

Implication of NMDA Receptors in the Antidyskinetic Activity of Cabergoline, CI-1041, and Ro 61-8048 in MPTP Monkeys with Levodopa-induced Dyskinesias

Bazoumana Ouattara · Samah Belkhir ·
Marc Morissette · Mehdi Dridi · Pershia Samadi ·
Laurent Grégoire · Leonard T. Meltzer ·
Thérèse Di Paolo

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Abstract This study assessed striatal N-methyl-D-aspartate (NMDA) glutamate receptors of 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkeys with levodopa (L-DOPA)-induced dyskinesias (LID). In a first experiment, four MPTP monkeys receiving L-DOPA/Benserazide alone developed dyskinesias. Four MPTP monkeys received L-DOPA/Benserazide plus CI-1041 an NMDA antagonist selective for NR1/NR2B and four were treated with L-DOPA/Benserazide plus a small dose of cabergoline; one monkey of each group developed mild dyskinesias at the end of treatment. In a second experiment, a kynurenine 3-hydroxylase inhibitor Ro 61-8048, combined with L-DOPA/Benserazide, reduced dyskinesias in MPTP monkeys. Drug-treated MPTP monkeys were compared to intact monkeys and saline-treated MPTP monkeys. Glutamate receptors were investigated by autoradiography using [³H]CGP-39653 (NR1/NR2A antagonist) and [³H]Ro25-6981 (NR1/NR2B antagonist). In general, striatal [³H]CGP-39653 specific binding was unaltered in all experimental groups. MPTP

lesion decreased striatal [³H]Ro25-6981 specific binding; these levels were enhanced in the L-DOPA-alone-treated MPTP monkeys and decreased in antidyskinetic drugs treated monkeys. Maximal dyskinesias scores of the MPTP monkeys correlated significantly with [³H]Ro25-6981 specific binding in the rostral and caudal striatum. Hence, MPTP lesion, L-DOPA treatment and prevention of LID with CI-1041 and cabergoline, or reduction with Ro 61-8048 were associated with modulation of NR2B/NMDA glutamate receptors.

Keywords NMDA receptor · L-DOPA · Dyskinesia · MPTP · Monkeys · Cabergoline · CI-1041 · Ro 61-8048

Abbreviations

DA	Dopamine
DAT	Dopamine Transporter
DOPAC	3, 4 dihydroxyphenylacetic acid
HVA	Homovanillic acid
L-DOPA	Levodopa
LID	L-DOPA-induced dyskinesias
LTD	long term depression
LTP	long term potentiation
3-MT	3-methoxytyramine
NMDA	N-methyl-D-aspartate
PD	Parkinson's disease

B. Ouattara · S. Belkhir · M. Morissette · M. Dridi · P. Samadi ·
L. Grégoire · T. Di Paolo (✉)
Molecular Endocrinology and Oncology Research Center,
Laval University Medical Center, CHUL,
2705 Laurier Blvd,
Quebec, QC G1V 4G2, Canada
e-mail: therese.dipaolo@crchul.ulaval.ca

B. Ouattara · S. Belkhir · M. Morissette · M. Dridi · P. Samadi ·
L. Grégoire · T. Di Paolo
Faculty of Pharmacy, Laval University,
Quebec, QC, Canada

L. T. Meltzer
Global Research & Development, Pfizer,
Ann Arbor, MI, USA

Introduction

Adverse effects such as dyskinesias compromise dopaminomimetic therapy of Parkinson disease (PD) patients (Brotchie et al. 2005). Several reports suggest that an

augmentation in the synaptic efficacy of striatal ionotropic glutamatergic receptors contribute to the appearance of these motor complications (Chase 2004; Bara-Jimenez et al. 2006). The synaptic efficacy of N-methyl-D-aspartate (NMDA) receptors increases, and the subsequent augmentation in cortico-striatal excitatory drive modifies output with dyskinesias as a consequence (Brotchie et al. 2005).

In accordance with this proposal, it has been shown that drugs that block NMDA receptors could diminish or prevent levodopa (L-DOPA)-induced motor response alteration in animal model of PD (Blanchet et al. 2004; Hadj Tahar et al. 2004; Gregoire et al. 2008). In the 6-hydroxydopamine (6-OHDA) rodent model of PD and the 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate model of PD, riluzole, an inhibitor of glutamate release was found to relieve or delay L-DOPA-induced dyskinesias (LID) (Gilgun-Sherki et al. 2003). However discordant results have been reported in respect to changes of NMDA receptors in dyskinesias. For instance in our previous study with 14 PD patients displaying motor complications, the NMDA receptors composed of the NR1/NR2B subunits increased in the putamen whereas those with the NR1/NR2A subunits remained unchanged (Calon et al. 2003). The development of dyskinesias following a D1 agonist (SKF-82958) treatment was associated with an up-regulation of NMDA receptors composed of NR1/NR2A and NR1/NR2B subunits (Calon et al. 2002). Other studies using [¹²⁵I]MK-801 binding reported that NMDA receptors did not change in the striatum of MPTP-lesioned monkeys and decreased in MPTP monkeys treated with L-DOPA (He et al. 2000). Conversely, Ulas and co-workers (1994) found an increase of NMDA-sensitive glutamate binding in the striatum of PD patients treated with L-DOPA; these authors did not indicate if patients displayed dyskinesias or other motor complications. Thus, the aim of this study was to evaluate by autoradiography changes of NMDA receptors composed of NR2A and NR2B subunits in MPTP primates treated with L-DOPA alone or with cabergoline, CI-1041, or Ro 61-8048. Cabergoline is a long-acting D2 dopamine (DA) receptor agonist (Calon et al. 2002) and CI-1041 is a specific NR2B/NMDA antagonist (Chen et al. 2003); these drugs administered with L-DOPA prevented the development of dyskinesias (Belanger et al. 2003). Ro 61-8048 is a kynurenine-3-hydroxylase inhibitor (Schwarcz and Pellicciari 2002) that was shown to reduce dyskinesias (Gregoire et al. 2008).

Materials and Methods

Animals and Drug Treatments

Experiments were carried out using 37 drug naive female ovariectomized monkeys (*Macaca fascicularis*) (2.5–

4.3 kg) in agreement with the standards of the Canadian Council on Animal Care. Animals were maintained in a temperature-controlled environment and artificial daylight (lights on 0600–1800 hours). The Laval University committee for the protection of animals approved this study.

This research was performed in two experiments. In the first experiment, monkeys weighing between 3.95–4.22 kg, aged 4–11 years, were divided into five experimental groups. One non-parkinsonian group ($n=4$) remained untreated and served as healthy controls (control group), and four groups (each $n=4$) were rendered parkinsonian with the neurotoxin MPTP (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) administered continuously using subcutaneous Alzet minipumps (0.5 mg/24 h in saline solution) until sustained parkinsonian features were achieved. Treatment was started after the bilateral parkinsonian syndrome had stabilized (after 1 to 6 months). Four of these MPTP-exposed monkeys remained untreated (MPTP group) while four others received a chronic daily oral treatment with L-DOPA/benserazide alone (100/25 mg; Prolopa®) (Hoffmann-La Roche limited, Mississauga, ON, Canada) and four of the eight remaining animals were treated simultaneously with L-DOPA (same dosage) plus CI-1041 (Pfizer, Ann Arbor, MI, USA; 10 mg/kg), a highly selective antagonist for NR2B containing NMDA receptor. CI-1041 was dissolved and administered *per os* at 9:30 A.M. and the spontaneous behavior of the animals was observed until the effect of a given drug was terminated. The last four other monkeys were treated with a combination of oral L-DOPA and a threshold dose of cabergoline administered by subcutaneous injection. The threshold dose of cabergoline was the minimum dose of cabergoline producing a small stimulatory effect but this change was not sufficient to modify the antiparkinsonian score obtained with the disability scale. Cabergoline (Adria laboratories, Columbus, OH, USA) was dissolved in 0.9% sterile saline (1 ml) acidified with 2% phosphoric acid (30 µl) and administered at doses ranging from 0.015 to 0.035 mg/kg. Drugs were administered in the morning (at 9:30 A.M.) for 4 weeks, and spontaneous behaviors were observed until the end of antiparkinsonian response (Belanger et al. 2003).

The second experiment included 17 monkeys weighing between 2.5–4.3 kg and aged 5–8 years. The animals were divided in four experimental groups. The control group ($n=4$) remained untreated. The 13 others received MPTP until a bilateral parkinsonian syndrome had stabilized (5–6 months). These monkeys were divided in three groups. An MPTP-untreated group ($n=4$), L-DOPA-treated group ($n=4$) received L-DOPA/benserazide alone (100/25 mg; Prolopa®), and five monkeys were treated with Ro 61-8048 plus L-DOPA (same dose). Ro 61-8048 (provided by Newron Pharmaceuticals, Italy) was homogenized and suspended in a solution of 0.1% (v/v) Tween80/sterile

water. A fresh solution was prepared every 2 days and was kept at 4°C under constant agitation. Animals of this experimental group received 20–25 ml of Ro 61-8048 suspension by nasogastric gavage; a dose of 50 mg/kg was given 3 h before L-DOPA administration, based on our previous acute experiment with this drug where a significant anti-dyskinetic response was obtained with 30 and 100 mg/kg of Ro 61-8048 (Samadi et al. 2005; Gregoire et al. 2008). MPTP and L-DOPA were administered as described in the first experiment.

In both experiments treatments lasted for 28 consecutive days; behavioral assessment of these treatments was previously reported (Belanger et al. 2003; Hadj Tahar et al. 2004; Gregoire et al. 2008).

Tissue Preparation

Animals were killed 24 h after the end of the chronic treatments by an over dose of sodium pentobarbital. The brains were then immersed in isopentane (−40°C) and stored at −80°C. Hemisected brains were cut into coronal sections of 12 μm on a cryostat (−18°C) at two different levels for the striatum corresponding approximately to levels A18–A22 (rostral striatum), A15–A18 (caudal striatum), in the atlas of Szabo and Cowan (Szabo and Cowan 1984). Sections were mounted onto Super Frost Plus (Fisher, Canada) slides, desiccated overnight at 4°C and stored at −80°C.

Biogenic Amine Assays

Small frozen tissue was taken for the biogenic amine assays. The pieces of coronal sections were homogenized in 250 μl of 0.1 M HClO₄ at 4°C. The homogenate was centrifuged at 10,000×g for 20 min. The supernatants were kept at −80°C. The pellets were dissolved in 100 μl of 0.1 M NaOH for assay of protein content. The concentration of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA) was measured by high-performance liquid chromatography with electrochemical detection (Morissette et al. 2006).

Receptor Autoradiography

The extent of denervation was also evaluated by measuring the DA transporter (DAT) with [¹²⁵I]RTI-121 (3β-(4-I-iodophenyl)tropane-2β-carboxylic acid isopropyl ester, 2,200 Ci/mmol; Mandel, Boston, MA, USA) binding autoradiography. Specific binding was measured using 25 pM of [¹²⁵I]RTI-121. Non-specific binding was determined by adding 100 nM of Mazindol (Sandoz Pharmaceuticals, Dorval, Quebec, Canada) to the incubation buffer (Morissette et al. 2006).

Autoradiography of NMDA receptors composed of NR1/NR2A and NR1/NR2B subunits was performed using

[³H]CGP-39653 (PerkinElmer 50 Ci /mmol) and [³H]Ro 25-6981 (F. Hoffman-Laroche Bazel, Switzerland; 25.7 Ci/mmol) respectively according to our previously published procedures (Calon et al. 2002). Briefly, sections were incubated with 20 nM of [³H]CGP-39653 or 5 nM [³H]Ro 25-6981 to estimate total binding to the NR1/NR2A and NR1/NR2B subunits respectively. Non-Specific binding was estimated by adding respectively 500 μM NMDA (NR1/NR2A subunit) or 10 μM Ro 04-5595 hydrochloride (F. Hoffman-Laroche) (NR1/NR2B subunit) to the incubation buffers. After postincubation washes, the slide-mounted tissue sections were dried overnight at room temperature and then exposed to [³H]-sensitive films (Kodak BIOMAX MR) along with tritium standards ([³H]-microscales, Amersham) during 10 weeks or 4 weeks at room temperature for [³H]CGP-39653 and [³H]Ro 25-6981 respectively. For each rostral and caudal brain regions investigated, we used 4 to 6 brain slices for each animal for autoradiographic analyses.

Image, Data, and Statistical Analysis

Quantification of all autoradiograms was performed on a G4 Macintosh connected to a Sony video camera (model XC-77) and a constant illumination light table using computerized densitometry with the software package NIH Image 1.63 (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). Optical grey densities were transformed into nCi/mg of tissue equivalent using a standard curve generated with tritium standards ([³H]-microscales, Amersham) and then converted into fmol/mg of tissue using the specific activity of the radioligand.

For analysis, the caudate nucleus and putamen were divided in four subregions (medial-lateral and dorsal-ventral) at two rostrocaudal coordinates. Statistical comparisons of data were performed with a one way analysis of variance with subregions and treatments as variables, for each autoradiographic assay, followed by post-hoc pairwise comparisons with Fisher's probability of least significant difference test. A simple regression model was used to determine coefficients of correlation and the significance of the degree of linear relationship between variables.

Results

Behavioral Assessment

The behavioral evaluation of these monkeys has previously been reported using parkinsonian and dyskinesia scores developed in our laboratory (Hadj Tahar et al. 2004; Gregoire et al. 2008). L-DOPA alone or combined with

CI-1041, cabergoline, or Ro 61-8048 significantly lowered the parkinsonian scores as compared to the controls. In the L-DOPA-treated group, the animals rapidly developed dyskinesias that were mainly choreic in nature. Conversely, co-administration of CI-1041 (Hadj Tahar et al. 2004) or cabergoline (Belanger et al. 2003) completely prevented the induction of dyskinesia in three animals in which no dyskinesia was noted until the end of the study, the fourth monkey developed mild choreic movements of the lower limbs at the end of the fourth week of treatment. In the group that received Ro 61-8048 with L-DOPA, dyskinesias were significantly reduced compared to MPTP monkeys that receive L-DOPA alone (Gregoire et al. 2008).

Denervation Assessment

Catecholamine concentrations were measured in the caudate nucleus and putamen. Results in each experiment were

similar in the caudate nucleus and putamen at the rostro-caudal regions analyzed. MPTP lesioning resulted in an extensive depletion of striatal DA concentration in all MPTP-treated monkeys with an average decrease of 99% compared to intact monkeys. Biogenic amine results of the first experiment were previously reported in a study focusing on subtype five of metabotropic glutamate receptors (Samadi et al. 2008). Assessment of denervation of the monkeys of the second experiment showed an extensive decrease of DA (anterior: caudate nucleus $F_{3,13}=61.0$, $p<0.0001$ and putamen $F_{3,13}=199.1$, $p<0.0001$; posterior: caudate $F_{3,12}=63.0$, $p<0.0001$ and putamen $F_{3,13}=121.9$, $p<0.0001$) and its metabolites DOPAC (anterior: caudate $F_{3,13}=27.5$, $p<0.0001$ and putamen $F_{3,13}=40.9$, $p<0.0001$; posterior: caudate $F_{3,12}=5.35$, $p=0.014$ and putamen $F_{3,13}=14.5$, $p=0.0002$), 3-MT (anterior: caudate $F_{3,13}=291.1$, $p<0.0001$ and putamen $F_{3,13}=100.9$, $p<0.0001$; posterior: caudate $F_{3,12}=59.3$,

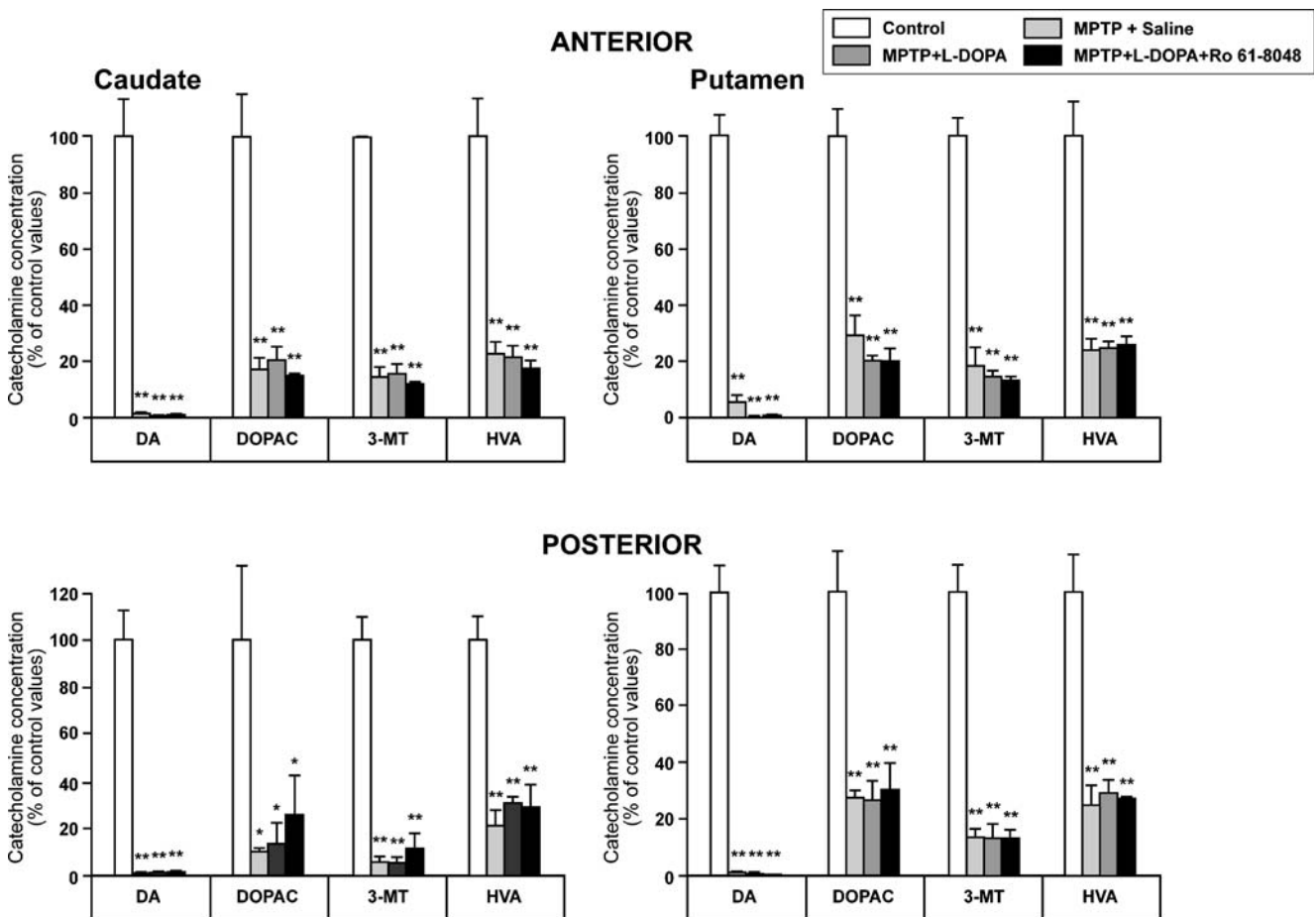


Figure 1 Effect of MPTP lesion and treatments on catecholamine concentrations in control monkeys ($n=4$), untreated MPTP monkeys ($n=4$), L-DOPA-treated MPTP monkeys ($n=4$), and MPTP monkeys treated with L-DOPA and Ro 61-8048 ($n=5$). Data are mean±SEM (bars) in the rostral and caudal parts of the caudate nucleus and putamen expressed in percentage of controls. Values are the mean±SEM (bars) in percent of control, where 100% control values were for

rostral caudate, DA=138.0, DOPAC=11.0, 3-MT=38.1, and HVA=285.6 ng/mg protein; for the caudal caudate, DA=87.9, DOPAC=7.0, 3-MT=25.2, and HVA=96.9; for rostral putamen, DA=160.9, DOPAC=11.4, 3-MT=44.9, and HVA=475.9; and for caudal putamen, DA=112.6, DOPAC=5.5, 3-MT=39.6, and HVA=289.4; * $p<0.01$ and ** $p<0.0001$ vs. control monkeys

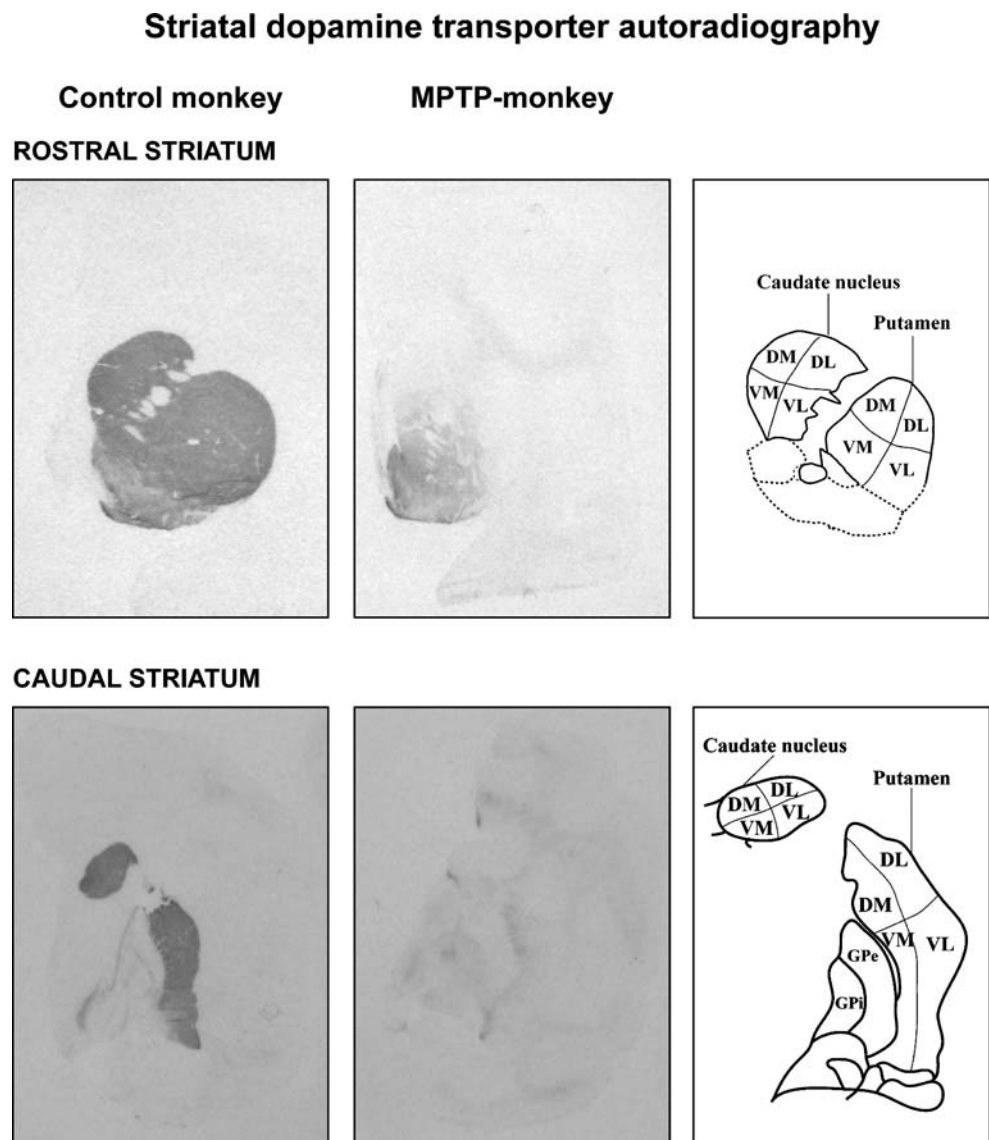
$p < 0.0001$ and putamen $F_{3,13} = 58.5$, $p < 0.0001$) and HVA (anterior: caudate $F_{3,13} = 29.0$, $p < 0.0001$ and putamen $F_{3,13} = 35.3$, $p < 0.0001$; posterior: caudate $F_{3,12} = 22.3$, $p < 0.0001$ and putamen $F_{3,13} = 24.2$, $p < 0.0001$), in MPTP monkeys, compared to the controls (Fig. 1). The DOPAC/DA and HVA/DA concentrations ratios were increased in MPTP-treated monkeys (data not shown). MPTP lesion also induced a decrease of [125 I]RTI-121 specific binding to the DAT in the lateral, medial, dorsal and ventral parts of the caudate nucleus (anterior: DM: $F_{3,13} = 68.3$, $p < 0.0001$; VM: $F_{3,13} = 28.0$, $p < 0.0001$; DL: $F_{3,13} = 236.4$, $p < 0.0001$; VL: $F_{3,13} = 115.6$, $p < 0.0001$; posterior: DM: $F_{3,13} = 127.3$, $p < 0.0001$; VM: $F_{3,13} = 235.1$, $p < 0.0001$; DL: $F_{3,13} = 129.1$, $p < 0.0001$; VL: $F_{3,13} = 223.6$, $p < 0.0001$) and putamen (anterior: DM: $F_{3,13} = 110.5$, $p < 0.0001$; VM: $F_{3,13} = 109.2$, $p < 0.0001$; DL: $F_{3,13} = 163.4$, $p < 0.0001$; VL: $F_{3,13} = 126.7$, $p < 0.0001$; posterior: DM: $F_{3,13} = 52.9$, $p < 0.0001$; VM:

$F_{3,13} = 39.7$, $p < 0.0001$; DL: $F_{3,13} = 67.1$, $p < 0.0001$; VL: $F_{3,13} = 57.9$, $p < 0.0001$) of all MPTP-treated monkeys (Figs. 2 and 3). Extent of denervation as assessed with DA and metabolites concentrations and DAT specific binding in the caudate and putamen was similar in all the MPTP-lesioned groups of monkeys (Figs. 1 and 3).

Distribution of [3 H]Radioligands Binding to Glutamate Receptors in the Basal Ganglia

[3 H]CGP-39653 and [3 H]Ro 25-6981 binding was observed in all parts of the rostral and caudal caudate-putamen of monkeys (Fig. 4). No lateral-medial or ventral-dorsal gradients of [3 H]CGP-39653 and [3 H]Ro 25-6981 total binding were observed in control monkeys in the rostral and caudal caudate nucleus as well putamen (Fig. 4).

Figure 2 Representative autoradiograms of coronal brain sections showing [125 I]RTI-121 binding to the dopamine transporter (DAT) in the rostral and caudal striatum of control and MPTP monkeys of the experimental groups investigated



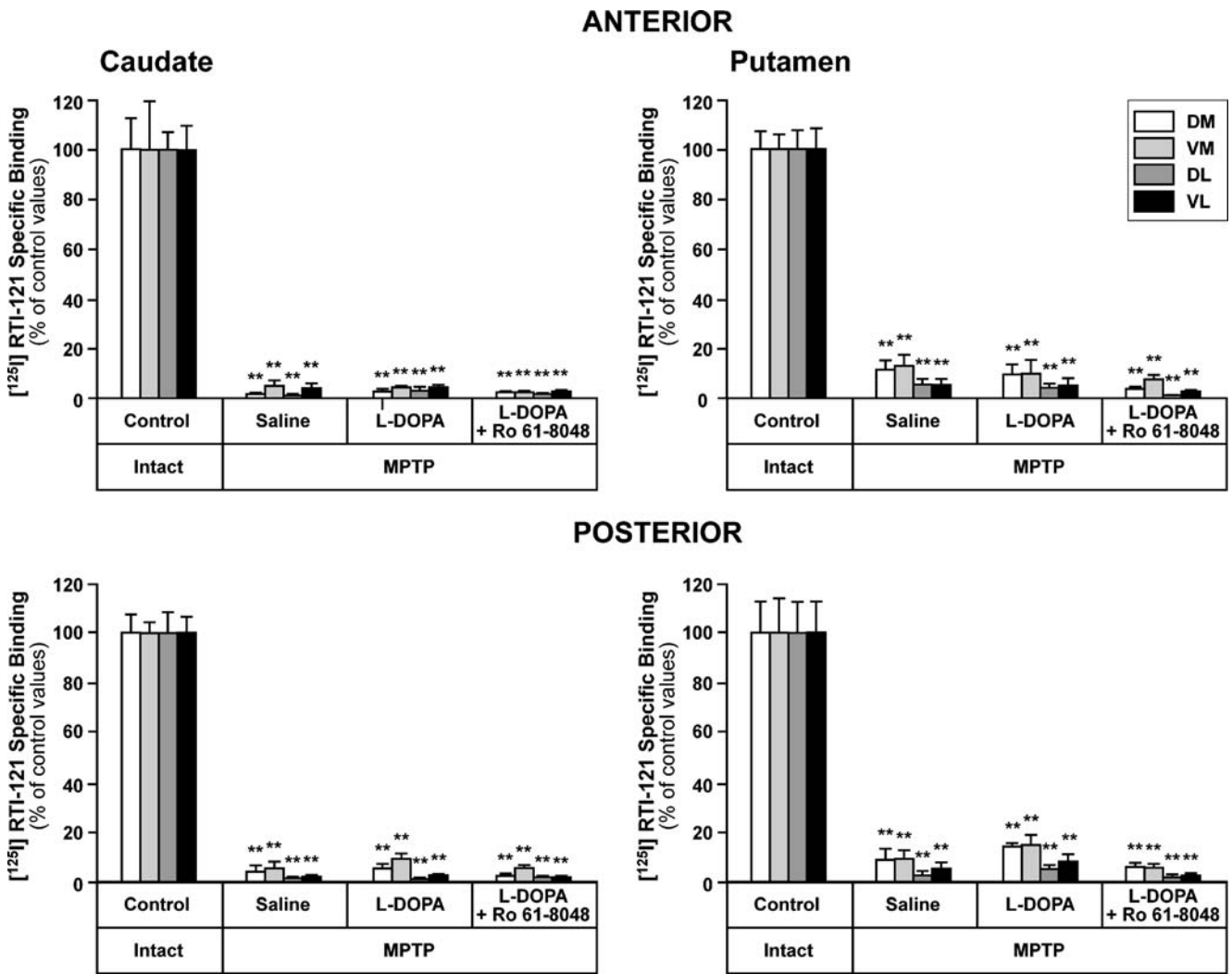


Figure 3 Effect of MPTP lesion and treatments on striatal dopamine transporter (DAT)-specific binding labelled with [¹²⁵I]RTI-121 in control monkeys (*n*=4), untreated MPTP monkeys (*n*=4), L-DOPA-treated MPTP monkeys (*n*=4), and MPTP monkeys treated with L-DOPA and Ro 61-8048 (*n*=5). Data are mean±SEM (bars) in the rostral and caudal parts of the caudate nucleus and putamen expressed in percentage of controls. One hundred percent control values (fmol/mg of

tissue) were for the rostral caudate, dorsomedial (DM)=1.1, ventromedial (VM)=1.1, dorsolateral (DL)=0.9 and ventrolateral (VL)=0.9; for the caudal caudate, DM=1.5, VM=1.3, DL=1.6, VL=1.6; for the anterior putamen DM=0.8, VM=0.8, DL=1.0, VL=1.1; for the caudal putamen DM=1.2, VM=1.2, DL=1.5, VL=1.4. ***p*<0.0001 vs. control monkeys

First Experiment

NR2A/NMDA Receptors

Striatal [³H]CGP-39653 specific binding to NMDA containing NR1/NR2A subunits did not significantly change with the lesion and treatments in the anterior and posterior regions of the caudate nucleus as well as the putamen (anterior caudate nucleus, DM: *F*_{4,13}=0.65, *p*=0.632; VM: *F*_{4,13}=0.87, *p*=0.504; DL: *F*_{4,13}=1.30, *p*=0.320; VL: *F*_{4,13}=2.16, *p*=0.131; posterior caudate nucleus, DM: *F*_{4,11}=1.23, *p*=0.352; VM: *F*_{4,11}=1.06, *p*=0.422; DL: *F*_{4,11}=0.69, *p*=0.609; VL: *F*_{4,11}=0.77, *p*=0.565) and (anterior putamen, DM: *F*_{4,13}=0.33, *p*=0.855; VM: *F*_{4,13}=0.54, *p*=0.709; DL:

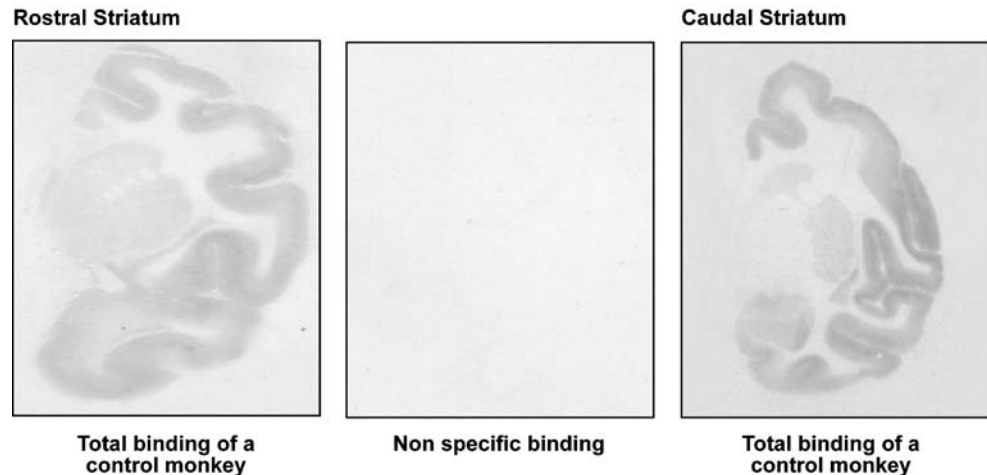
*F*_{4,13}=2.90, *p*=0.064; VL: *F*_{4,13}=0.68, *p*=0.619; posterior putamen, DM: *F*_{4,11}=1.01, *p*=0.445; VM: *F*_{4,11}=1.94, *p*=0.173; DL: *F*_{4,11}=2.89, *p*=0.073; VL: *F*_{4,11}=2.78, *p*=0.080) (Table 1). MPTP monkeys treated with L-DOPA alone or with CI-1041 or cabergoline also had striatal [³H]CGP-39653 specific binding unchanged (Table 1).

NR2B/NMDA Receptors

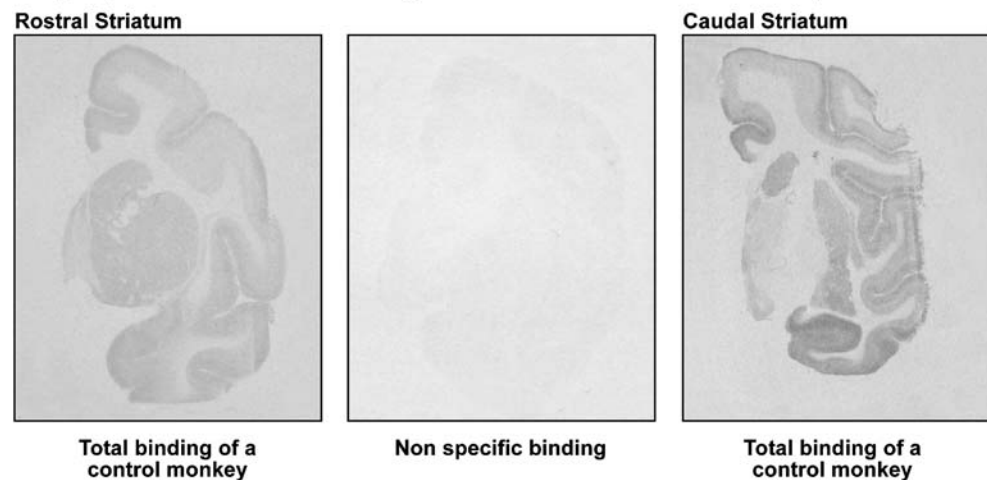
Striatal [³H]Ro 25-6981 specific binding to NMDA receptors containing NR1/NR2B subunits was changed following the MPTP lesion and treatments in the anterior caudate nucleus (DM: *F*_{4,14}=3.93, *p*=0.024; VM: *F*_{4,14}=3.35, *p*=0.040; DL: *F*_{4,14}=2.25, *p*=0.116; VL: *F*_{4,14}=2.53, *p*=0.087) and puta-

Figure 4 Representative autoradiograms of coronal brain sections showing **A** [^3H]CGP-39653 binding to NR1/NR2A and **B** [^3H]Ro 25-6981 binding to NR1/NR2B assembly of NMDA receptors of control monkeys. Total and non-specific binding, for the rostral and caudal caudate nucleus and putamen is shown

A: [^3H] CGP-39653 binding to NR1/NR2A NMDA receptors



B: [^3H] Ro 25-6981 binding to NR1/NR2B NMDA receptors



men (DM: $F_{4,14}=2.65$, $p=0.077$; VM: $F_{4,14}=4.29$, $p=0.018$; DL: $F_{4,14}=2.96$, $p=0.057$; VL: $F_{4,14}=6.28$, $p=0.004$). No significant effect of lesion and treatments was seen in the posterior striatum (caudate nucleus: DM: $F_{4,14}=1.28$, $p=0.325$; VM: $F_{4,14}=1.13$, $p=0.382$; DL: $F_{4,14}=0.81$, $p=0.540$; VL: $F_{4,14}=0.84$, $p=0.521$). putamen: DM: $F_{4,14}=0.56$, $p=0.697$; VM: $F_{4,14}=0.69$, $p=0.613$; DL: $F_{4,14}=0.84$, $p=0.523$; VL: $F_{4,14}=0.42$, $p=0.794$) (Fig. 5). A decrease of [^3H]Ro 25-6981 specific binding in the MPTP lesioned compared to the control monkeys was significant in the anterior medial caudate nucleus and in the ventral putamen (Fig. 5). [^3H]Ro 25-6981 specific binding in MPTP monkeys chronically treated with L-DOPA was significantly higher in the anterior dorso-medial caudate nucleus and in the ventro-lateral putamen compared to untreated MPTP monkeys (Fig. 5). The non-dyskinetic MPTP monkeys that received the combined L-DOPA+CI-1041 treatment had [^3H]Ro 25-6981 specific binding levels significantly lower than control monkeys and similar to those observed in untreated MPTP monkeys in the anterior striatum (Fig. 5). The other non-

dyskinetic MPTP monkeys that received L-DOPA plus cabergoline presented significant lower levels of [^3H]Ro 25-6981 specific binding in the medial part of the anterior caudate nucleus compared to the control group. This effect was more pronounced in the ventral part of the anterior putamen (Fig. 5). In the anterior putamen, this decrease was also significant when compared to L-DOPA-treated MPTP monkeys. In the posterior striatum the pattern was similar to that observed in the anterior striatal region but was not significant (Fig. 5).

Second Experiment

NMDA/NR2A Receptors

In both the anterior and posterior striatal regions, in general [^3H]CGP-39653 specific binding was not significantly changed by the lesion and treatments (anterior caudate nucleus DM: $F_{3,13}=0.49$, $p=0.694$; VM, $F_{3,13}=0.45$, $p=0.719$; DL: $F_{3,13}=0.83$, $p=0.502$; VL: $F_{3,13}=0.84$, $p=0.498$;

Table 1 [³H]CGP-39653 specific binding to NR1/NR2A subunit of the NMDA receptors in anterior and posterior subregions of the caudate-putamen of control (intact), MPTP+saline-treated, MPTP+L-DOPA-treated, and non-dyskinetic MPTP+L-DOPA+CI-1041 or + non-dyskinetic L-DOPA+cabergoline-treated monkeys

	Caudate				Putamen			
	DM	VM	VL	DL	DM	VM	VL	DL
	Rostral							
Control	100±7.9 ^a	100±5.2 ^a	100±10.6 ^a	100±14.6 ^a	100±4.9 ^a	100±5.7 ^a	100±4.6 ^a	100±4.2 ^a
MPTP + Saline	78.4±10.8	72.1±7.7	103.4±13.9	130.2±22.8	100.6±14.1	105.7±18.7	107.9±11.1	115.8±9.1
MPTP+L-DOPA	96.1±9.0	94.9±5.5	136.9±10.4	150.5±10.1	109.4±9.8	102.5±18.0	102.1±13.2	121.5±3.1
MPTP+L-DOPA+ CI-1041	78.2±19.7	80.4±15.0	110.8±12.5	127.7±21.9	98.5±19.4	91.7±15.9	106.5±10.1	116.1±12.0
MPTP+Cabergoline	98.7±22.7	86.7±27.3	90.1±13.4	117.5±14.5	84.5±27.7	70.9±29.5	75.5±31.7	83.6±15.4
	Caudal							
Control	100±2.3 ^a	100±2.1 ^a	100±1.1 ^a	100±4.2 ^a	100±3.1 ^a	100±2.4 ^a	100±2.3 ^a	100±4.7 ^a
MPTP + Saline	100.6±5.1	99.5±7.0	121.5±7.2	121.6±7.5	112.5±11.2	114.2±14.5	117.9±8.3	139.3±9.3
MPTP+L-DOPA	119.8±24.5	108.3±18.5	111.5±18.6	122.3±20.5	101.3±5.8	100.6±5.7	107.7±8.7	117.1±9.9
MPTP+L-DOPA +CI-1041	84.7±10.5	87.7±9.2	99.2±5.2	100.1±10.6	86.9±8.9	81.2±5.5	87.4±6.9	107.9±13.7
MPTP+Cabergoline	129.4±25.8	119.9±16.1	118.9±19.8	123.8±26.2	97.8±14.1	90.9±12.0	88.9±11.2	102.8±9.5

Results are expressed as percent of the mean of 4 control monkeys±SEM values

^a 100% control values in fmol/mg of tissue are rostral caudate nucleus: dorso-medial (DM)=71.9±7.0, dorso-lateral (DL)=66.8±10.6, ventro-medial (VM)=66.5±7.4, ventro-lateral (VL)=70.9±9.6; rostral putamen: DM=76.5±8.8, DL=87.6±5.4, VM=74.4±9.4, VL=87.0±8.1; caudal caudate nucleus: DM=6.5±0.3, DL=7.5±0.5, VM=6.4±0.2, VL=7.6±0.3; caudal putamen: DM=8.0±0.5, DL=8.8±0.7, VM=8.2±0.4, VL=10.4±0.6

posterior caudate nucleus DM: $F_{3,13}=0.75$, $p=0.538$; VM: $F_{3,13}=1.13$, $p=0.372$; DL: $F_{3,13}=1.25$, $p=0.332$; VL: $F_{3,13}=2.75$, $p=0.085$) and (anterior putamen DM: $F_{3,13}=0.43$, $p=0.738$; VM: $F_{3,13}=0.16$, $p=0.922$; DL: $F_{3,13}=0.31$, $p=0.816$; VL: $F_{3,13}=0.11$, $p=0.953$; posterior putamen DM: $F_{3,13}=3.85$, $p=0.034$; VM: $F_{3,13}=3.01$, $p=0.069$; DL: $F_{3,13}=1.88$, $p=0.183$; VL: $F_{3,13}=2.90$, $p=0.075$) (Table 2). In the L-DOPA-alone-treated group and L-DOPA+Ro 61-8048 group, [³H]CGP-39653 specific binding was unchanged in caudate nucleus of both regions. In the dorsomedial posterior putamen, [³H]CGP-39653 specific binding of L-DOPA+Ro 61-8048-treated MPTP monkeys was significantly higher than untreated MPTP monkeys and L-DOPA-treated MPTP monkeys (Table 2).

NMDA/NR2B Receptors

Striatal [³H]Ro 25-6981 specific binding was also changed by lesion and treatments in this experiment (anterior caudate nucleus DM: $F_{3,13}=5.06$, $p=0.015$; VM: $F_{3,13}=3.62$, $p=0.043$; DL: $F_{3,13}=4.88$, $p=0.017$; VL: $F_{3,13}=4.99$, $p=0.016$; posterior caudate nucleus DM: $F_{3,13}=4.94$, $p=0.017$; VM: $F_{3,13}=6.97$, $p=0.005$; DL: $F_{3,13}=3.63$, $p=0.042$; VL: $F_{3,13}=4.50$, $p=0.023$) and (anterior putamen DM: $F_{3,13}=3.21$, $p=0.059$; VM: $F_{3,13}=5.03$, $p=0.016$; DL: $F_{3,13}=4.51$, $p=0.022$; VL: $F_{3,13}=6.17$, $p=0.008$; posterior putamen DM: $F_{3,13}=3.19$, $p=0.060$; VM: $F_{3,13}=4.51$, $p=0.022$; DL: $F_{3,13}=3.49$, $p=0.047$; VL: $F_{3,13}=5.49$, $p=0.012$). A decrease of [³H]Ro

25-6981 specific binding was observed in subregions of the rostral and caudal caudate nucleus and putamen (Fig. 6). Following L-DOPA treatment, [³H]Ro 25-6981 specific binding increased significantly in all regions of the anterior and posterior caudate-putamen compared to untreated MPTP monkeys (Fig. 6). In the L-DOPA+Ro 61-8048-treated group [³H]Ro 25-6981 specific binding remained elevated (Fig. 6).

Relationship between Behavior and Biochemical Results

There were significant positive correlations between maximum dyskinesias scores for all the MPTP monkeys ($n=29$) and [³H]Ro 25-6981 specific binding (in percent of respective control values for each experiment). This correlation was more significant in the rostral (caudate nucleus: $R=0.711$, $p=0.0001$ and putamen: $R=0.683$, $p=0.0001$) than the caudal striatum, (caudate nucleus: $R=0.541$, $p=0.0024$ and putamen: $R=0.414$, $p=0.025$) (Fig. 7). Overall no significant correlation was measured between dyskinesia scores and striatal [³H]CGP-39653 specific binding (data not shown).

Discussion

A long time ago amantadine, a noncompetitive antagonist of NMDA receptors, was reported to improve both motor

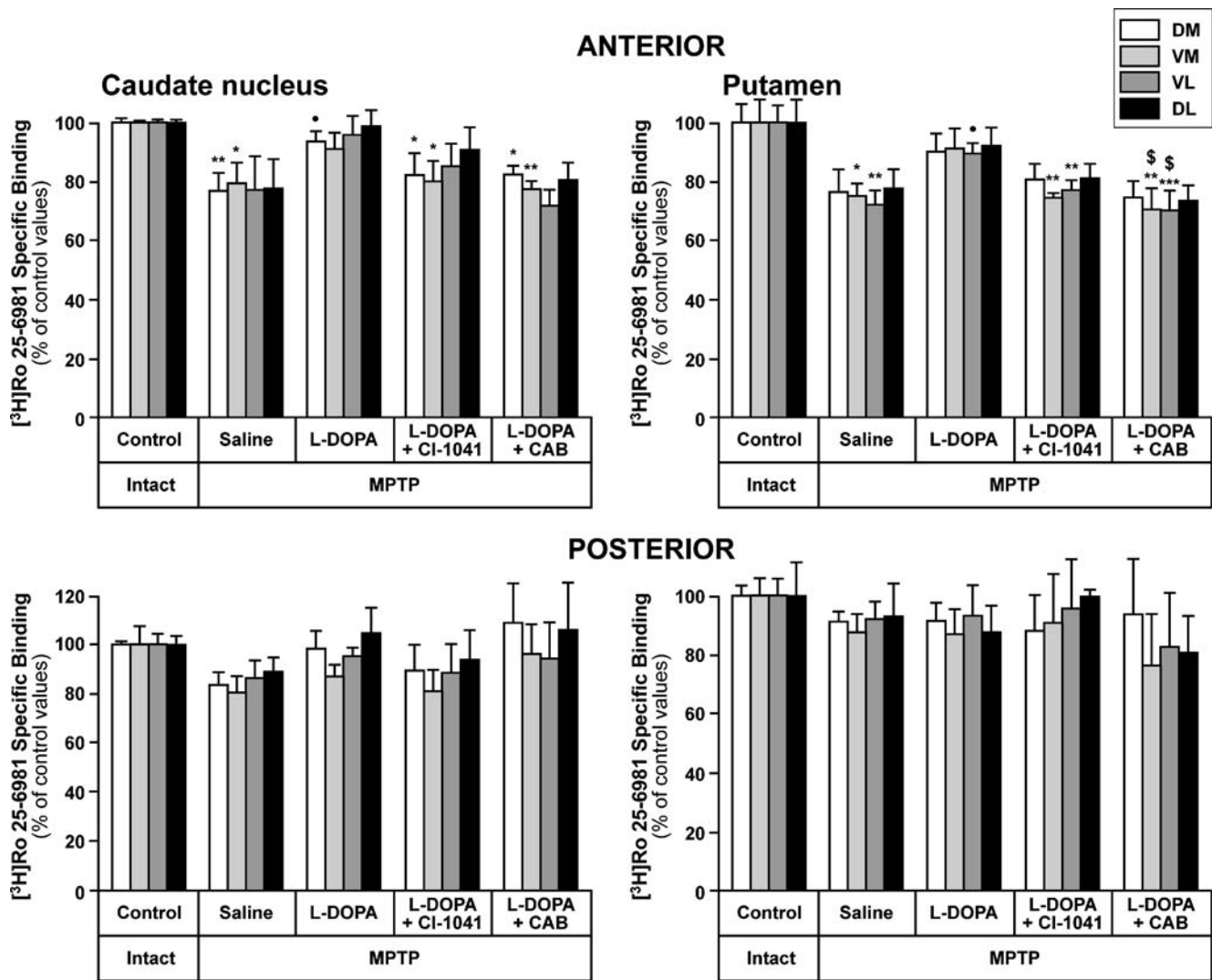


Figure 5 [^3H]Ro 25-6981 specific binding to NR1/NR2B subunit of NMDA receptors in anterior and posterior subregions of the caudate-putamen of control monkeys (intact, $n=4$), untreated MPTP monkeys ($n=4$), L-DOPA-treated MPTP monkeys ($n=4$), L-DOPA+CI-1041-treated MPTP monkeys ($n=3$) and L-DOPA+cabergoline-treated MPTP monkeys ($n=3$). Results are expressed as relative percent of the mean of 4 control monkeys \pm SEM (bars) values. One hundred percent control values in fmol/mg of tissue are rostral caudate: dorso-medial (DM)=

461.2 \pm 25.4, dorso-lateral (DL)=381.4 \pm 27.8, ventro-medial (VM)=426.6 \pm 21.2, ventro-lateral (VL)=367.4 \pm 32.1; rostral putamen: DM=384.6 \pm 32.3, DL=395.5 \pm 34.6, VM=432.3 \pm 36.2, VL=449.2 \pm 29.0; caudal caudate: DM=474.9 \pm 6.7, DL=404.3 \pm 14.8, VM=487.9 \pm 39.8, VL=407.6 \pm 24.9; caudal putamen: DM=308.5 \pm 18.3, DL=345.4 \pm 69.5, VM=364.1 \pm 23.4, VL=351.3 \pm 20.9. * p <0.05; ** p <0.01, and *** p <0.005 vs. control monkeys; * p <0.05 vs. untreated MPTP monkeys; \$ p <0.05 vs. L-DOPA-treated MPTP monkeys

fluctuations and dyskinesias in advanced PD, suggesting the implication of NMDA receptors in motor disorders (Schwab et al. 1969). Since then, it is now well-established that glutamate, the principal excitatory neurotransmitter, is involved in the development of PD, LID, and other motor complications associated with dopatherapy (Hallett et al. 2005). Nevertheless, studies concerning the regulation of these receptors in PD and LID are inconclusive. The present study sought the role of glutamate NR2A- and NR2B-containing NMDA receptors in a monkey model of PD and dyskinesias. All MPTP monkeys of this study were

extensively and similarly denervated, as assessed with striatal DA concentration and specific binding to the DAT.

The NMDA receptor is composed of several subunits (NR1, NR2A-D, and NR3C) (Erreger et al. 2007). This variability in NMDA receptor composition confers diversity for functional regulation of this receptor. In fact, there are multimeric NR1/NR2A-D-containing NMDA receptors, and Erreger et al. (Erreger et al. 2004, 2007) demonstrated that the NR1-NR2 heterodimer is a functional association. The present study evaluated NMDA receptor binding composed of NR2A and NR2B subunits.

Table 2 [³H]CGP-39653 specific binding to NR1/NR2A subunit of the NMDA receptors in anterior and posterior subregions of the caudate-putamen of control (intact), MPTP+saline-treated, MPTP+L-DOPA-treated, and MPTP+L-DOPA+Ro 61-8048-treated monkeys

	Caudate				Putamen			
	DM	VM	VL	DL	DM	VM	VL	DL
	Rostral							
Control	100±20.0 ^a	100±21.9 ^a	100±18.4 ^a	100±18.5 ^a	100±20.6 ^a	100±22.6 ^a	100±22.4 ^a	100±23.0 ^a
MPTP + Saline	77.5±25.7	79.9±28.8	71.7±25.3	76.4±22.9	81.1±26.2	87.3±29.4	98.9±22.9	100.4±26.53
MPTP+L-DOPA	75.7±14.7	68.5±13.3	72.1±14.1	77.1±16.2	95.1±12.3	89.4±10.3	102.9±15.9	113.9±16.4
MPTP+L-DOPA +Ro 61-8048	97.3±11.7	86.1±9.9	98.2±10.2	106.0±11.2	108.2±9.7	101.96±8.9	111.2±8.9	121.2±10.1
	Caudal							
Control	100±11.2 ^a	100±5.9 ^a	100±8.8 ^a	100±11.9 ^a	100±3.9 ^a	100±2.1 ^a	100±2.9 ^a	100±4.4 ^a
MPTP + Saline	107.8±12.8	98.7±9.1	110.9±8.1	114.9±11.6	85.1±12.9	95.3±11.9	105.5±13.1	113.5±20.3
MPTP +L-DOPA	130.1±25.1	121.1±17.6	136.8±18.9	130.7±20.4	83.4±13.3	88.95±9.8	107.6±13.4	132.1±18.4
MPTP +L-DOPA +Ro 61-8048	128.0±15.7	123.5±13.3	140.2±9.5	135.9±13.2	124.5±7.9 ^{*S}	123.7±9.3	138.8±10.3	145.9±12.7

Results are expressed as relative percent of the mean of four control monkeys ± SEM values.

^a 100% control values in fmol/mg of tissue are rostral caudate nucleus: dorso-medial (DM)=98.5±19.7, dorso-lateral (DL)=100.7±18.6, ventro-medial (VM)=91.1±19.9, ventro-lateral (VL)=93.8±17.2; rostral putamen: DM=82.5±17.0, DL=85.3±19.6, VM=78.3±17.7, VL=83.7±18.8; caudal caudate nucleus: DM=66.7±7.5, DL=73.5±8.7, VM=70.5±4.2, VL=74.4±6.5; caudal putamen: DM=8.0±3.3, DL=79.6±3.5, VM=88±3.3, VL=93.1±2.7

**p*<0.05 vs. saline-treated MPTP monkeys; ^S*p*<0.05 vs. L-DOPA-treated MPTP monkeys

In both experiments, NMDA receptors composed of NR2A subunits were generally unchanged in all treatment groups in the anterior and posterior parts of both the caudate nucleus and putamen. In contrast, the NR1/NR2B-containing NMDA receptors decreased with the MPTP lesion whereas these levels were enhanced in the L-DOPA-alone-treated MPTP monkeys. CI-1041 and cabergoline administered with L-DOPA were able to prevent dyskinesias and the augmentation of NMDA receptors containing NR2B. For the Ro 61-8048+L-DOPA-treated MPTP monkeys, NMDA receptors containing NR2B were not significantly reduced compared to untreated MPTP monkeys; the dyskinesia scores of these monkeys correlated positively with the specific binding to NR2B-containing NMDA receptors.

Effects of MPTP Lesion on NMDA Receptors

Our laboratory was the first to report a post-mortem study investigating alterations of glutamate receptors in the brain of L-DOPA-treated human PD patients in relation with the development of motor complications (Calon et al. 2003). Caudate nucleus and putamen [³H]CGP-39653 binding to NR2A/NMDA receptors were unchanged in subgroups of

PD patients compared to age-matched controls (Calon et al. 2003). Previously, we reported no effect in MPTP-lesioned monkeys on NMDA receptors composed of NR2A subunits in the total striatum (Calon et al. 2002). Accordingly, the present data suggest that NMDA receptors containing NR2A subunits are not altered by PD, in contrast to those composed of NR2B subunits that were decreased. The lesion-induced decrease of NR1/NR2B-containing NMDA receptors may be due to an augmentation of the cortico-striatal release of glutamate that down-regulated the receptor. A possible mechanism of NR1/NR2B-containing NMDA receptors reduction is the des-inhibition of glutamate cortico-striatal neurons as a result of depletion of DA and subsequent loss of inhibitory D2 effect localized post-synaptically on the cortical dendrites (Deutch 1993) and pre-synaptically on cortico-striatal terminals (Desce et al. 1992). In contrast, He et al. reported unchanged [¹²⁵I]MK-801 specific binding in the striatum of monkeys with MPTP-induced PD compared to controls (He et al. 2000). Others studies reported an increase of NMDA receptors in PD patients (Ulas et al. 1994) and in 6-OHDA-lesioned rats (Samuel et al. 1990). These discrepancies may be explained by different radioligands used and their specificities for the NMDA

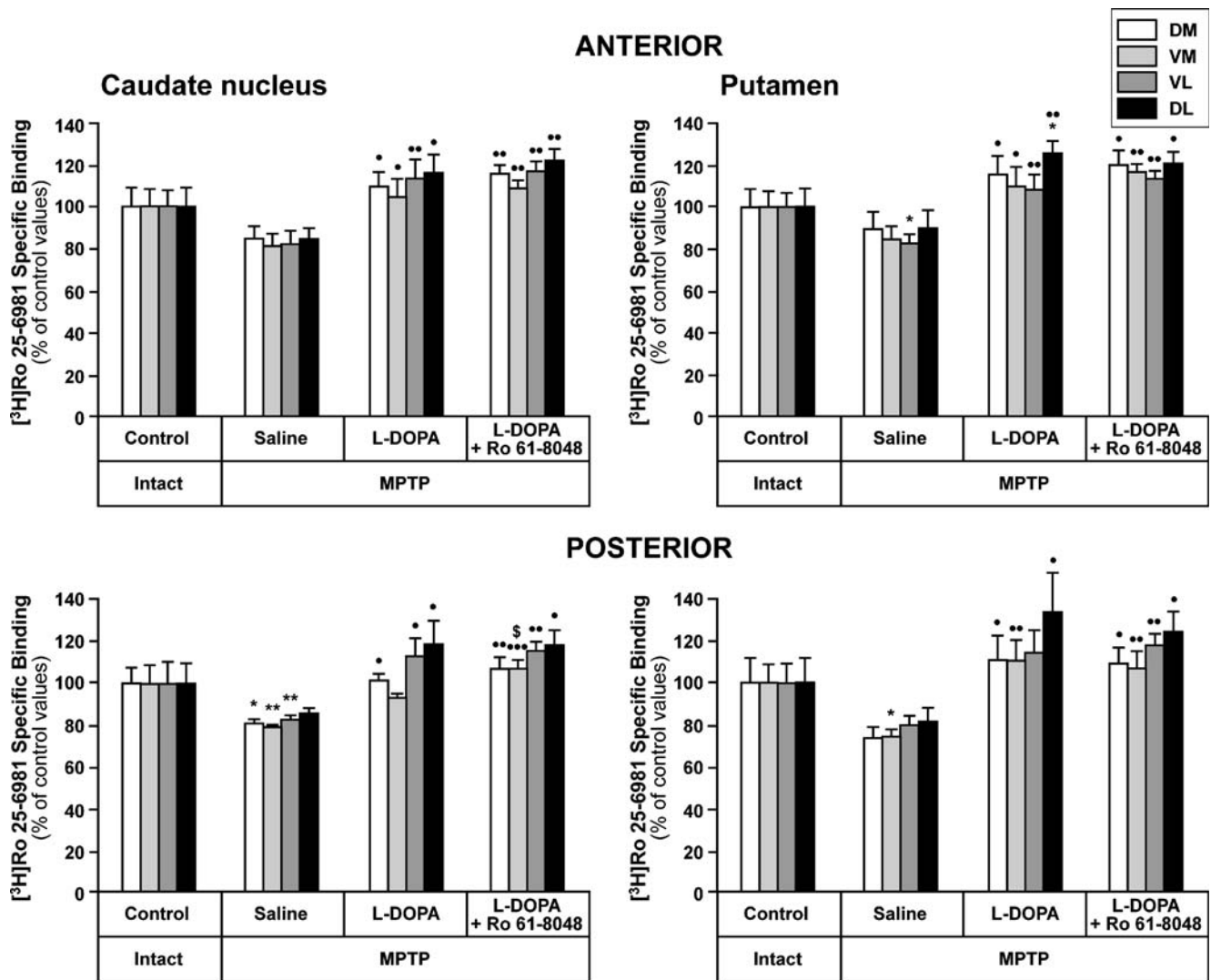


Figure 6 [^3H]Ro 25-6981 specific binding to NR1/NR2B subunit of the NMDA receptors in anterior and posterior subregions of the caudate-putamen of control (intact, $n=4$), untreated MPTP monkeys ($n=4$), L-DOPA-treated MPTP monkeys ($n=4$), and L-DOPA+Ro 61-8048-treated MPTP monkeys ($n=5$). Results are expressed as relative percent of the mean of four control monkeys \pm SEM (bars) values. One hundred percent control values in fmol/mg of tissue are: rostral caudate: dorso-medial (DM)=279.4 \pm 25.2, dorso-lateral (DL)=254.8 \pm

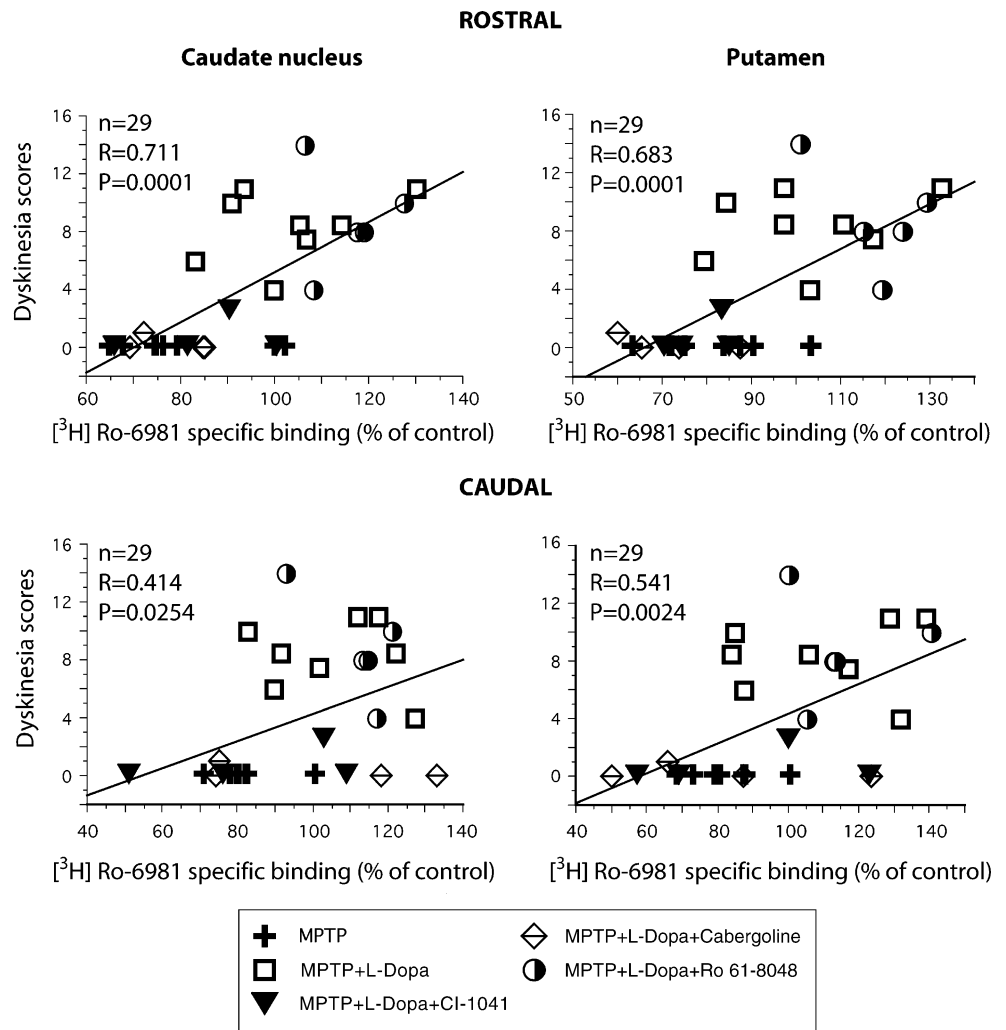
23.4, ventro-medial (VM)=285.4 \pm 24.1, ventro-lateral (VL)=258.6 \pm 22.3; rostral putamen: DM=232.7 \pm 27.2, DL=227.4 \pm 21.6, VM=256.2 \pm 19.2, VL=260.3 \pm 17.7; caudal caudate: DM=264.4 \pm 20.2, DL=218.6 \pm 20.7, VM=250.7 \pm 21.7, VL=216.9 \pm 22.4; caudal putamen: DM=167.6 \pm 19.4, DL=150.2 \pm 17.5, VM=17.2 \pm 15.5, VL=180.3 \pm 15.7 * p <0.05 and ** p <0.01 vs. control monkeys; • p <0.05; •• p <0.01 and ••• p <0.001 vs. untreated MPTP monkeys; \$ p <0.05 vs. L-DOPA-treated MPTP monkeys

subunits. In agreement with our autoradiographic studies, western blotting studies have shown a decrease of striatal NR2B/NMDA receptor levels with MPTP (Hallett et al. 2005) or 6-OHDA lesions (Dunah et al. 2000).

Changes in NR2 subunit composition of NMDA receptors within synapses can be triggered by mechanisms that include differences in insertion (Barria and Malinow 2002), internalization (Lavezzari et al. 2004), and lateral diffusion (Tovar and Westbrook 2002). The surface mobility of NMDA receptors depends of its NR2A or NR2B subunits composition (Groc et al. 2007); the NR2A/NMDA receptors being more stable than the NR2B-containing ones (Groc et al.

2007). In addition, following agonist binding the subsequent conformational transitory changes was found to be regulated by the identity of NR2 subunit; this process being more rapid for NR2A than NR2B (Erreger et al. 2005). The duration of activation for NR1/NR2B is longer than for NR1/NR2A (Erreger et al. 2005). The differences in time course (slower deactivation for NR1/NR2B) and frequency dependence of both current and total charge transfer, and downstream signaling molecules may explain how NR1/NR2A and NR1/NR2B make distinct contributions to synaptic plasticity (Erreger et al. 2005) and probably their localization in synaptic cleft at a given moment.

Figure 7 Correlation between maximum dyskinesias scores of MPTP monkeys from both experiments and [³H]Ro 25-6981 specific binding to NR1/NR2B subunit of the NMDA receptors expressed as relative percent of respective control monkeys from each experiment



Effects of Repeated L-DOPA Treatment on NMDA Receptors: Relation with Dyskinesias

We found an increase of NR2B-containing NMDA receptors in dyskinetic monkeys. A decrease of NMDA receptor levels in MPTP monkeys treated with L-DOPA and displaying dyskinesias was reported as measured with [¹²⁵I]MK-801 (He et al. 2000). However, [¹²⁵I]MK-801 does not discriminate the different subunits of these receptors (He et al. 2000). By western blot, Hallett et al. showed that LID increased levels of NR2A receptors in a synaptic membrane fraction while NR2B did not change (Hallett et al. 2005). A trend for a decline of NMDA receptors has also been observed in 6-OHDA-lesioned rats treated with L-DOPA (Dunah et al. 2000). In agreement with our results, Hurley et al. using immunoradiographic analysis showed that animals with high levels of dyskinesia had increased levels of NR2B receptors in the striatum (Hurley et al. 2005). Discrepancies observed in the modulation of NMDA receptors may be explained by the different assays used to measure the levels of these

receptors, and seems to depend on the activation state and subtype of striatal dopaminergic receptors (Cepeda et al. 1993). For example, we previously observed that there was a 111% increase in [³H]CGP-39653 binding in dyskinetic monkey striatum following SKF-82958 (D₁ receptors agonist) treatment (Calon et al. 2002). This is not observed with chronic L-DOPA treatment. In fact, DA can either potentiate or attenuate responses evoked by excitatory neurotransmitters (Cepeda et al. 1993). Activation of D₁ receptors potentiates responses evoked by NMDA receptors but D₂ activation primarily attenuates these responses (Cepeda et al. 1993). Picconi et al. demonstrated that chronic L-DOPA treatment is able to restore long term potentiation (LTP) in the denervated striata (Picconi et al. 2003) that can be reversed (depotentialiation) by low frequency stimulation (Picconi et al. 2003). This depotentialiation is lost in a LID rodent model (Picconi et al. 2003), suggesting an important modification of glutamate receptors function (Picconi et al. 2005) involving the NR2B-containing NMDA receptors. However, as mentioned above concerning synaptic plasticity, there are several potential

mechanisms by which NMDA receptors subtypes selectively activate signaling molecules; the differential localization of the two subunits on pre- and postsynaptic compartments also modify the synaptic plasticity (Gerkin et al. 2007). Alternatively, the synaptic or extrasynaptic localization of NR2A or NR2B can confer on them differential sensitivity to contrasting stimulus patterns leading to LTP or long term depression (LTD) (Bliss and Schoepfer 2004).

Effect of Repeated L-DOPA+CI-1041, Cabergoline, or Ro 61-8048 Treatment on NMDA Receptors: Relation with Prevention of Dyskinesias

The selective NMDA NR1/NR2B subunit antagonist (CI-1041) (Hadj Tahar et al. 2004) and cabergoline (Belanger et al. 2003) prevented dyskinesia. CI-1041 prevented the augmentation of NR2B/NMDA receptors. Thus antagonizing NR2B/NMDA receptors is efficient against LID. Alternatively, cabergoline by its action on cortico-striatal pre-synaptic D2 DA receptors (Maura et al. 1989) can reduce glutamate release. Both actions may contribute to prevent dyskinesias by reducing glutamatergic activity. Ro 61-8048 inhibits kynurenine 3-hydroxylase and diverts tryptophane degradation towards kynurenic acid (KYNA) production (Moroni et al. 2005). KYNA antagonizes glutamate receptors (Nemeth et al. 2005) and was reported to reduce dyskinesias (Gregoire et al. 2008). But why Ro 61-8048 did not significantly reduce [³H]Ro 25-6981 specific binding? This is possibly due to other pharmacological properties of Ro 61-8048 and KYNA. Low micromolar concentrations of KYNA are reported to antagonize the glycine_B site of NMDA receptors while higher concentrations (0.1–1 mM) are less discriminatory, inhibiting all ionotropic glutamate receptors (Stone 2000). Moreover, KYNA also blocks the alpha7 subtype of the nicotinic acetylcholine receptor at submicromolar concentrations (Hilmas et al. 2001). In PD, a reduction of nicotinic receptors occurs in several structures of the basal ganglia, such as the striatum (Calabresi et al. 2006). After DA depletion, the activation of nicotinic acetylcholine receptors is able either to reduce the threshold for the induction of LTD or prevent its loss in the corticostriatal pathway (Calabresi et al. 2006); this DA-acetylcholine interaction is able to modulate the final effect of sustained synaptic transmission (LTP) described as underlying dyskinesias (Picconi et al. 2003). There are likely multiple neurotransmitters implicated in the development of LID, however, considering the present results, there is likely a cause–effect link between dyskinesias and NR2B/NMDA specific binding in the striatum. This is supported by the high correlations observed between dyskinesia scores and NR2B/NMDA specific binding with a large number (29) of MPTP monkeys investigated here.

We observed that LID are associated with an alteration of NMDA receptors and significantly for the NR2B subunit containing NMDA receptors. According to our biochemical results reduction of NR2B/NMDA receptors are more involved in LID compared to NR2A/NMDA receptors. Therefore, drugs able to antagonize the function of the NR2B/NMDA receptors in adjunction to L-DOPA therapy may be carrying hope to prevent or alleviate dyskinesias (Thanvi et al. 2007).

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