Changes in glutamate receptors in dyskinetic parkinsonian monkeys after unilateral subthalamotomy

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OBJECT Unilateral subthalamotomy is a surgical procedure that may be used to alleviate disabling levodopa-induced dyskinesias (LIDs) in patients with Parkinson disease (PD). However, the mechanisms involved in LID remain largely unknown. The subthalamic nucleus (STN) is the sole glutamatergic nucleus within the basal ganglia, and its lesion may produce changes in glutamate receptors in various areas of the basal ganglia. The authors aimed to investigate the biochemical changes in glutamate receptors in striatal and pallidial regions of the basal ganglia after lesion of the STN in parkinsonian macaque monkeys.

METHODS The authors treated 12 female ovariectomized monkeys with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce PD-like symptoms, treated 8 of these animals with 3,4-dihydroxy-L-phenylalanine (L-DOPA; levodopa) to induce LID, and performed unilateral subthalamotomy in 4 of these 8 monkeys. Four additional monkeys were treated with saline only and were used as controls. The MPTP monkeys had previously been shown to respond behaviorally to lower doses of levodopa after the STN lesion. Autoradiography of slices from postmortem brain tissues was used to visualize changes in the specific binding of striatal and pallidal ionotropic glutamate receptors (that is, of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] and N-methyl-d-aspartate [NMDA] NR1/NR2B subunit receptors) and of metabotropic glutamate (mGlu) receptors (that is, mGlu2/3 and mGlu5 receptors). The specific binding and distribution of glutamate receptors in the basal ganglia of the levodopa-treated, STN-lesioned MPTP monkeys were compared with those in the saline-treated control monkeys and in the saline-treated and levodopa-treated MPTP monkeys.

RESULTS The autoradiographic results indicated that none of the pharmacological and surgical treatments produced changes in the specific binding of AMPA receptors in the basal ganglia. Levodopa treatment increased the specific binding of NMDA receptors in the basal ganglia. Subthalamotomy reversed these increases in the striatum, but in the globus pallidus (GP), the subthalamotomy reversed these increases only contralaterally. Levodopa treatment reversed MPTP-induced increases in mGlu2/3 receptors only in the GP. mGlu2/3 receptor–specific binding in the striatum and GP decreased bilaterally in the levodopa-treated, STN-lesioned MPTP monkeys compared with the other 3 groups. Compared with mGlu5 receptor–specific binding in the control monkeys, that of the levodopa-treated MPTP monkeys increased in the dorsal putamen and remained unchanged in the caudate nucleus and in the GP.

CONCLUSIONS These results implicate glutamate receptors in the previously observed benefits of unilateral subthalamotomy to improve motor control.

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KEY WORDS subthalamotomy; levodopa-induced dyskinesia; MPTP monkey; Parkinson disease; levodopa; glutamate receptor; functional neurosurgery

ABBREVIATIONS AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GP = globus pallidus; GPe = GP externus; GPI = GP internus; LID = levodopa-induced dyskinesia; mGlu = metabotropic glutamate; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NMDA = N-methyl-d-aspartate; PD = Parkinson disease; STN = subthalamic nucleus; 6-OHDA = 6-hydroxodopamine.


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DISCLOSURE Dr. Jourdain received a studentship from the Fonds d’Enseignement et de Recherche of the Faculté de Pharmacie of Université Laval and from the Centre de recherche en endocrinologie moléculaire et oncologique et en génomique humaine and currently holds a postdoctoral scholarship from the Fonds de la recherche en santé du Québec. Dr. Morin held a professional health care studentship from the Fonds de la recherche en santé du Québec.
There is strong evidence that glutamatergic neurotransmission is altered in Parkinson disease (PD) and also during the development and expression of levodopa-induced dyskinesias (LIDs). Reducing glutamate overactivity by blocking the ionotropic receptors for N-methyl-D-aspartate (NMDA) and for α-aminoadipic acid (NMDA) effectively reduces LID in parkinsonian primates. However, similar results in humans have not been conclusive.

Besides via the aforementioned 2 ionotropic glutamate receptors, the actions of glutamate are also mediated via G protein–coupled metabotropic glutamate (mGlu) receptors. Among the 8 known mGlu receptors, the mGlu2/3 and mGlu5 receptors have attracted much interest because of their distribution within the basal ganglia. Activation of mGlu2/3 receptors inhibits glutamate release in the striatum and in other basal ganglia nuclei involved in LID. Their specific role in motor control remains unclear because both mGlu2/3 receptor agonists and antagonists produce anti–parkinsonian-like effects in rodent models of PD. However, no clinical trials have investigated the use of drugs targeting the mGlu2/3 receptors to treat PD or LID. The use of negative allosteric modulators of the mGlu5 receptor has been reported for the treatment of LID in animal models and also in PD patients.

The primate basal ganglia receives massive glutamate afferents from several cortical areas, from the central–paraacaudal thalamic nuclei, and from the subthalamic nucleus (STN). The STN is currently the surgical target of choice in patients whose symptoms are refractory to medication or who have disabling LID. In fact, almost 60% of neurosurgeons still perform some kind of lesioning, and subthalamotomies are offered to the patient as often as other surgical lesions. Subthalamotomies are often used to treat patients who cannot afford deep brain stimulation. Moreover, transcerebral MR-guided focused ultrasound is a new technique for lesioning deep brain structures and has been recently introduced for thalamotomy, which may revive the interest in lesioning for treating movement disorders.

We recently demonstrated that unilateral subthalamotomy in MPTP-treated monkeys with LID had beneficial effects bilaterally on the parkinsonian scores both in monkeys receiving saline (baseline) or various doses of 3,4-dihydroxy-L-phenylalanine (L-DOPA; levodopa). Postmortem analysis showed only postsynaptic modifications in the dopaminergic system. We observed a reversal of the levodopa-induced decreases in D3 receptor–specific binding ipsilateral to the subthalamicotomy and no effects on the D3-related system. A reduction of STN glutamatergic activity had beneficial effects on PD symptoms, as predicted by the direct and indirect pathways model of the basal ganglia. Given the glutamatergic nature of the STN, subthalamotomy is hypothesized to induce changes in levels of glutamate receptors in the basal ganglia through direct STN projections or through a normalization of cortico- and thalamostriatal pathways. In the present study, ionotropic (AMPA and NMDA) receptors and metabotropic (mGlu2/3 and mGlu5) receptors, which are known to be modulated in PD and LID, were investigated using receptor-binding autoradiography in levodopa-treated, STN-lesioned MPTP monkeys with LID and compared with those in saline-treated controls and in saline-treated or levodopa-treated MPTP monkeys.

Methods

Animals and Experimental Treatments

The number of animals used in this study was kept to the minimum required for statistically valid analyses. Experiments were carried out with 16 female ovariectomized macaque monkeys (Macaca fascicularis) (weight range 3.4–5.4 kg) in agreement with the standards of the Canadian Council on Animal Care. The Laval University committee for protection of animals approved this study. Four saline-treated monkeys served as controls, and 12 monkeys were treated with systemic MPTP and developed a severe parkinsonian syndrome. Four of these MPTP-treated monkeys were treated with saline (saline-treated, MPTP monkeys). The other 8 MPTP-treated monkeys were chronically treated with levodopa/benserazide and developed LID. Four of these 8 monkeys did not receive subsequent treatments (levodopa-treated, MPTP monkeys), while the remaining 4 underwent unilateral subthalamotomy (levodopa-treated, STN-lesioned MPTP monkeys) by stereotactic injection of ibotenic acid into the subthalamus. A unilateral rather than a bilateral subthalamotomy was used to allow a comparison between the lesioned and nonlesioned sides in the same animal, which reduced interindividual variability. Moreover, bilateral lesioning may induce behavioral deficits, enhance LIDs, or even induce hemiballism.

All MPTP-treated monkeys in the present study displayed similar baseline parkinsonism scores (saline-treated MPTP monkeys 10.1 ± 1.1, levodopa-treated MPTP monkeys 9.7 ± 0.8, and levodopa-treated, STN-lesioned MPTP monkeys 11.1 ± 0.7) assessed according to the Laval University Disability Scale for MPTP monkeys. The detailed behavioral assessment and pharmacological/surgical treatments of these monkeys have been previously reported. Histopathology of the lesioned versus nonlesioned STN was included in the behavioral study, by visualizing the lesion with cytochrome oxidase and cresyl violet. Moreover, a rostrocaudal reconstruction of the lesion was also demonstrated.

Tissue Preparation

At the end of the experiments, all monkeys were killed with pentobarbital. All 12 MPTP monkeys were killed 24 hours after their last levodopa/benserazide or saline treatment. The animals’ brains were flash-frozen in isopentane (−45°C). Brains were cut into 12-μm-thick coronal sections on a cryostat (−18°C) at levels corresponding approximately to A18–A22, according to the atlas of Szabo and Cowan. Sections were mounted onto Super Frost Plus (Fisher) slides and stored at −80°C until use in assays. All measurements were made at the postcommisural levels.

AMPA Receptor Autoradiography

Tissue sections for AMPA receptor–binding assays were preincubated for 20 minutes in a 50 mM Tris-HCl...
buffer, pH 7.4, followed by incubation for 60 minutes at 4°C in the same buffer supplemented with 5 nM [3H]-Ro 48-8587 (1.92 x 10^2 Bq/mmol; Novartis). Nonspecific binding was determined in a set of adjacent tissue slides via incubation with 50 μM of the AMPA antagonist 1,4-dihydro-6-(1H-imidazol-1-yl)-7-nitro-2,3-quinoxalininedione hydrochloride (YM 90K, Tocris). After the incubations, the labeled sections were washed 2 times for 30 seconds in ice-cold buffer, followed by brief dipping in bidistilled water (4°C). Lastly, the slide-mounted tissue sections were dried overnight at room temperature and incubated with [3H]-sensitive films (BIOMAX MR Film, Kodak) for 4 weeks along with standards ([H]-microscales, GE Healthcare).

**NMDA NR1/NR2B Receptor Autoradiography**

Tissue sections for NMDA (containing NR1/NR2B subunits, hereafter called NMDA NR1/NR2B) receptor–binding assays were preincubated twice for 10 minutes at room temperature in a buffer of 50 mM Tris-HCl and 10 mM EDTA, pH 7.4. Sections were then incubated for 90 minutes at room temperature in the same buffer containing 5 nM [3H]-Ro 25-6981 (0.95 x 10^2 Bq/mmol; F. Hoffman-La Roche). Nonspecific binding was determined with 10 μM of the NMDA antagonist Ro 04-5595 (F. Hoffmann-La Roche). After two 5-minute washes and one 15-minute wash at 4°C in the Tris-EDTA buffer, sections were rinsed for 10 seconds in ice-cold distilled water. The slide-mounted tissue sections were dried and incubated with [3H]-sensitive films as described above.

**mGlu2/3 Receptor Autoradiography**

Tissue sections for mGlu2/3 receptor–binding assays were preincubated for 30 minutes at 4°C in a phosphate buffer (8.66 mM K2HPO4, 1.34 mM KH2PO4, and 100 mM KBr), pH 7.6. Sections were then incubated for 90 minutes at room temperature in the same buffer containing 5 nM [3H]-LY341495 (1.48 x 10^3 Bq/mmol; American Radiolabeled Chemicals). Nonspecific binding was determined in the presence of 20 mM glutamate. After two 30-second washes at 4°C in the phosphate buffer, sections were rinsed for 30 seconds in ice-cold distilled water. The slide-mounted tissue sections were dried and incubated with [3H]-sensitive films as described above.

**mGlu5 Receptor Autoradiography**

Tissue sections for mGlu5 receptor–binding assays were preincubated for 30 minutes at room temperature in a Krebs-Ringer HEPES buffer (20 mM HEPES, 118 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, and 10 mM NaOH), pH 7.4. Sections were then incubated for 15 minutes at room temperature in this buffer, containing 5 nM [3H]-ABP688 (3.02 x 10^2 Bq/mmol; Novartis) with 0.05 mg/ml bovine serum albumin. Nonspecific binding was determined in the presence of 10 μM 2-methyl-6-(phenylethynyl)-pyridine (Tocris). After three 20-minute washes at 4°C in the Krebs-Ringer HEPES buffer, sections were rinsed for 5 seconds in ice-cold distilled water. The slide-mounted tissue sections were dried and incubated with [3H]-sensitive films as described above.

**Data Analysis**

For each brain region examined, we performed autoradiographic analyses on 3–6 brain slices from each animal. The intensity of the radio labeling was quantified with computerized densitometry (NIH ImageJ 64-bit mode, v.1.46) analysis of autoradiographs placed on a constant illumination light table and photographed with a video camera (XC-77, Sony) connected to a Power Macintosh G4 computer. Signals on the autoradiographs were measured as optical density in the caudate nucleus, the putamen, and the pallidal complex and corrected for nonspecific binding in each experiment. Subsequently, optical gray densities were transformed into Bq per milligram of tissue equivalent using a standard curve generated with [3H]-standards ([H]-microscales, GE Healthcare). Results were then converted into femtomoles per milligram of tissue using specific activity of the radioligands.

Data in figures show the means and SEM. Statistical comparisons of data were performed by a 2-way ANOVA (brain subdivisions and pharmacological or surgical treatment) mixed model using paired and nonpaired values with “animal” considered a random effect, followed by post hoc pairwise comparisons with least squares means tests. Analyses were conducted using SAS software (version 9.3, SAS Institute, Inc.). A p value of ≤ 0.05 was considered statistically significant.

**Results**

**Effect of MPTP Denervation and Treatments on Glutamate Receptors**

Figure 1 shows a schematic of the subdivisions of the caudate nucleus and putamen and representative autoradiographs of AMPA, NMDA NR1/NR2B, mGlu2/3, and mGlu5 receptor binding in the postcommissural striatum of the levodopa-treated MPTP monkeys that underwent a unilateral subthalamotomy to alleviate their LID. For all 4 glutamate receptors investigated, autoradiographic labeling was high in the caudate nucleus and putamen, whereas it was much lower in both compartments of the globus pallidus (GP), namely, in the internal and external regions (that is, the GP internus [GPI] and GP externus [GPe], respectively). The changes in receptor-specific binding and distribution in response to the 3 experimental treatments are summarized in Table 1.

**AMPA Receptor Binding**

The MPTP treatment nonsignificantly increased specific binding of [3H]-Ro 48-8587 to the AMPA receptor in both the caudate nucleus and the putamen (Fig. 2). Administration of levodopa to the MPTP monkeys tended to decrease AMPA receptor–specific binding, but this decrease did not reach statistical significance. The subthalamotomy also produced no significant change in AMPA receptor binding. No specific binding of [3H]-Ro 48-8587 to AMPA receptors was detected in the 2 GP regions in all of the experimental groups examined (data not shown).

**NMDA NR1/NR2B Receptor Binding**

Specific binding of [3H]-Ro 25-6981 to NMDA NR1/NR2B receptors was unaffected by MPTP in all subdvi-
sions of the caudate nucleus and the putamen (Fig. 3A and B). Levodopa treatment significantly increased NMDA NR1/NR2B receptor–specific binding in both striatal regions. Lesion of the STN reversed these levodopa-induced increases bilaterally to control values in both the caudate nucleus and the putamen. No striatal difference was observed between the lesioned versus the nonlesioned side of the STN.

The MPTP treatment did not affect the binding of [3H]-Ro 25–6981 to the NMDA NR1/NR2B receptors in the GP (Fig. 3C). Levodopa treatment significantly increased the NMDA NR1/NR2B receptor binding in the GPe and showed a trend toward an increase in the GPi. These levodopa-induced increases were reversed contralaterally to the STN lesion, whereas the NMDA NR1/NR2B binding remained increased ipsilaterally.

mGlu2/3 Receptor Binding

Striatal [3H]-LY341495 binding to the mGlu2/3 receptor increased in the medial parts of the caudate nucleus and ventromedial putamen of saline-treated MPTP monkeys and remained unchanged in the lateral subregions of the striatum (Fig. 4A and B). Striatal mGlu2/3 receptor–specific binding in the levodopa-treated MPTP monkeys was similar to that in controls. In the ventral caudate nucleus and all subdivisions of the putamen, the subthalamotomy led to a bilateral decrease in mGlu2/3 receptor–specific binding compared with that in the control and in saline- or levodopa-treated MPTP monkeys. The subthalamotomy decreased mGlu2/3 receptor binding less in the dorsal than in the ventral caudate nucleus. The mGlu2/3 receptor binding was higher in the dorsomedial caudate nucleus ipsilateral to the STN lesion than in its contralateral counterpart (Fig. 4A).

Both compartments of the pallidal complex showed a mGlu2/3 receptor binding pattern similar to that in the striatum (Fig. 4C). The MPTP treatment increased mGlu2/3 receptor binding, which was reversed by the levodopa treatment. The STN lesioning and MPTP treatment extensively decreased pallidal mGlu2/3 receptor binding by >
nervous system, making glutamate the most abundant transmitter in the brain. Approximately 70% of the synaptic transmission in the central nervous system is glutamatergic.

**mGlu5 Receptor Binding**

In the caudate nucleus (Fig. 5A), [³⁵]H]-ABP688 binding to the mGlu5 receptor was similar in all treatment groups; however, we noted a trend toward an increase in binding among MPTP monkeys treated with levodopa with or without subthalamotomy. In the putamen (Fig. 5B), mGlu5 receptor–specific binding in saline-treated MPTP monkeys was similar to that in the control monkeys, but was higher in the levodopa-treated and STN-lesioned MPTP monkeys than in the controls in all striatal subdivisions except for the ventromedial putamen. Compared with mGlu5 receptor binding in the saline-treated MPTP monkeys, the binding in the lateral putamen was greater in the levodopa-treated MPTP monkeys. However, the mGlu5 receptor binding remained increased in the levodopa-treated, STN-lesioned MPTP monkeys in the dorsolateral and dorsomedial putamen contralateral and ipsilateral to the subthalamotomy, respectively. No change was observed in the ventromedial putamen across all experimental groups.

mGlu5 receptor binding in the GP was unaffected by any of the treatments (Fig. 5C).

### Discussion

Glutamate neurotransmission accounts for approximately 70% of the synaptic transmission in the central nervous system, making glutamate the most abundant excitatory neurotransmitter. The SN is the only excitatory structure intrinsic to the basal ganglia, and its lesion is hypothesized to induce changes in the binding activity of glutamate receptors. Therefore, we investigated the changes in glutamate receptors known to be involved in LID in monkeys after MPTP and levodopa treatments and after unilateral subthalamotomy. Lesion of the SN increases striatal glutamate levels in normal rats and decreases 6-hydroxydopamine (6-OHDA)–induced elevation of glutamate. Moreover, STN ablation in normal primates decreases pallidal 2-deoxyglucose uptake and expression of the gene for glutaic acid decarboxylase together, these observations indicate decreased cellular activity in SN efferent structures.

**AMPA Receptor Binding**

AMPA receptors are postsynaptic, glutamate-gated ion channel receptors involved in many brain functions, including neurological disorders. Some studies in monkeys have reported few or no changes in AMPA receptors in the striatum after MPTP or levodopa treatment, while others have noted striatal increases in these receptors in dyskinetic monkeys. A previous autoradiographic study has indicated that AMPA receptor–specific binding was higher in the lateral putamen of PD patients with motor complications (both wearing-off and dyskinesias) than in those without motor complications. The SN ablation in normal primates decreases pallidal 2-deoxyglucose uptake and expression of the gene for glutamic acid decarboxylase together, these observations indicate decreased cellular activity in SN efferent structures.

**Subthalamotomy and glutamate receptors**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>MPTP†</th>
<th>L-DOPA‡</th>
<th>Subthalamotomy§</th>
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<tbody>
<tr>
<td>AMPA</td>
<td>No change in putamen &amp; no detectable binding in GP</td>
<td>No change in putamen &amp; no detectable binding in GP</td>
<td>No change in putamen &amp; no detectable binding in GP</td>
</tr>
<tr>
<td>NMDA (containing NR1/NR2B subunits)</td>
<td>No change in putamen or GP</td>
<td>Increased in putamen &amp; GP</td>
<td>Reversed the L-DOPA–induced increases in putamen; in GP, no change ipsilateral &amp; reversed the L-DOPA–induced increases contralateral</td>
</tr>
<tr>
<td>mGlu2/3</td>
<td>No change in putamen &amp; increase in GP</td>
<td>No change in putamen &amp; returned to control levels in GP</td>
<td>Decreased in putamen &amp; decreased bilat in GP</td>
</tr>
<tr>
<td>mGlu5</td>
<td>No change in putamen or GP</td>
<td>Increased in putamen &amp; no significant change in GP</td>
<td>Remained increased in putamen &amp; no change in GP</td>
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* All animals whose results are shown in this table were treated with MPTP; a subgroup of these animals was also treated with levodopa, and 4 of the MPTP- and levodopa-treated animals underwent subthalamotomy.
† Changes in receptor binding reported in this column are from comparisons with receptor binding in the control monkeys.
‡ Changes in receptor binding reported in this column are from comparisons with receptor binding in the control and saline-treated MPTP monkeys.
§ Changes in receptor binding reported in this column are from comparisons with receptor binding in the levodopa-treated MPTP monkeys.

58% relative to that in control monkeys, by > 76% relative to that in saline-treated MPTP monkeys, and by > 65% relative to that in levodopa-treated MPTP monkeys.

mGlu5 Receptor Binding

In the caudate nucleus (Fig. 5A), [³⁵]H]-ABP688 binding to the mGlu5 receptor was similar in all treatment groups; however, we noted a trend toward an increase in binding among MPTP monkeys treated with levodopa with or without subthalamotomy. In the putamen (Fig. 5B), mGlu5 receptor–specific binding in saline-treated MPTP monkeys was similar to that in the control monkeys, but was higher in the levodopa-treated and STN-lesioned MPTP monkeys than in the controls in all striatal subdivisions except for the ventromedial putamen. Compared with mGlu5 receptor binding in the saline-treated MPTP monkeys, the binding in the lateral putamen was greater in the levodopa-treated MPTP monkeys. However, the mGlu5 receptor binding remained increased in the levodopa-treated, STN-lesioned MPTP monkeys in the dorsolateral and dorsomedial putamen contralateral and ipsilateral to the subthalamotomy, respectively. No change was observed in the ventromedial putamen across all experimental groups.

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unit trafficking, but not in the clinical effects of lesioning or stimulating interventions for PD treatment.

**NMDA NR1/NR2B Receptor Binding**

The NMDA receptor is the other major ionotropic postsynaptic glutamate receptor in the brain. Administration of the NMDA noncompetitive antagonist amantadine to MPTP-treated monkeys\(^7\) or to PD patients\(^42,84\) has been shown to reduce LID. It has been proposed that the NR2B subunit is involved in the development of LID because its binding sites increase both in dyskinetic patients and in MPTP-treated monkeys.\(^13, 27,46, 54\) Moreover, the activated, phosphorylated form of NR2B plays a more important role in dyskinesia than the nonphosphorylated NR2B.\(^9,25\) However, the application of NR2B-selective antagonists has yielded conflicting results. The compound CP-101,606 decreased peak-dose LID in a small cohort of PD patients,\(^51\) but increased LID in MPTP-treated marmosets;\(^49\) in addition, CP-101,606 showed antiparkinsonian activity in monkeys,\(^79\) but not in PD patients.\(^51\) While pharmacological blockage of NR2B with some agents (for example, CI-1041 and Co 101244) has antidysskinetic activity in primate models of PD,\(^9,25\) other compounds (Ro 25-6981 and Ro 63-1908) failed to reproduce such activity in 6-OHDA-treated rats.\(^87\)

In the present study, the NMDA NR1/NR2B-specific binding was unaffected by MPTP. By contrast, increases of NMDA NR1/NR2B-specific binding in the striatum were observed in the levodopa-treated MPTP monkeys, consistent with previous findings in dyskinetic primates and PD patients with motor complications.\(^13, 27,46,54\) In the levodopa-treated, STN-lesioned MPTP monkeys, bilateral levels of NMDA NR1/NR2B returned to normal values,
suggesting an effect of the subthalamotomy, most probably from a modulation of the corticostriatal overactive pathway in LID. These results are consistent with those from a study in rats showing that L-DOPA significantly increased both the phosphorylated and nonphosphorylated forms of the NMDA NR1/NR2B receptors. In the same study, high-frequency stimulation of the STN increased only the nonphosphorylated form of NMDA NR1/NR2B. However, the study did not report results of combining both treatments, that is, of L-DOPA treatment with subthalamic stimulation. Because the setting in that study differed from ours (for example, in the animal models used and in the combined pharmacological or surgical treatments), we cannot easily draw conclusions from our observation that alteration of the STN induced changes in the NMDA NR1/NR2B receptor binding.

In all experimental groups, the GP segments displayed a pattern of NMDA NR1/NR2B–specific binding similar to that in the striatum, except for the levodopa-treated, STN-lesioned MPTP monkeys in which the NMDA NR1/NR2B-specific binding remained elevated in the GP region ipsilateral to the subthalamotomy. The decrease in binding contralateral to the lesion may be explained by a compensatory mechanism from a decreased glutamatergic input from the STN. The STN neurons in primates also project to the contralateral GP via the dorsal supraoptic decussation. The strength of this efferent connection is currently unknown but may contribute to the results observed here. More studies are warranted to assess this

**Fig. 4.** mGlu2/3 receptor–specific binding in the caudate nucleus ($F_{12,45} = 3.08$, $p < 0.01$) (A), putamen ($F_{12,45} = 5.37$, $p < 0.001$) (B), and GP (GPi $F_{12,45} = 43.37$, $p < 0.001$ and GPe $F_{12,45} = 29.55$, $p < 0.001$) (C) of saline-treated control monkeys; saline-treated MPTP monkeys; levodopa-treated MPTP monkeys; and levodopa-treated, STN-lesioned MPTP monkeys. Various symbols indicate the following levels of statistical significance and comparisons: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus controls; ++$p < 0.01$, and +++$p < 0.001$ versus saline-treated MPTP monkeys; and Δ$p < 0.05$, ΔΔ$p < 0.01$, and ΔΔΔ$p < 0.001$ versus levodopa treatment.

**Fig. 5.** mGlu5 receptor–specific binding in the caudate nucleus ($F_{12,45} = 1.51$, $p = 0.180$) (A), putamen ($F_{12,45} = 3.37$, $p < 0.01$) (B), and GP (GPi $F_{12,45} = 0.41$, $p = 0.751$ and GPe $F_{12,45} = 0.49$, $p = 0.695$) (C) of saline-treated control monkeys; saline-treated MPTP monkeys; levodopa-treated MPTP monkeys; and levodopa-treated, STN-lesioned MPTP monkeys. Various symbols indicate the following levels of statistical significance and comparisons: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus controls; +$p < 0.05$ and ++$p < 0.01$ versus saline-treated MPTP monkeys.
observed discrepancy in outcomes between ipsi- and contralateral subthalamotomy. The NMDA receptor density decreases in the GP (equivalent to the GPi in primates) and in the substantia nigra pars reticulata ipsilateral to the STN lesion in normal rats.11,88 Our results add evidence to previous studies demonstrating the involvement of the NMDA receptors in LID.

**mGlu2/3 Receptor Binding**

The specific role of the mGlu2/3 receptors in the basal ganglia remains to be elucidated. They closely interact with the dopamine system, pre- and postsynaptically in the dopamine circuitry.1,5,35,47 Administration of mGlu2/3 receptor agonists after exposure to 6-OHDA in rats or before MPTP injection in mice have yielded interesting neuroprotective effects.4,17,48 and blockade of the mGlu2/3 receptors amplifies the MPTP insult.4

 Autoradiographic studies in monkeys reported that MPTP treatment resulted in no changes in the mGlu2/3 receptor-specific binding in the striatum.56,69 Similarly, we observed that MPTP treatment did not change mGlu2/3 receptor-specific binding in the putamen and in the lateral caudate nucleus but increased that in the medial caudate nucleus. Levodopa treatment causes either a decrease in striatal mGlu2/3 receptors46 or no change in parkinsonian monkeys69 or PD patients.71 Consistent with these results, we found that the striatal and pallidal levels of mGlu2/3 receptors were at normal levels in the saline- or levodopa-treated MPTP monkeys. However, mGlu2/3 receptor levels were strongly decreased in the levodopa-treated, STN-lesioned monkeys compared with levels of these receptors in the controls and in the saline- or levodopa-treated MPTP monkeys.

Group II metabotropic receptors (mGlu2/3) are located presynaptically on corticostriatal neurons and on subthalamic terminals.61 Their expression in both the GPe and the GPi suggests localization on subthalamopallidal neurons, but this has not been demonstrated yet. If they are indeed localized to subthalamopallidal neurons, a subthalamotomy would reduce mGlu2/3 receptors in both the GPe and GPe because both regions receive afferent glutamatergic neurons from the STN.39 The same observation could also apply to the reduction in the striatum, since the STN also projects to the striatum.39,32,34 This scenario does not exclude a reduction of the mGlu2/3 receptors on the corticostriatal efferents, which may express the mGlu2/3 receptors presynaptically.82 To our knowledge, no other reports have examined the effects of surgical alleviation of LID on the expression of mGlu2/3 receptors; therefore, more studies are warranted to fully address the mechanisms implicated in the effect of lesions of the STN.

**mGlu5 Receptor Binding**

mGlu5 receptors are mainly located postsynaptically on striatal projection neurons and interneurons23 and are expressed on few cortico- and thalamostriatal presynaptic terminals.59 Their wide distribution in the basal ganglia has generated interest to use them as a potential therapeutic target for treating PD and LID.24 Lesion of the nigrostrial pathway via MPTP treatment has been reported to increase mGlu5 receptor-specified binding, measured by autoradiography, in the striatum,45,56,70 whereas other studies55 and also the present one showed no significant increase in mGlu5 receptors in the striatum.

The most striking change in mGlu5 receptors is observed in MPTP-treated monkeys after chronic levodopa treatment. Motor complications induced by levodopa correlated with an increase of mGlu5 receptors in the posterior striatum.45,55,56,70 Consistent with these previous reports, striatal levels of the mGlu5 receptors were increased or tended to be increased in all MPTP-treated monkeys treated with levodopa, including those that underwent subthalamotomy.

One report studied the effects of systemic administration of an mGlu5 receptor antagonist and of subthalamic lesion on dyskinesia in 6-OHDA–treated rats.40 The authors concluded that the negative allosteric mGlu5 modulator 2-methyl-6-(phenylethynyl)-pyridine reduces LID better than subthalamotomy does and that this reduction is correlated with a greater decrease in the number of cells immunoreactive to the LID-associated marker deltaFosB/ FosB.40 Currently, no studies combining both deep brain stimulation and mGlu5 receptor manipulation are available, and evidence from subthalamotomy is too insufficient to draw solid conclusions about whether STN alteration may change mGlu5 receptor density. More studies are needed to fully assess the interactions between surgical procedures and the mGlu5 receptor, especially because mGlu5 receptors are located postsynaptically in the STN and perisynaptically in the GPi,34 structures that are both the main targets in PD surgery.31

**Conclusions**

AMPA-specific binding remained unchanged by the pharmacological and surgical treatments. The MPTP treatment increased the levels of mGlu2/3 receptors in the nonmotor striatum (that is, in the medial caudate nucleus and ventromedial putamen), as well as in both compartments of the GP. However, it had no effect on all of the other investigated glutamate receptors. Levodopa treatment in MPTP-treated monkeys increased the levels of NMDA NR1/NR2B receptors in the GP and striatum, and also of the mGlu5 receptors in the putamen. The levodopa-induced increases in striatal NMDA NR1/NR2B receptors were reversed by subthalamotomy. In the GP, this reversal was contralateral to the STN lesion. The mGlu5 receptors in the basal ganglia were unaffected by the subthalamotomy and remained increased in the striatum. mGlu2/3 receptors displayed an opposite pattern: their density in levodopa-treated MPTP monkeys was similar to that in the controls and decreased in the striatum and in the GP of levodopa-treated, STN-lesioned MPTP monkeys. Considering the presynaptic location of the mGlu2/3 receptors, their decrease in the GP and in the striatum may be the result of the subthalamic lesion and of a reduction in the corticostriatal and/or subthalamostriatal efferent connections, respectively. This decrease may contribute to the alleviation of parkinsonian symptoms and LID after subthalamotomy.

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