

Apomorphine-induced alterations in striatal and substantia nigra pars reticulata glutamate following unilateral loss of striatal dopamine

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Abstract

We have reported time-dependent changes in extracellular glutamate within the striatum at 1 and 3 months following a unilateral lesion of the nigrostriatal pathway using the neurotoxin, 6-hydroxydopamine (6-OHDA) (Meshul, C.K., Emre, N., Nakamura, C.M., Allen, C., Donohue, M.K., Buckman, J.F., 1999. Time-dependent changes in striatal glutamate synapses following a 6-hydroxydopamine lesion. *Neurosci.* 88, 1–16.). The aim of the present study was to determine the effects of such a lesion on glutamate within the substantia nigra pars reticulata (SN-PR) and the effect of subchronic administration of the dopamine D-1/D-2 agonist, apomorphine, on extracellular glutamate within both the striatum and the SN-PR using *in vivo* microdialysis. One month after the lesion, there is an increase in extracellular glutamate within the striatum and apomorphine treatment leads to a further increase. Within the SN-PR, a loss of striatal dopamine leads to a decrease in extracellular glutamate, while apomorphine treatment leads to a further decrease in nigral glutamate. Three months after a 6-OHDA lesion, there is a decrease in extracellular striatal glutamate, with apomorphine administration leading to essentially no further change in glutamate. The loss of striatal dopamine increased extracellular glutamate within the SN-PR while apomorphine administration resulted in a decrease in extracellular glutamate back to the value observed in the control group. The data suggests that the increase in striatal glutamate 1 month following a 6-OHDA lesion alone or following subchronic apomorphine is consistent with the hypothesis that a decrease in glutamate within the SN-PR leads to activation of the thalamo-cortico-striatal pathway. The decrease in striatal glutamate 3 months after a nigrostriatal lesion is also consistent with the observed increase in extracellular glutamate within the SN-PR, thus leading to a decrease in output of the thalamo-cortico-striatal pathway.

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Introduction

The major dopamine input into the striatum originates from the dopamine neurons located within the substantia nigra pars compacta (SN-PC). These dopamine terminals 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).

In Parkinson's disease, there is a slow and progressive loss of dopamine input to the striatum, one of the major

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nuclei of the basal ganglia. The loss of striatal dopamine affects the output neurons from this brain area that are part of the so-called direct and indirect pathways (Albin et al., 1989). Ultimately, these pathways merge at the level of both the SN-PR and motor thalamus. The motor thalamus is then the start of another excitatory projection, leading to the cortex and then back to the striatum (thalamo–cortico–striatal pathway). In animal models of Parkinson's disease, administration of AMPA or NMDA glutamate receptor antagonists decrease the behavioral effects of such a nigrostriatal lesion (Brotchie et al., 1991; Carlsson and Carlsson, 1989; Engber et al., 1994; Klockgether et al., 1991, 1993; Loschmann et al., 1991; Mitchell et al., 1995; Papa and Chase, 1996; Starr, 1995). In addition, intrastriatal injection of NMDA leads to parkinsonism (Klockgether and Turski, 1993), suggesting that glutamate synapses within the striatum play an important role in the development of behaviors associated with Parkinson's disease.

Another important area of the basal ganglia which receives a major excitatory (glutamate) input from both the subthalamic nucleus, cortex and pedunclopontine nucleus is the substantia nigra pars reticulata (SN-PR) (Wilson, 1998). The glutamate terminals originating from these brain areas make synaptic connections onto dopamine containing dendrites within the SN-PR (Kita and Kitai, 1987; Smith et al., 1996). A decrease in glutamate output from the subthalamic nucleus reverses the behavioral effects of an MPTP lesion in monkeys and significantly decreases the bradykinesia/akinesia in humans (Bergman et al., 1990; Obeso et al., 1997). Microinjection of NMDA or AMPA glutamate antagonists into the SN-PR, decreasing the inhibitory GABAergic drive from the SN-PR to the motor thalamus, results in enhanced behavioral activity in nigrostriatal-lesioned animals (McPherson and Marshall, 2000). A possible anatomical mechanism leading to this increased activity would be activation of the thalamo–cortico–striatal pathway. Dendrites from the overlying dopamine neurons in the SN-PC project down to the SN-PR, suggesting another site for dopamine–glutamate interactions (Gerffen et al., 1976; Rosales et al., 1997). Therefore, glutamate synapses within both the SN-PR and striatum play a critical role in Parkinson's disease.

We have reported time-dependent changes in striatal glutamate following a lesion of the nigrostriatal pathway with the neurotoxin, 6-OHDA. One month after the lesion, there was an increase in extracellular glutamate, as measured by in vivo microdialysis, while 3 months after the lesion, there was a decrease in extracellular striatal glutamate (Meshul et al., 1999). The increase in striatal glutamate 1 month after the lesion could be explained by an increase in activity of the thalamo–cortico–striatal pathway. However, the model of basal ganglia function would predict that following loss of striatal dopamine, there is an increase in output from the subthalamic nucleus to the SN-PR, leading to an increase in GABAergic inhibitory output to the thalamus and a decrease in thalamo–cortico–striatal function (Albin et al., 1989). Although dopaminergic denervation has

been reported to result in an increase in the frequency of subthalamic nucleus firing (Miller and DeLong, 1987) and subsequently an increase in firing of at least the (internal) globus pallidus (Bergman et al., 1994; Miller and DeLong, 1987), as the basal ganglia model predicts (Albin et al., 1989), there is growing evidence that does not support these previous observations (Raz et al., 2000; Wichmann and DeLong, 1999; Wichmann et al., 1999; Zhu et al., 2002). Because glutamate input to the SN-PR can influence the activity of the thalamo–cortico–striatal pathway, the aim of the current study was to determine if there were also time-dependent changes in the level of extracellular glutamate within the SN-PR 1 and 3 months following a nigrostriatal lesion. Since subchronic intermittent treatment of the unilateral 6-OHDA-lesioned rat with the dopamine agonist, apomorphine, produces a sensitized motor response (i.e., contralateral rotations) (Gancher and Mayer, 1995; Gancher et al., 1996), we hypothesized that such treatment would result in a further change in striatal and SN-PR glutamate compared to the saline-treated group.

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 Institute of Health Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 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; Touchon et al., 2004). For the control (sham) animals, a hole was drilled at the above coordinates for a MFB lesion and then the skin stapled together. The animals were placed in their home cage for either 1 or 3 months following the sham or 6-OHDA lesion of the MFB.

All rats were then tested for contralateral turning 2 weeks following the 6-OHDA or sham lesion. The animals were first placed in individual cages for 15 min to acclimate, injected subcutaneously with 0.05 mg/kg of apomorphine (Sigma, 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 (1st 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 for at least 3 months following the 6-OHDA lesion (Meshul and Allen, 2000; Meshul et al., 1999; Mura et al., 1998; Touchon et al., 2004). None of the sham-lesioned animals rotated using this dose of apomorphine (Meshul and Allen, 2000; Mura et al., 1998; Neve et al., 1982; Touchon et al., 2004) and were used as the control group.

Microdialysis was carried out either 1 or 3 months following the 6-OHDA or sham lesion. One week prior to the start of the microdialysis study, sham or 6-OHDA-lesioned rats were injected with either vehicle or apomorphine (0.05 mg/kg) for 6 days. The 7-day time period used in the current study for apomorphine administration was based on a previous report of increased contralateral rotations in 6-OHDA-lesioned rats even as early as 3–4 days of daily drug treatment (Gancher et al., 1996) and based on our previous findings (Meshul et al., 2002). For this study, apomorphine was chosen to be used versus a selective dopamine D-1 or D-2 agonist since (1) apomorphine was used to test the success of the 6-OHDA lesion and that the same drug should be used for the subchronic dopamine agonist study, and (2) it was premature to separately test dopamine D-1 or D-2 agonists with regards to SN-PR and striatal glutamate since it was uncertain as to what effect, if any, the nonselective dopamine D-1/D-2 agonist, apomorphine, would have on extracellular glutamate. On day 7, all the animals were tested for apomorphine-induced contralateral rotations (8th challenge with apomorphine, which includes the first apomorphine dose given 2 weeks after the 6-OHDA injection) as described above. The percent change in contralateral rotations between the 8th and 1st challenge (i.e., number of rotations at 8th challenge/number of rotations at 1st challenge) was then determined. An overall group mean was calculated (mean percentage \pm SEM) and the lesioned groups compared against each other using the Student's *t* test. Two days after the 8th apomorphine challenge, *in vivo* microdialysis was carried out (see below), allowing for sufficient washout of the drug.

In vivo microdialysis measurement of extracellular glutamate

Guide cannulae surgeries and dialysis probes (2 mm in length for the striatum, 1 mm for the SN-PR) were carried out and prepared as described by Robinson and Wishaw (1988) with modifications (Meshul et al., 1999, 2002). Separate groups of animals were used for the striatal and SN-PR microdialysis. Glutamate concentration in the dialysate samples was determined using a Hewlett Packard HPLC 1090 interfaced with a Hewlett Packard 1046A

Programmable Fluorescence Detector. Dialysates were derivatized with *o*-phthalaldehyde 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 or the SN-PR and perfused with artificial cerebrospinal fluid (aCSF) (0.2 μ l/min; 140 mM NaCl, 3.4 mM KCl, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 1.4 mM NaH₂PO₄, and 4.85 mM Na₂HPO₄, pH 7.4). The fluid was left to perfuse through the probe overnight at a rate of 0.2 μ l/min. The following morning, the pump speed was increased up to 2 μ l/min for 1 h and then four samples were 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 (2.5% glutaraldehyde/0.5% paraformaldehyde, 0.1% picric acid in 0.1 M HEPES, pH 7.3), vibratome sections (100 μ m thick) cut, stained with thionin and the site of the probe placement within the striatum or SN-PR verified histologically. Probe placement extended 2 mm along the dorsolateral quadrant of the rostral striatum (equivalent to 1.0 mm anterior of Bregma, 3.5 mm lateral, according to Paxinos and Watson, 1986) or 1 mm within the SN-PR (equivalent to -5.3 mm posterior of Bregma and 2.5 mm lateral, according to Paxinos and Watson, 1986). If the placement was not correct (i.e., outside the striatum or SN-PR), data from those animals were discarded. The 4 baseline data points for either the striatum or SN-PR were averaged separately at each time point (i.e., 15, 30, 45, 60 min) and then an overall mean was determined. The values are expressed as the mean \pm SEM in pmol/ μ l basal extracellular striatal or SN-PR glutamate within the dialysate sample. The mean probe recovery was 12.4 \pm 1.2%. All the 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 are well aware of the controversy regarding whether extracellular glutamate is derived from the calcium-dependent neuronal vesicular pool, the calcium-independent, but neuronal, glutamate/cystine antiporter, or the non-neuronal (glial) pool (Baker et al., 2002; Timmerman and Westerink, 1997). We and others have reported that about 30% of extracellular glutamate is calcium dependent (Baker et al., 2002; Meshul et al., 2002; Wolf et al., 2000; Xue et al., 1996) and that over 60% of the K⁺-depolarized extracellular level of glutamate is calcium dependent (Meshul et al., 1999), suggesting a role for the synaptic vesicle pool within the nerve terminal contributing to the extracellular level of glutamate. 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. It has also been reported that 60% of the basal extracellular level of glu-

Table 1
Apomorphine-induced contralateral rotations

Treatment groups	Percent change in rotations (8th challenge/1st challenge)	% of 6-OHDA/SAL group
6-OHDA/SAL: 1 month caudate group	152.0 ± 24.4	
6-OHDA/APO: 1 month caudate group	212.1 ± 22.2*	39.5%
6-OHDA/SAL: 1 month SN-PR group	131.1 ± 13	
6-OHDA/APO: 1 month SN-PR group	200.2 ± 20*	52.7%
6-OHDA/SAL: 3 months caudate group	109.6 ± 6	
6-OHDA/APO: 3 months caudate group	150.1 ± 12*	36.9%
6-OHDA/SAL: 3 months SN-PR group	125.7 ± 15	
6-OHDA/APO: 3 months SN-PR group	175.2 ± 13*	25.1%

Values are mean percentages ± SEM and the lesioned groups were compared against each other using the Student's *t* test.

* *P* < .05 compared to the 6-OHDA/SAL group using the Student's *t* test.

tamate is due to exchange with the glutamate/cystine antiporter, which has been shown to be calcium insensitive and most likely of cytoplasmic, but still of neuronal origin (Baker et al., 2002).

Results

Apomorphine-induced rotations

One month after the 6-OHDA lesion, there was either a 39.5% or 52.7% increase in the number of contralateral rotations in the apomorphine-treated group that either

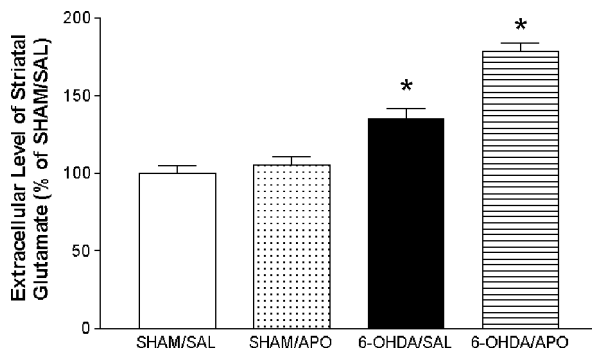


Fig. 1. In vivo microdialysis of the striatum 1 month following a sham or 6-OHDA lesion of the nigrostriatal pathway. Rats were injected for 6 days with saline (SAL: *N* = 7) or apomorphine (APO: *N* = 7) (0.05 mg/kg, sc) and then all groups were challenged with apomorphine on day 7 and the number of contralateral rotations determined. Two days later, in vivo microdialysis was carried out. There was an increase in the extracellular level of striatal glutamate in the 6-OHDA-lesioned rats injected with saline (6-OHDA/SAL: *N* = 8) compared to either the sham rats injected with saline (SHAM/SAL) or apomorphine (SHAM/APO). Following apomorphine treatment in the 6-OHDA-lesioned group (6-OHDA/APO: *N* = 9), there was a significant increase in the extracellular level of striatal glutamate compared to all other groups. Values are means ± SEM. Mean absolute concentration of glutamate within the dialysate samples for each group are as follows (values are pmol/μl ± SEM: SHAM/SAL: 0.71 ± .03; SHAM/APO: 0.75 ± .04; 6-OHDA/SAL: 0.96 ± .04; 6-OHDA/APO: 1.27 ± .05. **P* < .05 compared to all other groups as determined by Peritz' *f* test for comparison of multiple means.

underwent microdialysis of the caudate or SN-PR, respectively (Table 1). Three months after the lesion, there was a statistically significant increase (36.9% or 25.1%) in the number of contralateral rotations in the apomorphine-treated group that were used for microdialysis of either the caudate or SN-PR, respectively. The data suggest that all animals in the apomorphine-treated groups, regardless of the time point after the 6-OHDA lesion, were behaviorally sensitized to the effects of the dopamine D-1/D-2 agonist.

One month following a 6-OHDA lesion

One month after a 6-OHDA lesion, 7 days of saline treatment (6-OHDA/SAL) resulted in an increase extracellular striatal glutamate compared to both the sham-lesioned group treated with either saline (SHAM/SAL) or apomorphine (SHAM/APO) (Fig. 1). Seven days of apomorphine treatment in animals previously lesioned with 6-OHDA leads to a further increase in extracellular striatal glutamate (6-OHDA/APO) compared to all the other groups. In a separate, but similarly treated group of animals, there was a statistically significant decrease in extracellular glutamate within the SN-PR in the 6-OHDA/SAL compared to the SHAM/SAL group (Fig. 2).

There was a similar decrease in extracellular glutamate within the SN-PR in the sham-lesioned group treated with apomorphine (SHAM/APO) compared to the SHAM/SAL group. Apomorphine treatment in the 6-OHDA-lesioned group (6-OHDA/APO) resulted in a further decrease in

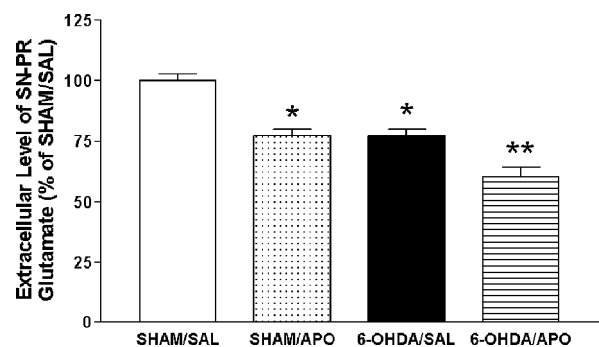


Fig. 2. In vivo microdialysis of the SN-PR 1 month following a sham or 6-OHDA lesion of the nigrostriatal pathway. Same procedure as in Fig. 1 except for a separate group of animals in which dialysis was carried out in the substantia nigra pars reticulata. There was a decrease in the extracellular level of glutamate within SN-PR in the 6-OHDA-lesioned group (6-OHDA/SAL: *N* = 9) compared to the sham group (SHAM/SAL: *N* = 7). Following apomorphine administration, there was a decrease in nigral glutamate in the sham group (SHAM/APO: *N* = 8) compared to the saline-treated group (SHAM/SAL). Treatment with this dopamine agonist in the lesioned group (6-OHDA/APO: *N* = 8) resulted in a further decrease in nigral glutamate compared to the saline, but 6-OHDA-lesioned, group. Values are means ± SEM. Mean absolute concentration of glutamate within the dialysate samples for each group are as follows (values are pmol/μl ± SEM: SHAM/SAL: 0.48 ± .007; SHAM/APO: 0.47 ± .01; 6-OHDA/SAL: 0.39 ± .01; 6-OHDA/APO: 0.32 ± .01. **P* < .05 compared to the SHAM/SAL and 6-OHDA/APO groups as determined by Peritz' *f* test for comparison of multiple means. ***P* < .05 compared to the other groups as determined by Peritz' *f* test for comparison of multiple means.

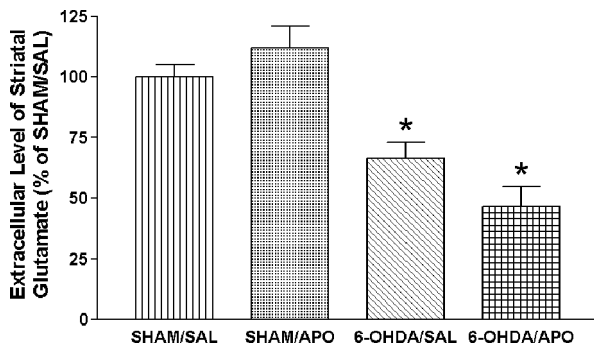


Fig. 3. In vivo microdialysis of the striatum 3 months following a sham or 6-OHDA lesion of the nigrostriatal pathway. Same procedure as Fig. 1 except that animals were injected with apomorphine for 6 days starting 2 months and 3 weeks after the 6-OHDA lesion. In the lesioned group treated with saline (6-OHDA/SAL: $N = 8$), there was a significant decrease in the extracellular level of striatal glutamate compared to the sham group treated either with saline (SHAM/SAL: $N = 7$) or apomorphine (SHAM/APO: $N = 7$). When the lesioned group was treated with apomorphine (6-OHDA/APO: $N = 8$), there was a similar decrease in the extracellular level of striatal glutamate as shown in the 6-OHDA/SAL group and this decrease was significantly different compared to both sham groups. Values are means \pm SEM. Mean absolute concentration of glutamate within the dialysate samples for each group are as follows (values are pmol/ μ l \pm SEM: SHAM/SAL: 2.39 ± 0.1 ; SHAM/APO: 2.68 ± 0.18 ; 6-OHDA/SAL: 1.59 ± 0.16 ; 6-OHDA/APO: 1.12 ± 0.2 . * $P < .05$ compared to the SHAM/SAL and SHAM/APO groups as determined by Peritz' f test for comparison of multiple means.

extracellular glutamate within the SN-PR compared to all other groups.

Three months following a 6-OHDA lesion

There was a similar increase in the number of apomorphine-induced contralateral rotations 3 months after the 6-OHDA lesion as compared to the 1-month time period (Table 1). We found that 3 months after a 6-OHDA lesion, there was a decrease in extracellular striatal glutamate (6-OHDA/SAL) compared to the sham group administered either saline (SHAM/SAL) or apomorphine (SHAM/APO) for 7 days (Fig. 3). Following administration of apomorphine for 7 days to the 6-OHDA-lesioned group (6-OHDA/APO), there continued to be a decrease in extracellular striatal glutamate compared to the sham groups. There was a small but statistically insignificant decrease in extracellular striatal glutamate in the 6-OHDA-lesioned groups treated with apomorphine (6-OHDA/APO) compared to the lesioned but saline (6-OHDA/SAL)-treated group.

A decrease in thalamo-cortico-striatal activity could result from an increase in glutamatergic activity of the pathway from the subthalamic nucleus to the SN-PR. Three months following a nigrostriatal lesion (6-OHDA/SAL), there was an increase in the extracellular level of glutamate within the SN-PR compared to the sham group administered either saline (SHAM/SAL) or apomorphine (SHAM/APO) (Fig. 4). There was also a significant decrease in extracellular glutamate within the SN-PR in the sham group given apomorphine (SHAM/APO) compared to the sham

group administered saline (SHAM/SAL). Apomorphine treatment to the 6-OHDA-lesioned group (6-OHDA/APO) resulted in a return of extracellular SN-PR glutamate to essentially the control level.

Discussion

The present series of studies were designed to test the hypothesis that the time-dependent changes in striatal glutamate in the nigrostriatal lesioned rat (Meshul et al., 1999) could be associated with alterations in glutamate within the SN-PR. We report that 1 month following a unilateral lesion of the nigrostriatal pathway with 6-OHDA, there is an increase in the extracellular level of striatal glutamate and a decrease in the extracellular level of glutamate within the SN-PR. Subchronic treatment with the dopamine D-1/D-2 agonist, apomorphine, results in a further increase in striatal extracellular glutamate and a further decrease in extracellular glutamate within the SN-PR. Three months following the unilateral lesion, there is a decrease in extracellular striatal glutamate and a marked increase within the SN-PR. Apomorphine administration resulted in no further change in striatal extracellular glutamate as compared to the saline, but 6-OHDA-lesioned, treated group. Within the SN-PR, apomorphine treatment resulted in a decrease in the extracellular level of glutamate essentially back to the control level.

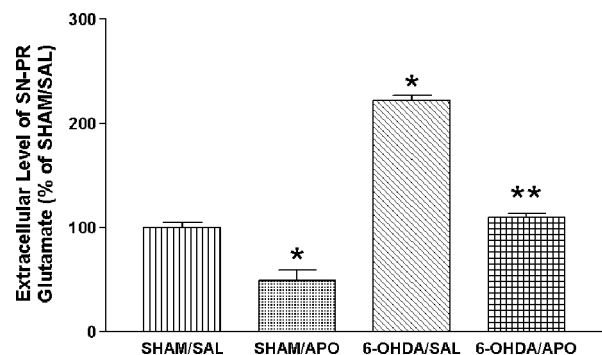


Fig. 4. In vivo microdialysis of the SN-PR 3 months following a sham or 6-OHDA lesion of the nigrostriatal pathway. Same procedure as in Fig. 3 except for a separate group of animals in which dialysis was carried out in the substantia nigra pars reticulata. Three months following the nigrostriatal lesion (6-OHDA/SAL: $N = 8$), there was a significant increase in the extracellular level of glutamate within the SN-PR compared to the sham group treated with saline (SHAM/SAL: $N = 8$) and to the other two groups treated with apomorphine. Following treatment with apomorphine, there was a significant decrease in the extracellular level of SN-PR glutamate in the sham group (SHAM/APO: $N = 8$) compared to all other groups. In the 6-OHDA-lesioned group injected with apomorphine (6-OHDA/APO: $N = 9$), there was a return of extracellular glutamate back to the control level. Values are means \pm SEM. Mean absolute concentration of glutamate within the dialysate samples for each group are as follows (values are pmol/ μ l \pm SEM: SHAM/SAL: $0.59 \pm .03$; SHAM/APO: $0.29 \pm .03$; 6-OHDA/SAL: $1.31 \pm .06$; 6-OHDA/APO: $0.65 \pm .02$. * $P < .05$ compared to all other groups as determined by Peritz' f test for comparison of multiple means. ** $P < .05$ compared to the SHAM/APO and 6-OHDA/SAL groups as determined by Peritz' f test for comparison of multiple means.

One month following a 6-OHDA lesion

Basal extracellular glutamate within the striatum and SN-PR

The increase in extracellular striatal glutamate following the loss of striatal dopamine is consistent with previous reports (Jonkers et al., 2002; Lindfors and Ungerstedt, 1990; Meshul et al., 1999; Tossman et al., 1986), but inconsistent with others (Abarca and Bustos, 1999; Bianchi et al., 2003; Marti et al., 2002; Reid et al., 1990). Why this discrepancy amongst the various reports is not known; however, the fact that we focus our 2-mm probes within the dorsolateral striatum may be important. This region of the striatum receives its input from the sensorimotor cortex (McGeorge and Faull, 1989) and all other regions of the striatum receive input from non-motor areas of the cortex. We have preliminary data that extracellular glutamate within the dorsolateral versus ventrolateral striatum are different, suggesting that probe placement may be even more critical than previously appreciated (McKee and Meshul, unpublished findings).

The increase in extracellular striatal glutamate in the dopamine-depleted striatum could be due to activation of the thalamo–cortico–striatal pathway. In agreement with this theory is the finding of an increase in cortical activity within the sensorimotor cortex of rats following a 6-OHDA lesion compared to the sham (non-lesioned) group as measured by the BOLD (blood oxygenation level-dependent) method (Pelled et al., 2002). We hypothesized that increased activation of this pathway may be due to a decrease in glutamate within the SN-PR, leading to a decrease in GABAergic output from the SN-PR to the motor thalamus. We have reported that activation of the motor thalamus results in an increase in extracellular striatal glutamate (Meshul et al., 1996). Indeed, we find that 1 month after a 6-OHDA lesion, there is a significant decrease in extracellular glutamate within the SN-PR compared to the sham group (Fig. 2). However, others have reported either no changes (Bianchi et al., 2003; Marti et al., 2000, 2002) or an increase in extracellular glutamate within the SN-PR in similarly nigrostriatal-lesioned rats at the 1-month time period (Abarca et al., 1995; Ochi et al., 2004a,b; You et al., 1996). This reason for this discrepancy is not apparent. Although we assume that the vast majority of the glutamatergic input to the SN-PR originates from the subthalamic nucleus (Kita and Kitai, 1987), other afferents from the cortex and pedunculopontine nucleus may be making a smaller, yet important, contribution.

However, the increase in striatal glutamate and the decrease in SN-PR glutamate following a 6-OHDA lesion are contrary to the current model of basal ganglia function (Albin et al., 1989), which would have predicted just the opposite finding. The changes in striatal and SN-PR glutamate found in the current study may be an initial compensatory response following the first month after the lesion. After the loss of the nigrostriatal pathway, there is an

initial increased glial response both within the striatum and the SN-PR due to the loss of dopamine nerve terminals and cell bodies (Stromberg et al., 1986). Although we have reported increased extracellular glutamate results in a depletion of glutamate within the nerve terminal 1 month following a 6-OHDA lesion (Meshul et al., 1999), an increase in the number of glial cells may also be contributing to the observed increase in striatal extracellular glutamate. However, within the SN-PR, since we observed a decrease in extracellular glutamate, the role of the glial cell in this region of the brain in terms of influencing extracellular glutamate levels is not clear.

Apomorphine-induced changes in glutamate within the striatum and SN-PR

Following subchronic treatment with the apomorphine, there was a continued increase and decrease in extracellular striatal and SN-PR glutamate, respectively, in the lesioned vs. sham group. This data is consistent with the hypothesis that the decrease in extracellular glutamate within the SN-PR could activate the thalamo–cortico–striatal pathway. The increase in striatal glutamate following dopamine D-1/D-2 agonist treatment is in agreement with the recent report of an increase in SN-PR or striatal glutamate following acute oral administration of L-dopa (Jonkers et al., 2002; Ochi et al., 2004a). At this early stage (i.e., 1 month) of dopamine loss within the nigrostriatal pathway, we speculate that the significance of the enhanced (either increase or decrease) striatal or SN-PR glutamate following apomorphine administration may be related to the increase in behavioral changes (i.e., contralateral rotations) following administration of this dopamine agonist compared to the vehicle-treated group (Table 1).

In the sham group, there was a decrease in extracellular glutamate within the SN-PR and not the striatum following apomorphine administration. According to the model of basal ganglia function (Albin et al., 1989), administration of a dopamine D-1/D-2 agonist to naïve animals would result in a decrease in GABAergic output from the striatum to the external globus pallidus (via primarily the dopamine D-2 indirect pathway) (Gerfen et al., 1990). This in turn would result in an increase in GABAergic activity from the globus pallidus to the subthalamic nucleus and a decrease in glutamate output from the subthalamic nucleus to the SN-PR. This hypothesis is consistent with the current data (Figs. 2 and 4).

Three months following a 6-OHDA lesion

Basal extracellular glutamate within the striatum and SN-PR

The decrease in striatal extracellular glutamate 3 months after a nigrostriatal lesion is consistent with the model of

basal ganglia function (Albin et al., 1989). The model predicts that with the loss of striatal dopamine, there is a decrease in activity associated with the direct pathway and an increase in the indirect pathway. With the indirect pathway, there would be an increase in activity of the pathway from the subthalamic nucleus to the SN-PR leading to a decrease in thalamo–cortico–striatal activity. The increase in output from the subthalamic nucleus would be reflected in an increase in glutamate within the SN-PR.

The question then becomes, How is it that over a 3-month time period, there is first a decrease at 1 month then an increase at 3 months in extracellular glutamate within the SN-PR following the loss of striatal glutamate? Although there is clearly no simple explanation, there are two possibilities. First, the activity of the (external) globus pallidus and how its inhibitory efferent GABAergic projection to the subthalamic nucleus is affected may be important. It has been reported that loss of striatal dopamine does not lead to decreased activity of the globus pallidus (Chesselet and Delfs, 1997) and that a lesion of the globus pallidus does not lead to increased activity of the subthalamic nucleus (Hassini et al., 1996), nor does it cause an increase in oscillatory bursting of the subthalamic nucleus as observed in the MPTP lesioned nonhuman primate (Soares et al., 2004). It has been recently reported that a 6-OHDA lesion results in an increase in mRNA levels of the enzyme associated with the synthesis of GABA, namely, glutamic acid decarboxylase 67 (GAD67), within the globus pallidus (Carta et al., 2003). An increase in GAD67 mRNA might suggest an increase in activity of the pathway from the globus pallidus to the subthalamic nucleus, leading to inhibition of this nucleus and a decrease in glutamate within the SN-PR (Fig. 2). It is possible that other inputs to either the globus pallidus or the subthalamic nucleus, for example, the excitatory input from cortex, may be playing an important role.

The second possibility is that the intense glial reaction observed within the striatum and the SN-PR 1 month after a 6-OHDA lesion subsides over time as the degenerating terminals and cell bodies are eliminated (Stromberg et al., 1986; Rataboul et al., 1986; Meshul, unpublished observations). If the glial cells are making a contribution to the extracellular glutamate levels at the 1-month time period, it is conceivable that after 3 months, when the glial cell numbers are back to the control level, then the decrease in extracellular striatal glutamate/increase in SN-PR glutamate observed at this time period would be in agreement with the model of basal ganglia function (Albin et al., 1989).

Apomorphine-induced changes in glutamate within the striatum and SN-PR

There was a small, but statistically, insignificant decrease ($P = .09$) in the extracellular level of striatal glutamate in the lesioned group treated with apomorphine compared to the lesion group treated with saline. However, the important

finding in the 3-month lesioned group was that apomorphine administration continued to result in a decrease in the extracellular level of striatal glutamate compared to the sham group. This is the opposite of the expected increase as predicted by the model of basal ganglia function (Albin et al., 1989) and as seen after local application of apomorphine into the striatum (Reid et al., 1990). At this later time point following the loss of striatal dopamine, apomorphine treatment essentially did not result in any further change in striatal glutamate but there was a significant decrease in SN-PR glutamate. This decrease in SN-PR glutamate at the 3-month time period is similar to that seen 1 month following a 6-OHDA lesion (Fig. 2) and is associated with the continued increase in motor behavior (i.e., contralateral rotations) following dopamine agonist administration. At this 3-month time point, compensatory synaptic changes in striatal glutamate may not be as important as at the 1-month time period and that alterations in the output of the SN-PR may be more central in terms of the apomorphine-induced contralateral rotations (Orosz and Bennett, 1992; Robertson and Robertson, 1988, 1989).

Within the SN-PR, administration of apomorphine to the lesioned group results in a significant decrease in extracellular glutamate compared to the lesioned group treated with saline. This should decrease the GABAergic output from the SN-PR to the thalamus, resulting in activation of the thalamo–cortico–striatal pathway, as predicted by the model. Since we actually observed a continued decrease in extracellular striatal glutamate in the lesioned group treated with apomorphine suggests that other factors may be controlling the extracellular level of glutamate. The glutamate transporter, GLT-1, is the major protein involved in the uptake of extracellular glutamate into glial cells (Maragakis and Rothstein, 2004). It has been reported that following the administration of L-dopa in nigrostriatal lesioned rats for 21 days, there was a significant increase in expression of both protein and mRNA for GLT-1 within the striatum compared to the lesioned group treated with vehicle (Lievens et al., 2001). It is possible that in the current study, treatment with apomorphine might also result in a similar increase in striatal GLT-1, leading to an increase in glutamate transport into glial cells and a decrease in extracellular glutamate. Such a decrease in extracellular striatal glutamate would be consistent with the findings in the present study. We are currently in the process of testing this hypothesis.

Functional implications

The time-dependent changes in striatal and SN-PR glutamate following a unilateral nigrostriatal lesion and the alterations in extracellular glutamate due to dopamine agonist treatment may be of interest in terms of behavioral changes associated with therapy used to treat patients with Parkinson's disease. The fact that extracellular glutamate levels are continuing to change over time in this nigrostriatal lesion

model may complicate what effect dopamine agonists are having on glutamate within both the striatum and SN-PR. If a similar degree of fluctuation of glutamate occurs in the Parkinson's disease patient, we speculate that the L-dopa- or dopamine-agonist-induced behavioral changes that are observed in these patients could be due to continued fluctuations in glutamate within either the striatum or SN-PR.

However, in the current study, the changes in striatal and SN-PR glutamate have been observed in a unilateral nigrostriatal lesion model, whereas in Parkinson's disease, there is bilateral loss of striatal dopamine. We have preliminary data showing time-dependent changes in striatal glutamate in the mouse MPTP model where there is bilateral loss of striatal glutamate. One month after acute MPTP treatment, there is an increase in extracellular striatal glutamate (Robinson et al., 2003), whereas with repeated acute MPTP administration once a month for 3 months, resulting in a continued loss of striatal tyrosine hydroxylase immunolabeling, there is now a decrease in extracellular striatal glutamate (Meshul et al., 2003). At least in terms of striatal glutamate, both the unilateral and bilateral nigrostriatal lesion models show time-dependent changes.

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