Behavioral and neurochemical effects of chronic L-DOPA treatment on non-motor sequelae in the hemiparkinsonian rat
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Depression and anxiety are the prevalent non-motor symptoms that worsen quality of life for Parkinson’s disease (PD) patients. Although dopamine (DA) cell loss is a commonly proposed mechanism, the reported efficacy of DA replacement therapy with L-DOPA on affective symptoms is inconsistent. To delineate the effects of DA denervation and chronic L-DOPA treatment on affective behaviors, male Sprague–Dawley rats received unilateral 6-hydroxydopamine or sham lesions and were treated daily with L-DOPA (12 mg/kg + benserazide, 15 mg/kg, subcutaneously) or vehicle (0.9% NaCl, 0.1% ascorbic acid) for 28 days before commencing investigations into anxiety (locomotor chambers, social interaction) and depression-like behaviors (forced swim test) during the OFF phase of L-DOPA. One hour after the final treatments, rats were killed and striatum, prefrontal cortex, hippocampus, and amygdala were analyzed through high-performance liquid chromatography for monoamine levels. In locomotor chambers and social interaction, DA lesions exerted mild anxiogenic effects. Surprisingly, chronic L-DOPA treatment did not improve these effects. Although DA lesion reduced climbing behaviors on day 2 of exposure to the forced swim test, chronic L-DOPA treatment did not reverse these effects. Neurochemically, L-DOPA treatment in hemiparkinsonian rats reduced norepinephrine levels in the prefrontal cortex, striatum, and hippocampus. Collectively, these data suggest that chronic L-DOPA therapy in severely DA-lesioned rats does not improve non-motor symptoms and may impair non-dopaminergic processes, indicating that long-term L-DOPA therapy does not exert necessary cause in neuroplastic changes for improving affect. Behavioural Pharmacology 00:000–000 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction
Parkinson’s disease (PD) is a progressive, neurodegenerative disorder characterized by the loss of dopamine (DA) neurons in the nigrostriatal pathway, resulting in motor symptoms, such as tremor, rigidity and bradykinesia. Though less acknowledged, PD patients also suffer from a variety of non-motor symptoms, including significant changes in affect that deleteriously impact quality of life (Schrag, 2006; Carod-Artal et al., 2008; McKinlay et al., 2008). For example, anxiety and/or depression are reported by more than 50% of PD patients, far exceeding rates in normal and chronic disease-affected populations (Wragg and Jeste, 1989; Richard et al., 1996; Beckman et al., 1999; Yamamoto, 2001; Barone et al., 2009). Therefore, understanding the neurobiological underpinnings of psychiatric disturbances in PD is a crucial but unmet goal.

Although the pathophysiological mechanism(s) underlying increased prevalence of anxiety and depression in PD are unclear, it is likely that both dopaminergic and non-dopaminergic systems contribute. For example, Remy et al. (2005) found a negative correlation between decreased binding to DA/norepinephrine (NE) transpor-
motor fluctuations, chronic L-DOPA may promote the
development of mood fluctuations, including alterations
in euphoria, anxiety and depression (Maricle et al., 1995;
Kulisevsky et al., 2007). Unfortunately, there is a paucity
of basic research regarding the impact of chronic L-DOPA
on mood-related behaviors and concomitant neurochem-
ical changes within the DA-depleted brain.

As such, the goals of this study were to determine the
effects of DA denervation and chronic L-DOPA treatment
on rodent measures of anxiety and depression, as well as
monoamine levels through high-performance liquid chromo-
matography with electrochemical detection (HPLC-ED)
within affect-related brain areas. Based on earlier research
(Maricle et al., 1995; Branchi et al., 2008), it was predicted
that DA lesion would promote anxiety and depression-
like symptoms whereas L-DOPA would improve aspects
of these behaviors. As DA, NE, and serotonin (5-HT) are
known to be involved in affect (Ressler and Nemeroff,
2000), neurochemical changes were predicted to parallel
chronic behavioral effects with reductions in DA, NE, and
5-HT function associated with increased anxiety and depression-like behaviors.

Methods

Subjects

Adult male Sprague–Dawley rats (225–250 g upon arrival;
Taconic Farms, Hudson, New York, USA) were housed in
plastic cages (22 cm high, 45 cm deep and 23 cm wide)
with free access to standard lab chow (Rodent Diet 5001;
Lab Diet, Brentwood, Missouri, USA) and water. The
colony was maintained on a 12/12 h light/dark cycle
(lights on 0700 h) at a temperature of 22–23°C. Animals
were maintained in accordance with the guidelines of the
Institutional Animal Care and Use Committee of
Binghamton University and the ‘Guide for the Care and
Use of Laboratory Animals’ (Institute of Laboratory
Animal Resources, National Academic Press 1996; NIH
publication number 85-23, revised 1996).

6-OHDA lesion surgeries

One week after arrival, rats received unilateral 6-OHDA or
sham lesions of the left medial forebrain bundle (AP:
−1.8 mm, ML: +2.0 mm, DV: −8.6 mm relative to bregma
with the incisor bar positioned 5 mm below the internal
line) to destroy DA neurons. Following pretreatment with
desipramine HCl (25 mg/kg, intraperitoneal; Sigma, St
Louis, Missouri, USA) to protect NE neurons, 6-OHDA
(12 μg; Sigma) dissolved in 0.9% NaCl + 0.1% ascorbic
acid was infused at a rate of 2 μl/min for a total volume
of 4 μl. The needle was withdrawn 5 min later and rats were
placed in clean cages on warming pads to recover from
surgery, after which they were returned to group-housing
(two rats/cage).

Pharmacological treatments

To test the behavioral effects of DA lesions and/or chronic
L-DOPA treatment, rats received either vehicle (0.9%
NaCl containing 0.1% ascorbic acid) or L-DOPA methyl
ester [L-DOPA; 12 mg/kg, subcutaneously (s.c.); Sigma] +
DL-Serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydro-
chloride (benserazide; 15 mg/kg, s.c.; Sigma) once daily
for 28 (n = 28) or 75 (n = 20) days, after 3 weeks of
recovery from 6-OHDA or sham lesions. L-DOPA and
benserazide were dissolved in vehicle (0.9% NaCl contain-
ing 0.1% ascorbic acid) and administered at a volume
of 1.0 ml/kg. Thus, four groups were formed: (i) sham-
lesioned, vehicle-treated (Sham-VEH), (ii) sham-lesioned,
L-DOPA-treated (Sham-LD), (iii) 6-OHDA-lesioned,
vessel-treated (Lesion-VEH), and (iv) 6-OHDA-lesioned,
L-DOPA-treated (Lesion-LD). To ensure that L-DOPA-
induced dyskinesia did not interfere with the behavioral
analyses, animals were tested 16 h after the earlier
treatment on day 24 (OFF phase). A subset of animals
was then killed on day 28 of treatment for neurochemical
tissue analyses through HPLC-ED, 60 min after treatment
with vehicle or L-DOPA. The remaining animals continued
their respective daily treatments during exposure to the
modified forced swim test, and social interaction with a
novel conspecific, each separated by at least 1 week. After
behavioral testing was completed (day 75), all remaining
animals were killed, 60 min after treatment with vehicle
or L-DOPA for neurochemical tissue analyses through
HPLC-ED.

Behavioral testing

Locomotor activity

Locomotor activity testing was conducted in six identical
acrylic chambers measuring 40 cm long, 40 cm wide and
30 cm high (Accuscan Instruments, Columbus, Ohio, USA).
Each chamber was surrounded by a 15 × 15 infrared pho-
tocell array interfaced with a computer that ran the Ver-
samax and Versadat programs, which tabulated and
processed behaviors in the test field. For this experiment,
the variables examined included locomotor measures
such as total distance traveled (cm) and anxiety measures
including center time spent (s), whole-body entries
(frequency), and vertical movements (frequency), for
twenty 6 min periods (total of 2 h). Rats had no earlier
experience in the test field before the first test day.

Modified forced swim test

The modified forced swim test was used as a well-
established measure of depressive behaviors in rats that is
sensitive to both the prodepressant and antidepressant
medications and manipulations (Detke et al., 1995; Lucki,
1997; Deak et al., 2005). In this study, test-naïve rats were
placed into a Plexiglas cylinder (45 × 20 cm) filled with
30 cm of warm water (25°C) and their responses were
recorded through video camera onto recordable DVD for
15 min. Twenty-four hours later and 16–20 h after their last
daily treatment, rats were placed into the cylinder and their responses were recorded for an additional 5 min. Recordings were later analyzed by a blinded, trained observer every 5 s for the prevailing behavior: climbing, swimming, or immobility. Climbing was defined as attempts to escape the chamber by struggling up the sides of the cylinder. Swimming was defined as mild paddling of the limbs around the cylinder and immobility as lack of limb movements (floating), except for those necessary to stay afloat.

**Social interaction test**

The social interaction test was used as a measure of anxiety-like behaviors in rats. The frequency of approaches and sniffing are sensitive to both anxiolytic and anxiogenic manipulations (File and Seth, 2003). Test and non-manipulated, novel conspecifics (stimulus rats) were habituated to a 25 × 40 cm chamber for 15 min in a darkened room. The next day, and 16–20 h after their last daily treatment, test rats were introduced to the chamber again with a stimulus rat and their interactions were recorded for 15 min through video camera onto recordable DVD. Recordings were later tallied by a blinded, trained observer for frequency of approaches, flights, following, and anogenital or other sniffing by the test rat towards the stimulus rat. Approaches were defined as purposeful movement of the test animal towards the stimulus animal, with flights being the opposite (purposeful movement of the test animal away from the stimulus animal). Any movements in which the test animal was following the stimulus animal as it moved away from the test animal were defined as following, sniffing was defined as visible sniffing motions of the test animal, either in contact with or close to the stimulus rat, with anogenital sniffing considered separate from other sniffing behaviors.

**High-performance liquid chromatography with electrochemical detection**

After 28 or 75 days of daily treatment, rats were killed by decapitation, 60 min after injection with either Vehicle or L-DOPA (12 mg/kg + benserazide, 15 mg/kg, s.c.). The striatum, hippocampus, amygdala, and prefrontal cortex ipsilateral to sham or DA lesion were collected, flash-frozen and stored at −80°C. Reverse-phase HPLC-EC was performed on tissue samples, obtained from all rats, according to the protocol of Kilpatrick et al. (1986), a method for semiautomated catecholamine and indoleamine analysis with coulometric detection. The system included an ESA autoinjector (Model 542), an ESA solvent delivery system (1582), an external pulse dampener (ESA), an ESA column and a C-18 (100 × 4.6 mm, 5 μm packing) column (ESA). Samples were homogenized in ice-cold perchloric acid (0.1 mol/l) with 1% ethanol and 0.02% ethylenediamine tetra-acetic acid. The homogenates were spun for 45 min at 14 400 g with the temperature maintained at 4°C. Aliquots of supernatant were then analyzed for abundance of DA, 5-HT, NE, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA). Samples were separated using a mobile phase composed of sodium phosphate (monobasic, anhydrous), 100 mmol/l; ethylenediamine tetra-acetic acid, 0.05 mmol/l; octane sulfonic acid, 1.4 mmol/l; and acetoniitrile, 9% adjusted to pH 3.0 with O-phosphoric acid. A coulometric detector configured with three electrodes (Coulonometric III; ESA) measured the content of monoamines and metabolites. An ESA model 5020 guard cell (+100 mV, second electrode at +250 mV) was located immediately after the column. The second analytical electrode emitted signals that were recorded and analyzed by EZChrom Elite software through Scientific Software Inc. module (SS420 ×). The final oxidation current values were plotted on a standard curve of known concentrations from 10⁻⁵ to 10⁻³ mol/l, adjusted to tissue weights and expressed as nanograms (ng) of monoamine or metabolite per milligram (mg) tissue (mean + SE).

**Data analyses**

Two-way analysis of variance (ANOVAs) and a limited number of planned comparisons were used for analyses of open field, social interaction, and neurochemical data. Post-hoc comparisons were completed through the least significant differences comparisons. Forced swim test data were analyzed by dependent samples t-tests to examine within-subjects variables. Alpha was set at P < 0.05. Statistical analyses were conducted with Statistica Software 98 (Statsoft Inc., Tulsa, Oklahoma, USA).

**Results**

**Effects of DA lesion and chronic L-DOPA treatment on motor and anxiety-like behaviors in locomotor chambers**

In this study, the locomotor chambers were used to examine the effect of lesion and treatment on overall movement and anxiety-like symptoms. Reduced total distance travelled is indicative of a parkinsonian effect on motor activity (Srinivasan and Schmidt, 2004) and as expected, a main effect of lesion on total distance was observed [Fig. 1a; F(1,46) = 9.43, P < 0.05]. Unilateral 6-OHDA lesion significantly reduced total distance travelled compared with sham lesions by approximately 50%. Moreover, as rats were tested 16–20 h after their last L-DOPA treatment (OFF L-DOPA), there was no difference on this measure between Lesion-VEH and Lesion-LD groups.

Reduced activities within the center of the test field (reduced entries, vertical movements, and time spent in the center) are traditionally considered evidence of enhanced anxiety-like behavior and are sensitive to treatment with anxiolytics (Prut and Belzung, 2003). There was a significant main effect of lesion on entries to center [Fig. 1b; F(1,46) = 23.88, P < 0.05], and vertical movements in center [Fig. 1c; F(1,46) = 22.27, P < 0.05], but not time in...
Both the DA-lesioned groups showed reduced center entries and center vertical movements versus sham-lesioned rats, likely associated with a reduction in total distance travelled. No significant main effect of treatment or interaction between lesion and treatment was observed on any measure for locomotor chamber behavior \( (P > 0.05) \), suggesting that chronic L-DOPA does not improve these measures of anxiety-like behavior. In fact, the anxiogenic effects of 6-OHDA lesion appeared to be stronger in rats that received chronic L-DOPA treatment, especially for center vertical movements (> 50% reduction compared with vehicle-treated rats).

Effects of DA lesion and chronic L-DOPA treatment on anxiety-like behaviors in social interaction test

Reduced interaction with a novel conspecific has been interpreted to indicate an anxiogenic state (File and Seth, 2003). For approaches, a significant main effect of lesion was observed \( [F(1,16) = 16.01, P < 0.05] \), with reductions in frequency to approach the stimulus animal in DA-lesioned rats compared with both sham-lesioned groups, regardless of treatment. A significant main effect of lesion was also observed for anogenital sniffing \( [F(1,26) = 8.07, P < 0.05] \), but not other sniffing, following, or flights \( (F < 0.05) \). No significant main effect of treatment or interaction between lesion and treatment was found in any measure of the social interaction test, though there was a further reduction in anogenital sniffing among hemiparkinsonian rats that received chronic L-DOPA treatment (approximately 50%) compared with those that received chronic vehicle treatment. Collectively, these data suggest that chronic L-DOPA treatment also does not improve, and may mildly exacerbate, anxiety-like behavior on these measures.

Effects of DA lesion and chronic L-DOPA treatment on depression-like behaviors in the forced swim test

The forced swim test was used in this study as a behavioral measure sensitive to both antidepressant and depression-inducing treatments (Lucki, 1997; Deak et al., 2005). Depressive behaviors are inferred by increases in immobility with concomitant decreases in climbing behavior on day 2 of exposure to the test conditions (Detke et al., 1995). Such effects were observed in this study in DA-lesioned rats, regardless of treatment. A significant main effect of day, but not lesion or treatment,
on climbing behaviors was revealed through 2-way ANOVA \(F(1,16) = 21.17, P < 0.05\). An interaction between lesion and day \(F(1,16) = 21.17, P < 0.05\) showed a significant reduction in climbing on day 2 of exposure in DA-lesioned rats compared with sham-lesioned rats (Fig. 3a; both \(P < 0.05\)). No significant effects were observed for swimming behavior, suggesting that motor performance on this task was equivalent across groups (Fig. 3b). A significant main effect of day on immobility behaviors was observed \(F(1,16) = 17.14, P < 0.05\), but neither main effects for lesion or treatment nor any interaction were significant.

Effects of DA lesion and chronic L-DOPA treatment on monoamine levels in affect-related brain areas

Tissue taken from the striatum, prefrontal cortex, hippocampus, and amygdala ipsilateral to lesion 1 h after either Vehicle or L-DOPA (12 mg/kg + benserazide, 15 mg/kg, s.c.) treatment was analyzed through HPLC-ED for monoamine content (Fig. 4). As there were no differences between rats killed on day 28 versus day 75 of L-DOPA or vehicle treatment, results include animals from both the sets.

As expected, 6-OHDA lesions effectively reduced striatal DA and DOPAC by > 98% [DA: \(F(1,26) = 105.48\); DOPAC: \(F(1,26) = 45.97\), both \(P < 0.05\); Fig. 5a and b], but no significant main effect of treatment or interaction were found. Although no significant effect of treatment or lesion was observed for DA or DOPAC within the prefrontal cortex, a significant main effect of treatment was observed for hippocampal DA \(F(1,23) = 15.88, P < 0.05\), with L-DOPA treatment increasing DA levels. Hippocampal DOPAC was reduced after 6-OHDA lesion \(F(1,28) = 11.92, P < 0.05\), regardless of treatment. For amygdala DA and DOPAC, there were significant main effects of lesion [DA: \(F(1,28) = 20.42\); DOPAC: \(F(1,28) = 6.18\), both \(P < 0.05\)] and treatment [DA: \(F(1,28) = 25.72\); DOPAC: \(F(1,28) = 4.92\), both \(P < 0.05\)], as well as their interaction [DA: \(F(1,28) = 8.61\); DOPAC: \(F(1,28) = 5.44\), both \(P < 0.05\)]. Upon chronic L-DOPA treatment, DA levels were increased in both the sham and DA-lesioned rats compared with vehicle-treated rats (both \(P < 0.05\)). L-DOPA treatment in sham-lesioned, but not DA-lesioned rats, significantly increased amygdalar DOPAC levels (both \(P < 0.05\)).

Although no significant main effects were observed on striatal NE, a significant interaction between the lesion and treatment was observed \(F(1,27) = 4.82, P < 0.05\), with DA lesions potently enhancing striatal NE (Fig. 5c). However, chronic L-DOPA squelched this increase (both \(P < 0.05\)). In the prefrontal cortex, a significant interaction was also revealed by two-way ANOVA on NE levels \(F(1,28) = 4.61, P < 0.05\). NE was significantly reduced in Lesion-LD rats compared with both the sham-lesioned and Lesion-VEH rats (both \(P < 0.05\)). Hippocampal NE was also altered by treatment \(F(1,30) = 11.88, P < 0.05\).
and a significant interaction between the treatment and lesion was revealed \( F(1,30) = 5.53, P < 0.05 \). Similar to striatal effects, DA lesions increased and L-DOPA treatment reduced NE levels. No significant main effects or interaction were observed within the amygdala, though there appeared to be a reduction in NE levels in Lesion-LD rats (approximately 50%).

As shown in Fig. 5d and e, no significant interactions between lesion and treatment were observed in any structure for 5-HT or its metabolite, 5-HIAA. However, a significant main effect of treatment was observed for 5-HT levels in the amygdala \( F(1,29) = 7.78, P < 0.05 \), which was likely driven by a reduction in 5-HT after L-DOPA treatment in both the sham and DA-lesioned rats. In contrast, DA lesion increased 5-HIAA within the striatum \( F(1,29) = 13.41, P < 0.05 \) and amygdala \( F(1,29) = 4.99, P < 0.05 \). Striatal 5-HIAA levels were also affected by L-DOPA treatment \( F(1,29) = 4.95, P < 0.05 \), as 5-HIAA was decreased in both the sham and DA-lesioned rats after L-DOPA treatment.

**Discussion**

The results of this study delineate the respective contributions of both the unilateral DA-depletion and L-DOPA treatment on affect-related behavioral changes and neurochemical alterations in a rat model of PD. As expected, the
unilateral DA lesions exerted anxiogenic effects on locomotor activity and social interaction, but did not alter depression-like behaviors in DA-lesioned rats as measured by the forced swim test. In contrast to our earlier prediction, chronic L-DOPA treatment did not appear to produce the necessary plasticity to alleviate these non-motor symptoms. Furthermore, chronic L-DOPA treatment modified 5-HT and NE levels in several key structures involved in the regulation of affect. Collectively, these results suggest a role for NE and 5-HT dysfunction induced by DA cell loss and subsequent chronic L-DOPA treatment in the expression of anxiety and depression in an animal model of PD.

Effects of DA lesion on affective behaviors

Earlier investigations into anxiety and depression-like behaviors in animal models of PD have been limited and contradictory. For example, Tadaiesky et al. (2008) observed that partial (59% striatal DA loss), bilateral 6-OHDA lesions of the striatum reduced sucrose consumption, increased immobility in the modified forced swim test, and decreased time spent in the open arms of an elevated plus maze. In contrast, a mild bilateral striatal DA lesion (36% striatal DA loss) exerted an anxiolytic effect in the elevated plus maze and during social interaction, whereas increasing immobility in the forced swim test without an effect of lesion on sucrose consumption (Branchi et al., 2008).

Thus, an important contribution of this study was to clarify the contribution of DA lesions on changes in affect by using a severe 6-OHDA lesion (> 95% DA loss in striatal tissue). To ensure the survival of rats with such lesions, a unilateral medial forebrain bundle lesion model was used.
Results from this study support a role for DA loss in the onset of affective disorders in PD. Compared with similarly-treated sham-lesioned rats, hemiparkinsonian rats made few entries into the center of the locomotor chamber (Fig. 1c) and approached a novel conspecific less in the social interaction test (Fig. 2a), suggesting an anxiogenic effect of unilateral DA lesions. Evidence of a depression-like behavioral profile in the modified forced swim test (Fig. 3a) was also observed. These findings corroborate earlier investigations (Tadaieky et al., 2008), including the depressogenic effect of substantia nigra pars compacta lesions observed within a learned helplessness paradigm (Winter et al., 2007).

Effects of L-DOPA on affective behaviors

Although Winter et al. (2008) successfully reversed their depression-like effects in DA-lesioned rats with DA replacement therapy, no benefit of chronic L-DOPA on anxiety or depression-like behaviors was established in this study. Although the present data provide no evidence of a depressogenic effect, behavioral tests of anxiety in-situ rate a mild anxiogenic effect of chronic L-DOPA treatment. Despite no differences in total distance travelled in the locomotor chambers (Fig. 1a), a reduction in exploratory activity in the center was observed for chronic L-DOPA-treated rats, beyond the effect of DA-lesion alone (Fig. 1c, approximately 50% reduction). This result was corroborated by another modest anxiogenic effect observed in the social interaction test (Fig. 2b, approximately 50% reduction). Given that DA depletion was similar between lesion groups, these results suggest that chronic L-DOPA in the hemiparkinsonian rat does not improve non-motor symptoms and may even mildly exacerbate anxiety-like behaviors.

It is noteworthy that all DA-lesioned animals receiving L-DOPA in this study displayed moderate-to-severe unilateral motor dyskinesias after daily treatment, lasting approximately 3–4 h. In fact, we found that these dyskinetic movements made behavioral testing during the OFF phase impossible. However, it is worth noting that such motor fluctuations may also coincide with fluctuations in mood in PD patients. For example, Maricle et al. (1995) found that L-DOPA dose dependently resulted in the development of mood fluctuations, where euphoria was associated with the ON phase whereas anxiety and depresed mood were associated with the OFF phase. Such effects appeared to depend upon disease progression, as L-DOPA dose only correlated with mood fluctuations in late PD but not early PD (Maricle et al., 1998), when dyskinesias would be more common and severe (Ahlskog and Muentner, 2001). In fact, mood fluctuations and affective disorders among the PD patients have been specifically correlated with increased severity of dyskinesia (Menza et al., 1990; Nègre-Pagès et al., 2010). However, alterations in anxiety and depression are not solely because of a psychological effect of changes in the motor symptoms of PD, as indicated by the lack of association between improved motor symptoms and affective states (Richard et al., 2001, 2004; Kulisevsky et al., 2007).

Effects of L-DOPA on neurochemistry

One explanation for the lack of improvement after chronic L-DOPA treatment may involve alterations in non-dopaminergic monoamines implicated in affective disorders, such as NE and 5-HT. Recent research has suggested that affective disorders are associated with a hyposensitive serotonergic system and a hypersensitive noradrenergic system (Ressler and Nemeroff, 2000). In both the animal models and PD patients, preclinical and clinical reports have suggested that L-DOPA treatment hinders 5-HT and NE function (Everett and Borcherding, 1970; Hashiguti et al., 1993; Borbely et al., 1999; Borah and Mohanakumar, 2007; Naivalles et al., 2010a, 2010b). This hypothesis is substantiated by the results of this study, as region-dependent alterations in monoamines were observed upon both DA lesion and L-DOPA treatment (Fig. 5). As expected, DA lesion of the nigrostriatal pathway profoundly reduced DA and DOPAC levels in the striatum, but had little effect in limbic areas which are largely innervated by dopaminergic neurons from the VTA, including the prefrontal cortex, hippocampus, and amygdala. Unilateral DA lesion alone did not reduce 5-HT levels in any structure, whereas L-DOPA treatment reduced 5-HT levels within the amygdala in both the sham and DA-lesioned rats, consistent with earlier studies (Borah and Mohanakumar, 2007). These results corroborate recent research by Naivalles et al. (2010a, 2010b) where L-DOPA treatment dose dependently impaired 5-HT release within the prefrontal cortex and hippocampus of anesthetized, hemiparkinsonian rats. Lesion and treatment effects on striatal 5-HIAA also indicated changes in 5-HT metabolism, as both the DA lesion and chronic L-DOPA treatment reduced 5-HIAA levels. On the other hand, amygdalar 5-HIAA was increased in DA-lesioned rats, likely signifying an increase in 5-HT turnover. There was no lesion-induced reduction in NE levels, likely because of the pretreatment with the selective NE reuptake inhibitor, desipramine before 6-OHDA infusion (Fulceri et al., 2006). However, lower NE levels within the striatum, prefrontal cortex, and hippocampus were observed following chronic L-DOPA treatment in DA-lesioned rats. Reduced NE activity is traditionally associated with anxiolytic effects. However, chronic L-DOPA treatment in the current study produced only mild anxiogenic effects, which could be explained by the development of supersensitized NE receptors implicated in affective disorders (i.e. α2, β receptors; Biegon and Israeli, 1988; Arango et al., 1990, 1993; García-Sevilla et al., 1999).

Several potential mechanisms could explain the alterations in non-dopaminergic function upon L-DOPA treatment in hemiparkinsonian rats. First, 6-OHDA itself
may have affected the integrity of other monoaminergic systems. However, this is unlikely given the use of desipramine before 6-OHDA lesion to block uptake into NE neurons, and its lack of specificity for 5-HT neurons opposes this suggestion (Fig. 5c and d). In addition, several studies have reported an increase in both the serotonergic dorsal raphe nuclei firing (Zhang et al., 2007; Kaya et al., 2008; Wang et al., 2009a) and locus coeruleus firing after 6-OHDA lesions in rat models of PD (Guiraud et al., 2008; Wang et al., 2009b). Such an effect may explain the increase in striatal and hippocampal NE observed in Lesion-VEH rats (Fig. 5c), but does not explain the dampening effect of chronic L-DOPA treatment. Second, L-DOPA itself may alter the synthesis of NE and/or 5-HT. This could account for the effects of L-DOPA on 5-HT as it is formed from tryptophan through tryptophan hydroxylase and aromatic amino acid decarboxylase, the same enzyme that transforms L-DOPA into DA. Exogenous L-DOPA administration may impair 5-HT function at two levels: (i) by inhibiting tryptophan hydroxylase (Hashiguti et al., 1993; Kuhn and Arthur, 1999) or (ii) by competing for conversion through aromatic amino acid decarboxylase. However, L-DOPA would be predicted to increase NE, as NE is formed directly from L-DOPA and would presumably be enhanced after exogenous L-DOPA treatment. Finally and perhaps more parsimonious with these findings, NE and 5-HT neurons act as surrogates for the DA system after severe DA loss, compromising normal monoaminergic function. For example, NE and 5-HT fibers form functional synapses that have been shown to take up exogenously administered L-DOPA and convert and release L-DOPA-derived DA into the striatum as a ‘false neurotransmitter’ (Ng et al., 1970; Kannari et al., 2001; Carta et al., 2007; Arai et al., 2008; Eskow et al., 2009). This phenotypic alteration has been suggested to result in reductions in 5-HT after L-DOPA treatment in hemiparkinsonian rats (Carta et al., 2008; Naivalles et al., 2010a, 2010b). However, no earlier studies have reported effects on NE or directly tested the mechanism underlying these findings.

One limitation of this study was the methodological dissociation between the neurochemical and behavioral data. Tissue for neurochemical analysis was obtained 60 min after respective treatments during the ON phase of L-DOPA, as it was expected that neurotransmitter changes would be strongest at the point of highest L-DOPA plasma levels (Sato et al., 1994). Therefore, behavioral analyses are likely indicative of durable alterations in brain function in response to chronic DA replacement therapy, whereas acute changes in neurotransmitter levels were measured through HPLC in this study. Furthermore, whole-tissue neurochemistry was obtained in this study, including both the extracellular and intracellular stores of monoamines. Owing to the nature of the lesion and the dyskinesias induced by L-DOPA treatment in all DA-lesioned rats, only OFF phase behavioral data could be assessed. However, this may be considered as strength of this research, as anxiety and depressed mood are more severe during this phase of L-DOPA treatment (Maricle et al., 1995). Therefore, future studies could allow for both OFF phase neurochemical analyses and for measures of functional extracellular efflux of neurotransmitter, such as microdialysis or in-vivo voltammetry (Young, 1993; Robinson et al., 2003).

The nature of the unilateral model should also be considered. Though issues with L-DOPA-related side effects have already been discussed, the unilateral 6-OHDA lesion may allow for contralateral compensation, thereby subverting the observed behavioral effects (Pierucci et al., 2009). In addition, the severity of the lesion resulted in the development of significant motor disability (Fig. 1a). As such, it is difficult to make extrapolations to affective processes because of the involvement of motor ability in many of the tasks used in this study. However, the addition of both the sham-lesioned and vehicle-treated controls clarifies this issue within the multiple testing procedures used. A bilateral lesion may be another informative alternative. However, a more moderate DA lesion would be required to ensure survival and could not address the involvement of L-DOPA-induced motor/mood fluctuations that is only evidenced after substantial DA denervation.

Conclusion
Monoaminergic impairment has been implicated in the development and expression of affective disorders (Ressler and Nemeroff, 2000). Though depression and anxiety in PD have traditionally been attributed to DA cell loss and may reportedly be alleviated by chronic L-DOPA treatment, our novel findings suggest that such treatment does not improve non-motor symptoms and insinuates a liability toward affective problems among PD patients through impairment of the function of other monoaminergic systems. Further knowledge of the mechanism underlying these effects is certainly essential to potentially improve the treatment of affective disorders, and ultimately quality of life, for the PD patient.

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References


L-DOPA and non-motor symptoms Eskow Jaunaraajs et al.
JOURNAL NAME: FBP
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