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UNILATERAL NIGROSTRIATAL LESIONS INDUCE A BILATERAL INCREASE IN GLUTAMATE DECARBOXYLASE MESSENGER RNA IN THE RETICULAR THALAMIC NUCLEUS

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Abstract—The reticular thalamic nucleus consists of densely packed neurons containing the neurotransmitter GABA. It surrounds the lateral border of the thalamus, has extensive reciprocal connections with thalamocortical neurons, and is thought to be involved in attentional processes. The reticular thalamic nucleus also receives direct and indirect inputs from the basal ganglia, suggesting that it may be involved in relaying motor information to the thalamus and cortex. We examined the possibility that decreased dopaminergic transmission in the basal ganglia indirectly affects the reticular thalamic nucleus. Rats received unilateral 6-hydroxydopamine lesions of the substantia nigra pars compacta and were killed two or three weeks after the lesion. Sections of the reticular thalamic nucleus were processed for *in situ* hybridization histochemistry at the single cell level with RNA probes for both isoforms of glutamate decarboxylase (M_r 65,000: glutamate decarboxylase 65 and M_r 67,000: glutamate decarboxylase 67), the rate limiting enzyme of GABA synthesis. Unilateral nigrostriatal dopaminergic lesions induced a topographically specific, bilateral increase in glutamate decarboxylase 67 messenger RNA in neurons of the lateral and ventral reticular thalamic nucleus. A much smaller increase in glutamate decarboxylase 65 messenger RNA was observed which was significant only ipsilateral to the lesion. Short- (seven day) and long-term (eight month) treatments with the antipsychotic drug haloperidol, in regimens that preferentially block D_2 dopamine receptors, induced catalepsy and orofacial dyskinesia, respectively, but did not alter glutamate decarboxylase 67 messenger RNA levels in the reticular thalamic nucleus. Thus, loss of dopaminergic terminals, but not blockade of D_2 dopamine receptors, induced the effects observed in the reticular thalamic nucleus.

The results reveal a novel bilateral effect of unilateral dopamine depletion. In view of the role of the reticular thalamic nucleus in tremor and attentional processes, which are altered in Parkinson's disease, this effect may contribute to the clinical manifestations of nigrostriatal dopamine depletion.

Key words: basal ganglia, GABA, Parkinson's disease, rat, dopamine, haloperidol.

The reticular thalamic nucleus (RTN) is a thin layer of densely packed neurons which encapsulates the anterior and lateral aspects of the dorsal thalamus.[32] The RTN projects to the dorsal thalamus and in return receives excitatory projections, in the form of collaterals, from nearly all thalamocortical and corticothalamic fibers.[32,44] Therefore, the RTN is in a strategic position to control the flow of neuronal information between the cortex and the thalamus.[13]

A series of elegant studies by Steriade and colleagues have demonstrated the importance of the RTN in the generation and synchronization of thalamocortical oscillatory rhythms observed during slow-wave sleep.[64] The RTN may also play a role in two other rhythmic phenomena, the neocortical spike activity characteristic of petit mal epilepsy and the resting tremor observed in Parkinson's disease.[7,46] The RTN has also been implicated in attentional and cognitive processes.[13,51]

Recent evidence indicates that, in addition to projections from the cortex and dorsal thalamus, the RTN receives substantial projections from the basal ganglia, specifically the external pallidum (globus pallidus in rats) and substantia nigra pars reticulata.[12,22,27,47] The globus pallidus projects to the output structures of the basal ganglia (internal pallidum/substantia nigra pars reticulata) both directly, and indirectly via the subthalamic nucleus.[2] These output nuclei then project to the cortex via the ventral anterior and ventral lateral thalamic nuclei.[2] The novel finding of a direct connection between the basal ganglia and the RTN suggests an additional route by which the basal ganglia can influence thalamocortical activity.[48]

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Abbreviations: DTT, dithiothreitol; GAD, glutamate decarboxylase; 6-OHDA, hydroxydopamine; RTN, reticular thalamic nucleus; 2 × SSC, 0.3 M NaCl/0.03 M sodium citrate.

Numerous studies have shown that nearly all neurons of the RTN contain the inhibitory amino acid neurotransmitter GABA.[30,43] The mRNAs encoding both isoforms of the enzyme of GABA synthesis, glutamate decarboxylase (GAD), GAD65 (M_r 65,000) and GAD67 (M_r 65,000), are present in neurons of the RTN.[9,19,20] Previous studies in other brain regions have shown that the levels of mRNA encoding GAD67 vary in relation to the electrophysiological activity of GABAergic neurons.[5,38,59,60] Alterations in GAD65 mRNA levels, however, are less commonly observed in the rat.[59,60]

Both unilateral dopamine depletion and short-term administration of the dopamine receptor antagonist haloperidol increase GAD67 mRNA levels in the globus pallidus.[14,15,35,59] In addition, they alter the electrophysiological activity of neurons in the globus pallidus and the substantia nigra pars reticulata.[21,42,45,65] The direct connections of the globus pallidus and substantia nigra pars reticulata with the RTN suggest that these changes in electrophysiological activity may affect the RTN. Indeed, stimulation of the substantia nigra or ventral pallidum has been shown to alter the firing of RTN neurons.[36,47,68] The effects of unilateral dopamine depletion and systemic haloperidol administration on neurons of the RTN, however, has not been explored.

In the present study, we have examined the effects of nigrostriatal lesions and haloperidol treatment on the level of GAD67 and GAD65 mRNAs in the RTN of adult rats.

EXPERIMENTAL PROCEDURES

Animals

All studies were performed on rats housed under standard conditions with food and water available *ad libitum*. All surgical procedures and behavioral testing were performed between 08.00 and 14.00, during the light cycle (07.00–19.00). All surgical procedures were performed under anesthesia with equithesin at a dose of 0.3 ml per 100 g body wt (prepared following protocol of Jensen-Salbutry Laboratories, Kansas City, MO). All procedures were done in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committees.

All analysis of GAD gene expression in the RTN was done on tissue from rats used in previous studies. Therefore, the injection protocols correspond to those used in these studies.[14–16,59]

6-Hydroxydopamine lesions

Three groups of rats with lesions induced by slightly different procedures were used in this study. All animals were male Sprague–Dawley rats (Charles River, initial weight 225–300 g). All injections were given over 2 min into the left substantia nigra. The stereotaxic coordinates of the injection site were AP, 3.4 mm from bregma; ML, 2.0 mm from midline; DV, +2.2 mm from interaural zero based on the atlas of Paxinos and Watson.[49] In all cases, 6-hydroxydopamine (6-OHDA, bromide salt, Sigma) was dissolved in 4 μl of 0.9% sterile saline solution containing 1% ascorbic acid.

In the first groups of animals (Group 1), five rats received an injection of 8 μg of 6-OHDA and five rats received an injection of 0.9% sterile saline/0.1% ascorbic acid (controls). Thirty minutes before the stereotaxic injection into the substantia nigra, both groups of rats received an injection of desipramine (25 mg/kg s.c., Sigma) to protect noradrenergic neurons.[67] All rats were injected with apomorphine one week after surgery to examine rotational behavior (see below). Rats from Group 1 were killed three weeks after lesion.

The second group of rats (Group 2) received unilateral substantia nigra lesions with 8 μg of 6-OHDA or saline injected as above ($n = 5$ lesioned and 5 controls), but none of these animals received desipramine or apomorphine treatments to ensure that the effects observed were not due to these drugs.[59] They were killed two weeks after lesion.

The third group of five animals (Group 3) received a unilateral injection of only 6 μg of 6-OHDA ($n = 5$) or saline ($n = 5$) into the substantia nigra with the same protocol as Group 1,[59] i.e. after pretreatment with desipramine. These animals received an injection of apomorphine one week later for behavioral testing as described below. They were killed three weeks after lesion.

Lesioned animals from both Groups 1 (8 μg) and 3 (6 μg) displayed consistent rotational behavior in response to apomorphine (0.5 mg/kg s.c.) one week after lesion.[15,59] In addition, [^{3}H]BTCP autoradiography on striatal sections from Group 1 demonstrated greater than 90% loss of dopamine uptake sites ipsilateral to the lesion.[15]

For all groups, sections from the substantia nigra were stained with hematoxylin and eosin, as well as with Nissl stain, and observed with bright-field microscopy to assess the location and extent of the lesions. As previously reported, all lesioned rats used in this study showed extensive cell loss in the substantia nigra pars compacta. There was a minimal involvement of the lateral part of the ventral tegmental area only in Group 1, which received the largest dose of 6-OHDA after desipramine treatment.[15,59]

Haloperidol treatments

For the short-term haloperidol experiments, 16 male Sprague–Dawley rats (Charles River, initial weight 225–250 g) received injections of either vehicle (Tween 80/deionized water, $n = 8$) or haloperidol (1 mg/kg s.c., $n = 8$) once daily for seven days. An additional 16 animals received injections of either the muscarinic antagonist scopolamine (1 mg/kg s.c., $n = 8$), or a combination of haloperidol and scopolamine (each 1 mg/kg s.c., $n = 8$) for seven days. All animals were tested for catalepsy using the horizontal bar test on injection days 1, 3 and 7 as reported in Delfs *et al.*[14] Animals were killed by decapitation 24 h after the final drug administration. This group of animals was used because this regimen of haloperidol was shown to induce catalepsy and increase GAD67 gene expression in the globus pallidus. Both effects were blocked by co-administration of scopolamine.[14]

For long-term administration of haloperidol, tissue from rats prepared by G. Ellison (UCLA) were used. For this study, 12 female Sprague–Dawley rats (Simonsen, Gilroy, CA, initial weight 200–250 g) received i.m. injections of haloperidol decanoate or vehicle (sesame oil) once every three weeks into the upper thigh muscle for seven months. As previously published by this group,[17,56] female rats were used because the behavioral model involves testing the animals over the course of many months, and since male rats are constantly growing it would necessitate habituating them to larger and larger tubes for computer-assisted recording of orofacial movements. The dose of haloperidol was 21 mg/kg, giving an average daily dose of 1 mg/kg per day. Due to the long clearance time of decanoate drugs, animals were switched to oral haloperidol for the final four weeks of drug administration (average intake 1.2–1.5 mg/kg per day). Animals were killed by decapitation one week after the final drug administration. This group of animals was used because this regimen of haloperidol has been shown to produce a tardive dyskinesia-like behavior in rodents[17,56] and to decrease GAD67 mRNA in the globus pallidus.[16]

In situ *hybridization histochemistry*

Brains were rapidly removed after decapitation, frozen on powdered dry ice and stored at −70°C until sectioning. Brains were cut into 10 μm sections on a Reichert–Jung cryostat, thaw-mounted on gelatin-coated slides and stored at −70°C until the day of the experiment.

The cDNAs encoding GAD65 and GAD67 were generously provided by Dr A. J. Tobin (UCLA, Los Angeles, CA). The cDNAs inserted into the transcription vectors Bluescript SK and PSP64/65, respectively, were linearized with appropriate restriction endonucleases. The GAD65 cDNA consists of a 2.7 kb sequence isolated from a rat hippocampal library. The GAD67 cDNA consists of a full-length 2.3 kb sequence isolated from a feline occipital cortex library. It shows a 97% sequence identity with the cloned rat GAD67 cDNA.[18,34]

The protocol for probe synthesis and *in situ* hybridization has been described previously.[9] Briefly, the synthesis mixture consisted of 10 μM UTP, 2.5 μM [^{35}S]UTP (1000 Ci/mmol, NEN/DuPont, Boston, MA), ATP, CTP and GTP in excess, SP6 or T7 RNA polymerase, dithiothreitol (DTT), ribonuclease inhibitor and 2 μg of linearized DNA containing the insert. Due to the large size of the GAD65 and GAD67 RNA probes, each was partially hydrolysed into 100–200 base pair fragments for increased tissue penetration.[9] Following the synthesis, the probes were extracted in phenol–chloroform–isoamyl alcohol and precipitated overnight in ethanol at −70°C.

For *in situ* hybridization histochemistry, experiments were performed on frontal sections through the RTN at the level of the entopeduncular nucleus (A-P −2.56 mm from bregma, Fig. 1) based on the atlas of Paxinos and Watson.[49] Sections were brought to room temperature from −70°C under a stream of cool air, postfixed in 3% paraformaldehyde containing 0.02% diethylpyrocarbonate (Sigma), acetylated and dehydrated. Sections were incubated with 3–5 ng probe (400,000 d.p.m./ng) in humid chambers at 50°C for 3.5 h. Post-hybridization treatments included three washes in 50% formamide/2 × SSC (0.3 M NaCl/0.03 M sodium citrate) at 52°C and a 30 min incubation in 100 μg/ml RNase A (Sigma Chemical Co., St Louis, MO) at 37°C. After an overnight rinse in 2 × SSC/0.05% Triton X-100, sections were dehydrated in graded ethanols, defatted in xylene and desiccated. Sections were coated with Kodak NTB3 emulsion diluted 1:1 with 300 mM ammonium acetate or undiluted Ilford K.5D (Polysciences) for single-cell analysis. Test slides were developed at regular intervals to determine optimal exposure time, i.e. when specific labeling was robust but not saturating based on previous emulsion experiments. This corresponded to five to 10 days for the RTN. Emulsion autoradiograms were developed in Kodak D-19 developer and fixed in Kodak Rapid Fix. Emulsion-coated sections were lightly counterstained with hematoxylin and eosin and coverslipped with Eukitt mounting media (Calibrated Instruments, Hawthorne, NY).

The anatomical specificity of autoradiographic labeling with each of the probes has been previously characterized and was verified in each individual experiment. No specific labeling was observed in control sections processed with sense RNA probes.[9]

Quantification

The level of labeling over individual neurons in the RTN was measured on emulsion-coated slides with a MORPHON image analysis system.[10] This system consists of a MTI 65

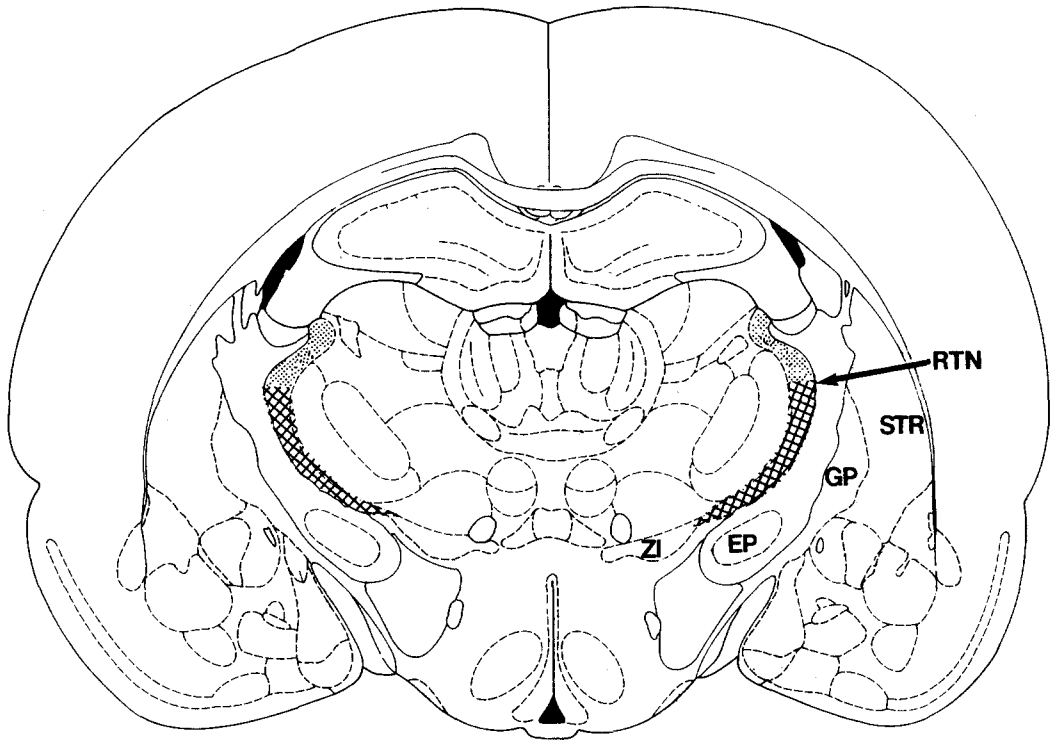

Fig. 1. Schematic illustration of a frontal section through the RTN at the level of the entopeduncular nucleus (EP). For quantification, the entire RTN was outlined and divided into two regions indicated by stippled and hatched areas, respectively. For all experiments, the number of pixels per neuron was measured in 50 neurons of the ventral two-thirds of the RTN (hatched area) on each side (lesion experiments) or one side (haloperidol experiments). In one lesion experiment, an additional 50 neurons was measured from the dorsal one-third of the RTN (stippled area) on both sides. GP, globus pallidus; STR, striatum; ZI, zona incerta.

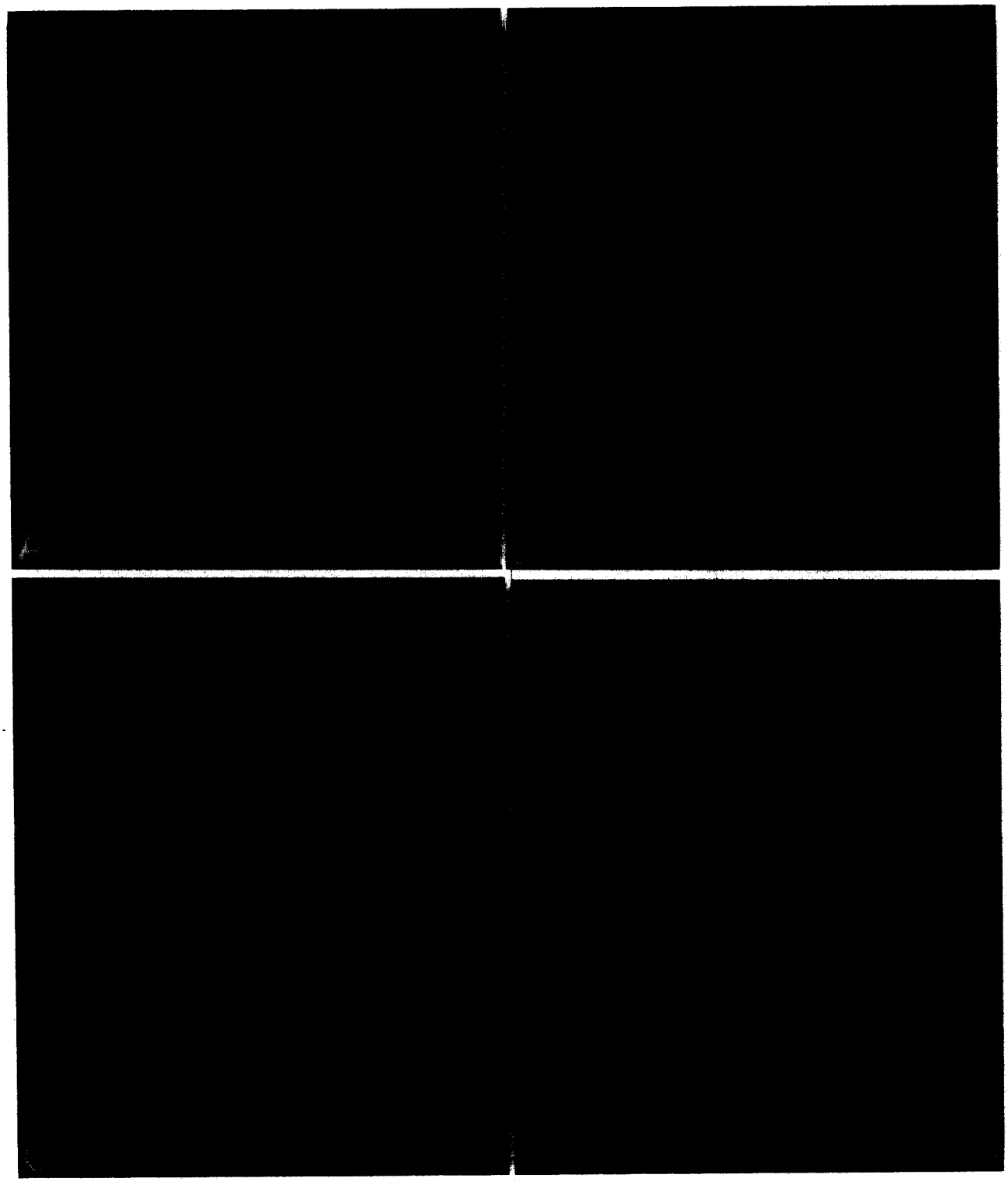

Fig. 2. Photomicrographs illustrating the bilateral increase in GAD67 mRNA in the ventral two-thirds of the RTN (white arrow) following a unilateral injection of 8 μg of 6-OHDA into the substantia nigra (Group 1). Sections of the RTN were processed for *in situ* hybridization and emulsion autoradiography as described in Experimental Procedures. Dark-field photomicrographs of neurons labeled with the GAD67 RNA probe on the ipsilateral side (A) and contralateral side (B) of the RTN in control animals. Note the bilateral increase in the number of silver grains over individual neurons on the side ipsilateral (C) and contralateral (D) to the 6-OHDA lesion. Scale bar = 250 μm.

video camera, a Leitz microscope, a Numonics GraphicMaster image digitizer and an IBM 212 computer. The anatomical structure of interest was outlined under low-power microscopy. Neurons were visualized under bright-field illumination with a 40× objective and projected onto a video monitor with a resulting magnification of 1200×. Each neuron of interest was outlined using the digitizing tablet and the threshold gray value was adjusted to distinguish grains from the cellular staining produced by hematoxylin–eosin. The portion of the enclosed area which was above threshold illumination was determined and expressed as the number of pixels occupied by silver grains. Linear regression analysis has shown a direct correlation (+0.96 for 40× magnification) between the number of pixels measured with the MORPHON system and visual grain counting.[10] For the present experiments, specific labeling was arbitrarily defined as the presence of 10 or more grains per neuron (approximately 10 pixels).

For all experiments, a random sample of 50 labeled neurons per animal was measured in the ventral two-thirds of the RTN on one side (haloperidol experiments) or both sides (lesion experiments; Fig. 1). In one lesion experiment, an additional 50 labeled neurons per animal were measured from the dorsal one-third of the RTN on both sides (Fig. 1). Random sampling was achieved by moving non-overlapping frames over the region, always starting at the same anatomical location, and measuring labeling in all neurons with at least 10 grains over the cell body until 50 labeled cells were analysed. We have previously verified that this sample size provides a very reliable estimate of the average level of labeling (J. J. Soghomonian and M. F. Chesselet, unpublished observations). Since the density of labeled cells did not change in controls versus treated animals in the conditions of the experiment (see Results), the same anatomical area was analysed in control and treated rats from each experiment. In the present experiments, autoradiographic background in areas devoid of labeled cells was extremely low and thus the values were not subtracted. A mean level of labeling per neuron was determined for each rat, and this value was used for statistical analysis. In addition, frequency distributions of the level of labeling were constructed for each group of rats. The density of labeled neurons in the RTN was determined from camera lucida drawings.

Data analysis

Only slides processed concurrently in individual experiments were compared statistically and all statistical analyses were done on absolute values with the Statview 512+ Interactive Statistics and Graphics Package (Version 1.0, Abacus Concepts). Comparisons were made between either the ipsilateral or the contralateral side of lesioned rats and the corresponding side of control rats using an unpaired two-tailed Student's t-test with $P < 0.05$ considered significant. Side-to-side comparisons in lesioned animals and comparisons between control and long-term haloperidol treated animals were also made using the Student's t-test. For short-term haloperidol experiments, ANOVA was used with the Dunnett's *post hoc* comparison with $P < 0.05$ considered significant.

RESULTS

Effects of unilateral nigrostriatal lesions on glutamate decarboxylase 67 messenger RNA expression in the reticular thalamic nucleus

A unilateral 6-OHDA lesion of the substantia nigra (8 μg, Group 1) resulted in a bilateral increase in the level of GAD67 mRNA in the RTN (Fig. 2C,D) as compared with controls (Fig. 2A,B). The magnitude of the increased mean level of labeling per neuron was similar ipsilateral and contralateral to the lesion (Table 1). There was no change in the number of labeled neurons detected under the conditions of the experiment, indicating that the changes in level of labeling are not altered by the inclusion of cells with levels of labeling below the threshold of detection in controls[10] (Table 1). Analysis of the frequency distributions of the level of labeling per neuron confirmed the bilateral increase in level of labeling by showing a shift to the right of the histogram (Fig. 3C,D) and an increase in the median of the distribution for lesioned animals (Table 1) as compared with the corresponding side of controls (Fig. 3A,B, Table 1).

This bilateral increase in GAD67 gene expression was only observed in the ventral two-thirds of the nucleus (Fig. 1) which has been shown to receive the most substantial innervation from basal ganglia structures in the rat.[12,22] There was no significant difference between the level of labeling per neuron for GAD67 in the dorsal one-third of this nucleus (Fig. 1) when lesioned animals were compared with control (ipsilateral control, 57.5 ± 1.9; contralateral control, 55.1 ± 1.7; ipsilateral 6-OHDA, 54.8 ± 0.8; contralateral control, 56.6 ± 2.3, mean level of labeling ± S.E.M.; $n = 4$). Similarly, there were no significant changes in the number of labeled neurons detected under the conditions of the experiment (ipsilateral control, 96 ± 11; contralateral control, 122 ± 17; ipsilateral 6-OHDA, 109 ± 9; contralateral control, 122 ± 13, mean ± S.E.M., $n = 4$). For the remainder of the experiments, only neurons in the ventral two-thirds of the RTN were analysed.

Table 1. Means and medians of the distributions of labeling and number of neurons labeled for glutamate decarboxylase 67 mRNA in the reticular thalamic nucleus after a unilateral 6-hydroxydopamine lesion of the substantia nigra (8 μg after desipramine treatment, Group 1)

	CONT	6-OHDA
Means†		
Ipsilateral RTN	79.4 ± 1.9	121.3 ± 6.0*
Contralteral STN	82.7 ± 5.4	118.9 ± 6.5*
Medians‡		
Ipsilateral RTN	73.0 ± 2.5	114.0 ± 8.8*
Contralateral RTN	77.0 ± 4.8	109.8 ± 6.2*
Number of labeled neurons§		
Ipsilateral RTN	94 ± 12	123 ± 4
Contralateral RTN	128 ± 22	121 ± 9

†Mean level of labeling for for GAD67 mRNA in the ventral two-thirds of the RTN (see Fig. 1) both ipsilateral and contralateral to a unilateral 6-OHDA lesion of the substantia nigra calculated as described in the Experimental Procedures.

‡Medians of frequency distributions. Frequency distributions of levels of labeling were constructed for individual animals and their medians were determined.

§Number of labeled neurons per mm² were measured using camera lucida drawings.

Data are mean ± S.E.M. ($n = 4$–5). *$P < 0.05$ when compared with the corresponding side of control animals using two-tailed unpaired Student's t-test.

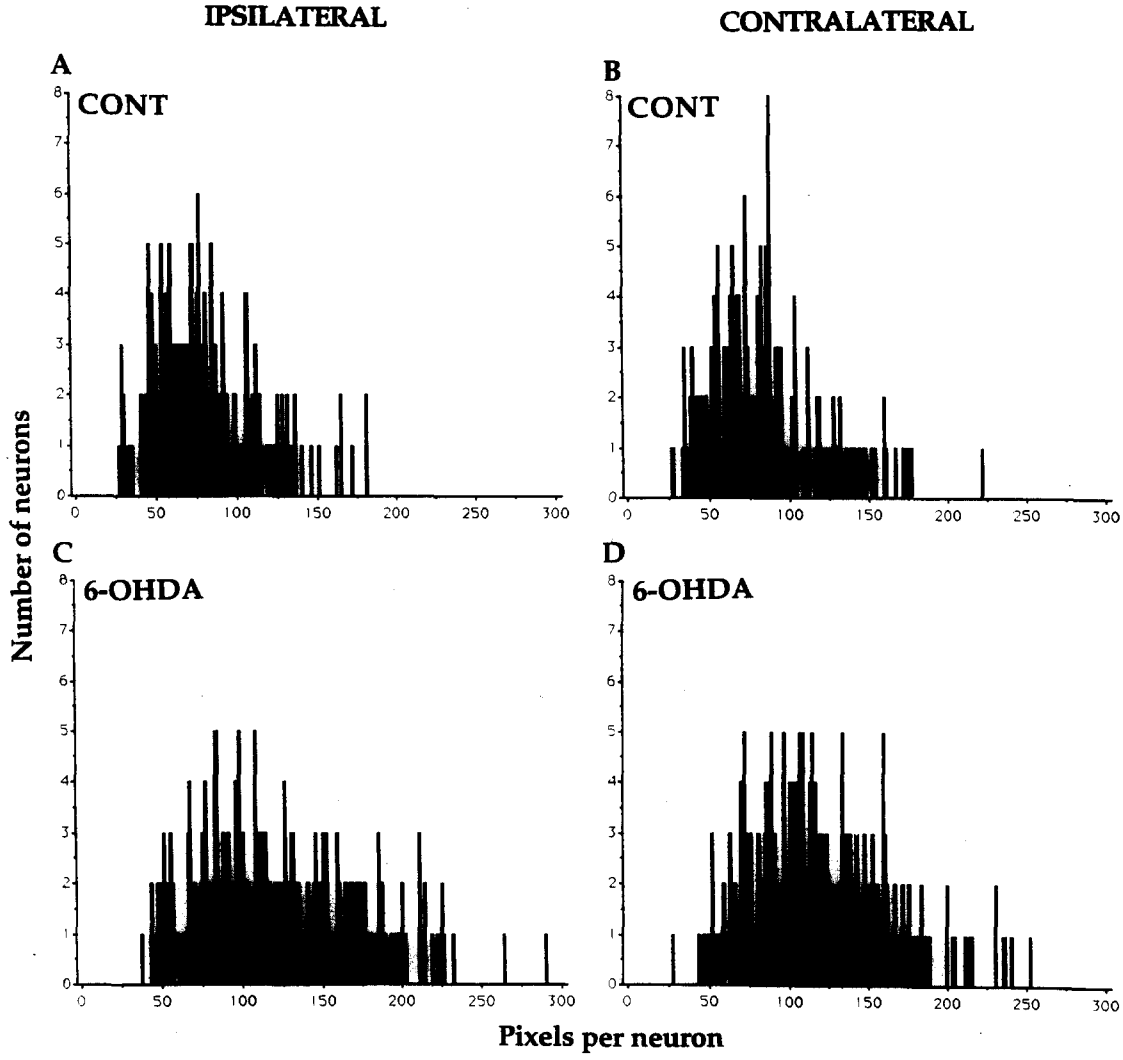

Fig. 3. Histograms of frequency distributions of labeling for GAD67 mRNA in the RTN of rats ipsilateral (left panels) and contralateral (right panels) to (A,B) a sham lesion (CONT) or (C,D) a unilateral 8 μg 6-OHDA lesion of the substantia nigra (Group 1) as described in Experimental Procedures. Quantification of silver grains over individual neurons was done with the MORPHON image analysis system on sections processed for emulsion autoradiography. Data include 50 neurons analysed per rat ($n = 4$–5) from the ventral two-thirds of the ipsilateral or contralateral RTN. The medians of the frequency distributions are shown in Table 1.

To comfirm these effects and rule out a role for desipramine and/or the dopamine agonist apomorphine, the level of labeling for GAD67 mRNA was measured in animals from another prior study.[59] One group of animals (Group 2) received an 8 μg infusion of the 6-OHDA into the substantia nigra as did Group 1, but without desipramine pretreatment or subsequent apomorphine treatment. The other group (Group 3) received a 6 μg infusion of the neurotoxin after desipramine pretreatment.[59] Confirming the results from our first experiment, rats from these two groups with a unilateral 6-OHDA lesion exhibited bilateral increases in GAD67 mRNA in the RTN (Tables 2, 3). As for Group 1, no change in the number of labeled neurons was observed in the conditions of the experiment for the lesioned rats (Tables 2, 3). Analysis of the frequency distributions of the level of labeling per neuron confirmed the bilateral increases observed in both groups by showing a shift to the right of the histogram in lesioned animals (not shown), and an increase in the medians (Tables 2, 3) compared with controls.

Effects of unilateral nigrostriatal lesions on glutamate decarboxylase 65 messenger RNA expression in the reticular thalamic nucleus

The level of mRNA encoding GAD65, the other isoform of the enzyme, was measured in the RTN of rats from Group 2 (8 μg 6-OHDA without desipramine). Although both the ipsilateral and contralateral sides of the RTN displayed a small increase in labeling in the lesioned animals, the effect only reached significance on the ipsilateral side, due to the greater variability of the contralateral effect (Table 2). This

Table 2. Medians of the distributions of labeling and number of neurons labeled for glutamate decarboxylase 65 and glutamate decarboxylase 67 mRNA in the reticular thalamic nucleus after a unilateral 6-hydroxydopamine lesion of the substantia nigra (8 μg without desipramine, Group 2)

	GAD65		GAD67	
	CONT	6-OHDA	CONT	6-OHDA
Means†				
Ipsilateral RTN	101.7 ± 3.2	126.1 ± 4.1*	74.7 ± 2.2	109.4 ± 5.2*
Contralateral RTN	102.3 ± 3.6	119.8 ± 6.6	70.2 ± 3.4	110.0 ± 1.4*
Medians‡				
Ipsilateral RTN	98.8 ± 3.9	115.8 ± 3.6*	71.3 ± 2.2	104.8 ± 6.3*
Contralateral RTN	93.8 ± 3.6	114.3 ± 8.1	64.8 ± 2.8	104.0 ± 1.7*
Number of labeled neurons§				
Ipsilateral RTN	117 ± 13	134 ± 19	102 ± 11	116 ± 4.8
Contralateral RTN	97 ± 13	119 ± 16	91 ± 11	115 ± 10

†Mean level of labeling for GAD65 and GAD67 mRNA in the ventral two-thirds of the RTN (see Fig. 1), both ipsilateral and contralateral to a unilateral 6-OHDA lesion of the substantia nigra calculated as described in Experimental Procedures.

‡Medians of frequency distributions. Frequency distributions of levels of labeling were constructed for individual animals and their medians were determined.

§Number of labeled neurons per mm^2 were measured using camera lucida drawings. Note that absolute values cannot be compared between GAD65 and GAD67 levels as the experiments were done separately.

Data are mean ± S.E.M. ($n = 4$–5). *$P < 0.05$ when compared with the corresponding side of control animals using two-tailed unpaired Student's t-test.

effect was confirmed by analysis of the frequency distributions of the level of labeling per neuron, which showed a shift to the right of the histogram and an increase in the median of the distribution on the ipsilateral, but not the contralateral side of lesioned animals (Fig. 4C,D) when compared with controls (Fig. 4A,B, Table 2). There were no significant changes in the number of labeled neurons detected under the conditions of the experiment (Table 2).

Table 3. Mean level of labeling, medians of the distributions of labeling and number of neurons labeled for glutamate decarboxylase 67 mRNA in the reticular thalamic nucleus after a unilateral 6-hydroxydopamine lesion of the substantia nigra (6 μg after desipramine treatment, Group 3)

	Control	6-OHDA
Means†		
Ipsilateral RTN	60.7 ± 3.1	93.9 ± 1.7*
Contralateral RTN	58.7 ± 1.4	84.9 ± 1.7*
Medians‡		
Ipsilateral RTN	56.0 ± 2.4	89.8 ± 2.6*
Contralateral RTN	57.3 ± 1.8	79.3 ± 1.4*
Number of labeled neurons§		
Ipsilateral RTN	55 ± 5	71 ± 10
Contralateral RTN	57 ± 9	80 ± 10

†Mean level of labeling for GAD67 mRNA in the ventral two-thirds of the RTN (see Fig. 1), both ipsilateral and contralateral to a unilateral 6-OHDA lesion of the substantia nigra calculated as described in Experimental Procedures.

‡Medians of frequency distributions. Frequency distributions of levels of labeling were constructed for individual animals and their medians were determined.

§Number of labeled neurons per mm^2 were measured using camera lucida drawings.

Data are mean ± S.E.M. ($n = 4$–5). *$P < 0.05$ when compared with the corresponding side of control animals using two-tailed unpaired Student's t-test.

Effects of short- and long-term haloperidol administration on glutamate decarboxylase 67 messenger RNA levels in the reticular thalamic nucleus

Short- and long-term haloperidol treatments, which cause catalepsy and orofacial dyskinesias, respectively, differentially alter the expression of GAD67 in the globus pallidus.[14,16] Short-term haloperidol treatments increase GAD67 mRNA levels in the globus pallidus, an effect also observed after unilateral dopamine depletion.[14,15,35,59] To determine whether the effects observed in the RTN after 6-OHDA lesions were related to the alterations in the pallidum seen after both 6-OHDA lesions of the nigrostriatal pathway and haloperidol administration, the level of GAD67 mRNA was also measured in the RTN of rats treated with haloperidol for seven days. This regimen of haloperidol did not alter the levels of GAD67 mRNA in the RTN (Table 4). The lack of effect on GAD67 mRNA was confirmed by analysis of the medians of the frequency distributions of the level of labeling (Table 4). Similarly, there were no changes in the number of labeled neurons (Table 4).

The RTN receives a substantial cholinergic projection from the basal forebrain and pedunculopontine tegmental nucleus.[26,58,63] However, the muscarinic antagonist, scopolamine, given at a dose (1 mg/kg s.c.) sufficient to block the catalepsy and the increase in pallidal GAD67 mRNA induced by haloperidol, did not alter GAD67 mRNA expression in the RTN when given either alone or in combination with haloperidol (1 mg/kg; Table 4). Similarly, there were no changes in the number of labeled neurons or the median of the frequency distributions (Table 4).

We have previously demonstrated that long-term administration of haloperidol decreases GAD67 gene

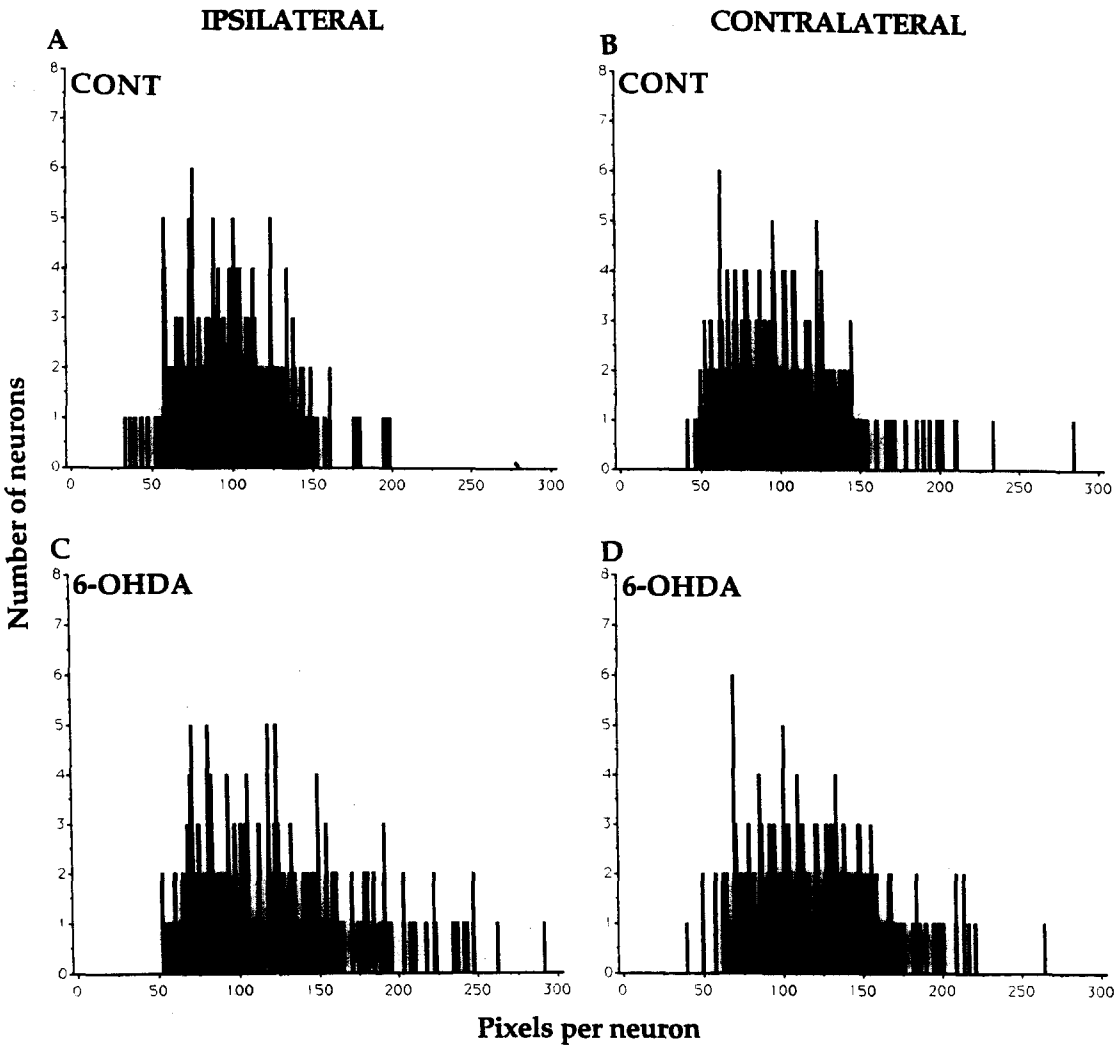

Fig. 4. Histograms of frequency distributions of labeling for GAD65 mRNA in the RTN of rats ipsilateral (left panels) and contralateral (right panels) to (A,B) a sham lesion (CONT) or (C,D) a unilateral 8 μg (6-OHDA) lesion of the substantia nigra (Group 2) as described in Experimental Procedures. Quantification of silver grains over individual neurons was done with the MORPHON image analysis system on sections processed for emulsion autoradiography. Data include 50 neurons analysed per rat ($n = 5$/group) from the ventral two-thirds of the ipsilateral or contralateral RTN. The medians of the frequency distributions are shown in Table 2.

Table 4. Mean level of labeling, medians of the distributions of labeling and number of neurons labeled for glutamate decarboxylase 67 mRNA in the reticular thalamic nucleus after haloperidol (1mg/kg) and scopolamine (1mg/kg) treatments

	Control	Haloperidol	Scopolamine	Combined
Means†				
RTN	82.1 ± 2.9	76.1 ± 2.6	77.9 ± 2.1	79.0 ± 2.6
Medians‡				
RTN	79.2 ± 3.1	73.3 ± 2.5	74.7 ± 2.6	75.3 ± 2.5
Number of labeled neurons§				
RTN	106.9 ± 8.9	90.0 ± 12.4	79.4 ± 12.0	88.1 ± 8.1

†Mean level of labeling for GAD67 mRNA in the ventral two-thirds of the RTN (see Fig. 1) after seven day treatment with haloperidol, scopolamine or a combination of the two drugs.

‡Medians of frequency distributions of labeling for GAD67 mRNA in the reticular thalamic nucleus. Frequency distributions of levels of labeling were constructed for individual animals and their medians were determined.

§Number of labeled neurons per mm^2 were measured using camera lucida drawings. All data are expressed as mean ± S.E.M. ($n = 6$–7).

*$P < 0.05$ as compared with control with ANOVA and Dunnett's *post hoc* test.

Table 5. Mean level of labeling, medians of the distributions of labeling and number of neurons labeled for glutamate decarboxylase 67 mRNA in the reticular thalamic nucleus after chronic haloperidol (eight month) treatment

	Control	Haloperidol
Means†		
RTN	91.6 ± 1.9	90.0 ± 4.9
Medians‡		
RTN	87.6 ± 1.4	84.8 ± 5.6
Number of labeled neurons§		
RTN	98.4 ± 12.2	98.6 ± 7.5

†Mean level of labeling for GAD67 mRNA in the ventral two-thirds of the RTN (see Fig. 1) following eight month administration of haloperidol (1 mg/kg).

‡Medians of frequency distributions of labeling for GAD67 mRNA in the RTN. Frequency distributions of levels of labeling were constructed for individual animals and their medians were determined.

§Number of labeled neurons per mm^2 were measured using camera lucida drawings. Data are expressed as mean ± S.E.M. ($n = 5$).

*$P < 0.05$ as compared with control using a two-tailed unpaired Student's t-test.

expression in the globus pallidus.[16] This regimen of haloperidol also did not alter the levels of GAD67 mRNA in the RTN (Table 5). Similarly, there were no changes in the number of labeled neurons or the medians of the frequency distributions of the level of labeling per neuron (Table 5).

DISCUSSION

The main finding of this study is that unilateral nigrostriatal lesions produce a bilateral increase in GAD67 mRNA in the lateral and ventral RTN. This effect was observed after nigrostriatal lesions performed under three different conditions and in animals killed two or three weeks after surgery. In two groups, dopamine depletion was achieved by local infusion of the neurotoxin 6-OHDA into the substantia nigra pars compacta after pretreatment with desipramine, an experimental model which has been shown to produce selective loss of dopaminergic neurons.[67] The alterations in GAD67 gene expression, however, were not attributable to desipramine administration, as one additional group of animals did not receive this drug.

Under these experimental conditions, the lesion primarily affected the substantia nigra pars compacta, and only produced minimal cell loss in the most lateral part of the ventral tegmental area after injection of 8 μg of the neurotoxin.[15] The magnitude of increases in GAD67 mRNA were similar in the three separate groups of 6-OHDA lesioned animals, even after injection of a smaller dose (6 μg) of 6-OHDA. Therefore, the effects observed in the RTN are most likely secondary to the loss of the nigrostriatal dopaminergic pathway. A preliminary report indicated that GAD mRNA levels increased in the RTN after an unspecified regimen of monoamine depletion.[29] The present data are consistent with this observation, and suggest that loss of nigrostriatal dopamine is responsible for the effect observed in the RTN.

Nigrostriatal lesions and regulation of messenger RNA encoding glutamate decarboxylase isoforms

Lesions of the nigrostriatal pathway have been shown to produce profound alterations in the level of expression of GAD67 mRNA in the striatum and pallidum of rats and primates.[15,35,37,59,60,61,66] As in the present study, these effects were either unique to GAD67 mRNA or accompanied by much smaller changes in GAD65 mRNA.[59,60] These differences are likely due to the distinct physiological functions of each of the isoforms.[18,40] It is postulated that GAD65, which is localized predominantly in nerve terminals, plays an important role in the rapid regulation of the GABA pool and is primarily regulated by its interaction with the cofactor, pyridoxal phosphate.[18,33,40] The GAD67 isoform, on the other hand, is distributed throughout the neuron, and is thought to be responsible for long-term regulation of GABA levels.[18,33,40] In contrast to GAD65, GAD67 is primarily regulated at the translational and transcriptional level.[40]

Neuronal pathways mediating the effects of nigrostriatal lesions on the reticular thalamic nucleus

There are no known direct connections between the nigrostriatal dopaminergic pathway and the RTN. However, many brain regions directly or indirectly influenced by nigrostriatal dopamine send topographically organized projections to the RTN. The most direct connections between nigrostriatal neurons and the RTN are by way of the recently described projections of the globus pallidus and substantia nigra pars reticulata to the RTN.[12,22,27,47] These projections specifically innervate the ventral two-thirds of the RTN in the rat,[12,22] the region in which the changes in GAD mRNA were observed.

Nigrostriatal lesions increase GAD67 mRNA levels[15,35,59] and burst firing activity in the globus pallidus,[21,45] suggesting that this brain region could mediate the effects observed in the RTN. This is unlikely, however, because short-term haloperidol treatments, which similarly increase GAD67 mRNA levels in the globus pallidus,[14] did not reproduce the effects of dopaminergic lesions in the RTN.

Alternatively, the substantia nigra pars reticulata may be involved in this effect either directly, through its projections to the RTN,[22,47] or indirectly, by way of the ventromedial nucleus of the thalamus or the pedunculopontine nucleus. These two brain regions receive inputs from the substantia nigra pars reticulata and also project to the lateral and ventral RTN.[4,26,28,31,58,62,63] Neurons of the substantia nigra pars reticulata are regulated by the descending striatonigral GABAergic pathway.[2] Release of GABA from these striatonigral neurons is modulated both by dopamine in the striatum, and by dopamine released from the dendrites of neurons of the substantia nigra pars compacta, which penetrate into the substantia

nigra pars reticulata.[8] In both cases, regulation of striatonigral neurons by dopamine is mediated by D_1 dopamine receptors.[1,23] A preferential involvement of D_1 receptors in the regulation of GAD mRNA in the RTN is compatible with our observation that this effect was not reproduced by haloperidol at a dose which preferentially blocks D_2 receptors.[52]

The activity of efferent neurons of the pars reticulata is increased after lesions of the nigrostriatal pathway.[2] This results from a decreased inhibition of these neurons secondary to both decreased activity in the GABAergic striatonigral pathway[23] and loss of stimulation of the presynaptic D_1 receptors, which normally increase GABA release from striatonigral terminals.[1] The result of this increased activation of nigral output neurons is an increased inhibition of target regions of the substantia nigra pars reticulata as demonstrated experimentally following electrical stimulation of the substantia nigra.[11,25,39,47]

Interestingly, electrical stimulation of the substantia nigra pars reticulata has been shown to increase cerebral glucose metabolism bilaterally in the RTN in rats.[54] Direct projections from the substantia nigra to the RTN and the ventromedial thalamic nucleus are mostly ipsilateral.[22,28,47] However, lesions of the ventromedial nucleus abolish the bilateral changes in glucose utilization induced by stimulation of the substantia nigra pars reticulata,[54] suggesting that the projection from the substantia nigra to the thalamus can mediate bilateral effects in the RTN. It is unknown whether the ventromedial thalamic nucleus projects bilaterally to the RTN, but the RTN can influence its counterpart either directly by way of cross-connections to the contralateral RTN[3] or indirectly, by cross-connections to the contralateral ventromedial thalamic nucleus.[50] Alternatively, cortical output pathways, which are affected by thalamocortical neurons, may be responsible for the contralateral effects through a cortico-cortical and cortico-thalamic circuit. Finally, the bilateral effects could be mediated by the pedunculopontine nucleus, which can influence the contralateral RTN by way of crossed connections with its contralateral homologue.[53,57]

Functional significance of increased glutamate decarboxylase 67 messenger RNA in the reticular thalamic nucleus

In many experimental models, changes in GAD67 mRNA levels parallel changes in either the level or the pattern of firing activity in GABAergic neurons.[5,38,59,60] In the globus pallidus, for example, GAD67 mRNA levels decrease when the pattern of firing becomes more regular, and increase when neurons fire in bursts.[15] This may represent an adaptive response to the increased neurotransmitter release which usually accompanies increased burst firing,[24] although this has not yet been demonstrated specifically for GABA.

The pattern of activity of RTN neurons after unilateral dopamine depletion is unknown. Evidence suggests that unilateral dopamine depletion leads to increased inhibition of thalamocortical neurons,[2] which send excitatory projections to the RTN.[32] The resulting inhibition of RTN however, may be masked by a decreased activity of cholinergic inputs from the pedunculopontine nucleus, which is also inhibited by the substantia nigra pars reticulata.[25] Decreased cholinergic inhibition of reticular thalamic neurons allows them to fire in an oscillatory mode, consisting of prolonged bursts of depolarizations followed by periods of inactivity.[41,64] This pattern of firing would be consistent with increased expression of GAD mRNA.[35,59] It is unclear, however, whether decreased cholinergic stimulation plays a role in the effects observed in the present study. The muscarinic antagonist scopolamine, at a dose sufficient to block catalepsy and changes in pallidal GAD mRNA induced by haloperidol,[14] failed to alter GAD67 mRNA levels in the RTN. However, other doses and durations of treatment need to be examined before ruling out a role for decreased cholinergic transmission.

Clinical implications

It has been proposed that oscillations in neurons of the RTN may underlie the resting tremor observed in Parkinson's disease.[7,46] The present data reveal a functional link between dopamine depletion and the RTN, which can provide a clue for the origin of tremor in patients with Parkinson's disease. Another consequence of alterations in GABAergic transmission in the RTN may be the cognitive and attentional deficits that have been observed in patients with Parkinson's disease and primates treated with low doses of MPTP.[6,55] Indeed, the RTN, which ultimately affects thalamocortical and corticothalamic processing, has been classically implicated in attentional and cognitive processes,[13,51] although direct evidence is still lacking.

In conclusion, these results demonstrate a novel bilateral effect of unilateral dopamine depletion. Considering that the RTN is integral in the control of thalamocortical and corticothalamic information flow, the data suggest an additional mechanism by which nigrostriatal dopamine depletion may have widespread effects on brain function. Changes in neuronal activity in the RTN deserve further attention in the study of experimental models of Parkinson's disease.

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REFERENCES

1. Aceves J., Floran B., Martinez-Fong D., Benitez J., Sierra A. and Flores G. (1992) Activation of D1 receptors stimulates accumulation of gamma-aminobutyric acid in slices of the pars reticulata of 6-hydoxydopamine-lesioned rats. *Neurosci. Lett.* **145,** 40–42.
2. Albin R. L., Young A. B. and Penney J. B. (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci.* **12,** 366–375.
3. Battaglia G., Lizier C., Colacitti C., Princivalle A. and Spreafico R. (1994) A reticuloreticular commissural pathway in the rat thalamus. *J. comp. Neurol.* **347,** 127–138.
4. Beckstead R. M., Domesick V. B. and Nauta W. J. H. (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res.* **175,** 191–217.
5. Benson D. L., Huntsman M. M. and Jones E. G. (1994) Activity dependent changes in GAD and preprotachykinin mRNAs in visual cortex of adult monkeys. *Cerebr. Cortex* **4,** 40–51.
6. Brown R. G. and Marsden C. D. (1988) Internal versus external cues and the control of attention in Parkinson's disease. *Brain* **111,** 323–345.
7. Buzsáki G., Smith A., Berger S., Fisher L. J. and Gage F. H. (1990) Petit mal epilepsy and parkinsonian tremor: hypothesis of a common pacemaker. *Neuroscience* **36,** 1–14.
8. Cheramy A., Leviel V. and Glowinski J. (1981) Dendritic release of dopamine in the substantia nigra. *Nature* **289,** 537–542.
9. Chesselet M.-F., Weiss L., Wuenschell C., Tobin A. J. and Affolter H. U. (1987) Comparative distributions of mRNAs encoding glutamic acid decarboxylase, tyrosine hydroxylase, and tachykinins in the basal ganglia: an *in situ* hybridization study in rodent brain. *J. comp. Neurol.* **262,** 125–140.
10. Chesselet M.-F. and Weiss-Wunder L. T. (1994) Quantification of *in situ* hybridization histochemistry. In *In Situ Hybridization in Neurobiology* (eds Eberwine J. H., Valentino K. L. and Barchas J. D.), pp. 114–123. Oxford Univ. Press, New York.
11. Chevalier G., Vacher S. and Deniau J. M. (1984) Inhibitory nigral influence on tectospinal neurons, possible implication of basal ganglia orienting behavior. *Expl Brain Res.* **53,** 320–326.
12. Cornwall J., Cooper J. D. and Phillipson O. T. (1990) Projections to the rostral reticular thalamic nucleus in the rat. *Expl Brain Res.* **80,** 151–171.
13. Crick F. (1984) Function of the thalamic reticular complex: the searchlight hypothesis. *Proc. natn. Acad. Sci. U.S.A.* **81,** 4586–4590.
14. Delfs J. M., Anegawa N. J. and Chesselet M.-F. (1995) Glutamate decarboxylase messenger RNA in rat pallidum: comparison of the effects of haloperidol, clozapine and combined haloperidol-scopolamine treatments. *Neuroscience* **66,** 67–80.
15. Delfs J. M., Ciaramitaro V. M., Parry T. J. and Chesselet M.-F. (1995) Subthalamic nucleus lesions: widespread effects on changes in gene expression induced by nigrostriatal dopamine depletion in rats. *J. Neurosci.* **15,** 6562–6575.
16. Delfs J. M., Ellison G. D., Mercugliano M. and Chesselet M.-F. (1995) Expression of glutamic acid decarboxylase mRNA in striatum and pallidum in an animal model of tardive dyskinesia. *Expl Neurol.* **133,** 175–188.
17. Ellison G. D. and See R. E. (1989) Rats administered chronic neuroleptics develop oral movements which are similar in form to those in humans with tardive dyskinesia. *Psychopharmacologia* **98,** 564–566.
18. Erlander M. G. and Tobin A. J. (1991) The structural and functional heterogeneity of glutamic acid decarboxylase: a review. *Neurochem. Res.* **16,** 215–226.
19. Esclapez M., Tillakaratne N. J. K., Tobin A. J. and Houser C. R. (1993) Comparative localization of mRNAs encoding two isoforms of glutamic acid decarboxylase with nonradioactive *in situ* hybridization methods. *J. comp. Neurol.* **331,** 339–362.
20. Feldblum S., Erlander M. G. and Tobin A. J. (1993) Different distributions of GAD65 and GAD67 mRNAs suggest that the two glutamate decarboxylases play distinctive functional roles. *J. Neurosci. Res.* **34,** 689–706.
21. Filion M. and Tremblay L. (1991) Abnormal spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced parkinsonism. *Brain Res.* **547,** 142–151.
22. Gandia J. A., De Las Heras S., Garcia M. and Gimenez-Amaya J. M. (1993) Afferent projections to the reticular thalamic nucleus from the globus pallidus and substantia nigra in the rat. *Brain Res. Bull.* **32,** 351–358.
23. Gerfen C. R., Engber T. M., Mahan L. C., Susel Z., Chase T. N., Monsma F. J. and Sibley D. R. (1990) D1 and D2 dopamine-receptor regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250,** 1429–1432.
24. Gonon F. G. (1988) Nonlinear relationship between impulse flow and dopamine release by midbrain neurons as studied by *in vivo* electrochemistry. *Neuroscience* **24,** 19–28.
25. Granata A. R. and Kitai S. T. (1991) Inhibitory substantia nigra inputs to the pedunculopontine neurons. *Expl Brain Res.* **86,** 459–466.
26. Hallanger A. E., Levey A. I., Lee H. J., Rye D. B. and Wainer B. H. (1987) The origins of cholinergic and other subcortical afferents to the thalamus in the rat. *J. comp. Neurol.* **262,** 105–124.
27. Hazrati L. N. and Parent A. (1991) Projection from the external pallidum to the reticular thalamic nucleus in the squirrel monkey. *Brain Res.* **550,** 142–146.
28. Herkenham M. (1979) The afferent and efferent connections of the ventromedial thalamic nucleus in the rat. *J. comp. Neurol.* **183,** 487–518.
29. Holm E. S. and Carlsson M. L. (1994) Effects of the NMDA receptor antagonist MK-801 on thalamic GAD mRNA. *Soc. Neurosci. Abstr.* **20,** 562.
30. Houser C. R., Vaughn J. E., Barber R. P. and Roberts E. (1980) GABA neurons are the major cell type of the nucleus reticularis thalami. *Brain Res.* **200,** 341–354.
31. Jackson A. and Crossman A. R. (1983) Nucleus tegmenti pedunculopontinus: efferent connections with special reference to the basal ganglia, studied in the rat by anterograde and retrograde transport of horseradish peroxidase. *Neuroscience* **10,** 725–765.
32. Jones E. G. (1975) Some aspects of the organization of the thalamic reticular complex. *J. comp. Neurol.* **162,** 285–308.
33. Kaufman D. L., Houser C. R. and Tobin A. J. (1991) Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. *J. Neurochem.* **56,** 720–723.

34. Kaufman D. L., McGinnis J. F., Krieger N. R. and Tobin A. J. (1986) Brain glutamate decarboxylase cloned in lambda gt11: fusion protein produces gamma aminobutyric acid. *Science* **232,** 1138–1140.
35. Kincaid A. E., Albin R. L., Newman S. W., Penney J. B. and Young A. B. (1992) 6-hydroxydopamine lesions of the nigrostriatal pathway alter the expression of glutamate decarboxylase messenger RNA in rat globus pallidus projection neurons. *Neuroscience* **51,** 705–718.
36. Lavin A. and Grace A. A. (1994) Modulation of the dorsal thalamic cell activity by the ventral pallidum: its role in the regulation of thalamocortical activity by the basal ganglia. *Synapse* **18,** 104–127.
37. Lindefors N., Brene S., Herrera-Marschitz M. and Persson H. (1989) Region specific regulation of glutamic acid decarboxylase mRNA expression by dopamine neurons in rat brain. *Expl Brain Res.* **77,** 611–620.
38. Litwak J., Mercugliano M., Chesselet M.-F. and Oltmans G. A. (1990) Increased glutamic acid decarboxylase (GAD) mRNA and GAD activity in cerebellar Purkinje cells following lesion-induced increases in cell firing. *Neurosci. Lett.* **116,** 179–183.
39. MacLeod N. K., Kilpatrick I. C. and Starr M. S. (1980) Evidence for a GABAergic nigrothalamic pathway in rat—II. Electrophysiological studies. *Expl Brain Res.* **40,** 55–61.
40. Martin D. L. and Rimvall K. (1993) Regulation of gamma-aminobutyric acid synthesis in the brain. *J. Neurochem.* **60,** 395–407.
41. McCormick D. A. (1989) Cholinergic and noradrenergic modulation of thalamocortical processing. *Trends Neurosci.* **12,** 215–221.
42. Miller W. C. and Delong M. R. (1987) Altered tonic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of parkinsonism. *Adv. Behav. Biol.* **32,** 415–427.
43. Oertel W. H., Graybiel A. M., Mugnaini E., Elde R. P., Schmechel D. E. and Kopin I. J. (1981) Coexistence of glutamic acid decarboxylase and somatostatin-like immunoreactivity in neurons of the feline nucleus reticularis thalami. *J. Neurosci.* **3,** 1322–1332.
44. Ohara P. T. and Lieberman A. R. (1985) The thalamic nucleus of the adult rat: experimental anatomical studies. *J. Neurocytol.* **14,** 365–411.
45. Pan H. S. and Walters J. R. (1988) Unilateral lesion of the nigrostriatal pathway decreases the firing rate and alters the firing pattern of globus pallidus neurons in the rat. *Synapse* **2,** 650–656.
46. Paré D., Curro Dossi R. and Steriade M. (1990) Neuronal basis of the parkinsonian resting tremor: a hypothesis and its implications for treatment. *Neuroscience* **35,** 217–226.
47. Paré D., Hazrati L. N., Parent A. and Steriade M. (1990) Substantia nigra pars reticulata projects to the reticular thalamic nucleus of the cat: a morphological and electrophysiological study. *Brain Res.* **535,** 139–146.
48. Parent A. and Hazrati L. N. (1995) Functional anatomy of the basal ganglia—II. The place of the subthalamic nucleus and external pallidum in basal ganglia circuitry. *Brain Res. Rev.* **20,** 128–154.
49. Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. Academic Press, San Diego.
50. Raos V. and Bentivoglio M. (1993) Crosstalk between the two sides of the thalamus through the reticular nucleus: a retrograde and anterograde tracing study in the rat. *J. comp. Neurol.* **332,** 145–154.
51. Ross D. T., Graham D. I. and Adams J. H. (1993) Selective loss of neurons from the thalamic reticular nucleus following severe human head injury. *J. Neurotrauma* **10,** 151–165.
52. Saller C. F. and Salama A. I. (1986) D-1 and D-2 dopamine receptor blockade: Interactive effects *in vitro* and *in vivo*. *J. Pharmac. exp. Ther.* **236,** 714–720.
53. Saper C. B. and Loewy A. D. (1982) Projections of the pedunculopontine tegmental nucleus in the rat: evidence for additional extrapyramidal circuitry. *Brain Res.* **252,** 367–372.
54. Savaki H. E., Girault J. A., Desban M., Glowinski J. and Besson M. J. (1985) Local cerebral metabolic effects induced by nigral stimulation following ventromedial thalamic lesions—II. Sensory motor, reticular and limbic systems. *Brain Res. Bull.* **14,** 287–296.
55. Schneider J. S. and Kovelowski C. J. (1990) Chronic exposure to low doses of MPTP—I. Cognitive deficits in motor asymptomatic monkeys. *Brain Res.* **519,** 122–128.
56. See R. E., Levin E. D. and Ellison G. D. (1988) Characteristics of oral movements in rats during and after chronic haloperidol and fluphenazine administration. *Psychopharmacologia* **94,** 421–427.
57. Semba K. and Fibiger H. C. (1992) Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: a retro- and anterograde transport and immunohistochemical study. *J. comp. Neurol.* **323,** 387–410.
58. Sofroniew M. V., Priestley J. V., Consolazione A., Eckenstein F. and Cuello A. C. (1985) Cholinergic projections from the midbrain and pons to the thalamus in the rat, identified by combined retrograde tracing and choline acetyltransferase immunohistochemistry. *Brain Res.* **329,** 213–223.
59. Soghomonian J. J. and Chesselet M.-F. (1992) Effects of nigrostriatal lesions on the levels of messenger RNAs encoding the two isoforms of glutamate decarboxylase in the globus pallidus and entopeduncular nucleus of the rat. *Synapse* **11,** 124–133.
60. Soghomonian J. J., Gonzales C. and Chesselet M.-F. (1992) Messenger RNAs encoding glutamate-decarboxylases are differentially affected by nigrostriatal lesions in subpopulations of striatal neurons. *Brain Res.* **576,** 68–79.
61. Soghomonian J. J., Pedneault S., Audet G. and Parent A. (1994) Increased glutamic acid decarboxylase mRNA levels in the striatum and pallidum of MPTP-treated primates. *J. Neurosci.* **14,** 6256–6265.
62. Spann B. M. and Grofova I. (1991) Nigropedunculopontine projection in the rat: an anterograde tracing study with *Phaseolus vulgaris*-leucoagglutinin (PHA-L). *J. comp. Neurol.* **311,** 375–388.
63. Spreafico R., Amadeo A., Angoscini P., Panzica F. and Battaglia G. (1993) Branching projections from the mesopontine nuclei to the nucleus reticularis and related thalamic nuclei: a double labelling study in the rat. *J. comp. Neurol.* **336,** 481–492.
64. Steriade M. (1992) Basic mechanism of sleep generation. *Neurology* **42,** 9–18.
65. Toan D. L. and Schultz W. (1985) Responses of rat pallidum cells to cortex stimulation and effects of altered dopaminergic activity. *Neuroscience* **15,** 683–694.
66. Vernier P., Julien J.-F., Rataboul P., Fourrier O., Feuerstein C. and Mallet J. (1988) Similar time course in striatal levels of glutamic acid decarboxylase and proenkephalin mRNA following dopaminergic deafferentiation in the rat. *J. Neurochem.* **51,** 1375–1380.

67. Wooten G. F. and Collins R. C. (1981) Metabolic effects of unilateral lesion of the substantia nigra. *J. Neurosci.* **1,** 285–291.
68. Yamamoto T., Hassler R., Huber C., Wagner A. and Sasaki K. (1983) Electrophysiological studies on the pallido- and cerebellothalamic projections in squirrel monkeys (*Saimiri sciureus*). *Expl Brain Res.* **51,** 77–87.

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