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Lesion of Subthalamic or Motor Thalamic Nucleus in 6-Hydroxydopamine-Treated Rats: Effects on Striatal Glutamate and Apomorphine-Induced Contralateral Rotations

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ABSTRACT A unilateral lesion of the rat nigrostriatal pathway with 6-hydroxydopamine (6-OHDA) results in a decrease in the basal extracellular level of striatal glutamate, a nearly complete loss of tyrosine hydroxylase (TH) immunolabeling, an increase in the density of glutamate immunogold labeling within nerve terminals making an asymmetrical synaptic contact, and an increase in the number of apomorphine-induced contralateral rotations. [Meshul et al. (1999) Neuroscience 88:1–16; Meshul and Allen (2000) Synapse 36:129–142]. In Parkinson's disease, a lesion of either the subthalamic nucleus (STN) or the motor thalamic nucleus relieves the patient of some of the motor difficulties associated with this disorder. In this rodent model, either the STN or motor thalamic nucleus was electrolytically destroyed 2 months following a unilateral 6-OHDA lesions. Following a lesion of either the STN or motor thalamic nucleus in 6-OHDA-treated rats, there was a significant decrease (40–60%) in the number of apomorphine-induced contralateral rotations compared to the 6-OHDA group. There was a significant decrease (<30%) in the basal extracellular level of striatal glutamate in all of the experimental groups compared to the sham group. Following an STN and/or 6-OHDA lesion, the decrease in striatal extracellular levels was inversely associated with an increase in the density of nerve terminal glutamate immunolabeling. There was no change in nerve terminal glutamate immunogold labeling in either the motor thalamic or motor thalamic plus 6-OHDA lesion groups compared to the sham group. The decrease in the number of apomorphine-induced rotations was not due to an increase in TH immunolabeling (i.e., sprouting) within the denervated striatum. This suggests that alterations in striatal glutamate appear not to be directly involved in the STN or motor thalamic lesion-induced reduction in contralateral rotations. **Synapse 51:287–298, 2004.** Published 2003 Wiley-Liss, Inc.[†]

INTRODUCTION

Within the striatum, the dopamine terminals originating from the substantia nigra pars compacta (SNpc) make synaptic connections with the necks of dendritic spines associated with medium spiny neurons, while glutamate terminals from the sensorimotor cortex, which project to the dorsolateral striatum, make contact with the heads of those same spines (Bouyer et al., 1984; Dube et al., 1988; Smith et al., 1994). Not only are dopamine and glutamate terminals anatomically located next to each other, these two neurotransmitters can control not only their own release but also the release from each other's nerve terminals (Morari et al., 1994, 1996; Yamamoto and Davy, 1992).

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We and others have reported that loss of dopamine neurons within the SNpc results in changes in glutamate synaptic input within the striatum (Lindefors and Ungerstedt, 1990; Ingham et al., 1998; Meshul et al., 1999; Jonkers et al., 2002). Electrophysiologically, such dopamine loss results in an increase in spontaneous activity of striatal neurons (Calabresi et al., 1993; Chang and Webster, 1997; Nieoullon and Kerkerian-LeGoff, 1992; Nisenbaum et al., 1986; Schultz and Ungerstedt, 1978). We have reported time-dependent changes in the basal extracellular level of striatal glutamate following a unilateral lesion of the nigrostriatal pathway using the neurotoxin 6-hydroxydopamine (6-OHDA), and have correlated this with alterations in the density of glutamate immunogold labeling within nerve terminals associated with an asymmetrical (excitatory) synaptic contact (Meshul et al., 1999). One month following the lesion there was an increase in the basal extracellular level of striatal glutamate and this was associated with a decrease in the density of nerve terminal glutamate immunolabeling. Three months following the lesion the decrease in the basal extracellular level of striatal glutamate was associated with an increase in the density of glutamate nerve terminal immunolabeling. This time-dependent reversal in striatal glutamate extracellular levels and nerve terminal glutamate immunolabeling may be due to changes in activity of the thalamo-cortico-striatal pathway. This time-dependent change could be due to alterations in the GABAergic input to the motor thalamus, which originates from both the substantia nigra pars reticulata (SNpr) and the internal globus pallidus (GPi), which could be influenced by the excitatory (glutamate) afferents to either of these structures from the subthalamic nucleus (STN) (Albin et al., 1989).

A behavioral method used to ascertain the success of a unilateral nigrostriatal lesion is to determine the number of contralateral rotations following the injection of the dopamine D1/D2 agonist apomorphine (Ungerstedt, 1971; Meshul et al., 1999). We have reported that following unilateral dopamine denervation of the striatum, subchronic administration of either the dopamine D2 antagonist, haloperidol, the noncompetitive glutamate receptor antagonist, MK-801, or nicotine, results in a decrease in the number of apomorphine-induced contralateral rotations (Meshul and Allen, 2000; Robinson et al., 2001; Meshul et al., 2002).

It has also been reported that in patients with Parkinson's disease, a lesion of either the STN or a subregion of the motor thalamus (ViM) results in a decrease in motor symptoms associated with this movement disorder (Benabid et al., 1987, 1991; Krack et al., 2000; Narabayashi et al., 1984). In the 6-OHDA rat model of Parkinson's disease in which there is a unilateral lesion of the nigrostriatal pathway, a lesion of either the STN or the motor thalamus reduces the number of apomorphine-induced contralateral rotations (STN: Blandini et al., 1997; Burbaud et al., 1995; Delfs et al., 1995; Garcia-Munoz et al., 1983; Henderson et al., 1999; Motor thalamus: Jenner et al., 1979; Reavill et al., 1981).

According to the model of basal ganglia function as hypothesized by Albin et al. (1989), a lesion of the nigrostriatal pathway should result in a decrease in the activity of the thalamocortical pathway. This model also suggests that such a lesion would produce a decrease in activity of the corticostriatal pathway, a result consistent with our 3-month 6-OHDA data (Meshul et al., 1999). Apomorphine administration leads to an increase in the number of contralateral rotations in a unilateral 6-OHDA lesioned animal compared to the nonlesioned control. Subchronic administration of this dopamine agonist (0.05 mg/kg/d for 28 days) also leads to an increase in the extracellular level of striatal glutamate compared to the saline-treated, but 6-OHDA-lesioned, group (Meshul et al., 2002). In addition, we have reported that administration of the glutamate antagonist, MK-801, leads to a decrease in the number of apomorphine-induced contralateral rotations (Robinson et al., 2001), suggesting that blockade of glutamate synaptic function appears to be associated with this behavior. We therefore investigated whether by decreasing the excitatory output from the STN or lesioning the motor thalamus would not only result in a decrease in the number of apomorphine-induced contralateral rotations, as reported by others (Blandini et al., 1997; Burbaud et al., 1995; Delfs et al., 1995; Garcia-Munoz et al., 1983; Henderson et al., 1999; Jenner et al., 1979; Reavill et al., 1981), but be capable of reversing the decrease in the basal extracellular level of striatal glutamate, as we reported in the unilateral nigrostriatal-lesioned animal (Meshul et al., 1999).

MATERIALS AND METHODS

Unilateral lesion of nigrostriatal pathway

Male Sprague-Dawley rats (n = 7–9 for each treatment group; 250–270 g, Harlan Labs, Indianapolis, IN) were maintained on a 12-h light/dark cycle with continuous access to food and water. All animal experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication 80-23, revised 1978) and all procedures were approved by the Portland VA Medical Center Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used. The rats were anesthetized (1 ml/kg of 5% ketamine, 2% xylazine, and 1% acepromazine) and the left medial forebrain bundle (MFB) was injected with 6-OHDA according to previously published methods (Meshul et al., 1999, 2000, 2002). For the control (sham) animals, a hole was drilled at the above coordinates for an MFB lesion and then the skin was stapled together.

All rats were then tested for contralateral turning 2 months following the lesion. The animals were first placed in individual cages for 15 min to acclimate, injected s.c. with 0.05 mg/kg of apomorphine (Sigma Chemical, St. Louis, MO), and then the number of contralateral rotations measured 15 min after the apomorphine injection. The number of rotations was counted over the next 5 min (first challenge with apomorphine). Only those lesioned animals showing robust contralateral turning (>7 turns/min) that were injected with 6-OHDA were used in subsequent experiments (O'Dell and Marshall, 1996). If lesioned animals rotated less than 7 turns/min they were not used in the study. It has been reported that animals that do show robust apomorphine-induced contralateral rotations have a >95% loss of striatal dopamine or tyrosine hydroxylase immunolabeling (Meshul et al., 1999; Meshul and Allen, 2000; Mura et al., 1998). None of the sham-lesioned animals rotated using this dose of apomorphine (Meshul and Allen, 2000; Mura et al., 1998; Neve et al., 1982) and were used as the control group.

Electrolytic lesion

Two weeks after testing the 6-OHDA-treated animals with apomorphine, the left STN or motor thalamic nucleus was electrolytically lesioned in both 6-OHDA and sham-lesioned animals. Due to the rostral-caudal length of both these nuclei, electrolytic lesions were carried out at two locations within the STN or motor thalamus at the following coordinates from bregma according to Paxinos and Watson (1986): subthalamic: anterior: −3.4 and −3.9 mm; lateral: 2.5 mm; dorsal/ventral: 8.1 mm; motor thalamus (VM): anterior: −2.6 and −3.0; lateral: 1.4 mm; dorsal/ventral: 7.4 mm). The electrolytic needle (Rhodes Medical Instruments, Woodland, Hills, CA), was attached to the stereotaxic apparatus and lowered to the STN or motor thalamic nucleus. The electrolytic apparatus (Biological Research Apparatus, Model 3500, 115V, 60 Hz, 0.25A), was set for a duration of 3 sec at 0.5 amps. After the lesion at the first location the needle was moved to the second set of coordinates, lowered, and then the second lesion carried out. To verify the correct location of the STN and motor thalamic lesion the glutaraldehyde fixed brains (see below) were serially cut (100 μm thick sections) with a vibratome through the site of the lesion. The results from an animal were discarded if the lesion was not located within the STN or motor thalamic nucleus. Immediately following the electrolytic lesion, an additional hole was drilled through the left side of the skull for placement of the guide cannula above the left dorsolateral striatum, as previously described (Meshul et al., 1999, 2002).

Two weeks after the electrolytic lesion, all the animals were tested for apomorphine-induced contralateral rotations (second challenge with apomorphine) as described above. The percent change in contralateral rotations between the second and first challenge (i.e., number of rotations at second challenge/number of rotations at first challenge) was then determined. An overall group mean was calculated (mean percentage ± SEM) and the lesioned groups compared with each other using Student's *t*-test. Two days after the second apomorphine challenge, in vivo microdialysis was carried out (see below).

In vivo microdialysis measurement of extracellular glutamate

Dialysis probes (2 mm in length) were prepared as described by Robinson and Wishaw (1988) with modifications (Meshul et al., 1999, 2002). Glutamate concentration in the dialysate was determined using a Hewlett Packard HPLC 1090 interfaced with a Hewlett Packard 1046A Programmable Fluorescence Detector. Dialysates were derivatized with o-phthalaldehyde (OPA) and chromatographed according to a modification of the method of Schuster (1988), as previously described (Meshul et al., 1999).

One day prior to the start of the microdialysis experiment, the probe was inserted through the guide cannula within the dorsolateral striatum and perfused with artificial cerebrospinal fluid (aCSF) (0.2 μl/min; 140 mM NaCl, 3.4 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.4 mM NaH_2PO_4, and 4.85 mM Na_2HPO_4, pH 7.4) and the fluid was left to perfuse through the probe overnight (0.2 μl/min). The following morning, the pump speed was increased up to 2 μl/min for 1 h and four samples were then collected every 15 min to determine the basal level of extracellular glutamate. At the conclusion of the experiment the animals were perfused with glutaraldehyde fixative (see below), vibratome sections (100 μm thick) cut, stained with thionin, and the site of the probe placement within the striatum and the success of the electrolytic lesions verified histologically. Probe placement extended 2 mm along the dorsolateral quadrant of the rostral striatum. If the placement was not correct (i.e., outside the striatum), data from those animals were discarded. The four baseline data points were averaged at each time point (i.e., 15, 30, 45, 60 min) and then an overall mean determined. The values are expressed as the mean ± SEM in picomoles/ml basal extracellular level of striatal glutamate. The mean probe recovery was 12.4 ± 1.2%. All data between groups were analyzed using a one-way ANOVA. Significant main effects were further characterized using Peritz' *f*-test for comparison of multiple means. We have previously verified that changes in the basal extracellular level of striatal glutamate are dependent on the presence of calcium within the aCSF. Replacement of calcium with the divalent chelating agent, EGTA, and increasing the aCSF concentration of magnesium resulted in a significant decrease in the basal level of glutamate (Meshul et al., 2002), suggesting that a significant proportion of

the resting level of striatal glutamate is of neuronal and not glial origin.

Light microscopic immunocytochemistry

A separate series of rats (n = 3 for each group: control, 6-OHDA, 6-OHDA/STN lesion, 6-OHDA/motor thalamic lesion) were used for the light microscopic immunocytochemical localization of tyrosine hydroxylase (TH) within the dorsolateral striatum. These experiments were carried out in order to determine if the STN or motor thalamic lesion resulted in a change in the relative density of TH immunolabeling within the dorsolateral striatum on the 6-OHDA lesioned (left) side compared to the 6-OHDA lesion group alone. Two days following the second challenge dose of apomorphine (see above), the rats were perfused with fixative (1% acrolein with 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3) and TH immunolabeling carried out as previously described (Meshul and Allen, 2000; Robinson et al., 2003). The TH antibody (monoclonal, Diasorin, Minneapolis, MN) was diluted 1:30,000. As a control, elimination of the primary antibody resulted in a complete lack of tissue immunolabeling (data not shown).

Optical density measurements were carried out by first capturing the image taken directly from the immunoreactive material using a Zeiss Axioplan light microscope (Carl Zeiss, West Germany) and transporting the image using a Polaroid Digital Microscope Camera (Cambridge, MA) at 1.25× magnification. Relative optical density measurements were calculated using Image-Pro Plus software (v. 3.01, Media Cybernetics, Silver Springs, MD). Six slices from each group were analyzed (alternate sections starting from +1.7 to +0.5 mm from bregma according to Paxinos and Watson (1986)). A computer-generated mean optical density for the dorsolateral striatum of both the lesioned (left) and unlesioned (right) sides was collected. The relative optical density of the left (lesioned) and right (unlesioned) dorsolateral striatum was determined and averaged for each slice. The dorsolateral striatum was analyzed since this is the major area of input of the corticostriatal pathway (McGeorge and Faull, 1989). The relative density of the overlying corpus callosum was taken as a background measure and subtracted from the value generated from the dorsolateral striatum. The data were analyzed using a one-way ANOVA followed by a post-hoc analysis using Peritz' *f*-test for comparison of multiple means (Meshul et al., 1999). Analysis of the immunolabeled tissue was carried out by an individual blinded to the experimental group. Measurements of the optical density of striatal TH immunolabeling in 6-OHDA-lesioned animals has been reported to be a valid index of dopamine fiber innervation and has been correlated with the apomorphine-induced contralateral rotation behavior (Burke et al., 1990).

Electron microscopy/immunocytochemistry

Due to the tissue damage of the dorsolateral striatum following in vivo microdialysis, the same rats could not be used for the electron microscopic or TH immunolabeling studies. Therefore, in a separate group of rats (n = 7–9 for each treatment group), 2 days after the second challenge dose, in which the number of rotations at second challenge/number of rotations at first challenge was also determined as previously described above, the rats were perfused with fixative (2.5% glutaraldehyde, 0.5% paraformaldehyde, 0.1% picric acid in 0.1 M HEPES buffer, pH 7.3), the left dorsolateral striatum cut (single, 2×2 mm^2 piece) and processed as previously described (Meshul et al., 1994, 1999, 2000, 2002). This corresponds to the same area, although in a separate group of rats, that was sampled for the in vivo microdialysis and the TH immunolabeling studies detailed above. All animals from each treatment group were perfused, cut, and processed on the same day in order to limit the variables from perfusing rats on different days. Postembedding immunogold electron microscopy was performed according to the method of Phend et al. (1992), as previously modified (Meshul et al., 1994). The glutamate antibody, (nonaffinity-purified, rabbit polyclonal; Sigma), as previously characterized by Hepler et al. (1988), was diluted 1:200,000 in TBST (pH) 7.6. Aspartate (1 mM) was added to the glutamate antibody mixture 24 h prior to incubation with the thin-sectioned tissue to prevent any cross-reactivity with aspartate within the tissue. Photographs (10/animal) were taken randomly throughout the entire section of the dorsolateral striatum at a final magnification of ×40,500 and within the area of the neuropil (region of highest synapse density) using a digital camera (AMT, Boston, MA). The images were directly captured and stored on the computer. The glutamate immunolabeling technique was carried out for all of the treatment groups on the same day in order to limit the variables that may occur by carrying out this procedure on different days.

For glutamate immunolabeling, the number of gold particles per nerve terminal associated with an asymmetrical synaptic contact was counted and the area of the nerve terminal determined using Image Pro Plus software (Media Cybernetics, Silver Springs, MD, v. 3.01). Glutamate containing nerve terminals were typically photographed making a synaptic contact on a dendritic spine, indicating that they most likely originated from the motor cortex (Dube et al., 1988; Smith et al., 1994). The gold particles contacting the synaptic vesicles within the nerve terminal were counted and considered part of the vesicular or neurotransmitter pool as previously established (Meshul et al., 1999). In addition, the mitochondrial pool of glutamate was analyzed within nerve terminals to determine if the nonvesicular pool of glutamate was affected by the lesion. The density of gold particles/μm^2 of nerve terminal

TABLE I. Number of synapses making an asymmetrical synaptic contact analyzed

Treatment groups	6-OHDA/motor thalamic lesion study	Treatment groups	6-OHDA/STN lesion study
Control	241	Control	219
6-OHDA	245	6-OHDA	237
Thalamic lesion	215	STN lesion	238
6-OHDA/thalamic lesion	185	6-OHDA/STN lesion	242

area was determined for each animal and the mean density for each treatment group calculated (mean density ± SEM). The differences between treatment groups were analyzed using a one-way ANOVA and significant main effects were further characterized using the Fisher post-hoc test for comparison of multiple means. The specificity of the immunolabeling for the glutamate antibody was previously established by incubating the antibody overnight with 3 mM glutamate (Meshul et al., 1994). This mixture was then applied to the sections as detailed above.

The total number of synapses making an asymmetrical synaptic contact that were analyzed in each of the studies is shown in Table I.

RESULTS

Apomorphine-induced rotations

Two months after first testing the rats with apomorphine to ensure the success of the 6-OHDA nigrostriatal lesion, and 2 weeks following a motor thalamic (THAL) or STN lesion, the animals were tested again with apomorphine. The lesion of the STN or motor thalamus was verified histologically (Fig. 1A,B, respectively). In the 6-OHDA/STN lesioned group there was a nearly 50% decrease in the percentage of contralateral rotations after the second challenge dose of apomorphine compared to the first challenge dose. Overall, there was a 54% decrease in the percentage of contralateral rotations when the 6-OHDA and 6-OHDA/STN lesion groups were compared (Table II, Behavioral Study). In the 6-OHDA/THAL lesioned group there was a nearly 30% decrease in the percentage of contralateral rotations after the second challenge dose of apomorphine compared to the first challenge dose. Overall, there was a 46% decrease in the percentage of contralateral rotations when the 6-OHDA and 6-OHDA/THAL lesion groups were compared (Table 2, Behavioral Study). Apomorphine injection into the sham, STN, or motor thalamic lesion-only groups did not result in any contralateral or ipsilateral rotations (data not shown).

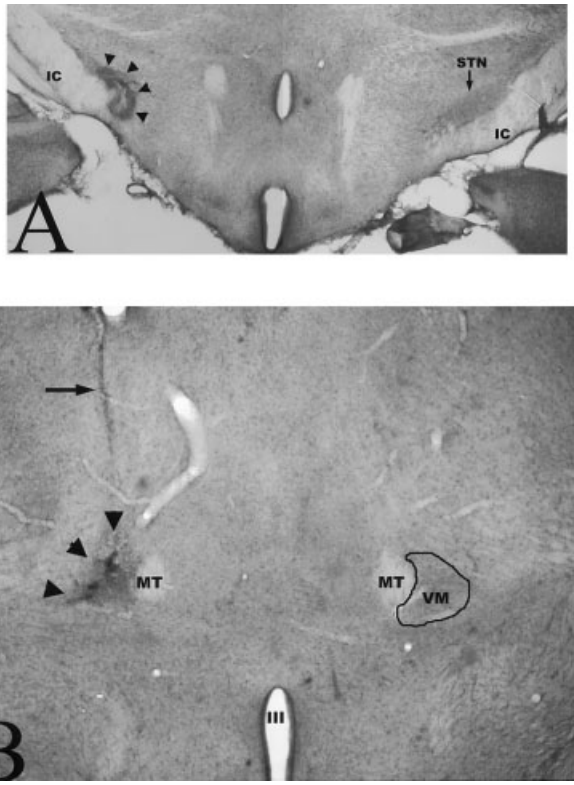


Fig. 1. **A:** Thionin-stained section illustrating an electrolytic lesion of the STN (left side, arrowheads) compared to the nonlesioned right STN (arrow). IC, internal capsule. **B:** Thionin-stained section illustrating an electrolytic lesion of the left motor thalamic nucleus (left side, arrowheads) compared to the nonlesioned right motor thalamus. MT, mammillothalamic tract. VM, ventromedial thalamic nucleus (motor thalamus). III, third ventricle. Arrow: needle track.

TABLE II. Apomorphine-induced contralateral rotations

Study	Treatment groups	Percent change in rotations (second challenge/first challenge)	Percent of 6-OHDA group
Behavior	6-OHDA	109.6 ± 6.0	
	6-OHDA/STN	50.1 ± 11.9*	54%
	6-OHDA	185.0 ± 24.0	
	6-OHDA/THAL	66.6 ± 10.2*	64%
Glutamate immunolabeling	6-OHDA	131.1 ± 13.1	
	6-OHDA/STN	71.4 ± 8.3*	46%
	6-OHDA	217.0 ± 48.1	
	6-OHDA/THAL	80.3 ± 34.9*	63%

*$P < 0.05$ compared to the 6-OHDA group using Student's *t*-test.
Two months following a 6-OHDA lesion, the animals were injected with apomorphine (0.05 mg/kg,s.c.) and the number of contralateral rotations counted (1st challenge with apomorphine). See Methods for details of the procedure. Two weeks after the electrolytic lesion, all the animals were tested for apomorphine-induced contralateral rotations (2nd challenge with apomorphine). The percent change in contralateral rotations between the 2nd and 1st challenge (i.e., number of rotations at 2nd challenge/number of rotations at 1st challenge) was then determined. An overall group mean was calculated (mean percentage ± SEM) and the lesioned groups compared against each other using Student's *t*-test.

In vivo microdialysis

Two days following the second apomorphine challenge, in vivo microdialysis was carried out. This time period between the last apomorphine injection and the microdialysis study allowed for sufficient washout of the drug, since only basal levels of extracellular glutamate were being assessed in the current study. The hypothesis was that a lesion of the STN or motor thalamus, resulting in a decrease in apomorphine-induced rotations, would also reverse the decrease in the basal

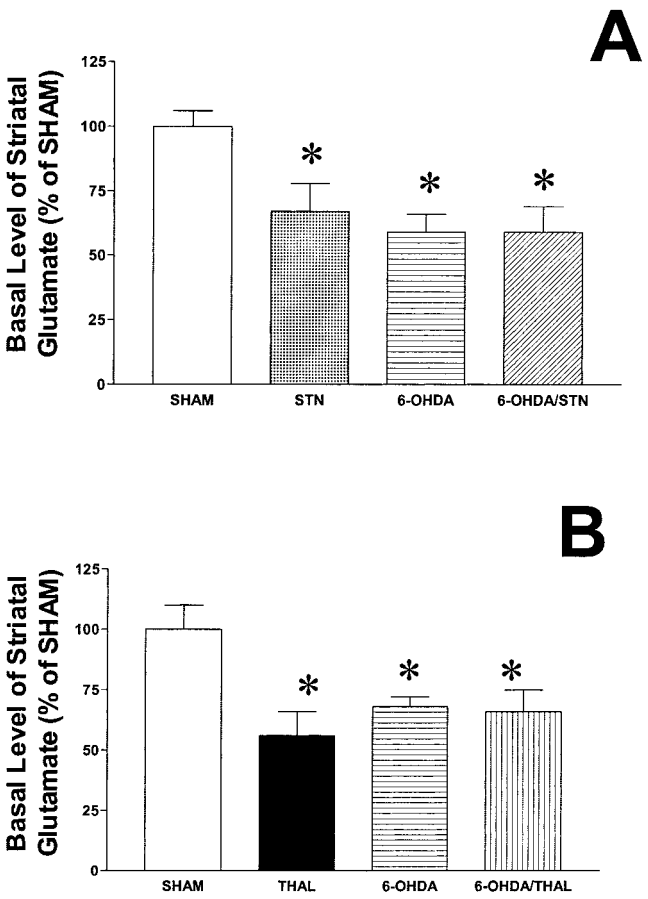


Fig. 2. **A:** In vivo microdialysis of the striatum 3 months following either a sham or 6-OHDA lesion of the nigrostriatal pathway alone (Sham, 6-OHDA), the subthalamic nucleus alone (STN), or a combined lesion of the nigrostriatal pathway and the subthalamic nucleus (6-OHDA/STN). There was a significant decrease in the basal extracellular levels of striatal glutamate in all of the lesioned groups compared to the sham group. **B:** In vivo microdialysis of the striatum 3 months following either a sham or 6-OHDA lesion of the nigrostriatal pathway alone (Sham, 6-OHDA), the motor thalamus alone (THAL), or a combined lesion of the nigrostriatal pathway and the motor thalamus (6-OHDA/THAL). There was a significant decrease in the basal extracellular levels of striatal glutamate in all of the lesioned groups compared to the sham group. $^*P < 0.05$ compared to the sham group as determined by Peritz' *f*-test for comparison of multiple means.

extracellular level of striatal glutamate due to a 6-OHDA lesion alone. A lesion of the medial forebrain bundle with 6-OHDA or a lesion of the STN or motor thalamus alone resulted in a decrease in striatal extracellular glutamate levels compared to the sham group (Fig. 2A,B, respectively). Although after the combined 6-OHDA/STN or 6-OHDA/THAL lesion there was a decrease in the apomorphine-induced contralateral rotations, the combined lesion resulted in a decrease in the basal extracellular level of striatal glutamate compared to the sham group.

Nerve terminal glutamate immunolabeling

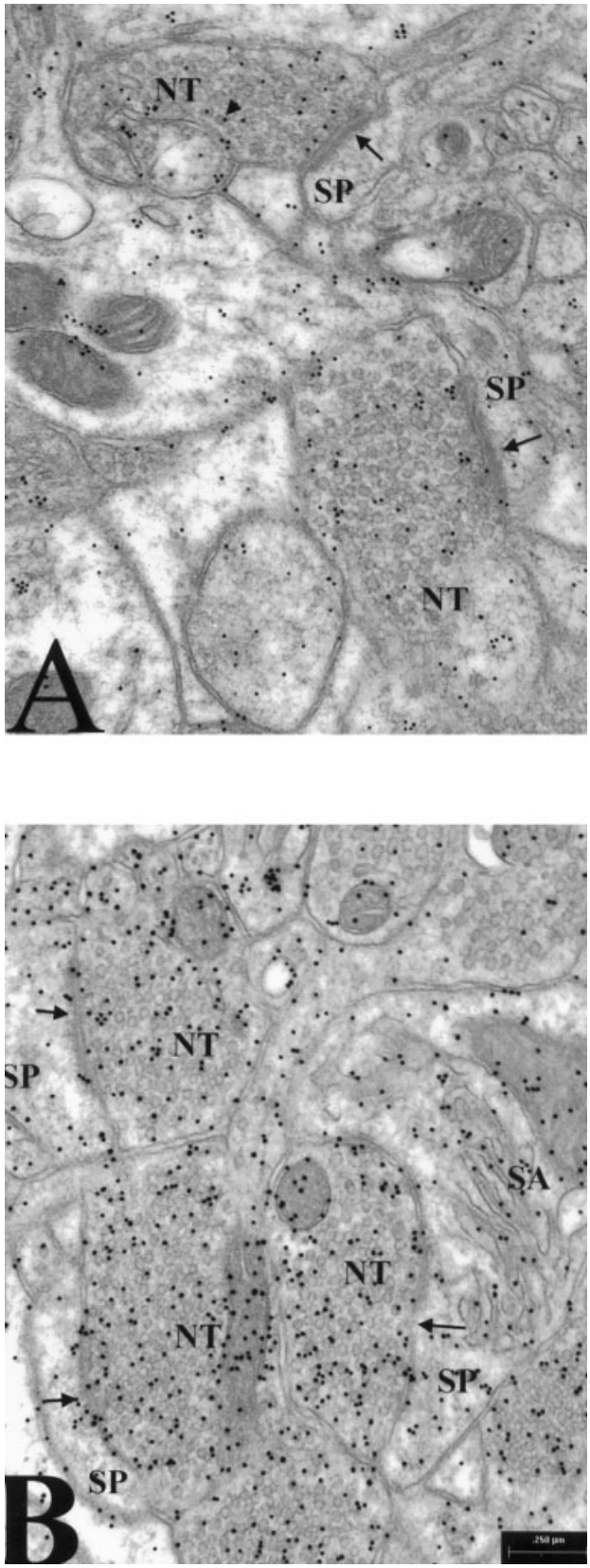
Since the left side of the striatum in the dialysis study was sufficiently damaged by the probe that well-preserved dorsolateral striatal tissue could not be taken, a separate series of rats were used for the electron microscopic studies (see Materials and Methods). These rats were similarly tested with apomorphine and there was a similar decrease (64%) in the number of apomorphine-induced contralateral rotations in the 6-OHDA/STN lesion group compared to the 6-OHDA group (Table II, Glutamate Immunolabeling). In the 6-OHDA/THAL lesioned group there was a decrease (63%) in the number of apomorphine-induced contralateral rotations compared to the 6-OHDA group (Table II, Glutamate Immunolabeling). Two days following the second challenge of apomorphine the animals were perfused with fixative for electron microscopic analysis. This time period allowed sufficient washout of the drug since the objective of this study was to determine the effects of either an STN or motor thalamic lesion on striatal glutamate nerve terminal immunolabeling in the 6-OHDA-lesioned rat. Glutamate immunolabeled nerve terminals from both the sham and 6-OHDA lesion groups are illustrated in Figure 3. A 6-OHDA lesion of the nigrostriatal pathway results in an increase in the density of glutamate immunolabeling within nerve terminals making an asymmetrical (excitatory) synaptic contact compared to the sham group (Fig. 4A). Following a lesion of the STN only or in the group with both a 6-OHDA and STN lesion, there continued to be an increase in the density of glutamate immunolabeling within nerve terminals making an asymmetrical synaptic contact (Fig. 4A). This immunogold data is inversely associated with the decrease in the extracellular levels of striatal glutamate (Fig. 2A).

Following a lesion alone of the motor thalamic nucleus, there was no change in the density of nerve terminal glutamate immunolabeling compared to the sham group (Fig. 4B). However, a motor thalamic lesion in animals previously treated with 6-OHDA resulted in a decrease in the density of nerve terminal glutamate immunolabeling compared to the 6-OHDA group (Fig. 4B). The density of glutamate immunolabeling within nerve terminals in the 6-OHDA/THAL group was similar to the sham group.

In both the STN and motor thalamic lesion studies, there was no change in the density of glutamate immunolabeling located within the mitochondrial pool between the experimental groups (data not shown). This suggests that the change in nerve terminal glutamate immunolabeling as shown in the 6-OHDA, 6-OHDA/STN, or STN only groups compared to the sham group (Fig. 4A,B) was specific for the vesicular vs. nonvesicular (i.e., mitochondrial) pool of glutamate.

Striatal TH immunolabeling

To determine whether the decrease in apomorphine-induced contralateral rotations in the 6-OHDA/STN or 6-OHDA/THAL groups compared to the 6-OHDA group



alone was potentially due to sprouting of new dopamine nerve terminals within the dopamine deafferented (left) striatum, the relative density of striatal TH immunolabeling was determined between the 6-OHDA and the combined lesion groups. An example of striatal TH labeling in the 6-OHDA and 6-OHDA/STN groups is illustrated in Figure 5. There was an equally significant decrease in the relative density of TH immunolabeling between the lesioned vs. the nonlesioned striatum in both the 6-OHDA and the 6-OHDA/STN groups (values are mean percent of nonlesioned side ± SEM: 6-OHDA: 13.7 ± 6.5%; 6-OHDA/STN lesion: 20.8 ± 6.6%, $P > 0.05$ using Student's t-test). A similar decrease in TH immunolabeling between the lesioned and nonlesioned striatum in the 6-OHDA and the 6-OHDA/THAL groups was also observed (6-OHDA and the 6-OHDA/THAL: values are mean percent of nonlesioned side ± SEM: 6-OHDA: 15.5 ± 8.5%; 6-OHDA/THAL: 20.4 ± 5.6%, $P > 0.05$ using Student's t-test). This finding suggests that following the lesion of the STN or motor thalamic nucleus, significant sprouting of dopamine fibers most likely had not taken place.

DISCUSSION

Following a unilateral loss of striatal dopamine using the neurotoxin 6-OHDA, a subsequent lesion of either the STN or motor thalamus results in a decrease in the apomorphine-induced contralateral rotations. Both an STN and motor thalamic lesion in animals previously treated with 6-OHDA resulted in a decrease in the extracellular levels of striatal glutamate. This decrease was similar to that observed following a 6-OHDA lesion alone. In this 6-OHDA group, together with the 6-OHDA/STN or STN-only group, the decrease in striatal extracellular levels was inversely associated with an increase in the density of glutamate immunolabeling in nerve terminals making an asymmetrical synaptic contact. However, in the motor thalamic lesion group alone or in combination with a 6-OHDA lesion there was no change in the density of nerve terminal glutamate immunolabeling compared to the control group. The decrease in the apomorphine-induced contralateral rotations following an STN or motor thalamic lesion in the group previously treated with 6-OHDA appeared not to be due to sprouting of new dopamine terminals within the striatum.

Fig. 3. Electron photomicrographs using the immunogold technique to localize an antibody against the neurotransmitter, glutamate, within the dorsolateral striatum. **A:** Sham group: within the nerve terminal (NT) there is an accumulation of small round synaptic vesicles and 10 nm gold particles indicating the location of the antibody (arrowhead). Both nerve terminals are making synaptic contact (arrow) with an underlying dendritic spine (SP). **B:** 6-OHDA lesioned group. Note the increased density of immunogold labeling in these nerve terminals compared to the sham preparation presented in **A**. SA, spine apparatus. Scale bar = 0.25 μm.

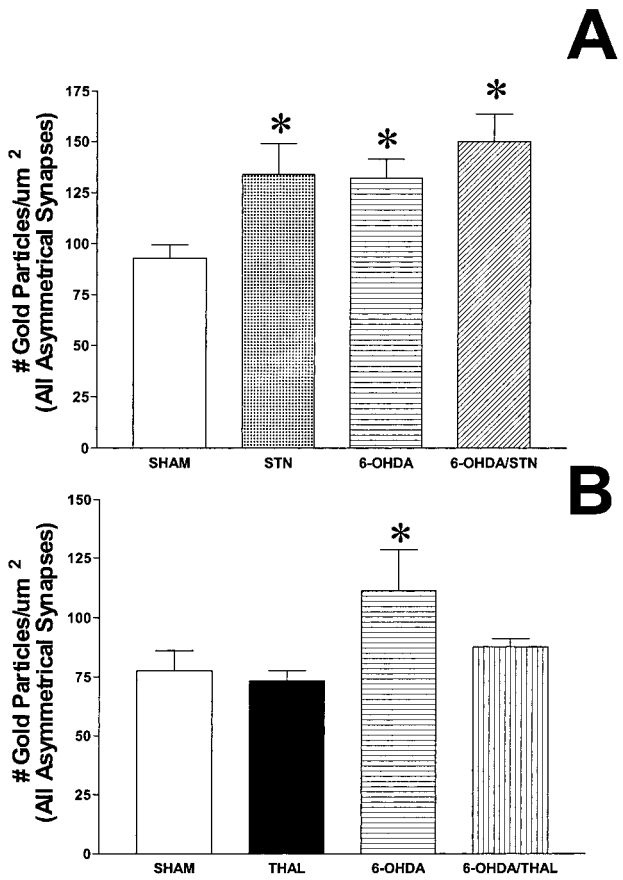


Fig. 4. **A:** Quantitative immunogold electron microscopy of the striatum 3 months following either a sham or 6-OHDA lesion of the nigrostriatal pathway alone (Sham, 6-OHDA), the subthalamic nucleus alone (STN), or a combined lesion of the nigrostriatal pathway and the subthalamic nucleus (6-OHDA/STN). There was a significant increase in the relative density of immunogold labeling within nerve terminals associated with an asymmetrical synaptic contact in all of the lesioned groups compared to the sham group. $*P < 0.05$ compared to the sham group as determined by the Fisher post-hoc test for comparison of multiple means. **B:** Quantitative immunogold electron microscopy of the striatum 3 months following either a sham or 6-OHDA lesion of the nigrostriatal pathway alone (Sham, 6-OHDA), the motor thalamus alone (THAL), or a combined lesion of the nigrostriatal pathway and the motor thalamus (6-OHDA/THAL). There was a significant increase in the relative density of immunogold labeling within nerve terminals associated with an asymmetrical synaptic contact in the 6-OHDA group alone compared to all the other groups. $*P < 0.05$ compared to all the other groups as determined by the Fisher post-hoc test for comparison of multiple means.

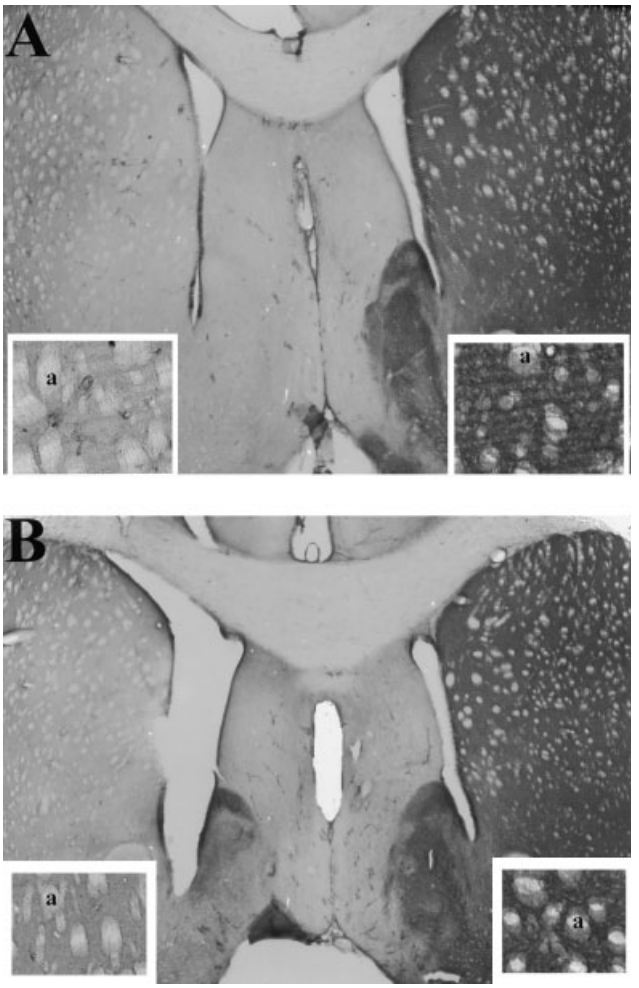


Fig. 5. Tyrosine hydroxylase (TH) immunolabeling within the striatum 3 months following either a 6-OHDA lesion alone (**A**) or following a combined 6-OHDA/STN lesion (**B**). The left side of the striatum was lesioned in both groups. Note the significant decrease in the relative density of TH immunolabeling on the lesioned (left) side compared to the nonlesioned (right) side in both groups of animals. Inset: higher magnification of the dorsolateral striatum from either the left or right sides for each treatment groups. The decrease in the relative density of TH immunolabeling on the lesioned side did not differ between the two treatment groups. a, myelinated axons.

Contralateral rotations

The decrease in the apomorphine-induced contralateral rotations in 6-OHDA-lesioned animals receiving either an STN or motor thalamic lesion compared to the nigrostriatal-lesioned group alone is in agreement with previous reports (STN: Blandini et al., 1997; Burbaud et al., 1995; Delfs et al., 1995; Henderson et al., 1999; motor thalamus: Garcia-Munoz et al., 1983; Jenner et al., 1979; Reavill et al., 1981). In the current study, electrolytic vs. a chemical/excitotoxic lesion was used to destroy either the STN or motor thalamus. Although it could be argued that an electrolytic lesion destroys the axons of passage compared to an excitotoxic lesion, it has been reported that the use of either method resulted in a similar decrease in apomorphine-induced contralateral rotations following a lesion of the motor thalamus (Garcia-Munoz et al., 1983). There were no apomorphine-induced ipsilateral rotations in naive animals receiving an STN or motor thalamic lesion alone. Although there have been reports of such rotations following an STN or motor thalamic lesion alone (Burbaud et al., 1995; Delfs et al., 1995; Garcia-Munoz et al., 1983), the dose of apomorphine used in these other studies was higher (0.15, 0.25, or 0.5 mg/kg) compared to the low dose of 0.05 mg/kg used in the current study. Similarly, it has been reported that, using this low dose of apomorphine, naive animals do

not rotate (Meshul and Allen, 2000; Meshul et al., 2002; Mura et al., 1998; Neve et al., 1982).

We hypothesized that the decrease in apomorphine-induced contralateral rotations following a lesion of either the STN or motor thalamus would be associated with a reversal in the decrease in striatal glutamate compared to the 6-OHDA-lesioned only group. Since the major glutamate input to the dorsolateral striatum originates from the motor cortex (McGeorge and Faull, 1989), it has been reported that either a lesion of the cortex in 6-OHDA-treated animals (Cenci and Bjorklund, 1993; Meshul et al., 2000; but see Crossman et al., 1977) or inactivation of striatal neuronal activity by the local application of lidocaine (Mura et al., 1998) results in a decrease in apomorphine-induced contralateral rotations. However, glutamate input from other brain areas, including the parafasicular/centromedian nuclei or the pedunculopontine nucleus, must also be taken into consideration (Dube et al., 1988; Charara et al., 1996). However, since over 90% of the nerve endings making an asymmetrical synaptic contact in the rodent are associated with dendritic spines (Meshul et al., 1999), this suggests that they most likely originate from the motor cortex (Dube et al., 1988). The involvement of the striatum in apomorphine-induced contralateral rotations is suggested by a decrease in such behavior following destruction of the output neurons of this basal ganglia structure with ibotenic acid (Barker and Dunnett, 1994). We have also reported that treatment of 6-OHDA-lesioned animals with the glutamate NMDA receptor antagonist MK-801 results in a similar decrease in apomorphine-induced contralateral rotations (Robinson et al., 2001).

Although it appears that elimination or blockade of the glutamatergic input to the striatum from the frontal cortex affects apomorphine-induced contralateral rotations, results from the current study suggest that other basal ganglia pathways/structures may be involved in this behavior. The substantia nigra pars reticulata (SNpr) plays an important role in influencing contralateral rotations (Orosz et al., 1992; Robertson and Robertson 1988; 1989). It has been reported that inactivation of the superior colliculus, either by a lesion or the direct injection of a GABA agonist, or inactivation of the deep cerebellar nuclei, resulted in a reduction in circling behavior (Kilpatrick et al., 1982; Speller and Westby, 1996).

In vivo microdialysis

Three months following a nigrostriatal lesion, there was a decrease in the basal extracellular level of striatal glutamate compared to the sham group (Meshul et al., 1999, 2002; Fig. 2). Basal glutamate levels are dependent on the presence of extracellular calcium, such that infusing a modified aCSF solution containing a high concentration of magnesium, no calcium, and including the calcium chelator, EGTA, results in a significant decrease in glutamate levels compared to the levels observed in the presence of normal aCSF (Meshul et al., 2002). Following either a lesion alone of the STN or motor thalamus, or a lesion of either structure in 6-OHDA-treated animals, there was also a decrease in the basal extracellular levels of striatal glutamate compared to the sham group. The decrease in the extracellular levels of striatal glutamate following either a 6-OHDA lesion alone or a motor thalamic lesion alone is consistent with the model of basal ganglia function as proposed by Albin et al. (1989). This model predicts that following a nigrostriatal lesion there is an increase in activity of the glutamate pathway from the STN to both the SNpr and the entopeduncular nucleus (GPi in the primate). The output from either the SNPR or the entopedunular nucleus to the motor thalamus is inhibitory (i.e., GABAergic). This GABAergic input would inhibit the motor thalamus, resulting in a decrease in activity of the thalamo-cortico-striatal pathway. This would result in a decrease in the extracellular levels of striatal glutamate (Fig. 2). The fact that a combined lesion of both the nigrostriatal pathway and the motor thalamus did not result in a reversal of the basal levels of striatal glutamate is also consistent with this model (i.e., a decrease in activity of the thalamo-cortico-striatal pathway).

However, the model would predict that a lesion of the STN alone or in combination with a nigrostriatal lesion should both result in an increase in activity of the thalamo-cortico-striatal pathway. As noted above, a lesion of the nigrostriatal tract is predicted to increase the activity of the STN, resulting in a decrease in activity of the thalamo-cortico-striatal pathway. Therefore, it would be reasonable to assume that a lesion of the STN in the 6-OHDA-lesioned rat should result in a decrease in the glutamate input to the SNpr and entopeduncular nucleus, resulting in an decrease in GABAergic input to the motor thalamus and an increase in activity of the thalamo-cortico-striatal pathway. The microdialysis data reported in the current study (Fig. 2) suggests that this is not the case.

There are two possible explanations for the results in the STN lesion study reported above. Besides the primary glutamate input from the STN to the SNpr, there is a small excitatory afferent projection from the frontal cortex to the substantia nigra (Naito and Kita, 1994). This could provide sufficient excitatory input to the SNpr, resulting in the continued decrease in activity of the thalamo-cortico-striatal input. This is somewhat unlikely, since we have preliminary evidence that following a 6-OHDA lesion there is actually a decrease in the extracellular levels of glutamate within the SNpr as measured by in vivo microdialysis (Frederickson and Meshul, unpubl. data). In support of this preliminary finding, it has been reported that there is a decrease in the firing rate of STN neurons after loss of nigrostriatal dopamine (Zhu et al., 2002). This would

most likely result in a decrease in the release of glutamate within the SNpr, a finding consistent with our preliminary microdialysis data. It has also been reported that following MPTP treatment in monkeys there was no change, while a second reported a decrease in the firing rate of GPi neurons (Raz et al., 2000; Wichmann and DeLong 1999). There was no significant change in the firing rate and only modest changes in the interspike interval and in the number of oscillatory cells in the SNpr in the MPTP-treated monkey (Wichmann et al., 1999). These findings suggest that the STN may not be overactive following the loss of striatal dopamine.

Although it has generally been assumed that the input from the SNpr to the motor thalamus utilizes the neurotransmitter GABA (Di Chiara et al., 1979; Kilpatrick et al., 1980), it has been reported that this projection also contains the neurotransmitters for acetylcholine and glutamate (Kha et al., 2001). In fact, only 25% of the projections from the SNpr to the motor thalamus contain GABA. Therefore, it is possible that with a lesion of the STN the decrease in excitatory input to the SNpr also results in a reduction in the glutamate input to the motor thalamus. This would result in a decrease in activity of the thalamo-corticostriatal pathway, a result consistent with data from the current study.

Nerve terminal glutamate immunolabeling

Following a lesion of the nigrostriatal pathway, the decrease in striatal extracellular levels of glutamate, as measured by in vivo microdialysis, was associated with an increase in the density of nerve terminal glutamate immunolabeling (Ingham et al., 1998; Meshul et al., 1999). Following a lesion of the STN or a combined 6-OHDA/STN lesion, the decrease in striatal extracellular glutamate levels was also associated with an increase in the density of nerve terminal glutamate immunolabeling compared to the sham group. The increase in the density of glutamate immunogold labeling within the presynaptic terminal was observed within the vesicular and not mitochondrial pool of glutamate. This suggests that the decrease in the extracellular levels of glutamate seen following either an STN, 6-OHDA, or combined STN/6-OHDA lesion most likely originated from the vesicular pool of neurotransmitter. This is supported by our previous finding of a decrease in the basal extracellular level of striatal glutamate when the probe is infused with aCSF containing no calcium (Meshul et al., 2002).

This inverse relationship between extracellular glutamate levels and the density of glutamate immunolabeling within nerve terminals making an asymmetrical synaptic contact was not observed following a lesion of the motor thalamus or in the combined 6-OHDA/motor thalamic lesion group. However, in the 6-OHDA group alone, the decrease in the extracellular level of striatal glutamate continued to be associated with an increase in the density of nerve terminal glutamate immunolabeling (Fig. 4B). The major excitatory thalamic input to the striatum originates from the parafasicular/centromedian nuclei (Dube et al., 1988) and not the motor thalamus. These nuclei are located caudal to that of the motor thalamic nucleus that was lesioned in the current study and therefore would most likely not affect the direct input from the parafasicular/centromedian nuclei to the striatum. We have also observed that in the mouse model of striatal dopamine depletion using the neurotoxin MPTP, the decrease in the extracellular level of striatal glutamate following subchronic toxin treatment resulted in no change in the density of nerve terminal glutamate immunolabeling (Robinson et al., 2003).

It is possible that the decrease in the extracellular glutamate level could be due to alterations in the release of glutamate, resulting in the nerve terminal compensating by decreasing the synthesis/uptake or increasing the breakdown of this neurotransmitter in order to maintain a steady-state pool of glutamate. This hypothesis would be consistent with our results showing a lack of change in the density of nerve terminal glutamate immunolabeling following either a motor thalamic lesion alone or combined with a 6-OHDA lesion. We reported a similar finding after subchronic vs. acute administration of MPTP (Robinson et al., 2003). As an interesting comparison, we have reported that subchronic administration of cocaine for 7 days results in no change in the density of glutamate immunogold labeling within the nucleus accumbens (Kozell and Meshul, 2001), although it has been reported that basal levels of extracellular glutamate are decreased in similarly treated animals (Pierce et al., 1996). This suggests that in some instances there is not an exact association between changes in the level of extracellular glutamate as measured by in vivo microdialysis and quantitative immunogold electron microscopy. In this case, other factors, such as alterations in uptake or synthesis of glutamate that serve to restore the system back to a baseline level, need to be taken into consideration. It is possible that following a motor thalamic compared to an STN lesion, the origin of the extracellular levels of striatal glutamate may be due to the cysteine-glutamate antiporter (Baker et al., 2002). In the current study, due to the methods used to process the tissue, only about 5–10% of the immunogold labeling is associated with the cytoplasmic pool of glutamate, and this pool did not change following a lesion of the nigrostriatal pathway (Meshul et al., 1999).

Although an STN or motor thalamic lesion decreased the apomorphine-induced contralateral rotations, this change in behavior was not associated with a reversal in the extracellular levels of striatal glutamate. This study questions the importance of the corticostriatal glutamate pathway in this particular series of experi-

ments. We and others have reported that either a lesion or blockade of the corticostriatal pathway results in a decrease in apomorphine-induced contralateral rotations (Cenci and Bjorklund, 1993; Meshul et al., 2000; Mura et al., 1998) suggesting that the glutamate input to the striatum appears to be important in this turning behavior. However, a lesion of either the STN or motor thalamus, while affecting extracellular glutamate levels as measured by in vivo microdialysis, may be affecting this turning behavior by influencing other pathways within and outside the basal ganglia, such as the nigrotectal track or the input from the deep cerebellar nuclei to the superior colliculus (Kilpatrick et al., 1982; Orosz et al., 1992; Robertson and Robertson 1988, 1989; Speller and Westby, 1996).

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