Levodopa enhances synaptic plasticity in the substantia nigra pars reticulata of Parkinson’s disease patients

I. A. Prescott,1 J. O. Dostrovsky,1,2 E. Moro,2 M. Hodaie,2 A. M. Lozano2 and W. D. Hutchison1,2

1 Department of Physiology, University of Toronto, Canada
2 Division of Neurosurgery, Department of Surgery, Toronto Western Research Institute and Krembil Neuroscience Centre, Canada

Correspondence to: W. D. Hutchison, PhD, Associate Professor of Surgery and Physiology, University of Toronto, and Senior Scientist, Toronto Western Research Institute, 399 Bathurst Street, MP11-308, Toronto, Canada M5T 2S8. E-mail: whutch@uhnres.utoronto.ca

Parkinson’s disease, caused by the loss of dopaminergic nigrostriatal projections, is a debilitating neurodegenerative disease characterized by bradykinesia, rigidity, tremor and postural instability. The dopamine precursor levodopa (L-dopa) is the most effective treatment for the amelioration of Parkinson’s disease signs and symptoms, but long-term administration can lead to disabling motor fluctuations and L-dopa-induced dyskinesias (LIDs). Studies in rat striatal slices have shown dopamine to be an essential component of activity-dependent synaptic plasticity at the input to the basal ganglia, but dopamine is also released from ventrally projecting dendrites of the substantia nigra pars compacta (SNc) on the substantia nigra pars reticulata (SNr), a major output structure of the basal ganglia. We characterized synaptic plasticity in the SNr using field potentials evoked with a nearby microelectrode (fEPs), in 18 Parkinson’s disease patients undergoing implantation of deep brain stimulating (DBS) electrodes in the subthalamic nucleus (STN). High frequency stimulation (HFS—four trains of 2 s at 100 Hz) in the SNr failed to induce a lasting change in test fEPs (1 Hz) amplitudes in patients OFF medication (decayed to baseline by 160 s). Following oral L-dopa administration, HFS induced a potentiation of the fEP amplitudes (+29.3% of baseline at 160 s following a plateau). Our findings suggest that extrastriatal dopamine modulates activity-dependent synaptic plasticity at basal ganglia output neurons. Dopamine medication state clearly impacts fEP amplitude, and the lasting nature of the increase is reminiscent of LTP-like changes, indicating that aberrant synaptic plasticity may play a role in the pathophysiology of Parkinson’s disease.

Keywords: Parkinson’s disease; substantia nigra; synaptic plasticity; microelectrode recordings; basal ganglia

Abbreviations: DBS = deep brain stimulating; fEP = field evoked potential; HFS = high frequency stimulation; LTP = long-term potentiation; SNc = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; STN = subthalamic nucleus

Introduction

Parkinson’s disease is a hypokinetic movement disorder characterized by the loss of dopaminergic projections from the substantia nigra pars compacta (SNc) to various targets, including the striatum, the input of the basal ganglia. Reduced dopaminergic input to the striatum is thought to ultimately result in increased neuronal firing of the inhibitory basal ganglia output and disturbed firing patterns with increased synchronization (Albin et al., 1989; DeLong, 1990; Levy et al., 2002; Brown, 2003). Such changes bring about bradykinesia, rigidity, tremor and postural instability, although the underlying mechanisms leading to these symptoms...
are still poorly understood. Currently, levodopa (L-dopa) administration is the most common and effective therapeutic treatment. However, long-term L-dopa treatment is not without its own serious side effects. Abnormal involuntary movements (dyskinesias) are motor complications that arise in the majority of Parkinson’s disease patients undergoing this treatment (Obeso et al., 2000a, b).

In addition to its dopaminergic nigrostriatal projections, the SNc also sends ventrally projecting dendrites to the substantia nigra pars reticulata (SNr) (Geffen et al., 1976; Korf et al., 1976; Cheramy et al., 1981; Robertson et al., 1991). However, little is known of the effects of dopamine released from these ventral SNc projections, either in animal models or humans, despite the fact that basal ganglia output structures seem intimately tied to dyskinesia. Deep brain stimulation (DBS) electrodes implanted in the subthalamic nucleus (STN), a basal ganglia structure that sends glutamatergic projections to the SNr and GPi, have proven remarkably efficacious as a treatment of Parkinson’s disease and L-dopa induced dyskinesia (Kleiner-Fisman et al., 2006; Perlmutt and Mink, 2006). While STN DBS does not provide a greater degree of benefit for Parkinson’s disease symptoms than optimal therapy with L-dopa (Krack et al., 2003; Pahwa et al., 2005), it does lessen the time a patient spends in the ‘OFF’ state when the benefit from an individual dose of medication has diminished, and permits the reduction of dopaminergic medications and their adverse side effects including dyskinesia (Moro et al., 1999; Jaggi et al., 2004; Kleiner-Fisman et al., 2006). DBS appears to mimic the effect of beneficial lesions instead of exacerbating the hyperactivity in the basal ganglia output neurons, but despite the discernible clinical benefits of STN DBS, its mechanism of action remains unclear.

Corticostriatal slice work suggests that abnormal involuntary movements such as dyskinesia are the result of alterations to synaptic plasticity at the basal ganglia input. Long-term potentiation (LTP) at the corticostriatal synapse is induced with high frequency stimulation (HFS) and reversed with low frequency stimulation (LFS) in healthy adult Wister rats (Picconi et al., 2003; Picconi et al., 2008). LTP is absent in dopamine lesioned (6-OHDA) rats, but can be restored with chronic L-dopa treatment. Additionally, several paired associative stimulation (PAS) studies have shown that motor evoked potential (MEP) amplitudes in the motor cortex of Parkinson’s disease patients are modulated by dopaminergic medication state and that these changes are LTP-like in nature (Morgante et al., 2006; Ueki et al., 2006). PAS increased MEP amplitude in controls but not in patients OFF medication irrespective of their dyskinesia state. L-dopa administration restored the potentiation of MEP amplitudes by PAS in non-dyskinetic but not dyskinetic patients (Morgante et al., 2006). These findings indicate that LTP-like plasticity is absent from the motor cortex in a dopamine deprived state and, taken together, these studies in cortex and striatum suggest that a lack of plasticity in the absence of dopamine may play an important role in the disabling motor symptoms of Parkinson’s disease. However, to this point, a suitable methodology for direct measures of synaptic plasticity in the human central nervous system has been lacking (Cooke and Bliss, 2006).

The aim of this study was to characterize synaptic plasticity at the basal ganglia output during in vivo recordings in Parkinson’s disease patients undergoing implantation of DBS electrodes in the STN. Employing a novel methodology for evoking and measuring field evoked potentials (FEPs) in SNr using a pair of microelectrodes, we found the amplitude of these positive FEPs were modulated both by tetanizing trains and L-dopa, implicating extrastriatal dopamine actions in the pathophysiology of Parkinson’s disease.

**Patients and methods**

Using intraoperative microelectrode recordings, we studied 18 patients undergoing stereotactic surgery for implantation of bilateral STN-DBS electrodes. The clinical characteristics of the patients and their daily doses of anti-Parkinson’s disease medications are shown in Table 1. The group, consisting of 14 men and four women, had a mean age (±SD) of 58.9 ± 6.8 years and mean disease duration (±SD) of 13.3 ± 4.4 years. Six patients were most affected on their right side, while nine patients were most affected on their left side. Three patients were severely affected bilaterally. Patients normally underwent a minimum of 12 h of anti-Parkinson’s disease medication withdrawal before testing, and were awake with local anesthesia for measures of synaptic plasticity in SNr following completion of the electrophysiological mapping of the STN. The UPDRS III OFF motor scores given in Table 1 are also following 12 h of anti-Parkinson’s disease medication withdrawal. Six patients were studied first in the ‘OFF’ state following 12 h withdrawal and then in the ‘ON’ state after oral administration of 100 mg of levodopa (Sinemet 100/25) in the contralateral hemisphere. An additional six patients were studied only in the OFF state in order to avoid the occurrence of severe dyskinesia during surgery. In four cases the patient was given one tablet of Sinemet 100/25 immediately before the procedure as it was deemed medically necessary for the patient (Patients 4, 6, 8 and 9 in Table 1). UPDRS motor scores indicate that all patients had some degree of motor improvement when ON L-dopa with an average improvement (±SD) of 61.5 ± 13.0% in the ON state. The experiments were approved by the University Health Network and University of Toronto Research Ethics Boards. Patients provided written informed consent prior to the procedure.

**Surgical procedure and microelectrode recordings**

Extracellular recordings were made with dual independently driven microelectrodes (about 25 µm tip length, axes 600 µm apart, 0.2–0.4 MΩ impedance at 1000 Hz) during the electrophysiological mapping procedure used to obtain physiological data for localizing the target for DBS quadripolar electrodes (Medtronic Model 3387, Minneapolis, MN). Recordings were amplified 5000–10000 times and filtered at 10–5000 Hz (analog Butterworth filters: high-pass, one pole; low-pass, two poles) using two Guideline System GS3000 amplifiers (Axon Instruments, Union City, CA). Microelectrode data were sampled and digitized at 12 kHz with a CED 1401 (Cambridge Electronic Design (CED), Cambridge, UK) and EMG of ipsi- and contralateral wrist and foot flexor and extensor was sampled at 500 Hz to monitor any dyskinetic movements.

Pre-surgery, the tentative STN target was identified by brain imaging (MRI) on the basis of the stereotactic coordinates and direct imaging of STN. Coordinates of the tentative target were 12 mm lateral to the midline, 2–4 mm posterior to the mid-commissural point.
Target nuclei were then localized via characteristic neuronal discharge patterns described elsewhere in detail (Hutchison et al., 1998). Briefly, after passing through thalamus and STN, the SNr was identified by the presence of neurons with a significantly higher discharge rate and more regular firing pattern (versus STN). The SNr neurons also displayed characteristically low thresholds (2–4 μA) for microstimulation-induced inhibition of firing (Dostrovsky et al., 2000). An example of a typical trajectory is shown in Fig. 1. All recording sites were deemed to be near the region of the soma and the spike amplitude was continuously monitored in order to confirm stability of electrode position.

fEPs were recorded from one electrode while stimulating with single pulses (100 μA, 0.3 ms biphasic pulse width) from a second electrode separated mediolaterally by 0.5–1.0 mm at the same dorsoventral level within the SNr. Depth profiles were examined in some cases by moving the stimulating electrode in 250 μm increments above and below the recording site for up to a 3 mm separation. Input–output curves were constructed by varying the pulse width (0.05, 0.1, 0.2, 0.3, 0.5 and 1 ms) in order to avoid saturation of the fEP amplitude during the tetanus. A paired pulse response (PPR) curve was constructed for one patient using a variety of paired pulse interstimulus intervals (20, 30, 50, 100 and 200 ms) by comparing the ratio of the peak amplitude of the second fEP to the first fEP.

After obtaining a stable baseline of peak fEP amplitudes at 1 Hz, HFS was given, consisting of four 100 Hz trains, 2 s in length, repeated four times every 10 s (100 μA, 0.3 ms pulse width). Blocks of ten pulses were tested every 30 s for at least 2 min, or until a stable plateau had occurred. Plasticity was quantified using fEP amplitudes in both OFF and ON dopaminergic medication states, with the first side being done after 12 h off medication and the second side following administration of Sinemet 100/25/C213. Typically, 25–30 min had elapsed between the time of administration (Sinemet 100/25/C213 was given as recording began on the ‘ON’ track) and SNr testing. The sites where synaptic plasticity was tested are shown in Fig. 1 and were determined by track reconstruction using neurophysiological landmarks and a customized brain atlas-based programme.

### Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/sex/ worst side</th>
<th>Disease duration (years)</th>
<th>Medication (daily dose)</th>
<th>L-dopa equivalence (mg/day)</th>
<th>UPDRS III (OFF/ON)</th>
<th>Improvements (%)</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61/M/L</td>
<td>16</td>
<td>l-dopa 800 mg, Pramipexole 2 mg, Amantadine 200 mg</td>
<td>1000</td>
<td>40/20.5</td>
<td>49</td>
<td>OFF and ON</td>
</tr>
<tr>
<td>2</td>
<td>68/M/R</td>
<td>21</td>
<td>l-dopa 1100 mg, Pergolide 2 mg, Selegiline 10 mg</td>
<td>1300</td>
<td>31.5/13</td>
<td>59</td>
<td>OFF</td>
</tr>
<tr>
<td>3</td>
<td>65/M/R</td>
<td>7</td>
<td>l-dopa 675 mg, Pramipexole 2 mg</td>
<td>875</td>
<td>48/19</td>
<td>60</td>
<td>OFF and ON</td>
</tr>
<tr>
<td>4</td>
<td>63/M/L</td>
<td>11</td>
<td>l-dopa 1400 mg, Amantadine 100 mg, Carbergoline 2 mg</td>
<td>1500</td>
<td>46/16.5</td>
<td>64</td>
<td>ON (2)</td>
</tr>
<tr>
<td>5</td>
<td>57/M/L</td>
<td>10</td>
<td>l-dopa 1500 mg</td>
<td>1500</td>
<td>33/6</td>
<td>82</td>
<td>OFF</td>
</tr>
<tr>
<td>6</td>
<td>54/F/L</td>
<td>10</td>
<td>l-dopa 300 mg, Requip 16 mg</td>
<td>566.66</td>
<td>41/18</td>
<td>56</td>
<td>ON</td>
</tr>
<tr>
<td>7</td>
<td>57/F/B</td>
<td>9</td>
<td>l-dopa 1450 mg</td>
<td>1450</td>
<td>48/18.5</td>
<td>61</td>
<td>OFF</td>
</tr>
<tr>
<td>8</td>
<td>70/F/L</td>
<td>15</td>
<td>l-dopa 412.5 mg, Amantadine 100 mg, Mirapex 3 mg</td>
<td>712.5</td>
<td>43.5/20.5</td>
<td>53</td>
<td>ON (2)</td>
</tr>
<tr>
<td>9</td>
<td>66/F/B</td>
<td>15</td>
<td>l-dopa 1150 mg, Requip 15 mg</td>
<td>1400</td>
<td>40/13</td>
<td>67</td>
<td>ON</td>
</tr>
<tr>
<td>10</td>
<td>56/M/L</td>
<td>24</td>
<td>l-dopa 1150 mg, Tasmor 300 mg, Amantidine 200 mg</td>
<td>1500</td>
<td>44/17.5</td>
<td>60</td>
<td>OFF</td>
</tr>
<tr>
<td>11</td>
<td>61/M/L</td>
<td>11</td>
<td>l-dopa 1100 mg</td>
<td>1100</td>
<td>40/10</td>
<td>75</td>
<td>OFF (2)</td>
</tr>
<tr>
<td>12</td>
<td>58/M/B</td>
<td>13</td>
<td>l-dopa 1600 mg, Comtan 800 mg</td>
<td>1920</td>
<td>32.5/24</td>
<td>26</td>
<td>OFF and ON</td>
</tr>
<tr>
<td>13</td>
<td>62/M/R</td>
<td>13</td>
<td>l-dopa 825 mg, Mirapex 2.25 mg</td>
<td>1150</td>
<td>28.5/10</td>
<td>65</td>
<td>OFF</td>
</tr>
<tr>
<td>14</td>
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<td>17</td>
<td>l-dopa 650 mg, Comtan 800 mg, Requip 20 mg</td>
<td>1300</td>
<td>19.5/7.5</td>
<td>62</td>
<td>OFF and ON</td>
</tr>
<tr>
<td>15</td>
<td>62/M/R</td>
<td>9</td>
<td>l-dopa 1950 mg, Amantidene 200 mg</td>
<td>1950</td>
<td>27.5/4.5</td>
<td>84</td>
<td>OFF and ON</td>
</tr>
<tr>
<td>16</td>
<td>46/M/R</td>
<td>10</td>
<td>l-dopa 1450 mg, Comtan 200 mg</td>
<td>1530</td>
<td>NA</td>
<td>NA</td>
<td>OFF and ON</td>
</tr>
<tr>
<td>17</td>
<td>57/M/L</td>
<td>15</td>
<td>l-dopa 1600 mg, Comtan 800 mg</td>
<td>1920</td>
<td>48/20.5</td>
<td>57</td>
<td>ON</td>
</tr>
<tr>
<td>18</td>
<td>44/M/R</td>
<td>14</td>
<td>l-dopa 500 mg, Mirapex 2.25 mg, Amantidine 300 mg</td>
<td>825</td>
<td>52.5/18.5</td>
<td>65</td>
<td>ON</td>
</tr>
<tr>
<td>Mean</td>
<td>58.9 ± 6.8</td>
<td>13.3 ± 4.4</td>
<td>–</td>
<td>1301 ± 409</td>
<td>39.0 ± 8.9/15.1 ± 5.5</td>
<td>61.5 ± 13.0</td>
<td></td>
</tr>
</tbody>
</table>

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The recordings were analyzed offline using Spike2 software version 5 (CED, Cambridge, UK). Post stimulus time histograms (PSTHs, 250 us bin width, time base 150 ms normalized to firing rate in hertz) were constructed of the high frequency spiking of putative GABAergic output neurons of SNr in two patients. Spike analysis was performed using a spike matching template algorithm in Spike2. For PSTHs, only those spikes identified as belonging to the same template were included, i.e. a single unit was used for analysis.

FEP amplitudes were normalized to a percent scale, with the average of baseline measures in each patient considered as 100%, and sorted by medication state (OFF versus ON). Synaptic potentiation was evaluated in each patient in all medication states by fitting an exponential function to the FEP amplitudes using Sigma Plot software (SPSS, Chicago, USA): \[ y = y_0 + ae^{-bx} \] where \( y_0 \) is the plateau value (relative to baseline FEP amplitude) to which the function decays, \( a \) is the difference of the maximum (first) value of the exponential curve to \( y_0 \), and \( b \) describes the steepness of the curve. Population data was fit with a regression line if the fit had a significance value of \( P < 0.05 \).

All statistical comparisons were conducted using Sigmastat software (Systat Software Inc., San Jose, USA). A two-way ANOVA was performed on the normalized data testing the main effects of DRUG (ON versus OFF) and TIME following HFS. A post hoc Bonferroni t-test
tested all pairwise comparisons between ON and OFF at each time point.

Results

fEP test site location

All sites tested for a field evoked response were located in the SNr. We tested a total of 23 SNr sites in 18 patients. The approximate location of test sites included in the study is shown in Fig. 1. Recordings took place in dorsolateral SNr and test locations were independent of medication state. fEPs could not be evoked in the STN region using the stimulation protocols described above.

Field potential characteristics

Blocks of 1 Hz test pulses at incrementally increased stimulation distance were conducted in three patients and revealed a positive field persisting for 2.5 mm dorsoventrally through the SNr with the peak field amplitude having a latency (±SD) of 5.5 ± 0.8 ms (Supplementary Fig. 1). Post stimulus time histograms of the cell firing were analyzed in two cases and both revealed that the positive peak of the fEP occurred during inhibition of SNr cell firing in both the OFF and ON states. Notice that lower firing rate is associated with a larger field.

Fig. 2 Post stimulus time histograms of SNr neuronal firing in Parkinson’s disease. Traces show the average of 10 raw fEPs overlaid on a PSTH of the same time course (150 ms). Traces on the top are from a patient in the OFF state, before (left trace) and following (right trace) high frequency stimulation. Traces on the bottom are from the other side on the same patient following administration of one tablet of Sinemet 100/25°. The positive peak of the fEP occurs during inhibition of SNr cell firing in both the OFF and ON states. Notice that lower firing rate is associated with a larger field.

A paired pulse response curve was constructed in a patient by comparing the paired pulse ratio before and after high frequency stimulation at a range of interstimulus intervals (Fig. 3). Paired pulse depression is most apparent at short (20 and 30 ms) interstimulus intervals, both before and after HFS, evidenced by small paired pulse ratios (PPR). Before HFS, the PPRs at 20 and 30 ms intervals were 0.48 ± 0.018 and 0.76 ± 0.014, respectively. Following HFS, paired pulse depression was similar in magnitude at short interstimulus intervals (20 ms = 0.31 ± 0.11;


**Fig. 3** Paired pulse measures (A) Raw traces of SNr neuronal activity and fEPs during paired pulse measurements following high frequency stimulation. Traces, from top to bottom, are taken during paired pulse measurements with interstimulus intervals of 100, 50 and 30 ms, respectively. Greater paired pulse depression is seen at smaller interstimulus intervals, as denoted by the arrow on the bottom trace. (B) PPR curve. Shown is the PPR before and after high frequency stimulation in one patient at increasing interstimulus intervals (20, 30, 50, 100 and 200 ms). Paired pulse depression is greatest at 20–50 ms, and is greater in all tests following HFS.

30 ms = 0.74 ± 0.19). However, as the interstimulus interval increased, there was a marked increase in PPR. Before HFS, the PPRs for intervals of 50, 100 and 200 ms were 1.10 ± 0.050, 1.12 ± 0.051 and 1.07 ± 0.072, respectively. Following HFS the PPRs at the same intervals were 0.88 ± 0.037, 0.94 ± 0.065 and 0.94 ± 0.072. Thus, paired pulse depression is greatest at 20–30 ms, and is significantly greater in all tests following HFS (P = 0.015). Interstimulus intervals causing maximal paired pulse depression (20 and 30 ms) are of the same time scale as the inhibition of SNr firing to the single pulses used for evoking the fEPs shown in Fig. 2.

### Dopaminergic modulation of synaptic plasticity in the SNr

L-dopa treatment of Parkinson’s disease patients markedly improved motor UPDRS in all patients preoperatively (Table 1). Note that the total daily L-dopa equivalences are approximately 10× greater than the dose administered intraoperatively. Thirteen measures of fEP amplitude were made in patients in the OFF state. In these patients (see Table 1 for daily medication use and L-dopa equivalence), HFS did not induce a lasting change in fEP amplitude (Fig. 4A). A typical example is shown in Fig. 4C (open circles), where a modest increase in fEP amplitude returned to baseline by ~50–100 s. However, in some patients a larger initial increase in fEP amplitudes was seen, with a subsequent rapid decay toward baseline (Supplementary Fig. 3). In this case, the patient reported that he was only about 50% of his worst OFF state. We found a close inverse linear relation (r² = 0.81, P < 0.001) between the patients’ clinical OFF rating based on UPDRS III motor subscale (high values indicate worse motor symptoms) and the peak of activity-dependent synaptic plasticity induced by HFS (Fig. 5A). Patients with a higher UPDRS OFF score underwent less change in fEP amplitudes following HFS. The population data for the OFF group shown in Fig. 5B (open circles) reveals a significant initial 28.9 ± 4.9% increase in fEP amplitude following HFS that then decayed by 100 s to baseline. Regression analysis on population data from the OFF group revealed a y₀, fEP amplitude plateau value no different than baseline (2.3 ± 3.8% above baseline).

Following administration of L-dopa, the same HFS protocol induced a much larger increase in fEP amplitudes (Fig. 4B). Such fEP amplitude increases persisted over several minutes of testing (Fig. 4C; closed circles). There was no significant correlation between patients’ clinical ON rating based on UPDRS III motor subscale and the maximum value of activity-dependent synaptic plasticity induced in the ON group (r² = 0.02, data not shown). Twelve measures of fEP amplitude were made in patients in the ON state (Fig. 5B; closed circles). The largest fEP amplitude measures occurred immediately following the tetanus (200.3 ± 19.5 %) with subsequent measures showing a decrease in fEP amplitude at each time point with an exponential decay function. Regression analysis on population data from the ON group’s fEP amplitude measures revealed a y₀, fEP amplitude plateau value of 29.3 ± 5.2% above baseline. The regression function for the OFF and ON groups was highly significant with plateau values at P < 0.001. Additionally, for the ON group, a b value describing the steepness of the curve was determined to be 0.019 ± 0.0036 (P < 0.05), which corresponds to a half-life (1/0.019) of 52.6 s for the decay function. The OFF group’s b value was slightly higher at 0.0258 ± 0.0152 (not significant), corresponding to shorter half life of 38.8 s.

A two-way ANOVA of population data revealed a highly significant difference between ON and OFF groups (df = 1, f = 799, P < 0.001) and a significant difference between time points (df = 5, f = 69, P < 0.001). It also revealed an interaction between medication state and time, i.e. the ON/OFF amplitude is also dependent on the time of measurement (df = 5, f = 17, P < 0.001).
Discussion

The present study describes the characteristics of the positive fEP in the SNr of Parkinson’s disease patients, both OFF and ON dopaminergic medication. It is unique in providing human data supporting dopamine regulation of synaptic plasticity in the human basal ganglia, and suggests an important role for activity-dependent synaptic plasticity in basal ganglia dysfunction.

The SNr receives numerous projections from a multitude of sources, chief among them the inhibitory GABAergic projection from medium spiny neurons of the striatum (Parent and Hazrati, 1995a, b; Bolam et al., 2000). The external segment of the globus pallidus (GPe) also sends a small, but significant, GABAergic contribution to the SNr (Smith and Bolam, 1989). Additionally, the STN sends excitatory projections to the SNr. These glutamatergic projections from the STN to the output structures of the basal ganglia have been shown to form asymmetric synapses (Ribak et al., 1981), primarily on the dendrites and shafts, but with a very small number of boutons terminating on the somata (Kita and Kitai, 1987). The vast majority of the terminals in the region form symmetric synapses with the somata and are GABAergic in nature (Ribak et al., 1979, 1981). The rapid inhibitory responses characteristic of GABAergic transmission in basal ganglia structures are mediated by the activation of GABA_{A} receptors, which are found exclusively at symmetric synapses (Galvan et al., 2006).

Based on several observations, our stimulation protocol is primarily activating the inhibitory GABAergic projections, either from the striatum or the GPe. During our field recording measurements, all of the field potential measurements in the SNr are positive. Precht and Yoshida demonstrated the inhibitory nature of a positive field in the SNr by observing that spontaneous activity of neurons located in the SNr was strongly suppressed conjointly with the occurrence of the caudate-evoked (GABAergic) positivity (Precht and Yoshida, 1971; Yoshida and Precht, 1971). They also demonstrated that the time course of the intracellularly measured IPSP was the same as the positive fEP, and that the potential was blocked in its entirety by the GABA antagonist picrotoxin. In the present study, we also saw a positive fEP and its time course was the same as the inhibition of SNr activity, suggesting that the observed stimulation-evoked positive fEP is associated with an inhibitory event, most likely local GABA release.

Our paired pulse studies also point to activation of the GABAergic projections. In the SNr, dopamine D1 receptors are present at the terminals of the GABAergic striatonigral projection (Altar and Hauser, 1987; Barone et al., 1987). Previous striatal studies have shown that paired pulse depression predominates at synapses under the influence of D1 receptors, whereas paired pulse facilitation predominates at synapses at which D2 receptors are active (Guzman et al., 2003). In this study, paired pulse depression was evident at short interstimulus intervals prior to HFS and at all interstimulus intervals following HFS, suggesting...
that stimulation was involving the presynaptic D1 receptors. Slice studies indicate that stimulation of D1 receptors found in the SNr increases extracellular GABA (Floran et al., 1990; Aceves et al., 1991; Aceves et al., 1995; Timmerman and Abercrombie, 1996) and that this facilitated GABA release in turn enhances GABAergic neurons of the SNr (Radnikow and Misgeld, 1998). Taken together, these observations suggest that our positive fEP is inhibitory and GABAergic in nature and that dopamine plays a role in presynaptic regulation of GABA release in this region. Dopamine action in the basal ganglia is usually considered in terms of its modulation (or lack thereof in Parkinson’s disease) of indirect and direct striatal output via the dopaminergic nigrostriatal projection. In this region, dopamine comitantly provides excitatory inputs mediated by D1 receptor activation in the direct pathway and inhibitory inputs mediated by D2 receptor activation in the indirect pathway (Albin et al., 1989; DeLong, 1990). However, dopamine can also have dramatic effects in other regions of the basal ganglia. Indeed, nigral dopamine depletion has been shown to impair motor performance independent of striatal dopamine neurotransmission, while increased nigral dopamine release can counteract striatal dopamine impairments (Andersson et al., 2006). Here, we posit that dopamine can also act directly in the SNr by influencing synaptic plasticity at striatoni- nigral synapses.

Previous studies have suggested a link between LTP and dopamine at the corticostriatal synapse. Indeed, LTP is absent in dopamine lesioned (6-OHDA) rats, but can be restored with chronic l-dopa treatment (Picconi et al., 2003). Here, we sought to characterize activity-dependent synaptic plasticity in basal ganglia output neurons in 18 Parkinson’s disease patients, all of whom experienced a significant improvement in motor function during their preoperatively measured ON state (Table 1).

HFS did not induce a lasting change in fEP amplitude in patients in the OFF state. However, there was a strong correlation between the patients’ clinical OFF rating based on the UPDRS (high values indicate worse motor symptoms) and the initial degree of activity-dependent synaptic plasticity that could be induced in the same 12 h defined OFF state. Although the long-duration response to l-dopa (Nutt et al., 1995) and the variable half-life of some dopamine agonists (Rinne et al., 1997) could have interfered with the severity of the OFF state, 12 h of anti-Parkinson’s disease medication withdrawal induced a noticeable increase in UPDRS III motor scores in the patients included in this study (Table 1), and all measures of fEP amplitudes were done following a similar period of anti-Parkinson’s disease medication withdrawal.

Comparatively, during their intraoperative ON state, l-dopa intake coupled with HFS caused an increased fEP amplitude response in a manner reminiscent of LTP-like changes, in addition to decreased SNr firing rates. When including all patients categorized as being ON, there was no correlation between patients’ clinical ON rating based on UPDRS III motor subscale and the maximum inducible activity-dependent synaptic plasticity. The lack of correlation in the ON group is likely the result of variability in intraoperative ON states. Such variability could be derived from a number of sources including, but not limited to, ineffectiveness of a single dose of l-dopa in patients taking high doses, the timing of the transient ON period of a single dose, and when the measurements were performed.
The rate model predicts that the administration of L-dopa reduces the elevated firing rates of basal ganglia output neurons in the OFF state (Hutchison et al., 1997). Our observations of lowered SNr firing rates, coupled with enhanced inhibitory synaptic plasticity are consistent with the rate model, and give further hints as to how the loss of dopamine can directly affect GABAergic striatonigral synapses.

Limitations of the current study prevented the testing of whether dopaminergic regulation of GABAergic activity was achieved by a pre or postsynaptic mechanism, but previous work suggests that such actions are likely presynaptic. Enhancement in miniature inhibitory post-synaptic currents (mIPSCs) in the SNr via D1 receptor activity has previously been shown to be coupled with the formation of cAMP in the pre synaptic terminal (Jaber et al., 1996). The enhancing effects of D1 receptor stimulation on mIPSC activity in the SNr can be mimicked by forskolin, which is known to activate adenylate cyclase (Radnikow and Misgeld, 1998). A more recent study has proposed that D1-receptor mediated GABA release involves the cAMP/PKA pathway, with PKA ultimately phosphorylating key targets involved with GABA exocytosis, such as P/Q-type voltage-activated Ca2+ channels (to enhance Ca2+ influx), synapsins (to enhance vesicle trafficking) and SNARE proteins (to enhance vesicle docking, priming, and fusion) (Arias-Montano et al., 2007). Nevertheless, rapid postsynaptic changes in the SNr may also affect GABAergic activity. Recent work has demonstrated that neuronal activity can directly regulate the number of cell surface GABAARs by modulating their ubiquitination and consequent proteosomal degradation in the secretory pathway (Saliba et al., 2007). However, a link between dopamine and the level of GABAAR insertion and subsequent post-synaptic accumulation has not been established to date, but demonstration of such a link would support a post-synaptic action of dopamine. Dopamine is thought to have diverse and complex actions on the physiological activity of the basal ganglia. It can both inhibit and enhance neuronal activity, depending on the level of membrane depolarization and physiological state of the neuron (Calabresi et al., 2007).

The results here indicate that synaptic plasticity can be measured in basal ganglia output neurons of Parkinson’s disease patients and that the presence of plasticity is sensitive to low doses of L-dopa. In the absence of dopaminergic medication, plasticity is lacking following HFS. Conversely, following administration of dopaminergic medication, synaptic plasticity is facilitated in the SNr by HFS. The close correlation between motor behaviour and the potential of nigral synapses to undergo activity-dependent changes suggests that dysfunction of direct dopaminergic action at the basal ganglia output plays an important role in Parkinson symptomatology.

**Supplementary material**

Supplementary material is available at Brain online.

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**References**


