

Effects of L-DOPA on Nigral Dopamine Neurons and Local Field Potential: Comparison with Apomorphine and Muscimol

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ABSTRACT

L-DOPA is more effective than direct dopamine (DA) agonists in relieving the motor deficits in Parkinson's disease. Using *in vivo* recording, we compared the effect of L-DOPA and the direct DA agonist apomorphine on DA neurons in rat substantia nigra (SN). L-DOPA (50–100 mg/kg *i.v.*) decreased the firing rate as well as the variability and slow oscillation (SO) of firing. All effects were blocked by raclopride and mimicked by quinpirole, suggesting that they are mediated through D2-like receptors. Autoreceptor-selective doses of apomorphine (5–20 μ g/kg *i.v.*) also inhibited all three parameters. The magnitude of the inhibition, however, was significantly greater than that induced by L-DOPA. Neither L-DOPA nor apomorphine had a consistent effect on SN local field potentials (LFPs). The GABA agonist muscimol, known to preferentially inhibit SN non-DA neurons,

consistently inhibited the SO in both DA cell firing and LFPs. These results suggest that SN LFPs mainly reflect the synaptic potentials in non-DA neurons, and L-DOPA and apomorphine, unlike muscimol, affect DA neurons primarily through DA autoreceptors. DA autoreceptor activation is known to hyperpolarize DA cells by increasing the membrane conductance to K⁺. This increase in membrane conductance would shunt synaptic input to DA neurons, thereby decreasing the variability and SO in DA cell firing. The low potency of L-DOPA to inhibit DA cell firing and reduce their responses to synaptic input may partially account for its superior therapeutic efficacy in Parkinson's disease compared with apomorphine and other direct DA agonists.

Introduction

Parkinson's disease (PD) is a movement disorder caused by the degeneration of dopamine (DA) neurons in the substantia nigra (SN). L-DOPA and direct DA agonists are the most common medications for PD. Compared with direct DA agonists, however, the DA precursor L-DOPA is more effective in relieving the motor symptoms of PD, although its long-term use is associated with an increased incidence of motor complications (see reviews by Mercuri and Bernardi, 2005; Cenci and Odin, 2009). The reason for the superior therapeutic efficacy of L-DOPA is still unclear (Mercuri and Bernardi, 2005; Cenci and Odin, 2009). One possible explanation is that L-DOPA, after conversion to DA, stimulates all DA receptor subtypes, whereas DA agonists used in treatment of PD preferentially bind to D2-like receptors (De Keyser et al.,

1995; Seeman, 2007). However, the role of D1-like receptors in PD remains controversial, and there is a lack of evidence that concurrent activation of D1 and D2-like receptors produces better efficacy than selective activation of D2-like receptors (Andringa et al., 1999; Gerlach et al., 2003; Seeman, 2007).

A more likely possibility might be that L-DOPA enhances the transmission of synaptic information from residual SN DA neurons to their terminal areas such as the striatum, whereas direct DA agonists lack this ability. DA neurons in the SN receive synaptic input from a wide range of brain regions. Information from these regions is processed and integrated in DA cell soma and dendrites in the SN, and then delivered, in the form of spike trains, to DA axon terminals to regulate DA release. This DA-mediated transmission is likely to be essential for normal movement control. L-DOPA may enhance the transmission by increasing DA synthesis and thus DA release evoked by each action potential fired by DA neurons (Keller et al., 1988; Wightman et al., 1988; Miller and Abercrombie, 1999). Direct DA agonists, on the other hand, bind to DA receptors directly, independent of the firing pattern of DA neurons. Consequently, the pattern of DA

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ABBREVIATIONS: PD, Parkinson's disease; CV, coefficient variation; DA, dopamine; ISI, interspike interval; LFP, local field potential; SKF-38393, 1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol; SN, substantia nigra; SO, slow oscillation; VTA, ventral tegmental area.

receptor stimulation induced by a direct DA agonist carries no information from the SN.

L-DOPA also has an inhibitory effect on the information transmission from the SN to DA terminal areas. Previous studies have shown that L-DOPA decreases DA cell firing (Bunney et al., 1973; Iwatsubo and Clouet, 1977). This feedback inhibition would limit DA release and offset the therapeutic effect of L-DOPA. Compared with direct DA agonists such as apomorphine, L-DOPA may be less effective in producing the inhibitory effect. This reduced inhibitory effect of L-DOPA on DA neurons could also contribute to L-DOPA's enhanced therapeutic efficacy. In this study, we tested this possibility by comparing the effect of L-DOPA and apomorphine on both the firing rate and pattern of DA neurons in the SN. We studied the firing pattern of DA neurons by measuring the variability and slow oscillation (SO) of firing. Both measurements are highly sensitive to synaptic input because they are inhibited in brain slices and in animals with forebrain input to DA neurons blocked (e.g., Shi, 2005). The fact that the two measurements are highly correlated with each other and covary under different conditions further suggests that the variability of firing in DA neurons is, at least partially, caused by the SO (Shi et al., 2004, 2005; Zhou et al., 2006).

We also recorded local field potentials (LFPs) in the SN. Previous studies in the ventral tegmental area (VTA) suggest that the SO is transferred from the forebrain to DA neurons through VTA GABA cells and LFPs in the VTA reflect the synchronized synaptic activities in these non-DA neurons (Gao et al., 2007). To test whether the SO in SN DA neurons is generated by a similar mechanism, we studied the effect of muscimol, a GABA_A agonist previously shown to preferentially inhibit VTA/SN non-DA neurons (MacNeil et al., 1978; Walters and Lakoski, 1978; Grace and Bunney, 1979; Waszczak and Walters, 1980). We hypothesized that muscimol, by inhibiting SN non-DA cells, inhibits the SO in both DA cell firing and SN LFPs, whereas L-DOPA produces its effect primarily through DA autoreceptors and therefore has little or no effect on SN LFPs. Some results have been reported previously in abstract form (Xu and Shi, 2009).

Materials and Methods

Single-Unit Recordings In Vivo. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee and in compliance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing between 190 and 340 g, were anesthetized with chloral hydrate (400 mg/kg i.p., followed by supplemental doses administered via a lateral tail vein). To minimize pain and discomfort, 0.125% bupivacaine was applied to all incision and pressure points. DA neurons in the SN were identified and recorded extracellularly using techniques described previously (Shi et al., 2004, 2005; Gao et al., 2007). LFPs were simultaneously recorded from the same electrodes by passing the signals through one band-pass filter for single-unit detection (300–2000 Hz) and another for spike-free LFP recording (0.1–100 Hz). Only one cell was studied in each rat. Throughout the experiment, body temperature was maintained at 36.5 to 37.5°C with a homeothermic blanket system (Harvard Apparatus Inc., Holliston, MA).

Drugs. All drugs were purchased from Sigma-Aldrich (St. Louis, MO), including L-DOPA methyl ester hydrochloride, apomorphine hydrochloride hemihydrates, SKF-38393 (1-phenyl-2,3,4,5-tetra-

hydro-1*H*-3-benzazepine-7,8-diol) hydrochloride, quinpirole dihydrochloride, muscimol hydrobromide, and raclopride tartrate. All were dissolved in distilled water and administered intravenously through a lateral tail vein. Doses are given in terms of the weight of the respective salts.

Data Analysis. All analyses were performed using Clampfit 10 (Molecular Devices, Sunnyvale, CA) and in-house VBA subroutines in Excel (Microsoft, Redmond, WA). Firing rate and the variability of firing were determined every 10 s. The latter was evaluated by the coefficient variation (CV) of interspike intervals (ISIs). Firing periodicity was analyzed using methods described previously (Shi et al., 2004, 2005; Zhou et al., 2006). In brief, rate histograms with a bin width of 50 ms were constructed based on 2-min recordings obtained under different conditions. After tapering using the Hanning-Tukey window function (15 windows with 50% overlap) and removal of the linear trend, a fast Fourier transform was performed to yield spectra with a resolution of 0.078 Hz. To help visualize slow changes, rate histograms were smoothed (Shi et al., 2004). All spectral analyses, however, were performed using nonsmoothed data. Autospectra of LFPs (down-sampled to 20 Hz) were obtained using similar methods (Gao et al., 2007). The degree of correlation between firing rate and LFP was evaluated using cross-spectral and coherence analysis (Gao et al., 2007). Coherence was calculated from the cross-spectral density between the two recordings normalized by the spectral density of each signal. Phase lags were calculated from portions of the phase spectrum encompassing the peak frequencies of cross spectra.

Effects of drugs were determined by comparing measures before and after the drug injection using paired *t* test. Differences between drugs were determined using analysis of covariance followed by post hoc Tukey tests. Baseline values were the covariates. Spectral data were log-transformed before being subjected to statistical comparison. All numerical data were expressed as the mean ± the S.E.M.

Results

Effects on DA Cell Firing Rate and CV. Systemic injection of L-DOPA (50–100 mg/kg i.v.) partially inhibited the firing and decreased the variability of ISIs (measured by CV) in 17 of 20 SN DA cells tested (Fig. 1). In the remaining three cells, L-DOPA produced no change (<5% of baseline) or an increase in one or both parameters (Fig. 1C). On average, the firing rate was reduced from 4.5 ± 0.5 to 3.6 ± 0.4 spikes/s by 50 mg/kg L-DOPA ($n = 16$, $p < 0.001$, paired *t* test) and from 4.6 ± 0.4 to 2.8 ± 0.3 spikes/s by 100 mg/kg L-DOPA ($n = 18$, $p < 0.001$) (Fig. 1B). CV was decreased from 50.7 ± 6.0 to $39.2 \pm 5.3\%$ ($n = 16$, $p < 0.001$) and from 47.9 ± 5.7 to $32.3 \pm 5.3\%$ ($n = 18$, $p < 0.001$) by 50 and 100 mg/kg L-DOPA, respectively (Fig. 1B). Subsequent injection of the D2-like receptor antagonist raclopride (50–100 µg/kg i.v.) reversed the inhibition induced by L-DOPA in 14 of 15 cells tested (Fig. 1A).

To rule out the possibility that raclopride reverses the inhibition induced by L-DOPA by exciting the cell, raclopride (0.1–0.2 mg/kg i.v.) was given before L-DOPA (100 mg/kg i.v.). In six of seven cells tested, L-DOPA, given after raclopride, produced no effect or a small increase in firing rate (Fig. 2). In the remaining cell, the firing rate was decreased from 2.9 to 1.5 spikes/s. On average, the firing rate was unchanged (from 4.68 ± 0.62 to 4.89 ± 0.88 spikes/s, $n = 7$, $p = 0.61$). L-DOPA also produced no significant effect on CV when given after raclopride (38.1 ± 6.9 to $47.6 \pm 8.1\%$, $n = 7$, $p = 0.13$). In four other rats, the D1 agonist SKF-38393 was administered (5 mg/kg i.v.). Unlike L-DOPA, SKF-38393 produced no effect on firing rate (from 5.61 ± 1.16 to 5.63 ± 1.28 spikes/s, $n = 4$, $p = 0.96$) and CV (from 41.2 ± 4.8 to $36.7 \pm$

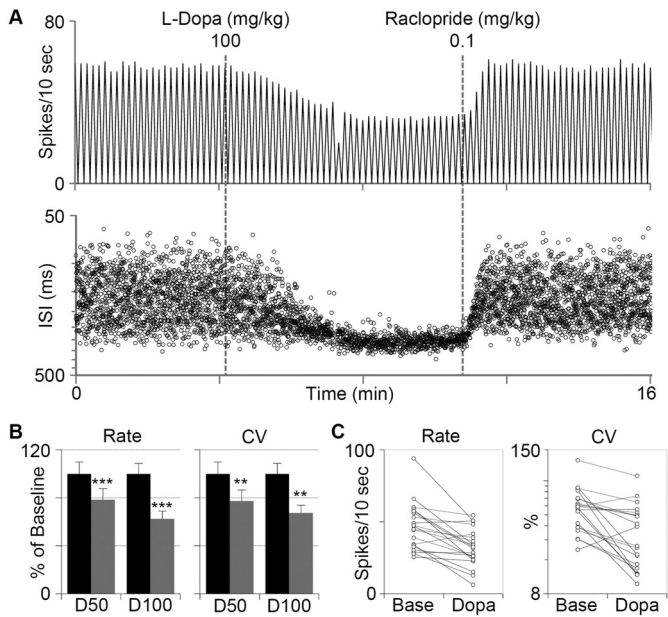


Fig. 1. Effects of L-DOPA on firing rate and CV of SN DA neurons. A, rate histogram (top) and ISI plots (bottom) of a typical SN DA neuron showing a decrease in both firing rate and the variability of ISIs by L-DOPA and the reversal of the effect by the D2 antagonist raclopride. Each dot in the ISI plots represents one ISI. B, summary bar graphs showing changes in firing rate (left) and CV (right) induced by 50 mg/kg L-DOPA (D50; $n = 16$) and 100 mg/kg L-DOPA (D100; $n = 18$), respectively. All values are expressed as percentages of predrug baseline. **, $p < 0.01$; ***, $p < 0.001$ compared with baseline (black bars). C, data from individual cells. Each pair of dots, linked by a single line, represents the firing rate (left) or CV (right) of a DA cell measured under baseline conditions (Base) and after L-DOPA injection (Dopa; 50 mg/kg, $n = 2$; 100 mg/kg, $n = 18$).

6.3%, $n = 4$, $p = 0.22$) (Fig. 2). In the same cells, subsequent injection of L-DOPA (100 mg/kg i.v., $n = 2$) or the D2 agonist quinpirole (5–20 $\mu\text{g/kg}$ i.v., $n = 2$) (Fig. 2B) inhibited both measurements. These results suggest that the effects of L-DOPA are mediated through D2-like receptors.

Apomorphine (5–20 $\mu\text{g/kg}$ i.v.) also inhibited both the firing rate and CV of DA neurons (Fig. 2). At 5 $\mu\text{g/kg}$ i.v., apomorphine reduced the firing rate from 6.1 ± 0.3 to 3.5 ± 0.3 spikes/s ($n = 10$, $p < 0.001$). This reduction was significantly greater than that induced by 50 mg/kg L-DOPA ($F_{1,23} = 6.05$, $p < 0.05$, analysis of covariance) (see Fig. 4) and was comparable with the effect produced by 100 mg/kg L-DOPA ($F_{1,25} = 0.05$, $p = 0.82$). The same dose of apomorphine decreased CV from 47.1 ± 5.1 to $20.0 \pm 3.8\%$ ($p < 0.001$). Again, the magnitude of the decrease was significantly larger than that produced by 50 mg/kg L-DOPA ($F_{1,23} = 12.91$, $p < 0.01$) and was not significantly different from the inhibition induced by 100 mg/kg L-DOPA ($F_{1,25} = 3.86$, $p = 0.06$) (see Fig. 4). Both changes in firing rate and CV induced by apomorphine were reversed by subsequent injection of raclopride (50–100 $\mu\text{g/kg}$ i.v.) (Fig. 2).

Effects on SO and LFP. L-DOPA decreased the SO (0.4–1.2 Hz) in 17 of 20 cells examined (Fig. 3) and increased it in the three remaining cells. On average, the SO in firing rate was reduced from 0.40 ± 0.11 to $0.26 \pm 0.08\%$ and from 0.36 ± 0.11 to $0.18 \pm 0.06\%$ by 50 mg/kg L-DOPA ($n = 16$, $p < 0.05$) and 100 mg/kg L-DOPA ($n = 18$, $p < 0.01$), respectively. In 16 cells, LFPs were concurrently recorded. Unlike the SO in firing rate, the SO in LFP showed inconsistent responses to L-DOPA. Thus, L-DOPA decreased LFP SO in eight cells,

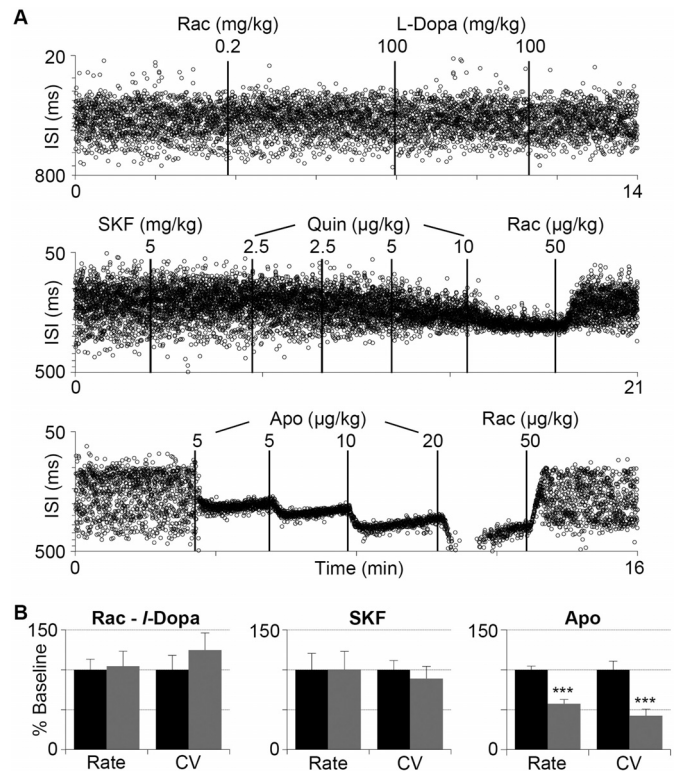


Fig. 2. Role of D2 autoreceptors in effects of L-DOPA. A, ISI plots from three different DA neurons showing that the inhibitory effect of L-DOPA on firing rate and CV was prevented by a pretreatment with the D2 antagonist raclopride (Rac, top) and mimicked by the D2 agonist quinpirole (Quin), but not by the D1 agonist SKF-38393 (SKF, middle). Low doses of apomorphine (Apo), known to be selective for DA autoreceptors, also mimicked the effects of L-DOPA (bottom). B, summary bar graphs showing the blockade of the inhibitory effect of L-DOPA (100 mg/kg) by raclopride (50–100 $\mu\text{g/kg}$; left), the lack of an effect of SKF-38393 on firing rate and CV (5 mg/kg; center), and the decrease in both firing rate and CV by 5 $\mu\text{g/kg}$ apomorphine (right). All values are expressed as percentages of baseline. ***, $p < 0.001$ compared with baseline (black bars).

increased it in six cells, and produced no effect in the remaining two cells. On average, LFP SO was not significantly altered by L-DOPA (50 mg/kg, from 4.37 ± 0.53 to $4.84 \pm 0.48\%$, $n = 16$, $p = 0.20$; 100 mg/kg, from 4.79 ± 0.49 to $4.35 \pm 0.39\%$, $n = 14$, $p = 0.22$) (see Fig. 4).

Figure 3C shows the correlation between CV and the SO in firing rate (left). Changes in CV induced by L-DOPA were also highly correlated with changes in the SO in firing rate (center). In contrast, changes in the SO in firing rate were uncorrelated with changes in LFP SO (right). As previously observed in the VTA (Gao et al., 2007), the SO in most SN DA neurons had a nearly antiphase relation with the SO in SN LFP (Fig. 3D). Together, these results support the suggestion that SN LFP reflects mainly the synchronized synaptic potentials in non-DA neurons.

Like L-DOPA, apomorphine suppressed the SO in firing rate in most DA cells tested (9/10) (Fig. 4). In one cell, the SO was slightly increased. On average, apomorphine at 5 $\mu\text{g/kg}$ decreased the SO in firing rate from $0.27 \pm 0.08\%$ to 0.07 ± 0.02 ($p < 0.001$, $n = 10$). The magnitude of the inhibition was significantly greater than that induced by 50 mg/kg L-DOPA ($F_{1,23} = 8.32$, $p < 0.01$) (Fig. 4C). Like L-DOPA, apomorphine produced inconsistent effects on SN LFPs. It decreased LFP SO in two cells, increased it in two other cells, and produced

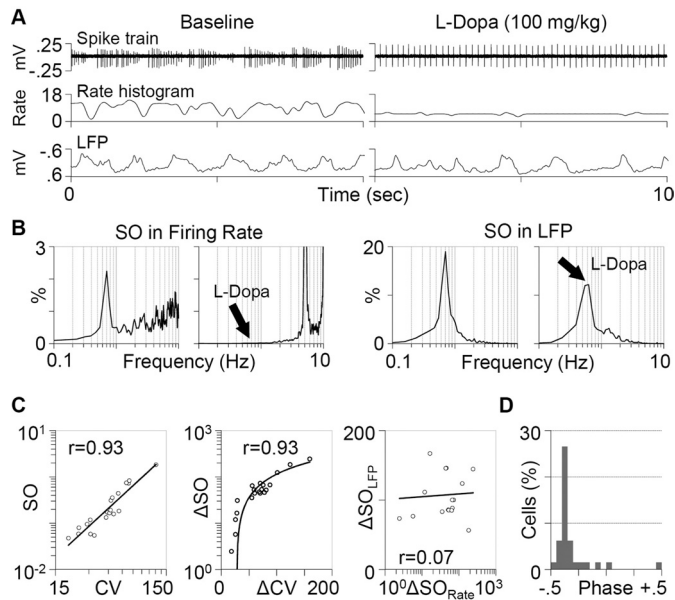


Fig. 3. Effects of L-DOPA on the SO in DA cell firing rate and SN LFP. A, segments of spike trains of a DA neuron (top), corresponding smoothed rate histograms (spikes/s, bin width 50 ms, middle), and concurrently recorded LFPs (bottom). L-DOPA injection (100 mg/kg i.v.) regularized the firing pattern and completely inhibited the SO in firing rate in this cell. The drug, however, only partially inhibited the SO in SN LFP. B, autospectra of rate histograms and LFPs confirming a total suppression of the SO in firing rate (left), but not in LFP by L-DOPA (right). C, charts showing the correlation between the SO in firing rate and CV under baseline conditions (left) and between changes of the two measurements induced by L-DOPA (expressed as percentage of baseline; middle). However, the effect of L-DOPA on the SO in firing rate and its effect on LFP SO were uncorrelated with each other (right). In all charts, each dot represents data from one cell. D, distribution of phase lags between the SO in firing rate and that in LFP (expressed as percentage of a cycle period). In most cells examined, the SO in firing rate had a phase lag more than 90° or 25% of a cycle period relative to the SO in LFP.

no effect in the remaining three cells. Overall, LFP SO was not significantly changed (from 2.84 ± 0.77 to $1.94 \pm 0.30\%$, $p = 0.1$, $n = 7$) (Fig. 4C).

Effects of Muscimol on SN DA Neurons and LFP. The GABA_A agonist muscimol is known to preferentially inhibit VTA/SN non-DA neurons when given systemically (MacNeil et al., 1978; Walters and Lakoski, 1978; Grace and Bunney, 1979; Waszczak and Walters, 1980). If SN LFPs represent synaptic activities in non-DA neurons, muscimol would differ from L-DOPA and apomorphine, inhibiting LFP SO in the SN. Confirming this prediction, muscimol markedly reduced LFP SO at all three doses tested (0.5, 1, and 2 mg/kg i.v.). After a cumulative dose of 2 mg/kg, muscimol reduced the size of LFP SO from 4.45 ± 0.37 to $2.18 \pm 0.40\%$ ($p < 0.01$, $n = 16$) (Fig. 5). All doses also significantly decreased the SO and CV in DA neurons. At 2 mg/kg, muscimol reduced CV from 43.3 ± 2.4 to $27.5 \pm 3.6\%$ ($p = 0.001$, $n = 16$) and the SO decreased from 0.23 ± 0.03 to $0.07 \pm 0.01\%$ ($p < 0.001$) (Fig. 5). These findings are consistent with the suggestion that the SO is relayed from the forebrain to DA cells through SN non-DA neurons. In contrast to the SO and CV, the firing rate of DA neurons was increased by muscimol. At 2 mg/kg, muscimol increased the firing from 4.83 ± 0.40 to 6.03 ± 0.38 spikes/s ($p < 0.01$) (Fig. 5). This increase is believed to be secondary to the inhibition of non-DA neurons by muscimol (MacNeil et al., 1978; Walters and Lakoski, 1978; Grace and Bunney, 1979; Waszczak and Walters, 1980).

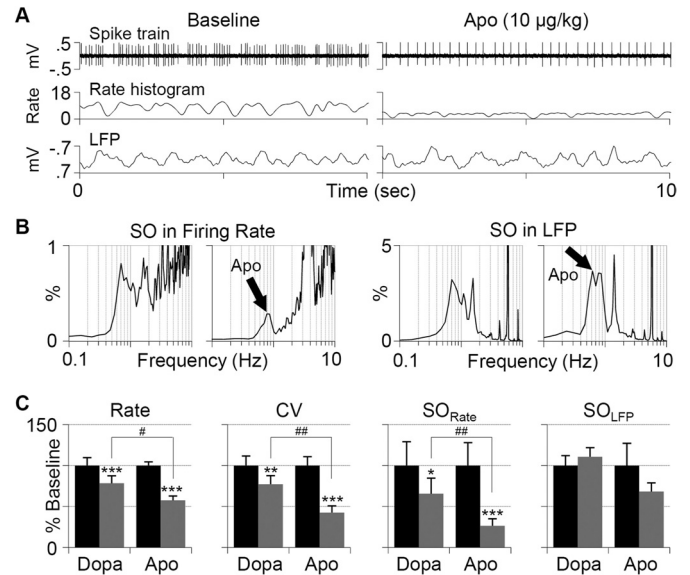


Fig. 4. Effects of apomorphine on the SO in DA cell firing rate and SN LFP. A, recordings from a DA neuron showing that apomorphine regularized the firing pattern (top), inhibited the SO in firing rate (middle), and slightly increased the SO in LFP (bottom). B, autospectra confirming a decrease in SO in firing rate (left) and a slight increase in SO in LFP (right) after apomorphine. C, bar graphs comparing effects between L-DOPA (DOPA) and apomorphine (Apo). At the lowest dose tested (5 $\mu\text{g}/\text{kg}$), apomorphine significantly decreased all three measures of DA cell activity (i.e., firing rate, CV, and SO). Furthermore, the decrease was significantly greater than that induced by a high dose of L-DOPA (50 mg/kg). Both apomorphine and L-DOPA produced no significant effect on the SO in LFP. All values are expressed as percentages of baseline. #, $p < 0.05$; ##, $p < 0.01$ L-DOPA versus apomorphine. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with baseline (black bars).

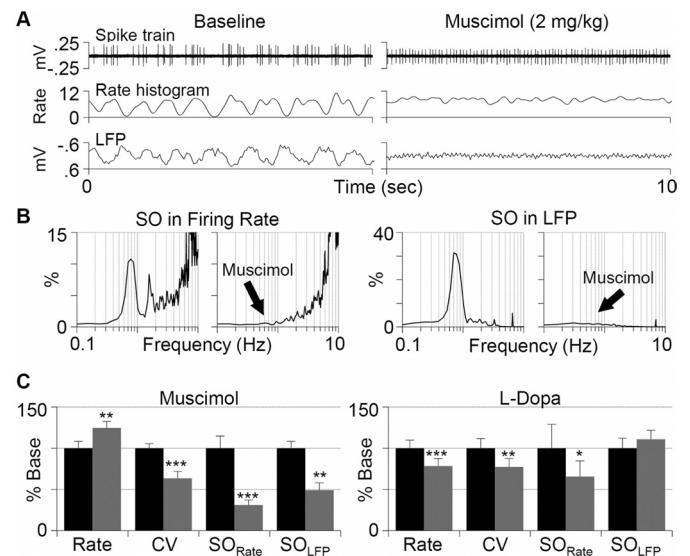


Fig. 5. Effects of muscimol on SN DA neurons and LFP. A, recordings from a DA neuron showing that muscimol increased the firing rate and regularized the firing pattern (top) and inhibited the SO in both DA cell firing (middle) and SN LFP (bottom). B, autospectra of rate histograms (left) and LFPs (right) confirming the inhibitory effect of muscimol on the SO in both DA neurons and LFP. C, bar graphs comparing effects between muscimol (2 mg/kg; left) and L-DOPA (50 mg/kg; right). All values are expressed as percentages of baseline (Base). **, $p < 0.01$; ***, $p < 0.001$ versus baseline (black bars).

Discussion

A main function of SN DA neurons is to process and transfer synaptic information from the SN to DA terminal areas

such as the striatum. This study suggests that L-DOPA has opposing effects on this DA-mediated transmission. As a DA precursor, L-DOPA facilitates the transmission by increasing DA synthesis and DA release. Because of the activation of feedback mechanisms, L-DOPA also hinders the transmission by slowing down the DA cell firing rate and reducing the responsiveness of DA neurons to synaptic input. Apomorphine at best partially mimics the facilitating effect of L-DOPA because its binding to DA receptors is independent of DA cell firing and does not encode the temporal and spatial information carried by synaptically released DA. Apomorphine, however, fully mimics the inhibitory effect of L-DOPA. In fact, it is much more potent than L-DOPA in inhibiting DA neurons. These differences may partially explain why apomorphine is not as effective as L-DOPA in improving the motor symptoms of PD.

Role of DA Autoreceptors. The inhibitory effect of L-DOPA on DA cell firing has been reported previously (Bunney et al., 1973; Iwatsubo and Clouet, 1977). As observed in the present study, L-DOPA only partially inhibited DA neurons at high doses and the effect was unaltered by anesthesia (Bunney et al., 1973; Iwatsubo and Clouet, 1977). Studies *in vitro* further suggest that the effect requires transport of L-DOPA into DA neurons (Sebastianelli et al., 2008) and its conversion to DA by the aromatic amino acid decarboxylase (Mercuri et al., 1990). DA, derived from L-DOPA, is then released in a Ca^{2+} -dependent manner and hyperpolarizes DA neurons by activating DA autoreceptors (Mercuri et al., 1990). The hyperpolarization is secondary to an increase in membrane conductance to K^+ and is blocked by D2-like receptor antagonists (Lacey et al., 1987, 1988). Consistent with these findings, the inhibitory effect of L-DOPA seen in this study was mimicked by the D2 agonist quinpirole and not by the D1 agonist SKF-38393 and was completely blocked by the D2 antagonist raclopride.

L-DOPA also decreased the variability and SO in firing. Similar results were previously obtained in the VTA (Zhou et al., 2006). Because both measures depend on synaptic input (Shi, 2005), our results suggest that L-DOPA inhibits synaptic terminals innervating DA neurons and/or reduces the responsiveness of DA neurons to synaptic input. Consistent with the latter possibility, the effect of L-DOPA on CV and SO was mimicked by autoreceptor-selective doses of apomorphine. As discussed above, DA autoreceptor activation increases the membrane conductance. This increase would shunt synaptic current, thereby reducing the response of DA neurons to synaptic input.

The results with LFP are also consistent with a role of DA autoreceptors in the effects of L-DOPA. We suggest that SN LFPs represent mainly the synaptic activities in SN non-DA neurons because the firing of most DA neurons had a nearly antiphase relation with SN LFP. Furthermore, muscimol, which preferentially inhibits SN non-DA neurons (MacNeil et al., 1978; Walters and Lakoski, 1978; Grace and Bunney, 1979; Waszczak and Walters, 1980), consistently suppressed the SO in SN LFP. The finding that muscimol also inhibits the SO in DA neurons (Gao et al., 2007 and present study) further supports the suggestion that the SO is relayed through SN/VTA non-DA neurons to DA cells. In contrast, L-DOPA lacked a consistent effect on SN LFP, suggesting that it differs from muscimol, affecting DA cells primarily through DA autoreceptors.

Role of Long Feedback Pathways. L-DOPA may also affect DA neurons indirectly through striatal projections to DA neurons. Several lines of evidence suggest, however, that long feedback pathways do not play a major role in the effects observed in the present study.

First, the results with apomorphine suggest that DA autoreceptor activation alone is enough to mimic all effects of L-DOPA. As discussed above, L-DOPA, by increasing DA synthesis, can induce DA release in the SN and cause activation of DA autoreceptors. Second, striatonigral neurons, including their terminals in the SN, express mainly D1-like receptors. All effects of L-DOPA observed in this study were blocked by raclopride and not mimicked by SKF-38393, suggesting that the direct striatonigral pathway does not contribute significantly to the observed effects of L-DOPA. Third, striatal D2-like receptor activation has been suggested to excite globus pallidus neurons (e.g., Carlson et al., 1987). Pallidal excitation inhibits SN GABA neurons, thereby increasing DA cell firing and bursting (Celada et al., 1999). Thus, if striatal D2-like receptors are involved in the effects of L-DOPA, their role would be opposite to that played by DA autoreceptors. The finding that this indirect striatonigral pathway is largely inactivated in anesthetized animals (Bergstrom et al., 1984) further supports the suggestion that the pathway is not critical to the effects of L-DOPA observed in the present study. Finally, SN non-DA neurons receive inputs from both the direct and indirect striatonigral pathways (Kravitz et al., 2010). We found that SN LFPs, which reflect the synaptic activities in SN non-DA neurons, were not consistently altered by L-DOPA.

It is important to point out that all our experiments were performed in anesthetized animals. Previous studies suggest that general anesthesia alters feedback control of DA neurons (Shi et al., 1997, 2000). Thus, whether in nonanesthetized animals L-DOPA influences DA neurons through both DA autoreceptors and long feedback pathways remains to be determined.

Normal Versus Parkinsonian Animals. All experiments in this study were performed in normal animals. Evidence suggests that L-DOPA may produce similar effects in parkinsonian animals. Thus, in 6-hydroxydopamine-lesioned rats, L-DOPA decreased both the firing rate and bursting in the remaining SN DA neurons (Harden and Grace, 1995). The decrease in bursting suggests that L-DOPA also inhibits synaptic input to DA cells in lesioned animals because burst firing in DA neurons depends on synaptic input and is partially correlated with CV and SO (Shi et al., 2004, 2005; Zhang et al., 2008). Apomorphine also inhibited DA cells in 6-hydroxydopamine-lesioned rats (Bilbao et al., 2006). In a PD mouse model, L-DOPA was initially reported to increase CV and bursting (Paladini et al., 2003). When re-examined using a nonanesthetized preparation, L-DOPA consistently inhibited both the firing rate and bursting (Robinson et al., 2004). Further investigation is needed to determine whether in parkinsonian animals apomorphine and other D2 agonists are also more potent than L-DOPA in inhibiting DA neurons. The role of long feedback pathways also needs to be re-examined in lesioned animals because L-DOPA has been shown to be more effective in inducing striatal DA release in lesioned animals than in control animals (Abercrombie et al., 1990; Miller and Abercrombie, 1999).

Effects of Apomorphine. Apomorphine is a mixed D1 and D2-like receptor agonist (Seeman, 2007). At low doses (≤ 50 $\mu\text{g}/\text{kg}$), apomorphine inhibits the firing of DA neu-

rons without significantly affecting cells postsynaptic to DA terminals (Skirboll et al., 1979; Bergstrom et al., 1984). Other D2-like receptor agonists tested exhibit similar properties, including the antiparkinsonian agents pergolide and pramipexole (Carlson et al., 1987; Piercey et al., 1997). These observations led to the suggestion that low doses of a D2 agonist selectively activate DA autoreceptors. Consistent with this suggestion, inactivation of DA autoreceptors by intranigra infusion of pertussis toxin blocks the inhibitory effect of apomorphine on DA neurons (Innis and Aghajanian, 1987).

In this study, low doses of apomorphine were found to mimic the effects of L-DOPA, decreasing all three parameters of DA cell firing. More importantly, the magnitude of decrease was significantly larger than that induced by a high dose of L-DOPA. Clinical doses of apomorphine range from below 1 mg to more than 10 mg per injection (e.g., Dewey et al., 2001). If the average human weight is 75 kg, a 5-mg injection would yield a dose of 67 $\mu\text{g}/\text{kg}$, which is equivalent to 411 $\mu\text{g}/\text{kg}$ in rats (Reagan-Shaw et al., 2008). At this dose, apomorphine would completely inhibit DA cell firing and thus stop the information transmission from the SN to DA terminal areas. This large inhibition may partially explain why apomorphine is not as effective as L-DOPA in reducing motor deficits in PD.

To summarize, this study suggests that L-DOPA and apomorphine decrease not only DA cell firing, but also their responsiveness to synaptic input. The latter effect is indicated by the decrease in CV and SO in DA neurons and is likely to be caused by synaptic shunting caused by DA autoreceptor activation. Both effects would impair information transmission from the SN to the striatum and thus be detrimental to PD. Compared with apomorphine, however, L-DOPA was much less potent in producing these effects, which may partially explain why L-DOPA is more effective than apomorphine in improving motor symptoms in PD. This study also showed that muscimol, but not L-DOPA and apomorphine, consistently suppressed the SO in SN LFP. This finding, together with those reported previously, suggests that SN LFP reflects mainly synaptic activities in non-DA neurons, and muscimol produces at least part of its effects on DA cells indirectly by inhibiting SN non-DA neurons. L-DOPA and apomorphine, on the other hand, may produce their effects primarily through DA autoreceptors.

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Authorship Contributions

Participated in research design: Xu and Shi.

Conducted experiments: Xu and Shi.

Performed data analysis: Xu, Karain, and Shi.

Wrote or contributed to the writing of the manuscript: Xu, Karain, Brantley, and Shi.

Other: Shi acquired funding for the research.

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