioned rats, but no toxins were infused. Sterispon was used to cover the holes in the bone, the scalp incision was sutured by Michel clips, and rats were monitored on a daily basis. The forepaws were covered with tape for 7 days to prevent the rats from self-injurious behavior. The behavioral procedure began 1 week after recovery from operation.

Apparatus and behavioral procedure

Behavioral testing was conducted in eight operant chambers (Campden Instruments, Loughborough, UK), housed in sound-attenuated boxes and equipped with a 3 W house light, a Sonalert module (Model SC 628) that could produce a 80 dB 2.8 kHz tone, and two retractable levers on either side of a food magazine (fitted with a 3 W magazine light), into which 45 mg Noyes precision food pellets (Noyes, Sandown Chemical Limited, Hampton, England) could be delivered. Access to the food magazine was through a hinged panel, the opening of which activated a microswitch. Equipment programming and data recording were computer controlled.

Prior to the beginning of the experiment, rats were handled for about 2 min daily for 5 days. A 22 h food restriction schedule began simultaneously with handling and continued throughout behavioral testing. Food was provided in the home cage at least half an hour after the end of the session. On the last 3 days, after handling, 20–30 food pellets used as reinforcement for operant training were introduced into the home cages on a tray. The tray was removed from the cage after each rat was observed to consume at least two pellets.

Post-training signal attenuation (PTSA)

The PTSA procedure included four stages.

Stage 1: magazine training. On Days 1–3, rats were trained to collect food pellets from the food magazine in the operant chamber, with the levers retracted. On each trial, a single food pellet was dropped into the food magazine, simultaneous with the onset of a compound stimulus consisting of the magazine light and the tone. The compound stimulus was turned off after the rat's head entered the food magazine or after 15 s had elapsed, and a 30-s intertrial interval began. On each day, each rat was trained until it completed 30 trials in which it inserted its head into the food magazine during stimulus presentation (collected trials), or until a total of 40 trials was reached. The number of collected trials and the total number of trials were recorded.

Stage 2: lever-press training. On Day 4, rats received a session of pre-training using a free-operant schedule. The house light was on and one lever was present in the operant box throughout the entire session. Responding on this lever [reinforced lever, (RL)] resulted in the delivery of a single food pellet into the magazine, accompanied by the presentation of the compound stimulus (magazine light and tone). The stimulus was turned off after the rat's head entered the food magazine or after 15 s from the rat's first lever-press had elapsed. The lever designated as RL was counterbalanced over subjects and remained the same for each rat over the entire experimental procedure. Each rat was trained until it completed 30 trials, that is, pressed the lever and inserted its head into the food magazine during stimulus presentation. Rats that failed to attain 30 completed trials within 30 min were returned to the test chamber at the end of the day for an additional session. On Days 5–7, rats were trained to lever-press in a discrete-trial procedure. On each trial, both levers were inserted into the chamber. Responding on the RL resulted in the delivery of a single food pellet into the magazine, accompanied by the presentation of the compound stimulus. The levers were retracted and the compound stimulus was turned off, after the rat's head entered the food magazine or after 15 s (on Day 5; 10 s on Days 6–7) from the rat's first lever-press had elapsed. Further lever-presses on the RL as well as responding on the other lever [non-reinforced lever (NRL)] had no programmed consequences. The lever designated as RL was counterbalanced over subjects and remained the same for each rat over the entire experimental procedure. Each trial was followed by a 30-s intertrial interval. Each rat was trained until it completed 40 trials, that is, pressed the lever and inserted its head into the food magazine during stimulus presentation, or for a total of 60 trials.

In order to assess acquisition of the lever-press response, the number of trials on which the rat did not press the RL (unpressed trials) and the number of trials on which the rat pressed the RL without inserting its head into the food magazine during stimulus presentation [that is, within 15 s (Day 5)] or 10 s (Days 6–7, Test) from the first lever-press] (uncompleted trials) were recorded in addition to the number of completed trials. In order to assess rats' tendency for excessive lever-pressing, the number of lever-presses on the NRL, and the number of lever-presses on the RL after the first response [extra lever-presses (ELP)] were recorded. The latter measure was further subdivided into ELP in uncompleted trials (that is, ELP not followed by insertion of the head into the food magazine during stimulus

presentation; ELP-U), and ELP in completed trials (that is, ELP that were followed by insertion of the head into the food magazine during stimulus presentation; ELP-C).

Stage 3: signal attenuation. On Days 8–10, with the levers retracted, rats were exposed to the presentation of the compound stimulus as on Days 1–3, but no food was delivered to the food magazine. Rats received 30 such trials on each day, and the number of collected trials was recorded. Rats that had more than 13 collected trials on Day 10 were returned to the test chamber at the end of the day for an additional session.

Stage 4: test. On the following day, rats were trained as in the lever-press training stage, except that no food was delivered to the food magazine, that is, pressing the lever resulted in the presentation of the compound stimulus only. The session lasted for 50 trials. The behavioral measures recorded included the same measures recorded in the lever-press training stage, as well as the number of nose-pokes during the entire test. The latter measure was recorded because previous studies have found that STN manipulations result in perseverative nose-poking (e.g. Baunez & Robbins, 1997, 1999), and changes in the tendency to nose-poke may affect the number of ELP-C and ELP-U, because operationally these two types of lever-presses differ only in whether they are or are not followed by a nose-poke while the stimulus is still on.

Compulsive lever-pressing is operationally defined as the number of ELP-U in the test stage of the PTSA procedure

Because in the PTSA procedure the effects of signal attenuation are assessed under extinction conditions, the effects of STN lesion were also assessed in rats undergoing a control procedure [termed 'regular extinction' (RE)], that is identical to the PTSA procedure but does not include a signal attenuation stage. (This experimental design enables a differentiation between the effects of a given manipulation on the behavioral response to signal attenuation and on extinction *per se*; for a detailed discussion of the use of this design see Joel, 2006.)

RE

Rats were run exactly as in the PTSA procedure, with the exception that they did not undergo the signal attenuation stage on Days 8–10. On these days, rats were brought to the laboratory and left in their home cages for a period equivalent to the average duration of the signal attenuation stage.

Statistical analysis

Rats' performance on the test stage was analysed using an analysis of variance (ANOVA) with main factors of Procedure (PTSA/RE) and Lesion (STN lesion/sham). Significant interactions were followed by *post hoc* least significant difference comparisons comparing, within each procedure, the sham and lesion groups. For all comparisons, significance was assumed at $P < 0.05$. Because the experiment was ran in two replications, data were first analysed using ANOVAs with Replication, Procedure and Lesion as main factors. Because the effect of Replication as well as its interactions with the other factors were not significant, data from the two replications were combined.

Performance on the magazine training, lever-press training and signal attenuation stages was also analysed, to ensure that differences in performance at the test stage were not a result of an earlier difference. Performance on the magazine training stage was analysed using mixed ANOVA with main factors of Lesion and Procedure and a repeated measurements factor of Session performed on the number of uncollected trials (i.e. trials on which the rat did not enter the magazine during stimulus presentation) on the three sessions of the magazine training stage. Performance on the lever-press training stage was analysed using mixed ANOVA with a main factor of Lesion and Procedure and a repeated measurements factor of Session performed on the number of unpressed trials and on the number of ELP-C on the three sessions of lever-press training (the variability of the other variables was too low to enable statistical analysis). Performance on the signal attenuation stage was analysed using a mixed ANOVA with main factors of Lesion and Procedure and a repeated measurements factor of Session performed on the number of collected trials (i.e. trials on which the rat entered the magazine during stimulus presentation) on the three sessions of the signal attenuation stage.

Histology and biochemistry

After the completion of behavioral testing, all rats were decapitated and their brains removed within seconds. One-millimeter-thick frozen coronal sections were prepared at the following coordinates with respect to bregma : +3.2 to +2.2, +1.7 to +0.7, −0.6 to −1.6 and −5.2 to −6.2 (coordinates in mm anterior–posterior according to Paxinos & Watson, 1998). Slices were stored at −80 °C for further neurochemical analysis. For verification of lesion location, 30-μm coronal sections of the STN (−3.4 to −4.3) were mounted and processed for Cresyl violet staining. The extent of the lesion was quantified by outlining the remaining STN on each section (the STN was identified by differences in morphology of cells) using a computer-based imaging system (Axio Vision 3.0, 2000, Carl Zeiss, Germany) as described previously (Paul *et al*., 2004; Winter *et al*., 2006). For neurochemical analyses, tissue samples of seven different brain regions [cingulate cortex area 1 (Cg1), prelimbic cortex, infralimbic cortex, nucleus accumbens, caudate-putamen, lateral globus pallidus and the substantia nigra/ventral tegmental area] from both hemispheres were quickly isolated by a punch of 1 mm in diameter. Tissue samples were collected from 11 sham-operated and 11 STN-lesioned rats (the remaining brains could not undergo the biochemical analysis because they were partly defrosted during the transportation from Tel Aviv to Berlin). Tissue samples were homogenized by ultrasonication in 250 μL (per punch) 0.1 N perchloric acid at 4 °C immediately after processing. One-hundred microliters of the homogenates was added to equal volumes of 1 N sodium hydroxide for measurement of protein content. The remaining homogenates were centrifuged at 17 000 ***g*** and 4 °C for 10 min. Aliquot of the supernatants were added to equal volumes (20 μL) of 0.5 M borate buffer and stored at −80 °C for subsequent analyses of amino acids. The remaining supernatants were used for immediate measurement of monoamines. The levels of monoamines [dopamine and serotonin (5-hydroxytryptamine), and their metabolites (3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxy indole acetic acid (5-HIAA)] were measured by high-performance liquid chromatography (HPLC) with electrochemical detection as previously described (Felice *et al*., 1978; Sperk *et al*., 1981; Sperk, 1982). Glutamate, GABA and taurine were determined using methods also described previously (Piepponen & Skujins, 2001). Briefly, amino acids were precolumn derivatized with o-phthalaldehyde-2-mercaptoethanol using a refrigerated autoinjector and then separated on a HPLC column (ProntoSil C18 ace-EPS) at a flow rate of 0.6 mL/min and a column temperature of 40 °C. The mobile phase was 50 mM sodium acetate pH 5.7 in a linear gradient from 5% to 21% acetonitrile. Derivatized amino acids were detected by their fluorescence at 450 nm after excitation at 330 nm.

Comparisons between groups were made by Student's *t*-test. A *P*-value of 0.05 was considered statistically significant.

Results

Anatomical

Figure 1A and B presents photomicrographs at several magnifications of a coronal section taken from representative sham operated and STN-lesioned rats, respectively. Figure 1C presents a schematic reconstruction of the lesions in the STN. Nine rats were excluded from the final analysis because their STN lesions were not complete. In all the remaining rats the lesions involved the entire STN bilaterally.

Behavioral

Seventy-three rats were randomly assigned to the four groups. Nine rats were excluded from the final analysis because their STN lesion was not complete, and three rats (one lesion–PTSA and two sham–RE) were excluded because in the test their score on one of the measures was more than four SD higher than their group's mean. Thus, the final analysis included 12, 19, 15 and 15 rats, in the PTSA–lesion, PTSA–sham, RE–lesion and RE–sham groups, respectively.

Magazine training

The number of uncollected trials (trials on which the rat did not enter the magazine during stimulus presentation) declined gradually throughout the three sessions of magazine training, reaching a mean level of less than one uncollected trial in the last session, in both the STN lesion and sham groups (data not shown; a Lesion × Procedure × Session mixed ANOVA yielded only a significant effect of Day: $F_{2,114} = 239.87$, $P < 0.0001$).

Lever-press training

STN-lesioned rats had more unpressed trials (trials on which the rat did not press the lever; Fig. 2A) and more ELP-C (Fig. 2B) compared with sham operated rats. The difference in the number of unpressed trials disappeared by the third day of lever-press training, whereas the difference in the number of ELP-C remained stable. In addition, there were differences between the two STN-lesioned groups, with the STN–PTSA group exhibiting more unpressed trials and more ELP-C compared with the STN–RE group, but these differences disappeared by the second and third days of lever-press training, respectively (see Fig. 2 for the results of the statistical analyses).

Signal attenuation

The number of collected trials (trials on which the rat entered the magazine during stimulus presentation) declined gradually throughout the three sessions of signal attenuation in the sham and STN lesion groups (Fig. 2C; the effect of Lesion and the Lesion–Session interaction were not significant, $P > 0.4$).

Test

Figure 3A and B presents the mean number of ELP-C and ELP-U, respectively, in STN-lesioned and sham operated rats undergoing the test stage of the PTSA or RE procedures. STN lesions did not affect the number of ELP-C in either procedure (Fig. 3A, ANOVA: Procedure, $F_{1,57} = 27.985$, $P < 0.0001$; Lesion, $F_{1,57} = 0.456$, $P = 0.502$; Procedure–Lesion interaction, $F_{1,57} = 0.003$, $P = 0.958$). In contrast, STN lesions increased the number of ELP-U, compared with sham rats, in rats undergoing PTSA but not in rats undergoing RE (Fig. 3B, ANOVA: Procedure, $F_{1,57} = 9.274$, $P < 0.005$; Lesion, $F_{1,57} = 2.180$, $P = 0.145$; Procedure–Lesion interaction, $F_{1,57} = 4.065$, $P < 0.05$). *Post hoc* least significant difference comparisons between the number of ELP-U in the sham and STN lesion groups within each procedure revealed a significant difference within the PTSA procedure only. Table 1 presents the mean (SEM) number of completed, uncompleted and unpressed trials as well as the mean (SEM) number of lever-presses on the NRL and of nose-pokes during the test in the four groups. There were no significant differences between lesion and sham groups that underwent the same behavioral procedure (i.e. PTSA or RE) on the number of completed, uncompleted and unpressed trials and on the number of lever-presses on the NRL. Regardless of the procedure, STN-lesioned rats tended to emit more nose-pokes compared with sham rats ($P = 0.0645$; see Table 1 for the full results of the statistical analyses).

Biochemical

Table 2 presents the content (μMol/g protein) of dopamine, serotonin, DOPAC, HVA, 5-HIAA, glutamate, GABA and taurine in the seven brain regions tested in STN-lesioned ($n = 11$) and sham operated ($n = 11$) rats. As can be seen, the main change following STN lesions was a decrease in the levels of serotonin in several brain regions, including the prelimbic and infralimbic cortices (but not Cg1), dorsal striatum (i.e. caudate-putamen but not nucleus accumbens), globus pallidus and substantia nigra-ventral tegmental area. Lesions to the STN have also resulted in an increase in glutamate levels in the prelimbic cortex and a decrease in dopamine levels in the caudate-putamen. It is noteworthy that STN lesions had no effect on the levels of GABA and taurine in any of the regions assessed. Notably, the decrease in serotonin was not paralleled by a decrease in the serotonergic metabolite 5-HIAA, and as a result an increased serotonergic turnover, reflected in the 5-HIAA/5-hydroxytryptamine ratio, was observed.

Discussion

The present study tested the effects of STN lesions on compulsive lever-pressing, assessed in the signal attenuation rat model of OCD. The main finding of the present study is that lesion of the STN caused a marked and selective elevation in the number of compulsive lever-presses, that is, in the number of excessive lever-presses that were not followed by magazine entry (ELP-U) in the PTSA procedure.

The increased number of compulsive lever-presses following STN lesion cannot be attributed to a decrease in the tendency of lesioned rats to enter the magazine, because STN-lesioned rats tended to emit more nose-pokes at the magazine compared with sham rats, in line with previous reports of perseverative nose-poking following STN lesions (Baunez & Robbins, 1997, 1999). Although STN-lesioned rats pressed the lever excessively during lever-press training (see below), the selective increase in compulsive lever-pressing during the test cannot be attributed to a non-specific perseveration or disinhibition of lever-press responding following the lesion, because STN-lesioned and sham rats exhibited a similar number of ELP-C in the test of the PTSA procedure, a similar number of ELP-U and ELP-C in the RE procedure, and a similar number of lever-presses on the NRL in the two procedures.

Lesions to the STN had no effect on the acquisition and extinction of magazine approach in the magazine training and signal attenuation stages, respectively, but did interfere with the acquisition of lever-press responding at the lever-press training stage. Specifically,

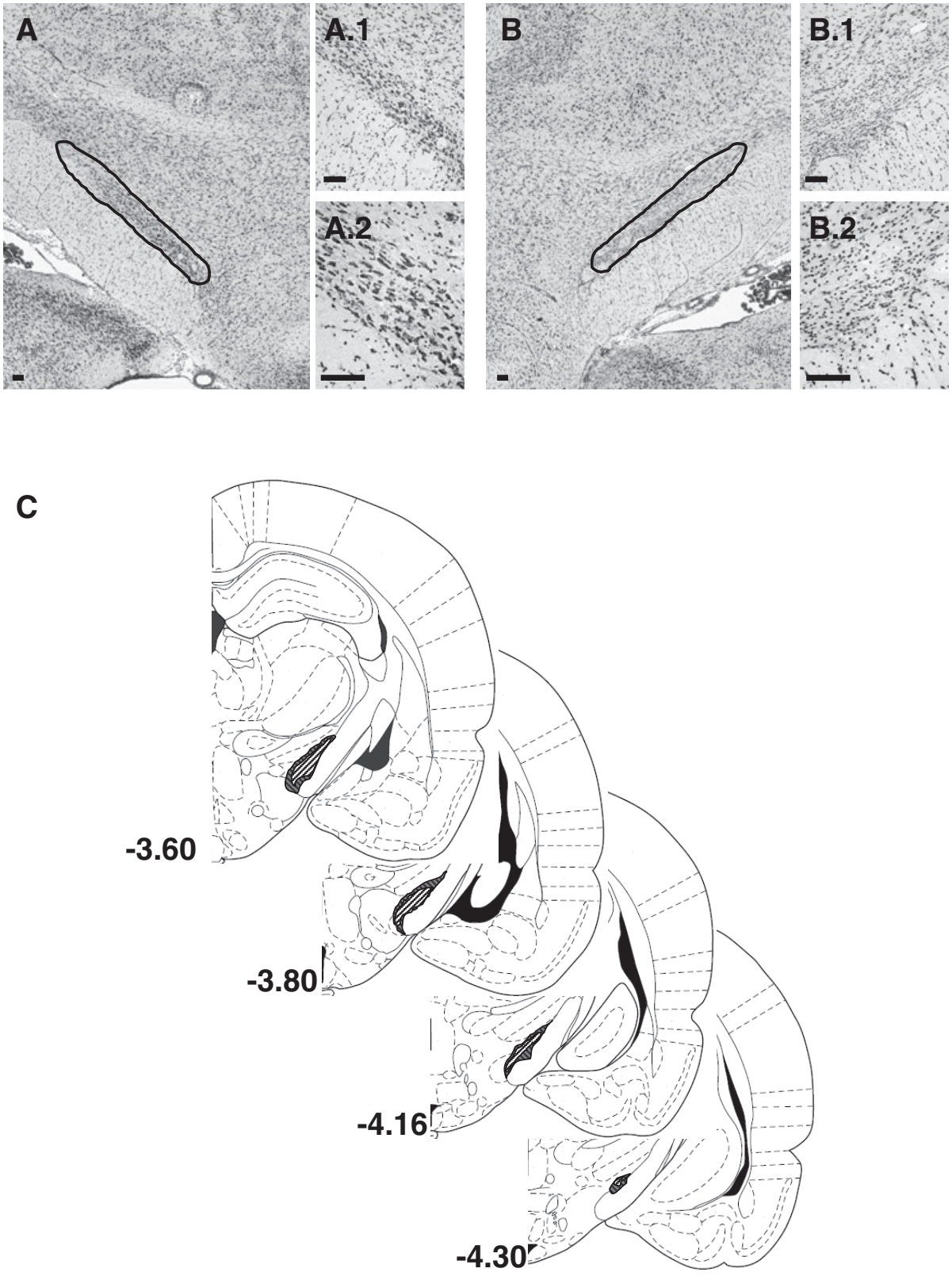

FIG. 1. A photomicrograph of a coronal section taken from a representative sham operated (A) and STN-lesioned (B) rat. (A, B, A.1, B.1, A.2 and B.2) The same section at a magnification of ×12.5, ×25 and ×100, respectively. (C) A schematic reconstruction of the lesions in the STN, showing the largest (gray) and smallest (black) lesions. In most animals, the lesion extended from −3.5 to −4.4 mm posterior to Bregma, and remained within the anatomical borders of the STN in the medio-lateral and dorso-ventral extents. Examination of the histological material from sham rats confirmed that the only visible damage in these rats was the cannulae tracts towards the STN. Coordinates of the coronal sections are indicated with reference to Bregma according to the stereotaxic atlas of Paxinos & Watson (1998).

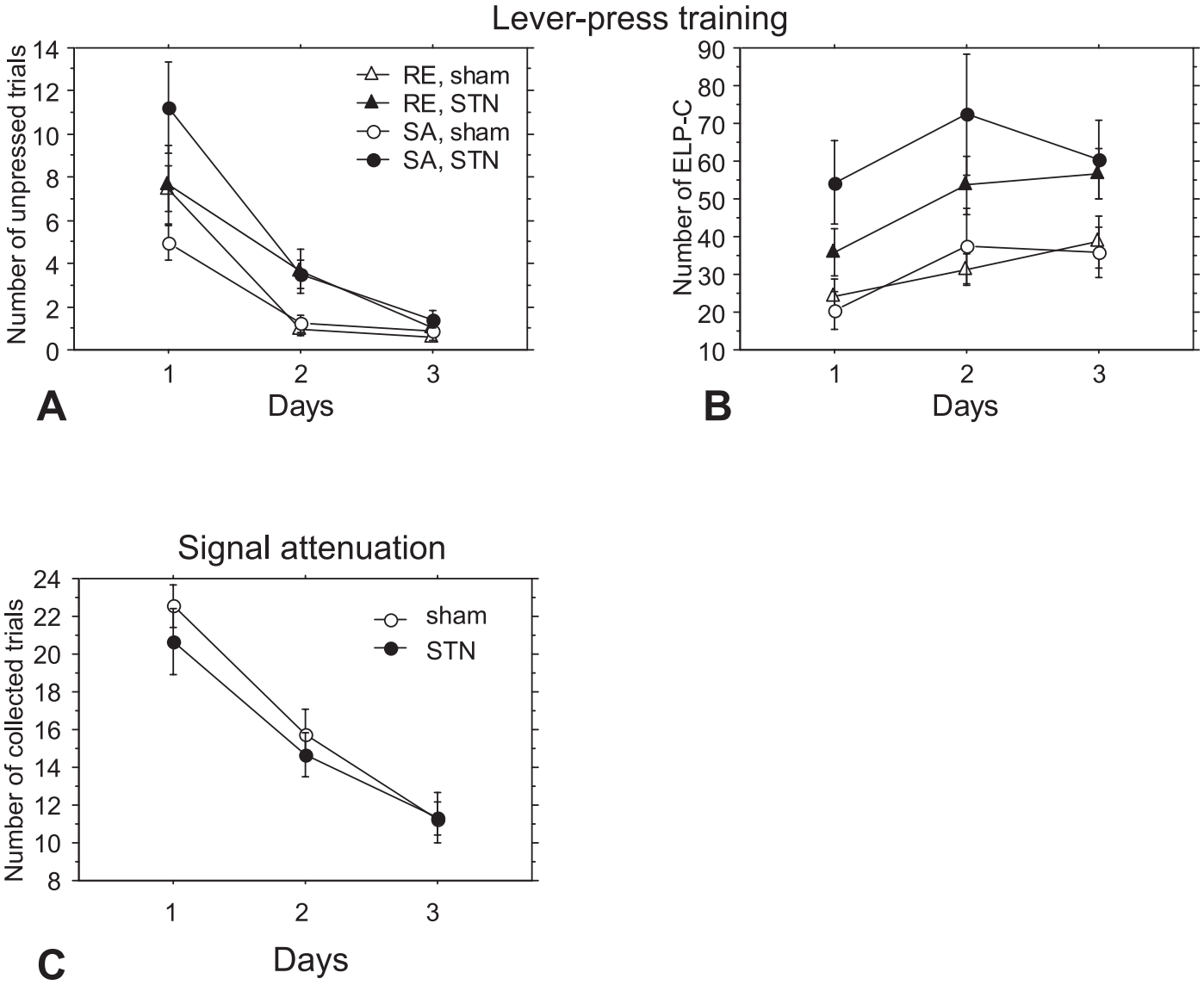

FIG. 2. (A) The mean and standard error of the mean number of unpressed trials on the 3 days of lever-press training of sham and lesioned rats. (A) Lesion × Procedure × Day mixed ANOVA yielded significant effects of Lesion, $F_{1,57} = 11.42$, $P < 0.005$, and Day, $F_{2,114} = 72.88$, $P < 0.0001$, as well as a significant Lesion × Procedure × Day interaction, $F_{2,114} = 4.5$, $P < 0.02$. (B) The mean and standard error of the mean number of extra lever-presses in completed trials (ELP-C) on the 3 days of lever-press training of sham and lesioned rats. A Lesion × Procedure × Day mixed ANOVA yielded significant effects of Lesion, $F_{1,57} = 11.24$, $P < 0.005$, and Day, $F_{2,114} = 12.72$, $P < 0.0001$, only. (C) The mean and standard error of the mean number of collected trials on the 3 days of signal attenuation of sham and lesioned rats. A Lesion × Day mixed ANOVA yielded a significant effect of Day, $F_{2,58} = 43.15$, $P < 0.0001$, only. SA, post-training signal attenuation; RE, regular extinction; STN, subthalamic nucleus.

compared with sham rats, STN-lesioned rats failed to press the lever on more trials (i.e. had a higher number of unpressed trials), but at the same time exhibited perseverative lever-pressing (i.e. exhibited a higher number of ELP-C). It should be noted that whereas the former difference disappeared by the third day of lever-press training, the latter did not. These findings are in agreement with previous reports that rats sustaining STN lesions exhibit an increased number of omissions and perseverative responses in simple and choice reaction time tasks (Baunez & Robbins, 1997, 1999), as well as higher rates of lever-pressing for food reward (Baunez *et al*., 2002).

Lesions to the STN had no effect on the extinction of lever-press responding in rats that underwent the RE procedure. This finding clearly demonstrates that STN-lesioned rats can suppress an instrumental behavior that is no longer rewarded. Although, to the best of our knowledge, there are no previous reports on the effects of STN lesions on extinction, Winstanley *et al*. (2005) found that STN-lesioned rats were able to suppress approach to a conditioned stimulus (CS) when an omission schedule was introduced (i.e. approach to the CS resulted in the cancellation of food delivery), and we have found here that lesioned rats were not impaired in extinguishing approach to the food magazine in the signal attenuation stage. The ability of STN-lesioned rats to suppress or withhold behavior that is no longer rewarded stands in marked contrast to their inability to inhibit a response that has already been initiated, as reflected in their impaired performance in the stop-signal reaction-time task (Eagle *et al*., 2007), and possibly also in the increased number of compulsive lever-pressing reported here. This dissociation provides further support to the notion that there are many forms of behavioral inhibition and that they are

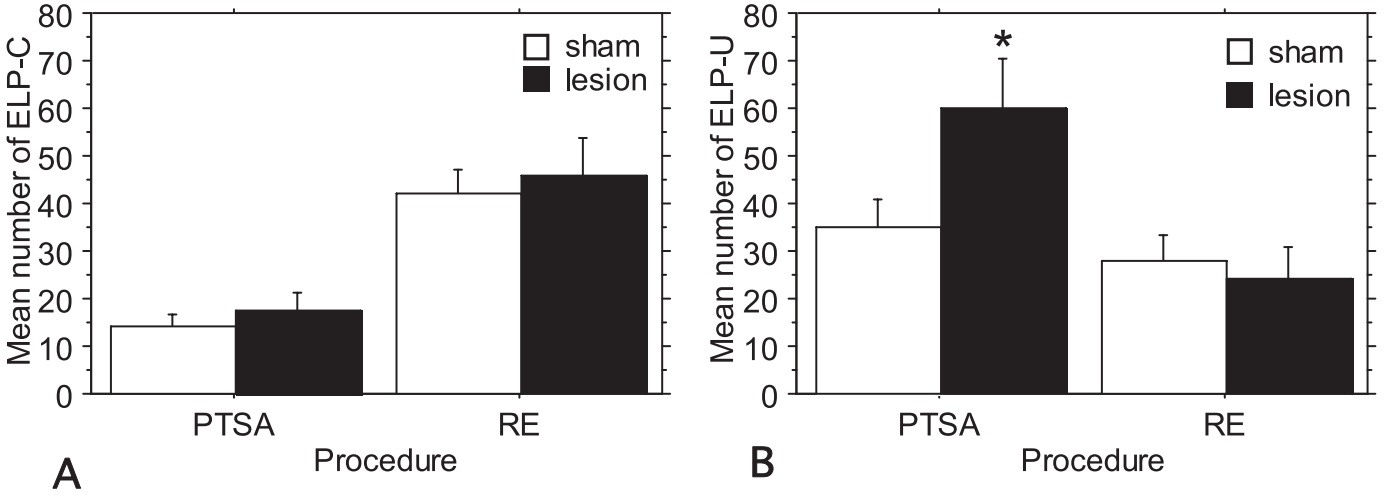

FIG. 3. The mean and standard error of the mean number of (A) ELP that were followed by an attempt to collect a reward (ELP-C) and (B) ELP that were not followed by an attempt to collect a reward (ELP-U) in the test of sham (empty bars) and STN-lesioned (filled bars) rats undergoing the regular extinction (RE) or post-training signal attenuation procedure (PTSA). *$P < 0.05$, compared with the sham group.

TABLE 1. Numbers of completed, uncompleted and unpressed trials, lever-presses on the non-reinforced lever (NRL) and nose-pokes on the test

	Completed trials	Uncompleted trials	Unpressed trials	Lever-presses on NRL	Nose-pokes
SA − Sham	9.368 ± 1.379	8.737 ± 1.121	31.895 ± 1.991	18.211 ± 3.761	71.579 ± 8.958
SA − Lesion	8.500 ± 1.725	9.167 ± 1.211	32.333 ± 1.994	15.667 ± 3.911	97.750 ± 26.232
RE − Sham	25.500 ± 2.221	6.200 ± 1.353	18.467 ± 1.366	23.667 ± 5.212	144.667 ± 21.042
RE − Lesion	25.133 ± 2.002	4.533 ± 0.975	20.333 ± 1.745	25.067 ± 3.948	191.333 ± 22.340
Procedure × Lesion ANOVA					
Procedure	$F_{1,57} = 80.661$ $P < 0.0001$	$F_{1,57} = 9.012$ $P < 0.005$	$F_{1,57} = 47.005$ $P < 0.0001$	$F_{1,57} = 2.95$ $P = 0.091$	$F_{1,57} = 18.604$ $P < 0.0001$
Lesion	$F_{1,57} = 0.087$ $P = 0.769$	$F_{1,57} = 0.268$ $P = 0.6066$	$F_{1,57} = 0.386$ $P = 0.537$	$F_{1,57} = 0.017$ $P = 0.895$	$F_{1,57} = 3.553$ $P = 0.0645$
Procedure × Lesion	$F_{1,57} = 0.034$ $P = 0.854$	$F_{1,57} = 0.770$ $P = 0.770$	$F_{1,57} = 0.148$ $P = 0.702$	$F_{1,57} = 0.208$ $P = 0.650$	$F_{1,57} = 0.281$ $P = 0.598$

Data are presented as mean ± SEM. NRL, non-reinforced lever; RE, regular extinction; SA, signal attenuation.

subserved by distinct, although partially overlapping, neural systems (e.g. Dias *et al.*, 1996, 1997; Birrell & Brown, 2000; Chudasama *et al.*, 2003a; McAlonan & Brown, 2003; Eagle *et al.*, 2007).

Taken together, the present results show that lesions to the rat STN lead to a selective increase in compulsive lever-pressing, in line with the hypothesis that disruption of the normal balance between activity in the direct and indirect pathways of basal ganglia-thalamocortical circuits underlies compulsive behaviors (Baxter *et al.*, 1996; Saxena *et al.*, 1998). Thus, it appears that the lesions resulted in a 'pro-compulsive' state, that is, the rats were more sensitive to a manipulation (signal attenuation) that induces compulsive responding. This finding is in line with the view that the STN may be particularly important in suppressing behavior when there are no external cues to direct behavior (Phillips & Brown, 2000), because signal attenuation would be expected to decrease the ability of the tone and light stimulus to direct behavior (that is, to suppress lever-pressing and initiate a nose-poke). This interpretation of the effects of STN lesions accords well with the observation from Szechtman & Woody (2004) that behaviors that may become compulsive are characterized by the lack of a clear external end signal.

A selective increase in compulsive lever-pressing has been previously reported following pre-training lesions to the orbitofrontal cortex (Joel *et al.*, 2005a,b). Similar effects of orbitofrontal and STN lesions have been reported in several other tasks, including autoshaping, reaction time and delayed discounting tasks (Baunez & Robbins, 1997; Chudasama & Robbins, 2003; Chudasama *et al.*, 2003a,b; Winstanley *et al.*, 2004, 2005), and have been taken to reflect the interaction between these two structures in the regulation of certain forms of behavior (Winstanley *et al.*, 2004, 2005). Indeed, there is evidence for direct projections from the orbitofrontal cortex to the STN in rats (Maurice *et al.*, 1997, 1998; Groenewegen, personal communication), and the STN is an important node in the circuits connecting the orbitofrontal cortex and the basal ganglia. The orbitofrontal cortex and STN have been previously suggested to play a role in inhibiting perseverative responding (Chudasama *et al.*, 2003a,b; Eagle *et al.*, 2007). Our present and past (Joel *et al.*, 2005a,b) results extend this view to suggest that these two regions also cooperate in the suppression of compulsive responding.

The *post mortem* biochemical assessment of the effects of the STN lesion revealed no change in the levels of GABA and taurine, which is considered an endogenous GABA analog that selectively activates $GABA_A$ (but not $GABA_B$) receptors (Bureau & Olsen, 1991; del Olmo *et al.*, 2000). These results are of particular interest given that exogenous administration of taurine has been shown to exert an anxiolytic-like effect in a number of animal models of anxiety (Chen *et al.*, 2004; Kong *et al.*, 2006). The present finding that GABA and taurine levels were not changed following a manipulation (STN

TABLE 2. Content dopamine, serotonin, glutamate, taurine and GABA in STN-lesioned and sham operated rats

Transmitter	Content (μmol/g protein) Controls	STN-lesioned	*P*-values
Cingulate cortex			
Dopamine	1.8 ± 0.1	2.0 ± 0.2	
DOPAC	4.2 ± 0.7	5.2 ± 0.8	
(DOPAC/DA)	2.5 ± 0.5	2.9 ± 0.5	
Serotonin	25.9 ± 1.6	25.6 ± 3.3	
5-HIAA	17.1 ± 3.8	23.9 ± 6.1	
5-HIAA/5HT	0.7 ± 0.1	0.9 ± 0.2	
Glutamate	83.8 ± 9.4	82.5 ± 4.6	
Taurine	47.0 ± 4.4	47.2 ± 2.9	
GABA	17.5 ± 2.4	20.2 ± 2.1	
Prelimbic cortex			
Dopamine	2.8 ± 0.3	3.6 ± 0.5	
DOPAC	4.4 ± 0.8	6.8 ± 1.1	
(DOPAC/DA)	1.9 ± 0.5	1.9 ± 0.4	
Serotonin	53.2 ± 5.7	21.1 ± 2.6	< 0.001
5-HIAA	27.6 ± 5.3	34.6 ± 7.1	
5-HIAA/5HT	0.6 ± 0.1	1.9 ± 0.5	< 0.01
Glutamate	63.3 ± 7.6	86.3 ± 3.4	< 0.001
Taurine	56.9 ± 6.7	44.9 ± 2.6	
GABA	20.4 ± 2.9	16.8 ± 1.2	
Infralimbic cortex			
Dopamine	5.4 ± 0.8	7.2 ± 1.7	
DOPAC	5.0 ± 0.9	5.9 ± 1.2	
(DOPAC/DA)	1.1 ± 0.2	1.2 ± 0.4	
Serotonin	47.7 ± 5.4	25.6 ± 4.1	< 0.001
5-HIAA	31.7 ± 8.2	27.7 ± 7.4	
5-HIAA/5HT	0.6 ± 0.1	1.0 ± 0.1	< 0.05
Glutamate	75.5 ± 9.0	85.8 ± 6.6	
Taurine	57.4 ± 6.2	45.1 ± 4.8	
GABA	21.7 ± 4.1	19.8 ± 2.3	
Nucleus accumbens			
Dopamine	386.5 ± 29.4	394.2 ± 17.9	
DOPAC	89.4 ± 6.9	91.8 ± 9.4	
(DOPAC/DA)	0.3 ± 0.03	0.2 ± 0.03	
Serotonin	82.6 ± 8.4	95.9 ± 19.1	
5-HIAA	64.1 ± 17.2	72.1 ± 15.9	
5-HIAA/5HT	1.0 ± 0.3	0.8 ± 0.3	
Glutamate	73.7 ± 2.6	67.1 ± 3.0	
Taurine	44.8 ± 3.3	33.8 ± 2.7	
GABA	25.7 ± 3.5	33.8 ± 2.7	
Caudate-putamen			
Dopamine	504.4 ± 22.7	419.8 ± 33.7	= 0.05
DOPAC	36.7 ± 3.0	32.9 ± 1.7	
(DOPAC/DA)	0.07 ± 0.004	0.08 ± 0.004	
Serotonin	31.2 ± 4.4	18.4 ± 2.0	< 0.001
5-HIAA	20.6 ± 6.2	52.9 ± 17.1	
5-HIAA/5HT	1.2 ± 0.3	1.6 ± 0.3	
Glutamate	80.5 ± 2.8	72.5 ± 3.2	
Taurine	60.7 ± 3.5	61.5 ± 3.4	
GABA	17.5 ± 1.5	19.4 ± 0.8	
Globus pallidus			
Dopamine	58.1 ± 7.0	62.6 ± 13.0	
DOPAC	10.7 ± 1.6	9.3 ± 1.0	
(DOPAC/DA)	0.2 ± 0.07	0.2 ± 0.05	
Serotonin	66.5 ± 6.1	49.4 ± 5.1	< 0.05
5-HIAA	37.6 ± 10.2	72.4 ± 17.9	
5-HIAA/5HT	0.6 ± 0.1	1.4 ± 0.3	< 0.05
Glutamate	50.5 ± 2.5	44.4 ± 2.3	
Taurine	42.1 ± 1.9	45.2 ± 4.1	
GABA	42.0 ± 4.7	45.5 ± 4.8	
Ventral tegmental area, substanti nigra			
Dopamine	32.4 ± 4.8	26.8 ± 4.1	
DOPAC	11.7 ± 1.0	8.0 ± 0.7	
(DOPAC/DA)	0.5 ± 0.08	0.3 ± 0.04	
Serotonin	102.2 ± 17.9	55.3 ± 9.2	< 0.05
5-HIAA	37.5 ± 9.1	45.3 ± 12.3	
5-HIAA/5HT	0.4 ± 0.06	0.9 ± 0.2	< 0.05
Glutamate	37.2 ± 1.7	34.3 ± 1.9	
Taurine	20.1 ± 1.7	21.7 ± 1.8	
GABA	42.5 ± 5.3	40.9 ± 5.1	

5-HIAA, 5-hydroxy indole acetic acid; 5HT, serotonin; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; GABA, γ-aminobutyric acid; STN, subthalamic nucleus.

lesion) that induced a selective increase in compulsive lever-pressing therefore supports the notion that compulsive behavior is not merely a consequence of anxiety (e.g. Bartz & Hollander, 2006), and our claim that the signal attenuation model is a model of OCD and not of anxiety-related behaviors (Joel *et al*., 2004; Joel, 2006).

STN lesions resulted in a decrease in dopamine content in the striatum, but not in the other regions assessed. A decrease in striatal dopamine in lesioned rats could be the result of the loss of subthalamic input to dopamine neurons in the midbrain (reviewed in Joel & Weiner, 2000), as these projections have been reported to exert an excitatory effect on dopamine neurons, and lesions of the STN have been reported to decrease firing of these neurons (Smith & Grace, 1992; Chang *et al*., 2003).

The main biochemical change following STN lesions was a significant decrease in serotonin content and an increase in serotonin turnover in the medial prefrontal cortex (prelimbic and infralimbic cortices), substantia nigra-ventral tegmental area, globus pallidus and striatum (although the increase in turnover was not significant in the latter). In contrast, no changes were noted in the nucleus accumbens and the dorsal region of the anterior cingulate (Cg1). Of the subcortical regions, the caudate-putamen and the globus pallidus receive serotonergic input solely from the dorsal raphe nuclei, whereas the nucleus accumbens is also innervated by the median raphe (Vertes, 1991; Vertes & Kocsis, 1994; Vertes *et al*., 1999), suggesting that the STN lesion affected mainly the functioning of the dorsal raphe. This is supported by the finding of decreased serotonin content in the medial prefrontal cortex, which receives its serotonergic input mainly from the dorsal raphe nuclei (Vertes, 1991; Vertes & Kocsis, 1994; Morin & Meyer-Bernstein, 1999; Vertes *et al*., 1999). Although the afferents to the dorsal and median raphe are very similar (Aghajanian & Wang, 1977), the serotonergic system in these two regions has been shown to be differentially modulated by several neurotransmitters, with the dorsal raphe being more under the control of dopaminergic and GABAergic tone, and the median raphe more under the control of glutamatergic tone (Ferre *et al*., 1994; Adell & Artigas, 1999; Tao & Auerbach, 2003). Although the STN projects directly to the dorsal raphe, these projections are sparse (Kita & Kitai, 1987). In contrast, the STN is in a position to affect the functioning of the lateral habenula, which provides one of the strongest projections to the dorsal raphe (Peyron *et al*., 1998), and which has been reported to exert a strong inhibition of serotonergic neurons in the dorsal raphe (Wang & Aghajanian, 1977; Park, 1987). Specifically, the STN projects to the entopeduncular nucleus, which innervates the lateral habenula. It is therefore possible that the lack of excitatory input to the entopeduncular nucleus following the STN lesions resulted in disinhibition of the lateral habenula and a consequent depression of the activity of

serotonin neurons in the dorsal raphe. Lesions to the STN may have also affected the dopaminergic innervation of the raphe; an effect that may be expected to affect more the functioning of the dorsal raphe (Ferre *et al*., 1994; Adell & Artigas, 1999).

The finding that STN lesions decreased the level of serotonin and dopamine in the striatum is of particular interest because a similar effect was evident following orbitofrontal cortex lesions, which also induce compulsive lever-pressing (Joel *et al*., 2005b; Schilman *et al*., unpublished observations). Specifically, orbitofrontal lesions were found to increase the density of the serotonin transporter in the striatum (Joel *et al*., 2005b), and to decrease the content of serotonin and dopamine in this nucleus (Schilman *et al*., unpublished observations), as was found here following STN lesions. It is noteworthy that in contrast to the marked changes in dopamine and serotonin neurotransmission in the caudate-putamen following STN and orbitofrontal lesions, these lesions did not induce changes in these neurotransmitter systems in the nucleus accumbens (present study; Schilman *et al*., unpublished observations). These results support the notion that compulsive behaviors depend on the dorsal but not the ventral striatum (e.g. Everitt & Robbins, 2005).

The behavioral and biochemical sequels of lesions to the STN and the orbitofrontal cortex suggest that there may be a final common pathway by which different brain pathologies lead to a pro-compulsive state. The possibility that this final common pathway involves the striatum and its serotonergic and dopaminergic systems is in agreement with many lines of evidence implicating these neural systems in the pathophysiology of OCD and in the response to treatment (for a recent review, see Friedlander & Desrocher, 2006).

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Abbreviations

5-HIAA, 5-hydroxy indole acetic acid; Cg1, cingulate cortex area 1; CS, conditioned stimulus; DOPAC, 3,4-dihydroxyphenylacetic acid; ELP, extra lever-presses; ELP-C, extra lever-presses in completed trials; ELP-U, extra lever-presses in uncompleted trials; GABA, γ-aminobutyric acid; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; NRL, non-reinforced lever; OCD, obsessive-compulsive disorder; PTSA, post-training signal attenuation; RE, regular extinction; RL, reinforced lever; STN, subthalamic nucleus.

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