Beyond dopamine (DA) loss, Parkinson’s disease is associated with many other monoamine alterations. While some monoaminergic systems benefit from l-3,4-dihydroxyphenylalanine (L-Dopa) treatment, others seem to be further altered, contributing to dyskinesia and non-motor symptoms. Surprisingly, the different contributions of parkinsonism and L-Dopa treatment on monoaminergic changes remain largely unknown. Here, both the consequences of vehicle or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposure and the subsequent effects of acute or chronic L-Dopa treatment were evaluated in macaques. Monoamine levels were measured in the putamen, the motor and prefrontal cortices, the hippocampus, and the amygdala using postmortem high-pressure liquid chromatography. In normal monkeys, L-Dopa treatment increased DA in the prefrontal cortex and hippocampus, but decreased serotonin levels in motor domains. Chronic L-Dopa treatment elevated monoamine levels in the prefrontal cortex, hippocampus, and amygdala in both normal and MPTP-treated monkeys. A substantial increase in DA levels in these regions, paralleled by a decrease in serotonin concentrations were related with dyskinesia severity, demonstrating that major changes in monoamine release also occur in nonmotor regions. Such monoaminergic dysregulation in limbic domains may also directly contribute to the expression of motor complications, such as dyskinesia, by impairing integrative processes upstream from motor execution.

Keywords: limbic, monkey, MPTP, Parkinson’s disease, serotonin

Introduction

In addition to the loss of mesencephalic dopaminergic neurons, Parkinson’s disease (PD) is associated with the degeneration of neurons in other nuclei, such as the nucleus basalis of Meynert, the dorsal raphe nucleus, the locus coeruleus, and the pedunculopontine nucleus (Ehringer and Hornykiewicz 1960; Halliday et al. 1990; Jellinger 1991; Zarow et al. 2003). The great therapeutic benefit afforded by l-3,4-dihydroxyphenylalanine (l-Dopa) has resulted in much focus upon dopamine (DA) loss, while noradrenergic (NA), serotonergic (5-HT), and cholinergic deficits are thought to ground most non-L-Dopa-responsive symptoms (Bonnet et al. 1987; Alldskog 2007) and to play a role in aberrant responses such as L-Dopa-induced dyskinesia (LID) and motor fluctuations (Carta et al. 2007; Navailles and De Deurwaerdere 2012a, 2012b). LIDs represent highly debilitating motor complications of long-term L-Dopa treatment, where structures belonging to the motor cortico-subcortico-cortical loops have received most experimental attention (for review see Bezard, Brochtrich, et al. 2001). Studies mapping monoaminergic depletion in different brain areas of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaques, showed that there were alterations in both motor and limbic domains (Pilf et al. 1991). However, this study did not investigate the effects of dopaminergic medication on monoamine levels (Pilf et al. 1991). Further work evaluating metabolic changes in response to dopaminergic treatments found an increased activity in both motor and “limbic/associative” components of the subthalamic nucleus (STN) (Mitchell et al. 1992). These findings suggested that segregated activation of the STN could account for differential pathophysiological markers of chorea and dyskinesia. Increased metabolic activity found in “limbic/associative” outputs of the basal ganglia suggests that LID should either be considered as being elicited through involvement of both motor and cognitive/affective networks or accompanied by dysregulated cognitive/affective manifestations (Guigoni et al. 2005). Recently, rat experiments demonstrated that chronic L-Dopa treatment induces a dysregulation of extra-striatal DA and 5-HT levels responsible for affective symptoms (Eskow Jaunarajs et al. 2012). In addition, other studies have shown abnormal transcriptional responses in many nonmotor regions outside the basal ganglia (Bastide et al. 2014).

Growing evidence in rodents suggests that chronic L-Dopa stimulation might disrupt monoamine transmission in PD (Eskow Jaunarajs et al. 2010; Navailles et al. 2011; Eskow Jaunarajs et al. 2012). However, no study thus far has differentiated the contribution of parkinsonism from the effect of L-Dopa treatment on monoaminergic changes in MPTP-treated monkeys. This experiment combined the study of the effects of the vehicle/MPTP exposure and the subsequent effects of acute and chronic treatment with L-Dopa upon the monoamines in 5 motor (putamen and motor cortex) and nonmotor (prefrontal cortex, hippocampus, and amygdala) structures.

Materials and Methods

Experimental Protocol

Experiments were conducted on a previously characterized brain bank (Fernagut et al. 2010; Santini et al. 2010; Porras et al. 2012). Animal groups were composed of forty-one female rhesus monkeys (Macaca mulatta, Xierxin, Beijing, PR of China; mean age = 5 ± 1 years; mean weight = 5.3 ± 0.8 kg) housed in individual primate cages allowing visual contacts and interaction with monkeys housed in the adjacent cages, under controlled conditions of humidity (50 ± 5%), temperature (24 ± 1 °C), and light (12 h light/12 h dark cycles, time lights on 8:00 am). Food and water were available ad libitum and animal care was supervised daily by veterinarians skilled in the healthcare and maintenance of nonhuman primates. Experiments were carried out in accordance with European Communities Council Directive (2010/63/ EU) for care of laboratory animals in an AAALAC-accredited facility following acceptance of study design by the Institute of Lab Animal Science IACUC (Chinese Academy of Medical Sciences, Beijing, China).
Briefly, 7 groups were constituted as follows (Fernagut et al. 2010; Santini et al. 2010; Porras et al. 2012): six untreateds controls (control group), 6 control animals receiving one single 20 mg/kg l-Dopa dose p.o. (control acute l-Dopa), 6 animals receiving twice daily 20 mg/kg l-Dopa p.o. for 3 months (control chronic l-Dopa). Twenty-three additional animals were rendered parkinsonian with MPTP hydrochloride (0.2 mg/kg, i.v., Sigma, St Louis, MO, USA) dissolved in saline according to a previously described protocol (Bezard et al. 1997; Bezard, Dovoero, et al. 2001). Following stabilization of the MPTP-induced syndrome (3 months), they received either: saline (MPTP group; n = 5), one single 20 mg/kg l-Dopa dose p.o. (MPTP acute l-Dopa; n = 6) or twice daily 20 mg/kg l-Dopa p.o. for a further 3 months (MPTP chronic l-Dopa; n = 12). At the end of the experiment, all animals were killed by sodium pentobarbital overdose (150 mg/kg, i.v.) 1 h after the last dose of vehicle or l-Dopa, and the brains were removed quickly after death. Each brain was bisected along the midline and the 2 hemispheres were immediately frozen by immersion in isopentane (−45 °C) and then stored at −80 °C.

**Behavioral Assessment of Parkinsonism and l-Dopa-Induced Dyskinesia**

Daily (9 AM) assessment of the parkinsonism degree was performed in home cages for 30 min by 2 blinded observers using a validated parkinsonian macaque clinical scale (Imbert et al. 2000; Bezard, Dovoero, et al. 2001) which rates tremor, variations in the general level of activity, body posture (flexion of spine), vocalization, freezing, and frequency of arm movements (reaching for food for each upper limb) and rigidity (for each upper limb). All MPTP groups displayed similar parkinsonian scores: MPTP: 8.5 ± 0.5, MPTP acute l-Dopa: 9.5 ± 0.5, MPTP chronic l-Dopa 9.33 ± 0.83, MPTP chronic l-Dopa (dysoptic): 9.83 ± 0.87, as previously reported (Fernagut et al. 2010). After chronic l-Dopa treatment, the severity of dyskinesia was rated as previously described (Fernagut et al. 2010) using the Dyskinesia Disability Scale (Hill et al. 2004; Fasano et al. 2010; Fox et al. 2012; Tison et al. 2013) detailed in legend to Figure 4. Daily behavioral assessments were performed before and after l-Dopa administration. Median rating scores and SEM were calculated daily for each group.

**HPLC Analysis**

Coronal 300 µm-thick sections were cryostat-cut and punches of brain tissue were taken for the following regions: (i.e., motor striatum (postcommissural dorsal putamen), motor cortex (precentral gyrus), prefrontal cortex (superior frontal gyrus), hippocampus (CA1), and amygdala (basolateral nucleus). An average sample size of 6 ± 2 mg was obtained (Santini et al. 2010). The tissues were homogenized in 60 µL of 0.1N HClO4 and centrifuged at 13 000 rpm for 30 min at 4 °C (De Deurwaerdere et al. 1995; Delavale et al. 2012). Supernatants were injected into the HPLC column (Chromasyl C8, 150 × 4.6 mm, 5 µm) protected by a Browlee-Newgaard precolumn (RP-8, 15 × 3.2, 7 µm). The mobile phase, delivered at 1.2 mL/min flow rate, was prepared as follows: 70 mM NaH2PO4, methanol 7%, triethylamine 100 µL/L, SOS 90 mg/L, adjusted to pH 4.2 with orthophosphoric acid and filtered through a 0.22-µm Millipore filter. Monoamine detection was performed with a coulometric detector (Coulochem II, ESA) coupled to a dual-electrode analytic cell (model 5011). The potential of the electrode was set at +350 and −270 mV. Tissue protein content was determined by the method of (Lowry et al. 1951). Very low NA concentrations in the striatum (Pift et al. 1991; Fitoussi et al. 2013) meant that these data were difficult to analyze in postmortem samples (De Deurwaerdere et al. 1998), especially in animals receiving l-Dopa. The pharmacological treatment can indeed contaminate the NA measures, rendering difficult the interpretation of the data. Striatal NA concentration were thus omitted from the analysis in all groups.

**Data Analysis**

Raw data were subjected to Student’s t-tests for “Control versus MPTP” comparisons and one-way ANOVA for l-Dopa treatments. Post hoc analyses were performed using Bonferroni’s multiple comparisons test on all groups for each condition. Linear regressions between LID and monoamine levels were performed. All data presented are means ± SEM with a threshold for statistical difference set at P<0.05.

**Results**

**MPTP-Induced Monoamine Depletion**

As previously reported (Pift et al. 1991; Bezard, Dovoero, et al. 2001; Fernagut et al. 2010), MPTP intoxication led to an almost total DA and 3,4 dihydroxphenylacetic acid (DOPAC) depletion in the striatum (−92.6% and −92.8%, respectively; Supplementary Fig. 1). Striatal 5-HT levels were also reduced by MPTP (−36.5%).

**Effects of Acute and Chronic l-Dopa Treatment on Monoamine Levels in Control Monkeys**

To date, no study has assessed l-Dopa-induced modifications of monoamine concentrations in normal monkeys. Administration of l-Dopa has produced opposite effects on 5-HT compared with DA and NA. In addition, l-Dopa-induced modifications of 5-HT levels occurred in motor areas, while changes in DA and NA concentrations occurred only in nonmotor regions (Figs 1 and 3). In the motor cortex, a 37% decrease in 5-HT levels was observed (one-way ANOVA, group effect: F2,n=14 = 10.37, P<0.01) and post hoc analysis revealed that the acutely treated group was significantly different from both untreated and chronically treated groups (P<0.05 for both). In the striatum, a significant difference in 5-HT levels was observed between the acute and chronic group (one-way ANOVA, group effect: F2,n=16 = 6.99, P<0.01). While chronic l-Dopa treatment did not modify 5-HT levels in control animals, it decreased 5-HT concentration by 24.5% in actively treated controls compared with untreated controls (Bonferroni: P=0.053). Conversely, l-Dopa elevated NA and DA levels in the prefrontal cortex (Fig. 1). NA levels were significantly increased after acute and chronic l-Dopa administration (55% and 52%, respectively, one-way ANOVA: F2,n=17 =6.19, P<0.05; Bonferroni: P<0.05 vs. control for both), while DA concentrations were significantly increased by 102% only after chronic l-Dopa treatment (one-way ANOVA: F2,n=16 = 5.8, P<0.05; Bonferroni: P<0.05 vs. control). In addition, chronic l-Dopa treatment also increased DA levels in the hippocampus by 146% (one-way ANOVA: F2,n=14 =9.86, P<0.01, Bonferroni: P<0.01 vs. untreated control and vs. acute l-Dopa). In the amygdala, DA levels were increased by 175% following acute l-Dopa administration (one-way ANOVA group effect: F2,n=17 = 3.82, P<0.05).

**Effects of Acute and Chronic l-Dopa Treatment on Monoamine Levels in MPTP-Treated Monkeys**

After a chronic l-Dopa treatment regimen, 2 behaviorally distinct populations arose as previously described (Fernagut et al. 2010; Santini et al. 2010; Porras et al. 2012): “Nondyskinetic” and “Dyskinetic” monkeys (scores presented in Fernagut et al. (2010)). Monoamine levels assessed following l-Dopa administration in the 3 groups of MPTP-treated animals (acute, chronic nondyskinetic, and chronic dyskinetic) are presented on Figure 2. Importantly, neither acute nor chronic l-Dopa was able to restore DA and DOPAC concentrations in the striatum (t-test comparing untreated controls with each MPTP + l-Dopa group, P>0.001 for all). One-way ANOVA comparing untreated MPTP monkeys to each treatment condition confirmed that striatal DA and DOPAC levels remained unaffected by...
L-Dopa treatment \( (F_{3, 22} = 2.99, \; P > 0.05 \text{ for DA and } F_{3, 22} = 2.05, \; P > 0.05 \text{ for DOPAC}) \). Acute and chronic L-Dopa treatments were also unable to restore the MPTP-induced 5-HT depletion (t-test comparing each of the 3 treated groups to untreated controls, \( P < 0.05 \) for all). Furthermore, in both nondyskinetic and dyskinetic monkeys, chronic L-Dopa treatment even worsened (by \( \sim 40\% \)) MPTP-induced striatal 5-HT loss compared with untreated MPTP animals (one-way ANOVA, \( F_{3, 22} = 10.23, \; P < 0.001 \); Bonferroni: \( P < 0.05 \)) and with acutely treated animals (Bonferroni: \( P < 0.01 \)). An L-Dopa-induced decrease of 5-HT levels was also found in the motor cortex, of dyskinetic monkeys (t-test, \( t = 2.96, \; df = 7, \; P < 0.05 \text{ vs. untreated controls} \)), while a slight but significant reduction of NA levels was found in nondyskinetic animals (t-test, \( t = 3.02, \; df = 10, \; P < 0.05 \)).

Contrary to the increased levels found in normal monkeys, NA levels decreased following both acute and chronic L-Dopa treatment in the prefrontal cortex of MPTP-treated animals (t-test comparing each of the 3 treated groups with untreated controls, \( P < 0.05 \) for all). Despite its inability to restore striatal DA and DOPAC levels, chronic L-Dopa was able to increase DA and DOPAC levels in the prefrontal cortex (t-test, \( t = 2.85, \)

### Table 1: Effect of an acute or a chronic L-Dopa treatment on monoamine levels in normal macaques: Noradrenaline (NA), dopamine (DA), serotonin (5-HT), and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in normal animals after acute or chronic L-Dopa treatment. Results are expressed as percentage of untreated controls as scatter plot featuring mean ± SEM. # \( P < 0.05 \); * \( P < 0.05 \) from chronic treatment.

![Figure 1. Effect of an acute or a chronic L-Dopa treatment on monoamine levels in normal macaques: Noradrenaline (NA), dopamine (DA), serotonin (5-HT), and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in normal animals after acute or chronic L-Dopa treatment. Results are expressed as percentage of untreated controls as scatter plot featuring mean ± SEM. # \( P < 0.05 \); * \( P < 0.05 \) from chronic treatment.](https://academic.oup.com/cercor/article-abstract/25/9/2783/2926091)
df = 10 for DA and \( t = 2.65, \) df = 9 for DOPAC, \( P < 0.05 \) vs. untreated controls). One-way ANOVA confirmed this increase in dyskinetic monkeys when compared with untreated MPTP (\( F_{3, 23} = 7.65, \) \( P < 0.01 \); Bonferroni: \( P < 0.01 \) for DA and \( F_{3, 22} = 8.82, \) \( P < 0.001 \); Bonferroni: \( P < 0.01 \) for DOPAC), acute (Bonferroni: \( P < 0.05 \) for both DA and DOPAC) and nondyskinetic animals (Bonferroni: \( P < 0.05 \) for both DA and DOPAC).

A similar effect was found in the hippocampus, where chronic \( \alpha \)-Dopa treatment induced an important increase of both DA and DOPAC concentrations exclusively in dyskinetic animals (\( t\)-test, \( t = 2.78, \) df = 9, for DA and \( t = 3.24, \) df = 10 for DOPAC, \( P < 0.05 \) vs. untreated control for both). This increase was also observed when compared with untreated MPTP (\( F_{3, 22} = 6.75, \) \( P < 0.01 \); Bonferroni: \( P < 0.01 \) for DA and \( F_{3, 22} = 9.37, \) \( P < 0.001 \); Bonferroni: \( P < 0.01 \) for DOPAC), acute (Bonferroni: \( P < 0.01 \) for both DA and DOPAC), and nondyskinetic (Bonferroni: \( P < 0.05 \) for both DA and DOPAC) groups. Interestingly, such increased hippocampal dopaminergic tone was mirrored by a decrease in 5-HT concentrations. One-way ANOVA (\( F_{3, 22} = 8.25, P < 0.001 \)) revealed that dyskinetic
animals were significantly different from untreated MPTP (Bonferroni: $P < 0.001$), acute (Bonferroni: $P < 0.05$), and nondyskinetic (Bonferroni: $P < 0.05$) animals.

Finally, in the amygdala, no treatment schedule managed to restore the trend toward NA depletion induced by MPTP. Thus, NA levels were significantly decreased in all MPTP groups compared with untreated controls ($P < 0.05$ for all). An increase in DA and DOPAC concentrations in dyskinetic compared with nondyskinetic monkeys was also observed (one-way ANOVA, $F_{3, 22} = 3.35$, $P < 0.05$ for DA and $F_{3, 22} = 7.04$, $P < 0.01$ for DOPAC; Bonferroni: $P < 0.05$ for both DA and DOPAC). Dyskinetic animals exhibited higher DA concentrations compared with untreated controls ($t$-test, $t = 4.22$, df = 10, $P < 0.01$), whereas nondyskinetic animals showed decreased DOPAC levels ($t$-test, $t = 2.69$, df = 10, $P < 0.05$ from untreated controls). Again, this increased dopaminergic tone in dyskinetic monkeys was concomitant with a decrease in 5-HT. Interestingly, the low 5-HT levels were present in both dyskinetic and nondyskinetic animals when compared with untreated controls ($t$-test, $P < 0.01$ for both), untreated MPTP (one-way ANOVA, $F_{3, 22} = 7.84$, $P < 0.01$; Bonferroni: $P < 0.05$ for both), and acute L-Dopa treatment (Bonferroni: $P < 0.01$ for both).

A graphic summary of these widespread monoaminergic changes produced by L-Dopa in both normal and MPTP-treated animals is provided in Figure 3 (see also Supplementary Table 1).

### Relations Between Dyskinesia and Monoamine Contents

Regression analyses were performed to investigate whether monoamine levels in the 5 brain regions varied with dyskinesia severity. There was no significant relation between NA levels and LID severity in any of the regions analyzed. There was a positive relation between DA levels and LID severity in the prefrontal cortex ($r^2 = 0.64$, $P < 0.01$), hippocampus ($r^2 = 0.39$, $P < 0.05$), and amygdala ($r^2 = 0.87$, $P < 0.0001$) (Fig. 4). Moreover, in the hippocampus, a negative relation was also found for 5-HT concentrations ($r^2 = 0.53$, $P < 0.01$). Finally, no relationships were reported between dyskinesia severity and monoamine levels in the striatum and motor cortex.

### Discussion

Over the years, L-Dopa has proven its efficacy in alleviating motor symptoms by reinstating dopaminergic function in the
basal ganglia (Meissner et al. 2011; Rascol et al. 2011). However, L-Dopa is not effective against all PD symptoms and wide detrimental effects are observed on both motor and non-motor functions (Chaudhuri et al. 2006), highlighting its broad influence upon various cerebral and neurochemical targets. Here, we report region-dependent effects of L-Dopa on tissue monoamine content in normal or MPTP-treated macaques, with and without dyskinesia. L-Dopa affected monoamine content in cortical and subcortical motor and nonmotor regions of both normal and MPTP-treated monkeys confirming its vast scope of action. Some alterations in limbic domains were observed exclusively in dyskinetic monkeys and were related to LID severity, further supporting the involvement of associative-limbic regions in LID (Guigoni et al. 2005).

The first goal of this study was to evaluate the effects of L-Dopa upon monoamine levels in normal monkeys. HPLC analysis revealed the ability of acutely administered L-Dopa to exert a metabolic substitution inside 5-HT terminals. By sharing similar decarboxylation pathways, 5-HT neurons can synthesize DA from L-Dopa at the expense of 5-HT (Tison et al. 1991; Arai et al. 1995) and release DA as a “false neurotransmitter” (Ng et al. 1970). This acute effect of L-Dopa on 5-HT function occurred only in motor areas which could highlight various and/or heterologous fiber tracts of the raphe nuclei (Azmitia and Segal 1978) or local interactions (Navailles and De Deurwaerdere 2012a, 2012b). Unaltered 5-HT levels in chronically treated normal monkeys suggest the occurrence of long-term adaptations possibly involving aromatic L-aminoacid decarboxylase (AADC) expression and/or activity in 5-HT, or other, cell-types (Melamed et al. 1981) to restore a “normal” 5-HT state. In addition, the lack of modifications in 5-HT levels after acute or chronic L-Dopa in limbic regions indicates that monoaminergic neurons projecting to these regions have greater adaptive capacities than those projecting to motor regions in supplying extra DA precursor.

As the metabolic precursor of catecholamines, L-Dopa was also able to increase NA content. Such an effect seems specific to NA terminals, as it requires DA β-hydroxylase, specifically expressed by NA neurons (Aston-Jones et al. 2004). Consistently, L-Dopa was shown to increase NA levels and its turnover.

Figure 4. Relations between monoamine contents and dyskinesia severity in the striatum and the cognitive/limbic structures. Noradrenaline (NA), dopamine (DA), serotonin (5-HT). Results are presented for MPTP-treated animals under chronic L-Dopa treatment. Monoamine levels are expressed in pg/mg tissue (or pg/mg protein for striatum). LID, levodopa-induced dyskinesia. LID scoring: 0 = no dyskinesia; 1 = mild and rare dyskinetic movements and postures; 2 = moderate abnormal movements not interfering significantly with normal behavior; 3 = marked, frequent and continuous dyskinesia intruding on the normal repertoire of activity.
in the brain (Chalmers et al. 1971; Romero et al. 1972), as well as the electrophysiological or behavioral responses to NA transporter inhibitors (Miguélez et al. 2013). Although the hippocampus, motor cortex, and striatum receive a similar innervation from the locus coeruleus (Aston-Jones et al. 2004), increased NA occurred only in the prefrontal cortex, highlighting a functional heterogeneity of NA innervation.

Interestingly, significant increases in DA content occurred only in the 3 studied associative-limbic domains receiving DA input from the mesolimbic pathway (Bjorklund and Dunnett 2007). Although the resulting increase in non DA-depleted animals should be masked by functional DA fibers (Wachtel and Abercrombie 1994), l-Dopa was shown to increase DA levels in intact rat limbic regions including the prefrontal cortex, hippocampus, and amygdala (Loeffler et al. 1998; Eskow Jaunarajs et al. 2010). Similar results are found here in monkeys, confirming that l-Dopa can significantly increase the basal dopaminergic tone of the limbic system. We did not, however, find DA accumulation in poor (motor cortex) and rich (putamen) DA-innervated motor areas. Nevertheless, there were differences between l-Dopa effects on DA levels in limbic structures according to the administration schedule, thereby illustrating different adaptation capacities to chronic l-Dopa exposure. Whether such effects are related to different expression levels and/or activities of AADC and DA-catabolic enzymes will require further investigation.

The second aim of this study was to identify neurochemical correlates of LIDs. In addition to the expected striatal DA and DOPAC depletion, MPTP intoxication induced a pattern of monoamine depletion consistent with prior studies (Perez-Otano et al. 1991; Pill et al. 1991), including a significant 5-HT reduction (Supplementary Fig. 1), albeit moderate compared with the seminal observations by Pill et al. (1991). In MPTP-treated monkeys, neither acute nor chronic l-Dopa administration rescued striatal DA levels as shown ex vivo in rats (Eskow Jaunarajs et al. 2012) and in vivo in macaques (Porras et al. 2014).

An important finding was that chronic l-Dopa treatment further exacerbated the MPTP-induced striatal 5-HT depletion. Such acute versus chronic difference was not found in control animals, suggesting that adaptive mechanisms occurring in normal animals are disrupted in MPTP-treated monkeys adding a deleterious effect of repeated l-Dopa administration onto the intrinsic effect of MPTP on 5-HT levels both in motor and limbic areas (Navailles et al. 2011). The demonstration that chronic l-Dopa treatment induces alterations of 5-HT neurons, including sprouting of 5-HT terminals in the striatum, together with increased synaptic contacts and evoked DA release (Rylander et al. 2010) further supports the notion that l-Dopa can profoundly alter both the structure and functioning of the 5-HT system in lesioned animals. Strikingly, the prefrontal cortex was the only structure where l-Dopa did not affect 5-HT content in dyskinetic monkeys. While in rats, a correlation was found between higher 5-HT content in the prefrontal cortex and LID (Carta et al. 2006), these low striatal and maintained prefrontal cortex 5-HT levels were found here both in dyskinetic and nondyskinetic animals, suggesting that such dysregulation is not involved in dyskinesia in monkeys (Brooks et al. 2000). Although, a difference between dyskinetic and nondyskinetic animals was found regarding 5-HT concentrations in the motor cortex indicating that cortical areas respond differently to chronic l-Dopa treatment. This raises the possibility that altered cortical 5-HT modulation (Aghajanian and Marek 1997; Ostock et al. 2011) may contribute to the differential motor cortex activations as reported in dyskinetic versus nondyskinetic patients (Rascol et al. 1998). These results are also in accordance with previous data demonstrating that chronic l-Dopa treatment in DA-depleted animals promotes region-dependent decreases in 5-HT content (Navailles et al. 2011). Hence, dyskinesia could be related to distinct responses of cortical areas to l-Dopa, creating an imbalance of cortical inputs to striatal subregions.

We also found an imbalance in cortical NA responses to l-Dopa in dyskinetic monkeys in which NA content was unaltered in the motor cortex but decreased in the prefrontal cortex. This phenomenon remains poorly understood, though similar observations have been made in the prefrontal cortex of hemiparkinsonian rats (Eskow Jaunarajs et al. 2010). This opposite pattern of cortical responses of NA and 5-HT may participate in the DA cortical imbalance reported specifically in the prefrontal cortex of dyskinetic monkeys. Indeed, while 5-HT neurons are responsible for the release of unregulated DA from l-Dopa, NA fibers, via their transporter, are responsible for the clearance of extracellular DA (Chotibut et al. 2012; Navailles et al. 2013). On the other hand, prefrontal dysfunctions would not be solely drug-related and could underlie a broader network dysfunction associated with LID.

Besides inducing monoaminergic alterations between dyskinetic and nondyskinetic animals in motor regions, l-Dopa also increased DA levels in the prefrontal cortex, the hippocampus and the amygdala of dyskinetic monkeys and linear regression analysis suggests a possible link between DA content in these structures and dyskinesia severity. Even though the MPTP-induced preferential degeneration of the nigrostriatal over the mesolimbic pathway (Fernagut et al. 2010) may promote an imbalance between both circuits under l-Dopa regimen, with a residual biosynthetic capacity at mesolimbic dopaminergic terminals potentially contributing to “overdose” the mesolimbic pathway, such a situation recalls the pattern of nigrostriatal versus mesolimbic denervation occurring in the human disease. Accumulating evidence from studies measuring metabolic changes (Guigoni et al. 2005) or transcriptional responses to l-Dopa (Bastide et al. 2014) indicate an involvement of structures outside the basal ganglia network in LID. While no study has directly assessed their contribution in dyskinesia as limbic-motor interfaces, these structures are involved in many integrative and associative processes that are ultimately critical for the correct execution of goal-directed behaviors, including movements and motor tasks. Thus, the hippocampus has been shown to support behavioral sensitization (Lodge and Grace 2008), while the PFC and basolateral amygdala modulate locomotion induced by dopaminergic agents (Rouillon et al. 2008). In addition, these structures control motivated behaviors (Robbins and Everitt 1996; Blanquet et al. 2013) and behavioral inhibition (Chudasama et al. 2012). The prefrontal cortex, which is involved in suppressing response interferences or impulses (Mink 1996; Gaugel et al. 2004; Wylie et al. 2010), promotes “go-bias” in procedural tasks responding when under dopaminergic medication (Frank et al. 2004). The rise in prefrontal DA levels observed along with increased dyskinesia severity might thus be involved in LID expression by promoting unwanted behaviors or by causing irrepressible actions through corticostral pathways (Graybiel et al. 2000). Considering that dyskinesias are also triggered or
enhanced by stress and emotional situations (Voon et al. 2009), modifications in amygdala and hippocampal DA might participate in LID expression. Altogether, altered integration and action selection/planning might contribute to LID. Their specific involvement in LID induction and expression will necessitate further investigation to provide a better understanding of the limbic component of dyskinesias. Although not assessed in the present study, alterations of monoamine levels in limbic regions are also compatible with the appearance of L-Dopa-induced psychiatric side effects (Carey et al. 1995; Forsaa et al. 2010; Lee and Weinerbaum 2012) and will deserve to be specifically investigated in future studies.

Altogether, these data set the pathophysiological monoamine framework in the gold-standard experimental model of PD. Of particular interest, chronic L-Dopa treatment elevated monoamine levels in cognitive and limbic regions both in normal and MPTP-treated monkeys. A noticeable increase in DA levels linked to LID severity is observed further supporting that LID might also possess nonmotor components that may also directly contribute to the expression of motor complications such as LID.

Authors’ Contributions
E.B. designed and organized the experiments; M.E., P.D.D., Q.L., and P-O.F. performed experiments; M.E., P.D.D., E.B., and P-O.F. analyzed the data and wrote the paper.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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