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In vivo evidence of D_3 dopamine receptor sensitization in parkinsonian primates and rodents with L-DOPA-induced dyskinesias

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A growing body of evidence indicates a role for D_3 receptors in L-DOPA-induced dyskinesias. This involvement could be amenable to non-invasive *in vivo* analysis using functional neuroimaging. With this goal, we examined the hemodynamic response to the dopamine D_3-preferring agonist 7-hydroxy-*N*,*N*-di-*n*-propyl-2 aminotetralin (7-OHDPAT) in naïve, parkinsonian and L-DOPA-treated, dyskinetic rodents and primates using pharmacological MRI (phMRI) and relative cerebral blood volume (rCBV) mapping. Administration of 7-OHDPAT induced minor negative changes of rCBV in the basal ganglia in naïve and parkinsonian animals. Remarkably, the hemodynamic response was reversed (increased rCBV) in the striatum of parkinsonian animals rendered dyskinetic by repeated L-DOPA treatment. Such increase in rCBV is consistent with D_1 receptor-like signaling occurring in response to D_3 stimulation, demonstrates a dysregulation of dopamine receptor function in dyskinesia and provides a potentially novel means for the characterization and treatment of L-DOPA-induced dyskinesia in patients.

Keywords: Dyskinesia; Parkinson's disease; Dopamine; Dopamine receptor; D_3; Striatum; phMRI; Primate

Long-term dopamine (DA) replacement therapy in Parkinson's disease (PD) often leads to development of abnormal motor response and dyskinesia (Olanow et al., 2004) through poorly understood mechanisms. DA modulates the basal ganglia output through opposite effects on the postsynaptic DA receptors: D_1 facilitation and D_2-like (D_2 and D_3) inhibition (Beaulieu et al., 2005). Anatomically, D_1 and D_2-like receptors are partially segregated into the striatonigral ("direct") and striatopallidal ("indirect") projections or pathways (Gerfen et al., 1990). However, there is evidence of substantial co-localization of functional D_1- and D_2-like receptors on striatal medium spiny projection neurons (Aizman et al., 2000; Pollack, 2004) implying that cross-talk may occur both at circuitry and intracellular levels. DA receptors are 7 transmembrane G-protein-coupled receptors: D_1 receptors are coupled to G αs/olf, increase cAMP levels and phosphorylation of DARPP-32 and proteins downstream (Bonci and Hopf, 2005). D_2 are linked to Gαi, inhibit adenyl cyclases and activate G-protein-coupled inward-rectifying potassium channels (Girk) and phosphatases (Bonci and Hopf, 2005). D_3 have no net effect on cAMP levels and may couple to both Gs and i proteins (Ilani et al., 2002). Notably, D_1 and D_2/D_3 agonists induce opposite hemodynamic changes in the striatum measured by functional pharmacologic (ph)MRI; D_1 agonists increase relative cerebral blood volume (rCBV) while D_2 and D_3 agonists decrease it (Chen et al., 2005; Choi et al., 2006). Pramipexole, a D_3-preferring agonist, has been shown to reduce cerebral blood flow in cingulate and orbitofrontal areas in monkeys in a PET study (Black et al., 2002). These opposite effects correlate well with the D_1-mediated facilitation and D_2 gating roles on glutamate transmission in the striatum.

While D_3 receptors are not highly expressed in the motor regions of the striatum (Murray et al., 1994; Sokoloff et al., 1990), there is compelling evidence from postmortem studies, of L-DOPA induction of ectopic D_3 receptor expression in D_1-expressing medium spiny neurons in the striatum of parkinsonian rats (Bordet et al., 1997; Bordet et al., 2000) and macaques (Bezard et al., 2003; Quik et al., 2000). Furthermore, both the presence of L-DOPA-induced dyskinesias in primates (Bezard et al., 2003) and sensitization to L-DOPA in rats (Bordet et al., 1997; Bordet et al., 2000; Guillin et al., 2003) have been correlated with changes in D_3 receptor expression in postmortem analyses. In this study we examined *in vivo* hemodynamic changes in response to D_3 activation using ph MRI and (7-hydroxy-*N*,*N*-di-*n*-propyl-2 aminotetralin) 7-OHDPAT, in naïve, parkinsonian and L-DOPA-treated rats and primates. 7-OHDPAT has a 10-fold higher affinity for the D_3 (Missale et al., 1998; Sokoloff et al., 1990) compared to the D_2 receptor.

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Methods

Rodent studies

All animal studies were performed following NIH guidelines and were approved by the IACUC at McLean Hospital and Harvard Medical Area. Naïve and 6-OHDA-lesioned adult female Sprague-Dawley rats (200–250 g) were purchased from Charles River Laboratories and Taconic. The animals were unilaterally lesioned by 6-OHDA injection (4 μl; 2 μg/μl) into the medial forebrain bundle. An appropriate level of denervation was evaluated by the rotational response to apomorphine (0.05 mg/kg) and amphetamine (4 mg/kg) at least 6 weeks after the lesion as described (Ferrari et al., 2006). A group of 10 animals showing good rotational response to apomorphine, 9±1.4 contralateral turns/min for 30 min, and to amphetamine, 11.6±1.5 ipsilateral turns/min for 90 min, was selected. In this group 6 animals were treated twice a day with i.m. injections of L-DOPA methylester (12 mg/kg) and benserazide (a peripheral inhibitor of L-aromatic amino acid decarboxylase, 2.5 mg/kg) for 2 weeks and 5 of them developed abnormal involuntary movements (AIMs). The response to L-DOPA was scored in a standard acute challenge session 5–7 days after the induction phase was completed, according to Cenci et al. (1998); the average AIMs score was 33.9±1.2.

Pharmacological magnetic resonance imaging

Studies were performed in naïve ($n=7$) and 6-OHDA-lesioned rats ($n=10$) as described (Chen et al., 2005) in a 4.7-T magnet (Bruker, Billerica, MA). Briefly, the rats were anesthetized with a 1% halothane in 1:1 mixture of O_2 and NO_2 and positioned in a stereotaxic frame. Eight 1.5-mm-thick coronal slices were acquired using a gradient echo sequence (600/20). After baseline acquisition, animals received an i.v. injection of a superparamagnetic agent (MION, 10 mg/kg, synthesized in-house (Mandeville et al., 2001)) through the tail vein to sensitize images to cerebral blood volume (CBV) changes (Chen et al., 2001). After collecting images for 20 min, the animals received an i.v. injection of 7-OHDPAT; 1.3–3 mg/kg) and image acquisition continued for 60 to 90 min. Analyses of the changes was performed as described (Chen et al., 2001) by generating CBV maps and analyzing the changes over time (0–40 min) in the regions of interest by curve fitting. Analysis of the effect of L-DOPA on regional CBV changes across groups was performed using nonparametric analysis of variance, because the signal change did not follow a normal distribution. Imaging was performed at least 3 days after the last L-DOPA administration.

Primate studies

Twelve adult male macaques (*Macaca fascicularis*) were included in the study. Animals were single-housed at the New England Regional Primate Research Center. Parkinsonism was induced by systemic administration of MPTP (Jenkins et al., 2004) ($n=8$). Three months after the last dose of MPTP animals showed a stable parkinsonian syndrome (Jenkins et al., 2004) and corresponding decrease in locomotor activity and loss of dopamine transporter binding sites [measured by PET and ^{11}C (2β-carbomethoxy-3β-(4-fluorophenyl) tropane) (CFT) binding potential] in the posterior putamen as described (Jenkins et al., 2004) (Table 1). Four parkinsonian animals were dosed chronically with oral L-DOPA/carbidopa (Sinemet® 100/10) for 12–15 weeks and three of them developed dyskinesias in response to L-DOPA. Dyskinesias were rated after a single i.m. administration of L-DOPA (methylester, 45 mg/kg) and benserazide (10 mg/kg) at least 1 week after the last dose. The abnormal movements were classified as *chorea* (rapid, random flicking movements), *athetosis* (sinuous, writhing distal limb movements) *dystonia* (sustained twisting movements resulting in abnormal posturing), *myoclonus* (jerky) or *stereotypy* (repetitive purposeless behavior) and the severity was rated from 1 to 4, based on frequency and interference with normal behavior (Pearce et al., 1995) as described for macaques by Bezard et al. (2003) (1: mild, fleeting and rare dyskinetic movements and postures, >5 in 10 min; 2: moderate, more prominent dyskinesias but not interfering with normal behavior, 5–20 in 10 min; 3: marked, frequent dyskinesias intruding on normal behavior, 21–50 in 10 min, and 4: severe, virtually continuous dyskinesias, disabling the animal and replacing normal behavior). The severity was scored independently by two observers at 30, 45, 60 and 90 min post injection, for each region (orofacial, right and left arm, right and left leg, axial) and composite scores (sum of scores) were obtained at 45 min (Table 2). Disability (interference with normal behavior) was calculated by dividing the composite score by the number of affected regions. Imaging was performed at least 3 days after the last L-DOPA administration.

Pharmacological magnetic resonance imaging

Studies were performed on a Siemens' 3-T Trio system using an in-house built transmitter/receiver 3-in. surface coil under halothane anesthesia as described (Jenkins et al., 2004) with collection of serial gradient echo images (TR/TE 500/10 ms; FOV=120 mm, 1 mm trans-axial slices, 128×128 matrix). After collection of 6–10 baseline images, 10–15 more images were

Table 1
Primate descriptive statistics

Group	Naïve, $n=4$	MPTP-PD, $n=5$	MPTP-Dysk, $n=3$
Age (years)	5±0.5	7±0.5	7±0.3
Weight (kg)	6.3±0.3	8.6±0.8	6.2±0.5
MPTP (total mg)	NA	22±5	13±5
MPTP (doses)	NA	17±3.4	11±4
Parkinson rating scale	0	13.6±3.4	16±2.5
Locomotor activity	42±7.5	18±3 * (57%)	23±3.8 * (45%)
^{11}C CFT BP (% loss)	3.14±0.1	1.31±0.05 * (58%)	1.42±0.1 * (55%)

BP: binding potential. All results are mean±SEM, (%): average decrease from naive baseline.

* MPTP-lesioned animals showed a significant reduction of locomotor activity compared to naive animals (ANOVA, $F_{2,9}=6.2$, $p<0.05$, with no difference between dyskinetic and non-dyskinetic groups) and a significant loss of dopamine terminals in the posterior putamen measured by PET and the dopamine transporter ligand ^{11}C-CFT (ANOVA, $F_{2,9}=98$, $p<0.0001$, with no difference between dyskinetic and non-dyskinetic groups).

Table 2
L-DOPA-induced dyskinesias in primates

Primate	Mf1	Mf2	Mf3	Mf4
Daily L-DOPA (mg)	424±16	482±12	462±12	437±14
Time on L-DOPA (days)	85	90	77	85
Dyskinesia: severity	0	1.85	3.6	3.5
Dyskinesia: type	Stereotypic	Dyst/chorea	Dyst/chorea	Dyst/chorea
Dyskinesia: peak score	2	9	18	14
Putamen rCBV	−4.66	15.23	6.08	−0.43
Putamen corrected rCBV	0.65	7.89	14.98	8.59

Dyskinesia was induced using high doses of L-DOPA in order to shorten the length of the induction phase (Parkinson's disease patients usually developed dyskinesias only after several years of daily treatment). Dyskinesia was evaluated 45 min after the i.m. administration of a standard dose of L-DOPA methylester with benserazide, at least 1 week after completion of the induction phase. rCBV: relative cerebral blood volume; corrected rCBV was obtained by subtracting whole brain CBV changes to eliminate the effect of CO_2 changes. Corrected rCBV values in the putamen were correlated with dyskinesia peak scores ($R^2=0.93$, $p<0.05$).

acquired after i.v. administration of 10 mg/kg of MION (Jenkins et al., 2004). 7-OHDPAT (0.25 mg/kg) was administered i.v. and imaging continued for 40–60 min. Continuous monitoring of end tidal (Et) CO_2 was made through tracheal intubation with a gas monitor (Puritan-Bennett Model 254, Pleasanton, CA) and heart rate was monitored using EKG leads (Siemens). Data analysis was performed using region of interest (ROI)-based analyses of regional changes in rCBV as described (Jenkins et al., 2004). We used the same ROIs as outlined in our previous study describing amphetamine stimulation (Jenkins et al., 2004), as those are most likely responsive to changes in DA signals. Data are presented as mean±SEM. One-way ANOVA and repeated measures ANOVA were used to examine the effect of DA lesion and L-DOPA treatment as well as physiological changes. Comparison of CBV changes between groups was performed using a nonparametric test (Kruskal–Wallis). All statistical analyses were performed using Statview software (SAS Institute Inc., Carny, NC).

Results

Effects of DA denervation and chronic L-DOPA administration on the rCBV response to 7-OHDPAT in 6-OHDA-lesioned hemiparkinsonian rats

In naïve anesthetized rats 7-OHDPAT administration induced a negative change in rCBV in the nucleus accumbens (Figs. 1a–b) (Choi et al., 2006) corresponding to the anatomical distribution of the D_3 receptor (Murray et al., 1994), which is highly expressed in the ventromedial shell of the nucleus accumbens, olfactory tubercle and islands of Calleja and low in the motor striatum (Sokoloff et al., 1990). The hemodynamic changes induced by 7-OHDPAT in the nucleus accumbens were larger than the insignificant response elicited in the caudate–putamen (<5%, Fig. 1b) and returned to baseline ~60 min after the injection.

Unilateral DA denervation by 6-OHDA did not induce major changes in the response to 7-OHDPAT (Fig. 1c). Ipsilateral to the side of the lesion, hemiparkinsonian animals showed a small enhancement in the magnitude of the rCBV response in the nucleus accumbens with no significant differences between sides (Fig. 1e). In contrast, in the 6-OHDA-lesioned animals chronically treated with L-DOPA, there was a rapid positive activation in response to 7-OHDPAT both in the caudate–putamen and accumbens nuclei, ipsilateral to the side of the 6-OHDA lesion (Figs. 1d–f). Analysis of the rCBV changes over time by curve fitting to a gamma-variate model demonstrated a significant effect of L-DOPA treatment ($p\leq0.001$) on the hemodynamic response to 7-OHDPAT in the caudate–putamen (Fig. 1f). A non-parametric analysis of variance revealed a significant effect of L-DOPA in the rCBV response (0–40 min) (*U* test, $z=-4.37$, $p\leq0.001$). Regional analysis showed significant changes in the ipsilateral caudate–putamen ($p\leq0.01$), thalamus ($p\leq0.05$) and cingulate cortex ($p\leq0.05$) (Fig. 1e). These results show that the hemodynamic response to D_3 DA agonist administration was altered (sensitized) by chronic L-DOPA administration in hemiparkinsonian rodents.

Effects of DA denervation and L-DOPA administration on the rCBV response to 7-OHDPAT in MPTP-lesioned parkinsonian primates

We next studied the effect of 7-OHDPAT, in naïve ($n=4$) and MPTP-lesioned primates ($n=8$) showing a stable, moderate parkinsonism, defined by a parkinsonian score of 14.5±1.9 (PRS: 0–24) and a 57% loss of dopamine transporter binding in the putamen measured by PET and the dopamine transporter tracer ^{11}C CFT (Table 1). For induction of dyskinesias, 4 parkinsonian animals were treated repeatedly with L-DOPA (Mf1–4, Table 2) and 3 of them developed typical L-DOPA-induced dyskinesias. The dyskinesias consisted on choreiform and dystonic movements predominantly affecting limbs, tail, and orolingual muscles, peaked ~45 min after L-DOPA injection and were moderate to severe, according to interference with normal behavior (Bezard et al., 2003). There were no significant differences between the L-DOPA-treated and not treated MPTP-lesioned PD primates with respect to MPTP dose or to the severity of parkinsonism, as determined by parkinsonian score, decrease in locomotor activity and ^{11}C CFT binding potential in the posterior putamen (ANOVA, $p>0.05$, Table 1).

In the anesthetized primates, 7-OHDPAT had a potent systemic physiological effect, inducing a significant decrease of $EtCO_2$ immediately after injection, from 38±0.9 to 32.7±2 (repeated-measures ANOVA, $p\leq0.05$) and returned to 38.8±2.2 at the end of the imaging session. There was also a significant drop in heart rate (from 121±4 to 98±6, $p\leq0.001$) which remained slow until the end of the study (99±5; $p\leq0.05$). These measures did not differ between groups.

In the brain, naïve animals showed minor changes in rCBV in striatal motor regions in response to 7-OHDPAT administration and mild (0–10%) global negative hemodynamic responses that were correlated with the decrease in $EtCO_2$ ($R=0.76$; $p\leq0.01$). As a group, MPTP-lesioned animals (MPTP-PD) did not differ from naïve animals. After removing the effect of CO_2 by fitting the

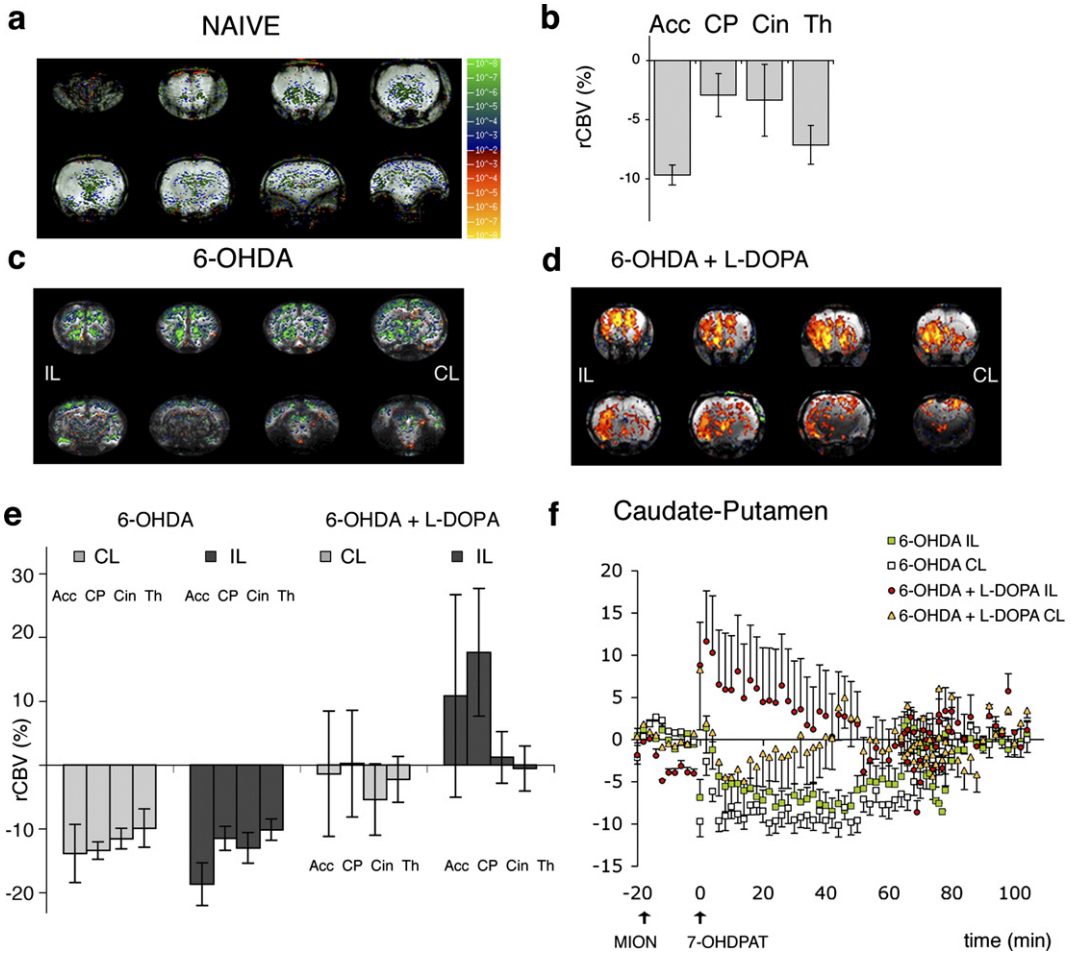

Fig. 1. (a–b) In naive rats, 7-OHDPAT administration induced mild negative changes in regional relative cerebral blood volume (rCBV), more pronounced in limbic than in motor regions: (a) representative rCBV map in a naive rat showing the anatomical distribution of signal changes on coronal brain slices (all maps are color-coded by significance level according to the color scale bar shown on the right); (b) the hemodynamic response in the nucleus accumbens was larger than in the caudate–putamen ($n=7$), as predicted by the low expression of D_3 receptors in the motor regions of the striatum. (c) Effect of 6-OHDA lesion on the rCBV response to 7-OHDPAT: rCBV map of a representative 6-OHDA-lesioned rat showing a small enhancement of the response, at the level of the nucleus accumbens ipsilateral to the lesion (IL). (d) Animals treated with L-DOPA showed different distribution and opposite sign of the hemodynamic response to 7-OHDPAT: rCBV map in a representative lesioned animal treated with L-DOPA. (e) Quantification of the average % signal change from 0 to 40 min in the nucleus accumbens (Acc), caudate–putamen (CP), cingulate cortex (Cin) and thalamus (Th) of hemiparkinsonian animals with and without L-DOPA chronic administration; ipsilateral rCBV changes were significantly affected by L-DOPA in the caudate–putamen (Mann–Whitney U test, $P \leq 0.01$) and also in the cingulate cortex and thalamus ($p \leq 0.05$); in the nucleus accumbens, two dyskinetic animals showed a negative response and the average change was not significant. (f) Time course of rCBV signal change in the caudate–putamen of 6-OHDA-lesioned animals with and without L-DOPA treatment revealed a significant effect of L-DOPA treatment, more marked on the side ipsilateral to the 6-OHDA lesion. Time 0 represents the injection time of 7-OHDPAT, approximately 20 min after the administration of an iron superparamagnetic agent (MION, 10 mg/kg) to sensitize the images to vascular changes. Acc: nucleus accumbens; CP: caudate–putamen; Cin: cingulate cortex; Th: thalamus; IL: ipsilateral to lesion (6-OHDA injection in the midbrain forebrain bundle); CL: contralateral to lesion.

rCBV values to a general linear model using $EtCO_2$ as a regressor (Fig. 2), both naïve and MPTP-PD showed a small decrease in rCBV in the basal ganglia (Figs. 2a–b). In contrast, the parkinsonian animals that developed dyskinesias, MPTP-Dysk, showed a selective, significant increase in rCBV in the putamen (Figs. 2c–d). This increase occurred in spite of the decrease in whole brain rCBV and the decrease in $EtCO_2$; without using the CO_2 as a regressor, the rCBV change in putamen was still positive for the dyskinetic group. ROI group analysis demonstrated significant changes between groups only in the putamen (Kruskal–Wallis, $H=6.23$, $p \leq 0.05$) (Fig. 2e). The putaminal changes in rCBV were not correlated with whole brain CBV changes whereas changes in other brain regions examined showed a strong correlation with whole brain rCBV changes and $EtCO_2$. After removing the contributions from whole brain CBV changes, there were significant correlations only between putamen, caudate, accumbens and dentate nucleus of the cerebellum, using Pearson product moment correlations. Putaminal changes in rCBV corrected for the effect of $EtCO_2$ by subtracting the whole brain CBV changes (Table 2) were correlated with the severity of dyskinesia ($R^2=0.93$, $F=26.7$, $p \leq 0.05$).

Discussion

In this study we examined *in vivo* dynamic changes in response to 7-OHDPAT using phMRI in naïve, parkinsonian and L-DOPA-

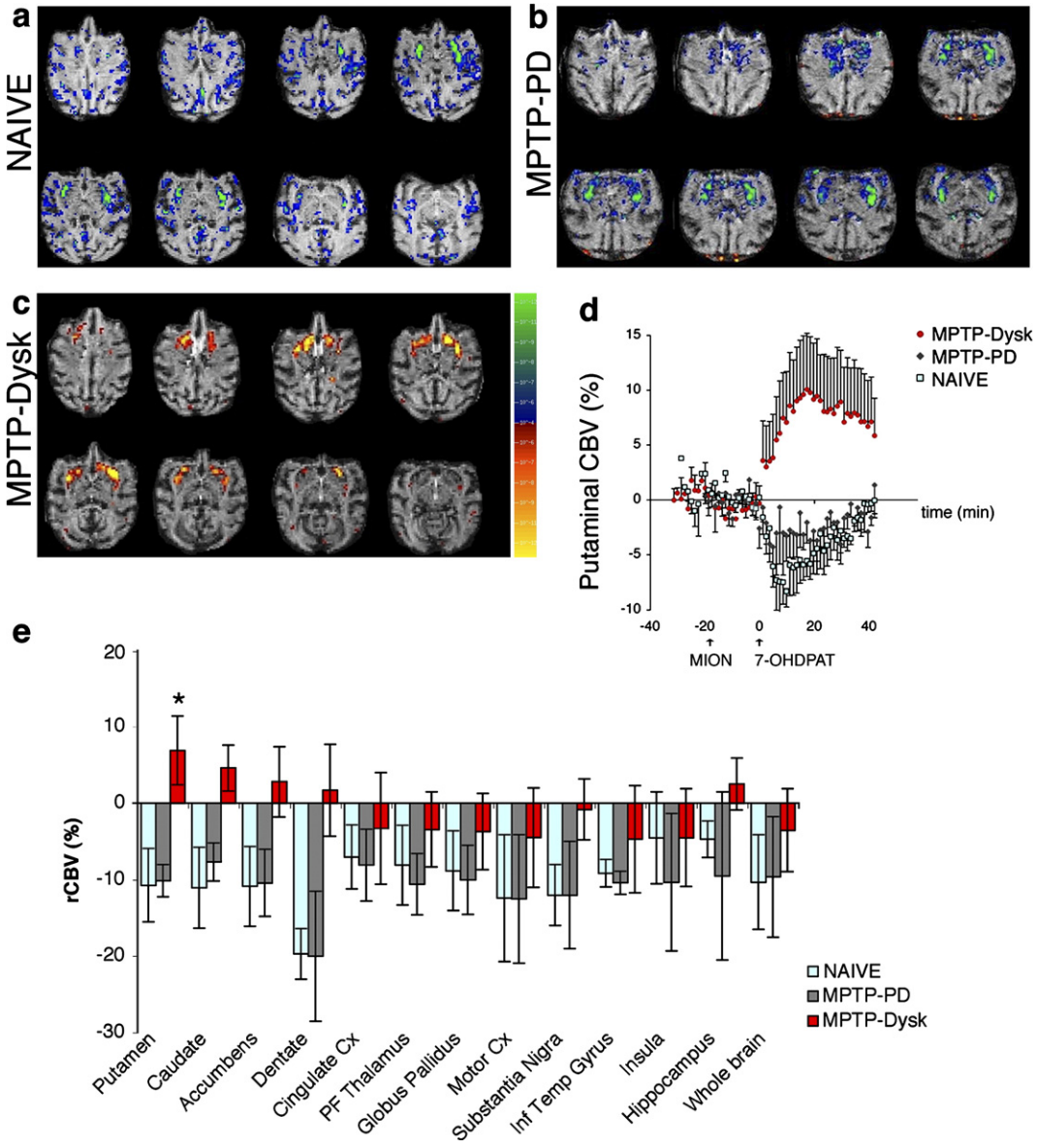

Fig. 2. (a–c) Representative maps of significant hemodynamic changes (p values) in response to 7-OHDPAT are shown overlayed on axial echo gradient brain images. These maps were derived by fitting the cerebral blood volume (CBV) p values to a general linear model using the $EtCO_2$ as a regressor to remove the effect of CO_2. In the basal ganglia, a small reduction in CBV was observed in both naive (a) and MPTP-PD animals (b), without differences in signal intensity or anatomical distribution between these groups. MPTP-dyskinetic primates (c) showed a significant increase in CBV in the putamen. Color scale bar for a–c: significance (p) values for t test comparisons of CBV before and after 7-OHDPAT (increase from baseline is coded red–yellow and decrease is blue–green). (d) Time course of rCBV change in the putamen in the three groups of primates. Time 0 marks the injection time of 7-OHDPAT, approximately 10–15 min after the administration of an iron superparamagnetic agent (MION, 10 mg/kg) to sensitize the images to vascular changes and baseline collection. (e) Regional analysis of rCBV changes in response to 7-OHDPAT showed a significant increase in the putamen of L-DOPA-treated primates (Kruskal–Wallis $H=6.23$, $p \leq 0.05$) with respect to MPTP-treated only and naive animals. Regression analysis of rCBV changes showed a lack of significant correlation between putaminal and whole brain signal change ($r=0.37$, n.s.), in contrast with other brain regions such as cingulate cortex ($r=0.8$, $p \leq 0.01$), globus pallidus ($r=0.73$, $p \leq 0.01$) and insular cortex ($r=0.65$, $p \leq 0.05$).

treated, dyskinetic animals. We used 2 complementary models: the unilateral acute 6-OHDA rodent model that, although not without limitations as a model for dyskinesia, is quite informative and allow us to compare this study with our previous studies mapping normal distribution of DA receptors in the rodent brain, and a bilateral primate model that closely resembles PD and the L-DOPA-related motor complications that most Parkinson patients develop during the course of the disease. Both naïve rodents and primates had a negative hemodynamic response to D_3 consistent with previous studies (Black et al., 2002; Choi et al., 2006) that was only marginally affected by DA denervation. In contrast, 7-OHDPAT induced a significant increase in rCBV, i.e. a D_1-like response (Choi et al., 2006; Jenkins et al., 2004), in the L-DOPA-treated, dyskinetic rodents and primates. This reversed, sensitized hemodynamic response was mainly restricted to the putamen and was independent of CO_2 effects.

The D_3 selectivity of 7-OHDPAT *in vivo* is not well characterized (Pritchard et al., 2003) and in the present study we used a

relatively high dose in order to induce a measurable hemodynamic response in naïve anesthetized animals. However, we have carefully examined the selectivity of D_3 and D_2 preferring agonists in our recently published studies (Chen et al., 2005; Choi et al., 2006). The D_2 preferring agonists quinpirole and norpropylapomorphine elicit negative rCBV changes over the entire striatum (motor and limbic); thus, using a ratio of the induced rCBV change in caudate–putamen over nucleus accumbens, the D_2 preferring agonists have a ratio close to 1 while the ratio for 7-OHDPAT is 0.2–0.7 (depending upon the dose). These data clearly indicate a D_3 preference of 7-OHDPAT *in vivo*. Moreover, in another recent study (Delfino et al., 2007), quinpirole (a D_2 preferring agonist) did not increase BOLD signal, or induce sensitization – strongly arguing against a sensitization of D_2 receptors in dyskinetic rats. Lastly, we have some preliminary evidence using the selective D_3 antagonist SB277011 supporting D_3 involvement in the rCBV change observed in the striatum of dyskinetic animals (Sanchez-Pernaute and Jenkins, unpublished observation) as the aberrant activation reported here was suppressed on the ipsilateral side by the D_3 antagonist, while 6-OHDA-lesioned rats showed bilateral medial activation similar to what we have shown previously with eticlopride, a D_3/D_2 antagonist (Chen et al., 2005).

Sensitization of the inhibitory response to DA involving D_3 receptors has been proposed to enhance D_1 receptor signaling (Bordet et al., 1997; Bordet et al., 2000) and thus facilitate the activation of the striatonigral (direct) pathway. Several recent studies (Aubert et al., 2005; Picconi et al., 2003; Tong et al., 2004) have indicated a preferential (or unbalanced) activation of the direct (D_1) pathways in dyskinesia in patients and animal models, as shown by an increase in adenyl cyclase activity in the striatum of parkinsonian patients (Tong et al., 2004), increased D_1 sensitivity and phosphorylation of proteins downstream the D_1 receptor in MPTP-treated dyskinetic primates (Aubert et al., 2005). D_3 and D_1 can have opposite or synergistic effects (Schwartz et al., 1998). It has been proposed that tolerance at the D_3 receptor underlies motor sensitization to indirect DA agonists, including transporter blockers (Richtand et al., 2003). G-protein-coupled receptors are typically regulated at multiple levels (receptor affinity, coupling, phosphorylation, regulators of G-protein signaling proteins, receptor internalization and degradation, clustering of adaptor and scaffolding proteins) (Pierce et al., 2002). Because DA has a higher affinity for D_3 than for D_2 receptor, adaptive changes may occur faster at the D_3 DA receptor (Richtand et al., 2003). In our study, dyskinetic animals showed a D_1-like pattern (Choi et al., 2006; Jenkins et al., 2004) of phMRI activation in response to a D_3-preferring agonist, suggesting that either directly, by abnormal G-coupling, or indirectly (i.e. by release of inhibition) sensitized D_3 signaling involves downstream activation of D_1 transduction pathways.

The observation of a complete reversal of the CBV changes after administration of 7-OHDPAT to the dyskinetic animals compared to the non-dyskinetic parkinsonian animals raises a number of interesting questions with regards to the mechanism underlying CBV changes. We previously showed that D_1 agonists produce large positive CBV changes in the striatum, while D_2/D_3 agonists (norpropylapomorphine, quinpirole and 7-OHDPAT) produce negative CBV changes in rats (Chen et al., 2005; Choi et al., 2006). Using BOLD a recent study in dyskinetic rodents has reported similar positive changes with D_1 (or non-selective) agonists (Delfino et al., 2007) while D_2 did not produce positive BOLD changes. Thus, since the CBV change observed in the dyskinetic animals is positive it is unlikely due to lack of specificity of the 7-OHDPAT for D_3 over D_2 receptors as the D_2 receptor activation should produce a negative CBV change. In contrast, if the post-synaptic D_3 signaling in the putamen occurs through activation of the D_1 pathway as discussed above, an increase in rCBV, as observed, may result. The fact that this increase occurred in spite of the decrease in $EtCO_2$ means that the increase in CBV was quite strong, as we have observed for D_1 agonists (Choi et al., 2006). These results are consistent with a number of studies showing that regulation of CBV and CBF consequent to neuronal activity (neurovascular coupling) is associated with post-synaptic signaling, not energy deficits (Attwell and Iadecola, 2002). Recent studies point to a key role of astrocytes in the regulation of the microvasculature, via release of vasoactive substances, such as prostaglandins (Takano et al., 2006), triggered by calcium waves after purinergic or glutamatergic stimuli (Mulligan and MacVicar, 2004). We cannot rule out a direct effect of 7-OHDPAT on astrocytes, as we recently showed that astrocytes express D_3 receptors (Choi et al., 2006), a fact also demonstrated by Miyazaki et al. (2004), and could therefore contribute to the hemodynamic response. However, while this possibility highlights how complicated is the interpretation of hemodynamic events related to neuronal activity, it does not lessen the relevance of D_3 sensitization in dyskinesia.

In this study, one of the monkeys did not develop dyskinesia after L-DOPA and did not show an increase in putaminal CBV changes further suggesting that the observed hemodynamic changes are linked to dyskinesia not to L-DOPA treatment itself. In naïve rhesus monkeys, acute administration of L-DOPA methylester (300 mg, i.m.) failed to induce fMRI changes in the forebrain (Chen et al., 1999). Although our imaging technique and experimental paradigm are different, a wealth of experimental and clinical data support the notion that in normal individuals with intact buffering capacity (uptake and storage) for exogenous L-DOPA, administration of L-DOPA does not alter the levels of DA or availability of receptors, thus being highly unlikely to modify the response to 7-OHDPAT or other DA agonists. For example, experimental studies using microdialysis have shown that the extracellular DA concentration in the intact striatum is not greatly (and only briefly) augmented by L-DOPA administration (Abercrombie et al., 1990; Miller and Abercrombie, 1999; Sanchez-Pernaute et al., 2001) in the rodent. In addition, chronic administration of L-DOPA does not affect DA receptor density or affinity in the normal striatum (Schneider et al., 1984) (Rouillard et al., 1987). On the other hand, in Parkinson patients, DA increase after L-DOPA administration measured by raclopride displacement and PET is larger in patients with more severe disease (Pavese et al., 2006) – probably related to failure to transport and store the neurotransmitter – and dyskinesias are correlated with larger increases in DA. Finally, Bezard et al. (2003) showed that postmortem changes in D_3 receptor expression in putamen and internal segment of the globus pallidus (GPi) were associated with dyskinesia, and not with L-DOPA per se.

Our results emphasize that repeated L-DOPA administration in a system lacking proper uptake and storage presynaptic capacity can lead to dysregulation of synaptic DA (Olanow et al., 2004) (de la Fuente-Fernandez et al., 2004) and persistent postsynaptic receptor changes, and support a role of D_3 receptor sensitization in the pathophysiology of dyskinesias. Further, and more importantly, this study provides a methodology for mapping the L-DOPA-

induced dyskinesias that is readily translatable to human studies and may lead to novel or improved therapeutic approaches.

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