

# Effect of the D3 Dopamine Receptor Partial Agonist BP897 [N-[4-(4-(2-Methoxyphenyl)piperazinyl)butyl]-2-naphthamide] on L-3,4-Dihydroxyphenylalanine-Induced Dyskinesias and Parkinsonism in Squirrel Monkeys

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## ABSTRACT

Although L-3,4-dihydroxyphenylalanine (L-dopa) is one of the most effective therapies for Parkinson's disease, continued treatment may result in excessive involuntary movements known as L-dopa-induced dyskinesias (LIDs). Because LIDs can become dose-limiting, there is great interest in finding ways to ameliorate or prevent this troubling side effect of L-dopa therapy. It was recently reported that the D3 receptor partial agonist BP897 [N-[4-(4-(2-methoxyphenyl)piperazinyl)butyl]-2-naphthamide] reduces LIDs without diminishing antiparkinsonian effects of L-dopa in macaques. In the present study, we tested the effects of BP897 on LIDs in squirrel monkeys, a nonhuman primate particularly prone to dyskinesias. Parkinsonism was induced using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Animals were then gavaged with L-dopa/carbidopa (7.5 or 15 mg/kg/dose) without and with BP897. The effects of BP897 treatment were evaluated on several components of

LIDs, including time course, peak dyskinesias, and area under the curve (AUC), a measure that encompasses both peak and duration of the response. Analyses of the time course and overall dyskinetic response (AUC) showed that BP897 significantly reduced LIDs but at the expense of the antiparkinsonian effect of L-dopa. BP897 had no significant effect on peak dyskinesias. Correlation studies showed that beneficial effects of BP897 on dyskinesias were linked to a decline in the antiparkinsonian action of L-dopa. Analyses of a subgroup of animals with mild/moderate parkinsonism yielded comparable results. Thus, in squirrel monkeys in contrast to macaques, BP897 fails to exert an antidyskinetic effect without diminishing the antiparkinsonian effects of L-dopa. These results suggest that BP897 may be less effective than originally anticipated for treating LIDs in Parkinson's disease.

L-Dopa therapy remains the most effective treatment for Parkinson's disease. However, long-term administration typically leads to motor complications, including motor fluctuations and involuntary choreoathetoid movements known as L-dopa-induced dyskinesias (LIDs) (Marsden, 1994; Olanow and Tatton, 1999; Calon et al., 2000; Obeso et al., 2000; Ball, 2001; Bezard et al., 2001; Tintner and Jankovic, 2002). These motor abnormalities may occur within a few years of initiation of treatment and are generally considered to be a major

limitation in Parkinson's disease management (Blanchet et al., 1996; Ahlskog and Muentner, 2001).

Although the pathophysiology of LIDs is still poorly understood, a number of investigators have proposed that an imbalance in activity of the two major striatal output pathways plays a role in their pathophysiology, possibly through activation of D1 and inhibition of D2 dopamine receptors on the direct and indirect pathway, respectively (Marsden, 1994; Olanow and Tatton, 1999; Calon et al., 2000; Obeso et al., 2000; Ball, 2001; Bezard et al., 2001; Tintner and Jankovic, 2002). Despite numerous experimental investigations, however, a clear relationship between dyskinesias and D1 or D2 receptor expression has yet to be established.

In addition to the D1 and D2 dopamine receptor subtypes, D3 receptors are present in the striatum and appear to be

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**ABBREVIATIONS:** L-dopa, L-3,4-dihydroxyphenylalanine; LID, L-dopa-induced dyskinesia; BP897, N-[4-(4-(2-methoxyphenyl)piperazinyl)butyl]-2-naphthamide; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ANOVA, analysis of variance; AUC, area under the curve.

involved in modulating locomotor activity (Pugsley et al., 1995; Sautel et al., 1995; Levant, 1997; Missale et al., 1998; Schwartz et al., 1998). The hypothesis that D3 receptors play a role in LIDs was initially proposed based on the observation that striatal expression of this receptor is highly dependent on afferent dopamine innervation (Sokoloff et al., 1990; Levesque et al., 1995). Administration of L-dopa to 6-hydroxydopamine-lesioned rats enhanced D3 receptor expression in the motor striatum, an area where D3 receptor density is usually <1% that of the D1 and D2 receptors (Levesque et al., 1992; Bordet et al., 1997). The observation that this ectopic induction of striatal D3 receptors parallels the development of L-dopa-induced behavioral sensitivity in rodents (a model for LIDs) has raised the possibility that D3 receptor activation might be relevant to dyskinesias in primates (Bordet et al., 1997).

D3 receptor expression is more prominent in the primate than the rodent nigrostriatal system with a relatively robust receptor expression in the caudate and putamen (Hurley et al., 1996; Morissette et al., 1998; Quik et al., 2000). As in the rat, these striatal receptors are decreased with nigrostriatal damage. Subsequent L-dopa treatment results in a partial reversal of striatal D3 receptor declines in squirrel monkeys and an overexpression in macaques (Hurley et al., 1996; Quik et al., 2000; Bezard et al., 2003). The fact that L-dopa treatment induces dyskinesias and also modulates D3 receptor expression has raised the question whether there may be a link between the D3 receptors and LIDs. Recent results showing a significant reversal of dyskinesias by the D3 receptor partial agonist BP897 in macaques support this hypothesis (Bezard et al., 2003). To further investigate this possibility, we studied the effects of BP897 in squirrel monkeys, another nonhuman primate species that develops dyskinesias after L-dopa treatment that are similar to those in Parkinson's disease. Preliminary results of these studies have been presented in abstract form (Hsu et al., 2003).

## Materials and Methods

**Animals.** Ten female squirrel monkeys (*Saimiri sciureus*) were purchased from Osage Research Primates (Osage Beach, MO). Females were only used because of the availability of mature to aged animals that may represent a more relevant parkinsonian animal model. Additionally, previous studies in our laboratory (Langston et al., 2000; Quik et al., 2000, 2002) did not yield significant differences between male and female animals with MPTP or L-dopa treatments. Monkeys were housed individually and maintained on a 13-/11-h light/dark cycle in a temperature-controlled room ( $27 \pm 1^\circ\text{C}$ ) with a relative humidity of ~30 to 40%. They were given fruits in the morning and food pellets in the afternoon, 3 h after the afternoon drug administration to optimize gastrointestinal absorption of L-dopa. Water was provided ad libitum. All procedures conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee.

**Experimental Treatments.** After an initial acclimatization period, all animals were injected subcutaneously with MPTP hydrochloride (Sigma-Aldrich, St. Louis, MO) dissolved in sterile saline at a dose of 1.9 mg/kg. Three weeks after MPTP injection, animals were videotaped daily for 1 week. Parkinsonism was assessed from videotapes as described below. Animals were treated with MPTP up to five times at 1.7 to 2.1 mg/kg/dose until they were stably parkinsonian for at least 1 month.

After assessment of baseline activity, animals were administered

either 7.5 mg/kg or 15 mg/kg L-dopa together with carbidopa prepared from a crushed tablet of Sinemet CR 25/100 (DuPont, Wilmington, DE) dissolved in water. Thus, the maximal daily L-dopa dose was within the upper range of that administered to Parkinson's disease patients. Moreover, they were similar to those administered to parkinsonian nonhuman primates in other studies (Hurley et al., 1996; Quik et al., 2002; Bezard et al., 2003). The monkeys were gavaged once or twice daily at 4-h intervals for a 2-week period, using a 5-day-on/2-day-off schedule (Quik et al., 2002). They were subsequently gavaged with both L-dopa and a given dose of BP897 (0, 5, 10, and 15 mg/kg) (Bioprojet, Paris, France) for 1 week each (Monday to Friday). BP897 was dissolved in warm water and administered in combination with L-dopa. Coadministration of BP897 and L-dopa was followed by a week of L-dopa treatment alone to reduce any carry-over effects of BP897. Subsequent doses of BP897 were tested following the same procedure of 1 week with BP897 plus L-dopa, followed by 1 week of L-dopa alone.

In some experiments, BP897 was gavaged together with the initial dose of L-dopa to evaluate whether the D3 receptor partial agonist could prevent the onset of dyskinesias. The results from these studies were similar to those obtained in animals that had been treated with L-dopa prior to BP897. Therefore, data from these sets of experiments were pooled.

**Behavioral Assessment.** All animals were monitored by videotape recording on each treatment day, which included 1 h of baseline activity (no drugs) from 8:00 to 9:00 AM, followed by two 4-h treatment periods starting at 9:00 AM and 1:00 PM. Entry into the monitoring room was restricted during videotaping. Parkinsonism and dyskinesias were rated from videotapes, a standard procedure in our laboratory (Togasaki et al., 2001; Tan et al., 2002), for 2-min periods at 30-min intervals throughout the day by two independent raters blinded to treatment using the scales described below.

Parkinsonism was scored from videotapes using measures of several independent behavioral abnormalities: abnormal posture of the head (0–1), trunk (0–1), and limbs (0–1); bradykinesia (0–3); and action tremor (0–3). The minimum score was 0 and a maximum total score was 9. This scale was optimized for rating parkinsonism from videotapes. A comparison of this scale with our previously published scale (Langston et al., 2000) showed a significant correlation between the degrees of parkinsonism ( $r = 0.54$ ,  $p < 0.05$ ). Parkinsonism was classified as mild/moderate if the total score range was 1 to 4 and severe if 5 to 9.

Dyskinesias were rated on a scale of 0 (no dyskinesias) to 4 using a well characterized global dyskinesia rating scale, which has been previously published, and tested for both validity and reliability using videotape analysis (Togasaki et al., 2001; Tan et al., 2002). Briefly, a score of 1 reflects subtle dyskinesias that are not sustained; 2, mild dyskinesias that are sustained; 3, moderate dyskinesias that impair the ability to remain stationary; and 4, severe dyskinesias that are generalized and incapacitating.

**Radioreceptor Assay for Measurement of Plasma BP897 Levels.** Monkeys were gavaged with 10 mg/kg BP897 in combination with L-dopa (7.5 mg/kg) to mimic experimental conditions described above. Blood samples were then collected from each animal 60 to 75 min after BP897 administration. To evaluate baseline BP897 levels, plasma samples were collected from the same animals gavaged only with L-dopa that had not received BP897 for at least 1 week. Two milliliters of blood was drawn from each animal and centrifuged at 2100 rpm (~1000g) at  $4^\circ\text{C}$  for 12 min. Approximately 600  $\mu\text{l}$  of plasma (supernatant) was collected and stored at  $-80^\circ\text{C}$ . Plasma concentrations of BP897 were measured using a radioreceptor assay with human recombinant D3 receptors (Sokoloff et al., 1992).

**Data and Statistical Analysis.** Because our standard treatment protocol involves a 5-day-on/2-day-off (Saturday and Sunday) schedule, animals were allowed to acclimatize to the drug treatment on Monday and Tuesday, and data from these 2 days were not used; rather behavioral responses to drug treatment for each monkey were determined by averaging the animal's response from Wednesday

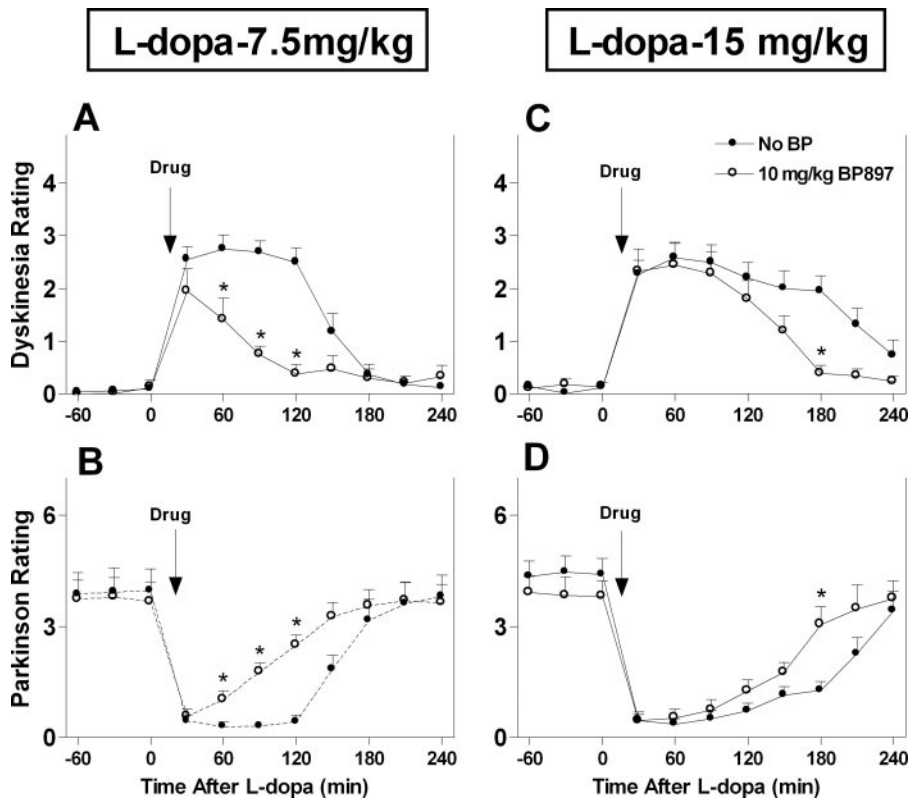
through Friday of each week. All values are expressed as the mean  $\pm$  S.E.M. of the indicated number of animals. Results were compared using one-way analysis of variance (ANOVA) with repeated measures, followed by Bonferroni multiple comparison test. For the BP897 radioreceptor assay, values were interpolated by nonlinear regression analysis using a one-site competition model. All statistical analyses were done using the Prism program (GraphPad Software Inc., San Diego, CA) at a significance level of 0.05.

## Results

A time course of the effect of BP897 on L-dopa-induced dyskinesias is shown in Fig. 1, A and C. Monkeys were gavaged with 7.5 or 15 mg/kg L-dopa together with 10 mg/kg BP897. Dyskinesias and parkinsonism were rated every 30 min over a 5-h time period (1-h baseline and 4-h morning treatment period). In the absence of BP897, dyskinesias developed by 30 min after L-dopa administration with both doses, were maximal by 30 to 120 min (at the 7.5 mg/kg dose) and 30 to 180 min (at the 15 mg/kg dose) after dosing, and declined thereafter. In animals administered BP897 (10 mg/kg) together with L-dopa (7.5 mg/kg), there was a statistically significant reduction in dyskinesias as compared with animals receiving only L-dopa. Repeated measures ANOVA yielded overall significant main effects of BP897 on treatment [ $F(1,165) = 42.56, p < 0.001$ ], indicating the drug reduced dyskinesias, and time [ $F(10,165) = 32.70, p < 0.001$ ], demonstrating the effect of BP897 was dependent on time after its administration. There was also a significant BP897 treatment  $\times$  time interaction [ $F(10,165) = 7.54, p < 0.001$ ], that is, BP897 modified the time course of dyskinesias as compared with control. Bonferroni post hoc tests showed that the dyskinesia scores at  $t = 60, 90,$  and  $120$  min were significantly reduced with BP897 + 7.5 mg/kg L-dopa treatment compared with L-dopa alone ( $p < 0.001$ ). Animals treated

with BP897 at the higher dose of L-dopa (15 mg/kg) also showed a statistically significant reduction in dyskinesias. Repeated-measures ANOVA yielded overall significant main effects of BP897 on treatment [ $F(1,176) = 12.26, p < 0.001$ ] and time [ $F(10,176) = 25.60, p < 0.001$ ], with a significant BP897 treatment  $\times$  time interaction [ $F(10,176) = 1.916, p < 0.05$ ]. Bonferroni post hoc tests showed that the dyskinesia scores at  $t = 180$  min were significantly reduced with BP897 treatment ( $p < 0.001$ ).

The results depicted in Fig. 1, B and D, demonstrate the effect of L-dopa (7.5 and 15 mg/kg) without and with BP897 treatment on parkinsonism. The response of the animals to L-dopa exhibited a similar time course as L-dopa's dyskinesic effects, with a maximal improvement 30 to 120 min at 7.5 mg/kg L-dopa and 30 to 180 min at 15 mg/kg after dosing, with re-emergence of parkinsonism as the effect wore off. When BP897 was gavaged together with L-dopa, the antiparkinsonian effects of L-dopa were diminished. There were significant main effects of BP897 and L-dopa (7.5 mg/kg) on parkinsonism [ $F(1,165) = 7.049, p < 0.01$ ] and time [ $F(10,165) = 19.47, p < 0.001$ ], with no significant interaction between BP897 treatment  $\times$  time [ $F(10,165) = 1.63, p = 0.10$ ]. Bonferroni post hoc tests showed that the parkinsonian scores at  $t = 120$  min were significantly worse with BP897 treatment ( $p < 0.05$ ). However, no significant main effects were observed with BP897 and the higher dose of L-dopa (15 mg/kg) on parkinsonism [ $F(1,176) = 3.387, p = 0.0674$ ], although significant effects were seen with time [ $F(10,176) = 34.87, p < 0.0001$ ] and between BP897 treatment  $\times$  time [ $F(10,176) = 1.982, p < 0.05$ ]. Bonferroni post hoc tests showed that the parkinsonian scores at  $t = 180$  min were significantly worse with BP897 treatment ( $p < 0.05$ ). These results indicate that there is a reciprocal change in dyskinesia



**Fig. 1.** Time course of dyskinesia and parkinsonian ratings in MPTP-lesioned monkeys gavaged with L-dopa (7.5 and 15 mg/kg) without or with 10 mg/kg BP897 (A–D). Treatment with L-dopa (7.5 mg/kg) in combination with BP897 reduced L-dopa-induced dyskinesias ( $F = 42.56, p < 0.001$ ) but also significantly ( $F = 7.05, p < 0.01$ ) reversed the antiparkinsonian effect of L-dopa (A and B). Administration of BP897 with the higher dose of L-dopa (15 mg/kg) also reduced L-dopa-induced dyskinesias ( $F = 12.26, p < 0.001$ ), and no significant differences were observed on parkinsonism ( $F = 3.387, p = 0.0674$ ) (C and D). Behavioral ratings were performed by videotape observation for 2-min periods at 30-min intervals over a 5-h period. Data points are the monkeys' weekly score, which represents the average ratings from Wednesday through Friday. Each symbol represents the mean  $\pm$  S.E.M. of 8 to 10 monkeys. \*,  $p < 0.05$ , compared with no BP.

sias and parkinsonism with BP897 treatment, that is, an improvement in dyskinesias is associated with a worsening of parkinsonism.

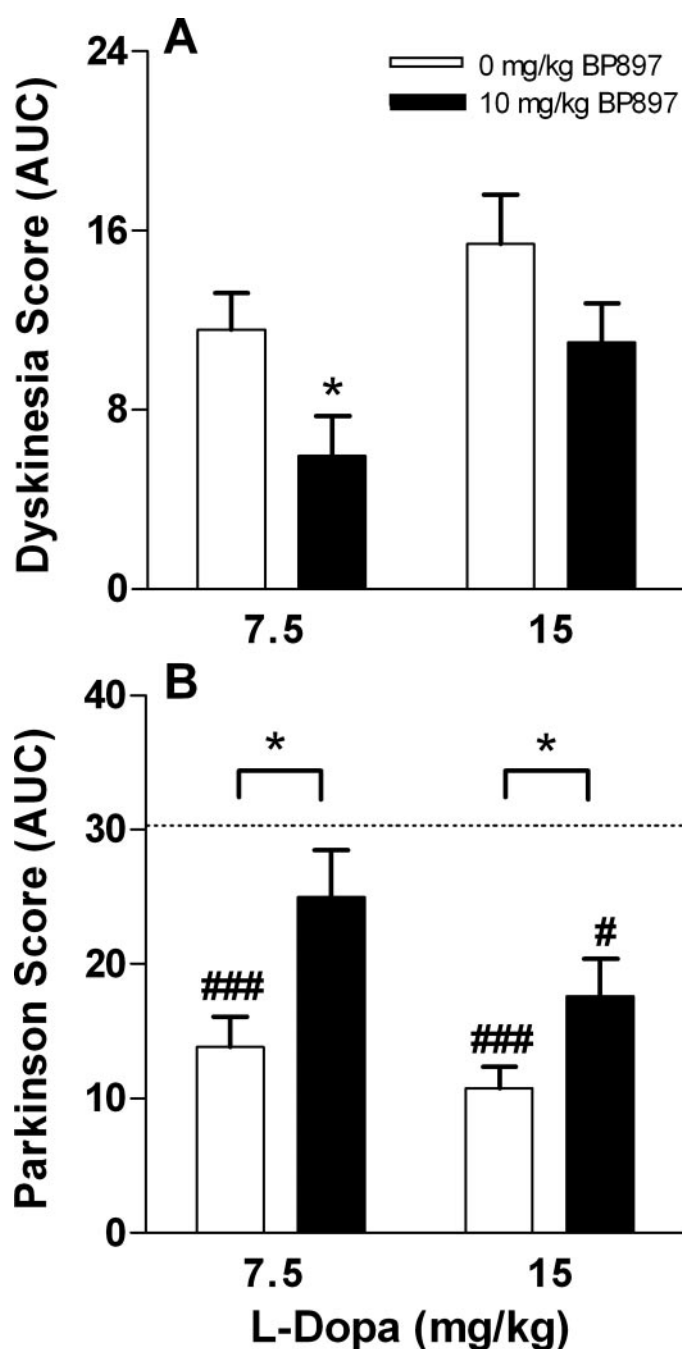
The effect of BP897 on L-dopa-induced dyskinesias was then examined on the total dyskinetic response defined as area under curve (AUC), which encompasses both magnitude and duration of the response (Fig. 2). When analyzed in this manner, BP897 induced a significant reduction ( $p < 0.05$ ) in AUC dyskinesias, but only in animals treated with 7.5 mg/kg and not the 15 mg/kg L-dopa dose. However, at both doses of L-dopa, BP897 induced a significant reduction in the antiparkinsonian effects of L-dopa ( $p < 0.05$ ) (Fig. 2B).

We next determined the effect of BP897 treatment on peak dyskinesias to evaluate whether there might be preferential effects of the D3 receptor partial agonist on specific characteristics of L-dopa-induced dyskinesias (Fig. 3). Although there was a small decline in peak dyskinesias at the 7.5 mg/kg dose of L-dopa after BP treatment, this was not significant, and there was not a significant change in peak dyskinetic movements or parkinsonism with BP897 at 15 mg/kg L-dopa.

Correlation analyses were done comparing the effect of BP897 treatment on LIDs and on L-dopa's antiparkinsonian action (Fig. 4). There was a significant correlation between the development of L-dopa-induced dyskinesias and improvement in parkinsonism in animals treated only with L-dopa (7.5 mg/kg) (Fig. 4, top panel). There was also a correlation between these two measures in animals treated with BP897 (10 mg/kg) together with L-dopa (Fig. 4, lower panel). No difference was observed in the slopes of the regression lines in animals treated only with L-dopa as compared with those treated with L-dopa in combination with BP897. These data indicate that BP897-induced declines in dyskinesias are associated with a diminution of the antiparkinsonian effects of L-dopa.

We also considered the possibility that BP897 may be more effective in animals with a mild to moderate parkinsonian syndrome. This condition is generally associated with less severe nigrostriatal damage and possibly a reduced tendency to develop dyskinesias (Di Monte et al., 2000). Correlation analyses (Fig. 5) showed that the slopes of the regression lines were not different in mild/moderate parkinsonian animals treated with 7.5 mg/kg L-dopa alone or together with BP897. Thus, there appear to be no differential effects of BP897 on dyskinesias and parkinsonism in animals with less severe nigrostriatal damage.

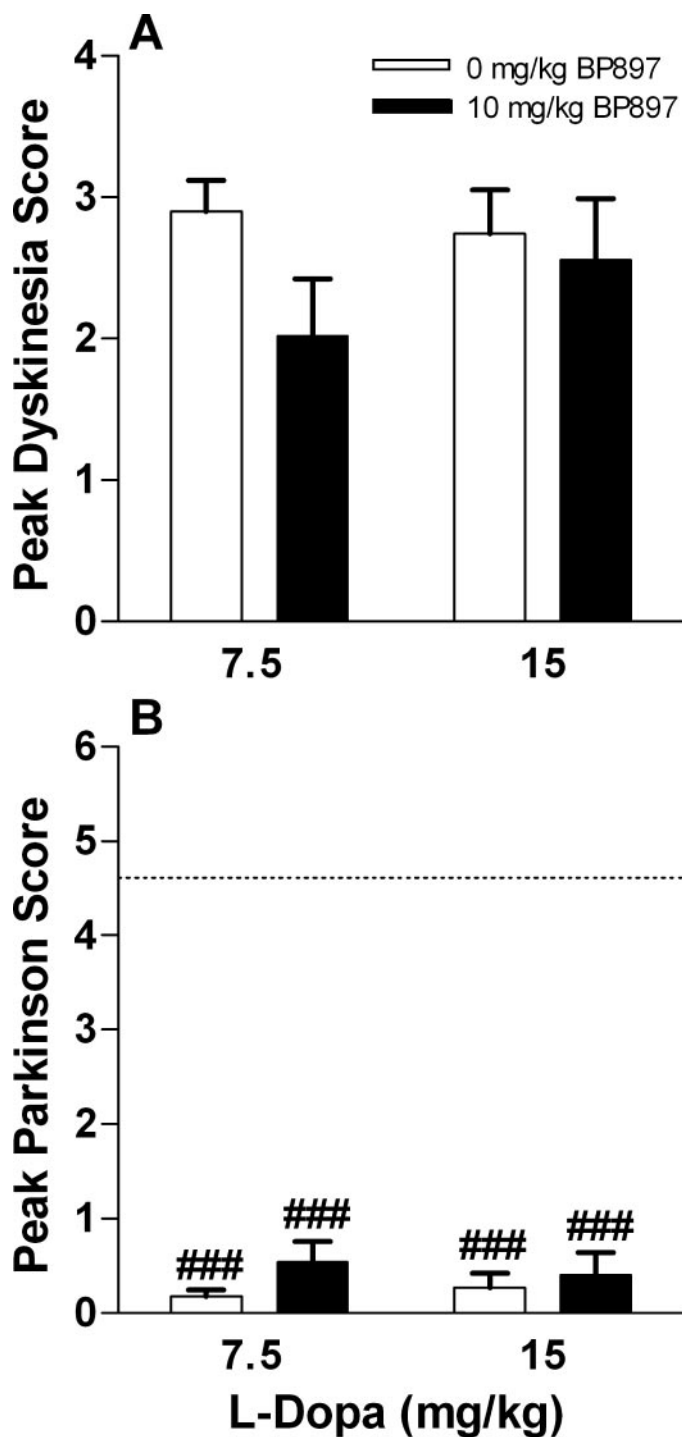
In addition to the studies with 10 mg/kg BP897 described above, we also tested 5 and 15 mg/kg BP897 in combination with L-dopa to determine whether there may be preferential declines in LIDs with only a minimal worsening of parkinsonism. For these experiments, 7.5 mg/kg L-dopa was used since there was a decline in dyskinesias with 10 mg/kg BP897 at this dose of L-dopa, although at the expense of the antiparkinsonian effects of L-dopa. (Fig. 2). The overall dyskinetic score using the AUC score at 7.5 mg/kg L-dopa alone was  $16.0 \pm 0.9$  ( $n = 3$ ). When 5 mg/kg BP897 was given together with L-dopa, dyskinesias were reduced by 34% (AUC:  $10.6 \pm 2.9$ ,  $n = 3$ ) but with a corresponding worsening of parkinsonism (27%). Thus, the effect of 5 mg/kg BP897 on LIDs appeared similar to, although somewhat less pronounced than, 10 mg/kg BP897. At the highest dose of BP897 tested (15 mg/kg), we observed an 83% reduction in dyskinesias (AUC,



**Fig. 2.** Overall dyskinetic and antiparkinsonian effect of L-dopa (7.5 or 15 mg/kg) alone or in combination with BP897 (10 mg/kg). AUC includes both peak and duration of response for each monkey. A decline in the overall dyskinetic response (A) was observed with 10 mg/kg BP897 treatment at the 7.5 mg/kg L-dopa but at the expense of the antiparkinsonian action (B) of L-dopa. Values represent the average weekly scores from Wednesday through Friday. Baseline parkinsonian rating scores for each monkey were averaged from the 1st h of recording (stippled line). Each bar represents the mean  $\pm$  S.E.M. of 8 to 10 animals. \*,  $p < 0.05$ , compared with 0 mg/kg BP897; #,  $p < 0.05$ ; ###,  $p < 0.001$  compared with baseline.

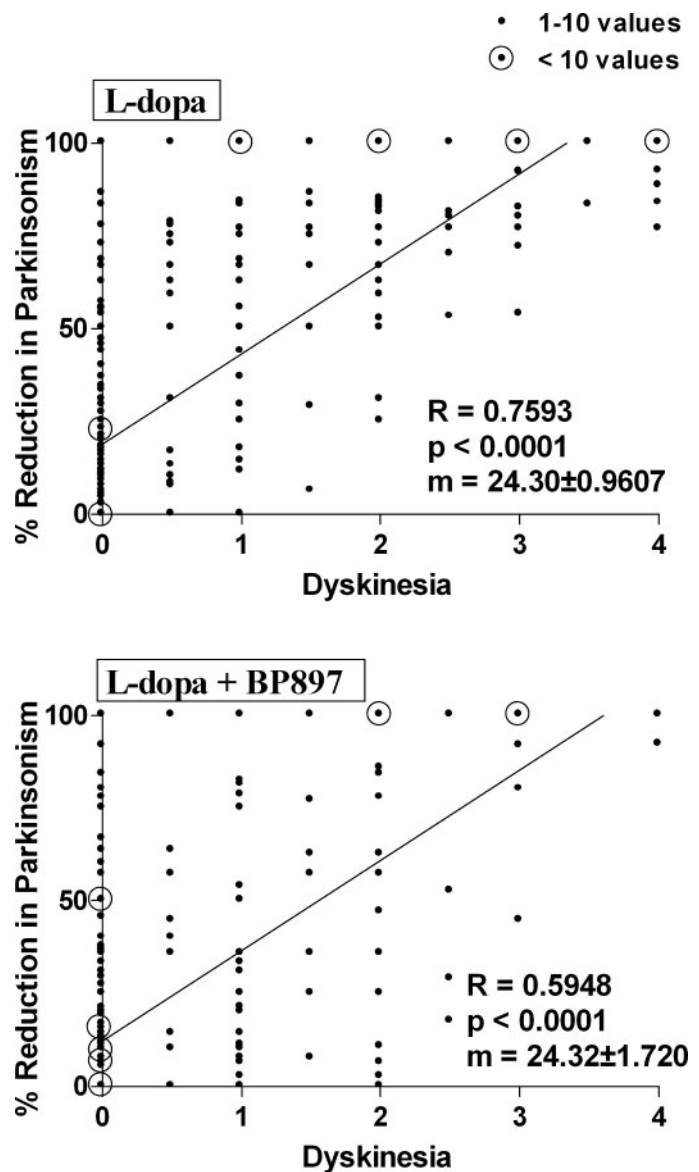
$2.8 \pm 1.9$ ,  $n = 3$ ). However, this effect was associated with a complete blockade of the antiparkinsonian effects of L-dopa.

The plasma concentration of BP897 may reflect brain levels and, thus, provide an index of concentration of the drug in the basal ganglia (Bezard et al., 2003). For this reason, we measured BP897 levels in blood samples after administra-



**Fig. 3.** Peak dyskinetic and antiparkinsonian responses to L-dopa (7.5 or 15 mg/kg) alone or in combination with BP897. There was no significant reduction in peak dyskinesias with BP897 (10 mg/kg) administration. Values represent minimum parkinsonian and maximum dyskinetic scores that were determined using the monkey's average weekly scores (rating scores from Wednesday through Friday) for the morning treatment period. Baseline activity scores for each monkey were averaged from the 1st h of recording (stippled line). Each bar represents the mean  $\pm$  S.E.M. of 8 to 10 animals. ###,  $p < 0.001$  compared with baseline.

tion of 10 mg/kg BP897. For the control condition, monkeys were treated only with L-dopa (7.5 mg/kg), and a blood sample was taken 60 to 75 min after treatment. The next day, the same animals were administered BP897 (10 mg/kg) plus L-dopa (7.5 mg/kg) to allow for an accurate assessment of

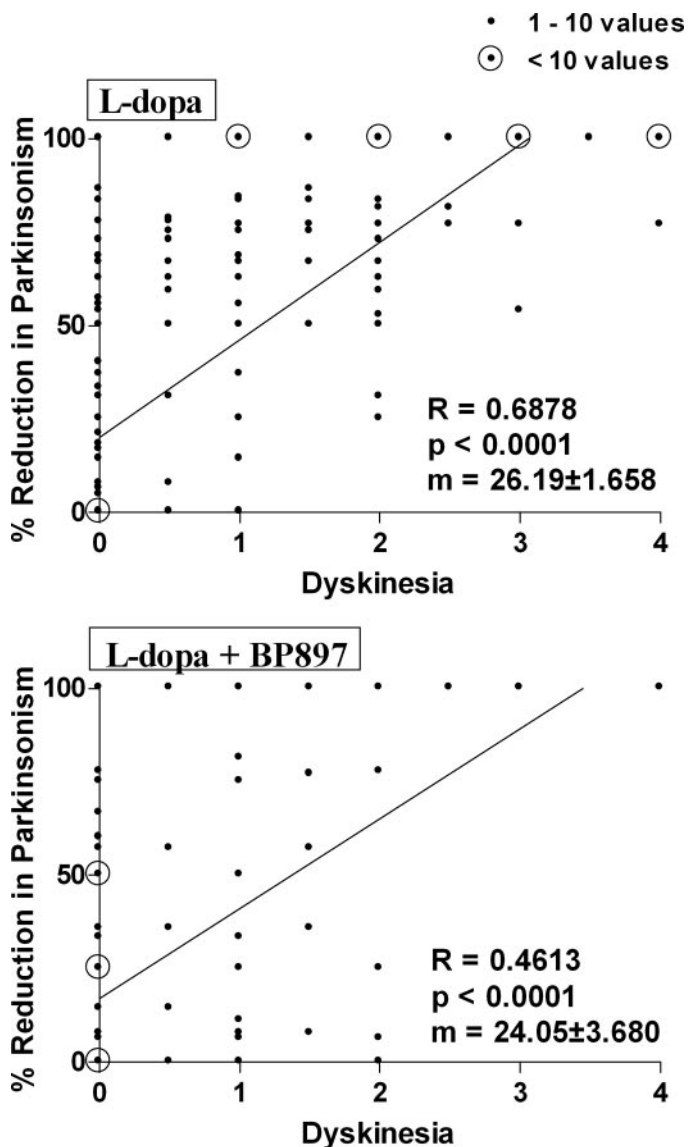


**Fig. 4.** Correlation between the percentage reduction in parkinsonism and L-dopa-induced dyskinesias in animals treated without and with 10 mg/kg BP897. Dyskinesia rating scores ( $n = 8-9$ ) taken every 30 min from the 4-h morning treatment period were plotted against the percentage reduction in parkinsonism for each treatment period of the animals. In the upper panel, animals were treated with L-dopa alone (7.5 mg/kg), and in the lower panel, monkeys were treated with L-dopa plus BP897 (10 mg/kg). Each point on the scatterplot represents the indicated number of values. The slopes ( $m$ ) of the regression lines without and with BP897 treatment were similar, indicating that the BP897-induced reduction in dyskinesias was associated with a corresponding worsening of parkinsonism.

BP897 plasma levels. Again, blood was collected 60 to 75 min after treatment. Samples were collected 60 to 75 min after treatment because BP897 was observed to have the greatest effect during this period. BP897 plasma levels were measured using a radioreceptor assay. Plasma concentrations averaged  $910 \pm 250$  nM ( $n = 5$ ) after BP 897 administration and  $0 \pm 0$  nM under control condition.

## Discussion

In the present experiments, we investigated the ability of the D3 receptor partial agonist BP897 to attenuate LIDs, as



**Fig. 5.** Correlation between the percentage reduction in parkinsonism and L-dopa-induced dyskinesias in the subset of animals with mild/moderate parkinsonism. Average dyskinesia rating scores taken every 30 min from the 4-h morning treatment period were plotted against the percentage reduction in parkinsonism in monkeys ( $n = 6$ ) with a parkinsonism rating score ranging from 1 to 4. In the upper panel, animals were treated with L-dopa alone (7.5 mg/kg), and in the lower panel, monkeys were treated with L-dopa plus BP897 (10 mg/kg). Each point on the scatterplot represents the indicated number of values. Similar regression slopes ( $m$ ) were obtained without and with BP897, suggesting that the BP897-induced reduction in dyskinesias was associated with a corresponding worsening of parkinsonism in the subset of animals with mild/moderate parkinsonism.

well as its effects on the antiparkinsonian action of L-dopa, in MPTP-lesioned squirrel monkeys. To approach this, we performed several different types of analyses including evaluating time courses, peak effects, and AUC, a measure that incorporates time course as well as peak effect. Our results show that there was a significant reduction in dyskinesias (AUC) when BP897 was administered with 7.5 but not 15 mg/kg L-dopa. However, this reduction in abnormal movements occurred at the expense of the antiparkinsonian action of L-dopa. These conclusions were reinforced by the results of correlation analyses showing that the regression slopes were

similar without and with BP897 treatment. These data indicate that declines in dyskinesias, when they occurred, were consistently associated with a diminution in the antiparkinsonian effects of L-dopa.

These results are at odds with previous data showing that BP897 decreases dyskinesias in macaques without exacerbating the antiparkinsonian effect of L-dopa (Bezard et al., 2003). A possible explanation may relate to the  $\sim 3\times$  greater plasma levels of BP897 in squirrel monkeys compared with those in macaques at doses at which BP897 was most effective (Bezard et al., 2003). It has been shown that BP897 is a potent D3 receptor partial agonist and a weak D2 receptor antagonist that displays a high affinity at the D3 and a 70 times lower affinity at the D2 receptor (Pilla et al., 1999). The higher plasma levels could result in increased brain levels of BP897, which might in turn lead to a greater blockade of D2 receptors and a resulting increase in parkinsonism, while alleviating dyskinesias. These observations suggest that administration of a lower dose of BP897 may be more favorable. However, treatment with 5 mg/kg BP897 was less beneficial than 10 mg/kg in suppressing LIDs.

Another possibility is that the beneficial effects of BP897 are dependent on an action at presynaptic D3 dopamine receptors. If this were the case, a larger response might be expected in animals with only mild/moderate damage, presumably because there would be more remaining presynaptic terminals (Levant, 1997). Conversely, a small or negligible response might be anticipated when the dopamine terminals are severely reduced (Alexander et al., 1992; Quik et al., 2001). In fact, in the earlier study, the animals exhibited moderate parkinsonism (Bezard et al., 2003). Therefore, to investigate whether there might be more robust effects of BP897 in monkeys with mild/moderate parkinsonism, we reanalyzed the data from this subgroup of animals. However, correlation analyses showed that there were no differential antidyskinetic effects of BP897 in squirrel monkeys with a less, as compared with a more severe parkinsonian syndrome. Thus, the drug response was similar regardless of the degree of parkinsonism.

In addition to the above, numerous other variables may influence assessment of drug effects. One important difference between the two studies concerns the specific parkinsonian features used to rate movement dysfunction. Although similar, the scales to assess parkinsonism in the two studies are not identical. Discrepancies in rating may relate in part to the ability to evaluate differences in squirrel monkeys not detected in macaques, or vice versa. For instance, the antiparkinsonian effects of L-dopa diminished somewhat more rapidly in the presence of the D3 receptor partial agonist in squirrel monkeys than in macaques. This could reflect an earlier return of bradykinesia or a slight increase in detectability of abnormal posture of the head, trunk, or limbs, or of action tremor, which may have been masked by the dyskinesias, thus reducing dyskinesias and aggravating the parkinsonian symptoms in squirrel monkeys. In fact, the idea that different scales highlight varying parkinsonian features is discussed in a paper by Imbert et al. (2000) comparing eight different clinical rating scales for the assessment of parkinsonism in monkeys. Another possibility is that the rating scale for parkinsonian symptoms, although adequate for detecting antiparkinsonian effects of a single drug, may be less effective for determining whether a reduction in the degree of

dyskinesias is accompanied by an increase in parkinsonian symptoms. However, similar correlations between the anti-parkinsonian effect and LID severity without and with BP897 treatment suggests that we are observing a phenomenon that is independent of the application of the rating scale under different conditions. The question of gender differences also arises since we used only female monkeys. However, the previous study also only used female animals (Bezard et al., 2003). Further research using different nonhuman primate species is needed to resolve the differential efficacy of BP897 to minimize LIDs without worsening parkinsonism.

The above considerations also raise the question of which parkinsonian animal model is most relevant for drug efficacy studies. Although both macaques and squirrel monkeys represent excellent models for Parkinson's disease, the etiology of this disorder and the molecular basis for its progressive nature are presently unknown. MPTP-induced parkinsonism most likely differs from the neurodegenerative process in Parkinson's disease; moreover, human locomotor behavior is not identical to that in nonhuman primates. Thus, these models may not reproduce the disease but simply model certain aspects, and results in any one species must be interpreted with caution. For instance, there are differences between the present and previous study (Bezard et al., 2003) with respect to the development of LIDs. These abnormal movements took several months to develop in macaques but occurred rapidly in squirrel monkeys. Another difference relates to changes in the relative proportion of dopamine receptor subtypes after denervation and L-dopa treatment in different nonhuman primate species. Previous work in macaques had shown that MPTP-induced denervation decreased striatal D3 receptors but that L-dopa treatment enhanced D3 receptor expression to levels higher than in unlesioned animals (Bezard et al., 2003). These findings contrast with those in squirrel monkeys and marmosets that showed that MPTP treatment decreased striatal D3 receptors, but treatment with L-dopa only partially reversed D3 receptor expression (Hurley et al., 1996; Quik et al., 2000). Therefore the nature and pathophysiological mechanisms of LIDs may differ in the two species, possibly accounting for the difference in efficacy of the D3 receptor partial agonist BP897.

The ultimate question relates to how useful BP897 will be in treating LIDs in Parkinson's disease patients. At present, the status of D3 dopamine receptors in human basal ganglia is still uncertain. An initial report showed that D3 receptors are decreased in the striatum of pathologically-defined Parkinson's disease cases treated with L-dopa (Ryoo et al., 1998). In a subsequent study, cases were subdivided into those with a robust and poor response to L-dopa therapy (Joyce et al., 2002). In the responders, there was an elevated level of D3 receptors in some striatal areas, globus pallidus, and nucleus accumbens, relative to those in the nonresponding group, although the levels were not above the control values. These results suggest that D3 receptors might be a factor contributing to dopaminergic drug responsiveness in Parkinson's disease but do not shed light on their relevance to LIDs.

To conclude, in this study, BP897 reduced LIDs in squirrel monkeys, but only at the expense of diminishing the antiparkinsonian effects of L-dopa. The lack of selectivity of BP897 against LIDs in squirrel monkeys as compared with macaques may relate to differences in BP897 metabolism, in D3

regulatory mechanisms after denervation and dopamine precursor treatments, in the evaluation of parkinsonism, and/or to the different nature and cause of LIDs in the two studies. Overall, the present results emphasize the need for additional studies to determine the usefulness of D3 receptor partial agonists for the treatment of LIDs in clinical practice.

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#### References

- Ahlskog JE and Muentner MD (2001) Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord* **16**: 448–458.
- Alexander GM, Schwartzman RJ, Brainard L, Gordon SW, and Grothusen JR (1992) Changes in brain catecholamines and dopamine uptake sites at different stages of MPTP parkinsonism in monkeys. *Brain Res* **588**:261–269.
- Ball J (2001) Current advances in Parkinson's disease. *Trends Neurosci* **24**:367–369.
- Bezard E, Brotchie JM, and Gross CE (2001) Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. *Nat Rev Neurosci* **2**:577–588.
- Bezard E, Ferry S, Mach U, Stark H, Leriche L, Boraud T, Gross C, and Sokoloff P (2003) Attenuation of levodopa-induced dyskinesia by normalizing dopamine D3 receptor function. *Nat Med* **9**:762–767.
- Blanchet PJ, Allard P, Gregoire L, Tardif F, and Bedard PJ (1996) Risk factors for peak dose dyskinesia in 100 levodopa-treated parkinsonian patients. *Can J Neurol Sci* **23**:189–193.
- Bordet R, Ridray S, Carboni S, Diaz J, Sokoloff P, and Schwartz JC (1997) Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa. *Proc Natl Acad Sci USA* **94**:3363–3367.
- Calon F, Grondin R, Morissette M, Goulet M, Blanchet PJ, Di Paolo T, and Bedard PJ (2000) Molecular basis of levodopa-induced dyskinesias. *Ann Neurol* **47**:S70–78.
- Di Monte DA, McCormack A, Petzinger G, Janson AM, Quik M, and Langston JW (2000) Relationship among nigrostriatal denervation, parkinsonism and dyskinesias in the MPTP primate model. *Mov Disord* **15**:459–466.
- Hsu A, Togasaki DM, Bezard E, Sokoloff P, Langston JW, Di Monte DA, and Quik M (2003) The D3 partial agonist BP897 attenuates dyskinesias in squirrel monkeys. Program No. 733.8. *Abstract Viewer/Itinerary Planner*. Washington, DC: Society for Neuroscience, 2003. Online.
- Hurley MJ, Stubbs CM, Jenner P, and Marsden CD (1996) D3 receptor expression within the basal ganglia is not affected by Parkinson's disease. *Neurosci Lett* **214**:75–78.
- Imbert C, Bezard E, Guitraud S, Boraud T, and Gross CE (2000) Comparison of eight clinical rating scales used for the assessment of MPTP-induced parkinsonism in the Macaque monkey. *J Neurosci Methods* **96**:71–76.
- Joyce JN, Ryoo HL, Beach TB, Caviness JN, Stacy M, Gurevich EV, Reiser M, and Adler CH (2002) Loss of response to levodopa in Parkinson's disease and co-occurrence with dementia: role of D3 and not D2 receptors. *Brain Res* **955**:138–152.
- Langston JW, Quik M, Petzinger G, Jakowec M, and Di Monte DA (2000) Investigating levodopa-induced dyskinesias in the parkinsonian primate. *Ann Neurol* **47**:S79–S89.
- Levant B (1997) The D3 dopamine receptor: neurobiology and potential clinical relevance. *Pharmacol Rev* **49**:231–252.
- Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, and Sokoloff P (1992) Identification, characterization and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. *Proc Natl Acad Sci USA* **89**:8155–8159.
- Levesque D, Martres MP, Diaz J, Griffon N, Lammers CH, Sokoloff P, and Schwartz JC (1995) A paradoxical regulation of the dopamine D3 receptor expression suggests the involvement of an anterograde factor from dopamine neurons. *Proc Natl Acad Sci USA* **92**:1719–1723.
- Marsden CD (1994) Problems with long-term levodopa therapy for Parkinson's disease. *Clin Neuropharmacol* **17** (Suppl 2):S32–S44.
- Missale C, Nash SR, Robinson SW, Jaber M, and Caron MG (1998) Dopamine receptors: from structure to function. *Physiol Rev* **78**:189–225.
- Morissette M, Goulet M, Grondin R, Blanchet P, Bedard PJ, Di Paolo T, and Levesque D (1998) Associative and limbic regions of monkey striatum express high levels of dopamine D3 receptors: effects of MPTP and dopamine agonist replacement therapies. *Eur J Neurosci* **10**:2565–2573.
- Obeso JA, Olanow CW, and Nutt JG (2000) Levodopa motor complications in Parkinson's disease. *Trends Neurosci* **23**:S2–7.
- Olanow CW and Tatton WG (1999) Etiology and pathogenesis of Parkinson's disease. *Annu Rev Neurosci* **22**:123–144.
- Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz JC, Everitt BJ, and Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature (Lond)* **400**:371–375.
- Pugsley TA, Davis MD, Akunne HC, MacKenzie RG, Shih YH, Damsma G, Wikstrom H, Whetzel SZ, Georgic LM, Cooke LW, et al. (1995) Neurochemical and functional characterization of the preferentially selective dopamine D3 agonist PD 128907. *J Pharmacol Exp Ther* **275**:1355–1366.
- Quik M, Police S, He L, Di Monte DA, and Langston JW (2000) Expression of D(3) receptor messenger RNA and binding sites in monkey striatum and substantia

- nigra after nigrostriatal degeneration: effect of levodopa treatment. *Neuroscience* **98**:263–273.
- Quik M, Police S, Langston JW, and Di Monte DA (2002) Increases in striatal preproenkephalin gene expression are associated with nigrostriatal damage but not L-DOPA-induced dyskinesias in the squirrel monkey. *Neuroscience* **113**:213–220.
- Quik M, Polonskaya Y, Kulak JM, and McIntosh JM (2001) Vulnerability of 125I-alpha-conotoxin MII binding sites to nigrostriatal damage in monkey. *J Neurosci* **21**:5494–5500.
- Ryoo HL, Pierrotti D, and Joyce JN (1998) Dopamine D3 receptor is decreased and D2 receptor is elevated in the striatum of Parkinson's disease. *Mov Disord* **13**:788–797.
- Sautel F, Griffon N, Sokoloff P, Schwartz JC, Launay C, Simon P, Costentin J, Schoenfelder A, Garrido F, Mann A, et al. (1995) Nafadotride, a potent preferential dopamine D3 receptor antagonist, activates locomotion in rodents. *J Pharmacol Exp Ther* **275**:1239–1246.
- Schwartz JC, Diaz J, Bordet R, Griffon N, Perachon S, Pilon C, Ridray S, and Sokoloff P (1998) Functional implications of multiple dopamine receptor subtypes: the D1/D3 receptor coexistence. *Brain Res Brain Res Rev* **26**:236–242.
- Sokoloff P, Andrieux M, Besancon R, Pilon C, Martres MP, Giros B, and Schwartz JC (1992) Pharmacology of human dopamine D3 receptor expressed in a mammalian cell line: comparison with D2 receptor. *Eur J Pharmacol* **225**:331–337.
- Sokoloff P, Giros B, Martres MP, Bouthenet ML, and Schwartz JC (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature (Lond)* **347**:146–151.
- Tan LC, Protell PH, Langston JW, and Togasaki DM (2002) The hyperkinetic abnormal movements scale: a tool for measuring levodopa-induced abnormal movements in squirrel monkeys. *Mov Disord* **17**:902–909.
- Tintner R and Jankovic J (2002) Treatment options for Parkinson's disease. *Curr Opin Neurol* **15**:467–476.
- Togasaki DM, Tan L, Protell P, Di Monte DA, Quik M, and Langston JW (2001) Levodopa induces dyskinesias in normal squirrel monkeys. *Ann Neurol* **50**:254–257.

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