Effects of Apomorphine on Subthalamic Nucleus and Globus Pallidus Internus Neurons in Patients With Parkinson’s Disease

R. LEVY, 1 J. O. DOSTROVSKY, 1,3 A. E. LANG, 3,4 E. SIME, 3 W. D. HUTCHISON, 1,3 AND A. M. LOZANO 2,3

1Department of Physiology, Faculty of Medicine, University of Toronto, Toronto M5S 1A8; and 2Department of Surgery, University of Toronto, Division of Neurosurgery, 3The Toronto Western Research Institute, and 4Department of Medicine, University of Toronto, Division of Neurology, The Toronto Western Hospital, Toronto, Ontario M5T 2S8, Canada

Received 12 June 2000; accepted in final form 20 March 2001

Levy, R., J. O. Dostrovsky, A. E. Lang, E. Sime, W. D. Hutchison, and A. M. Lozano. Effects of apomorphine on subthalamic nucleus and globus pallidus internus neurons in patients with Parkinson’s disease. J Neurophysiol 86; 249–260, 2001. This study examines the effect of apomorphine (APO), a nonselective D1- and D2-dopamine receptor agonist, on the firing activity of neurons in the subthalamic nucleus (STN) and internal segment of the globus pallidus (GPi) in patients with Parkinson’s disease (PD). Single-unit microelectrode recordings were conducted in 13 patients undergoing implantation of deep brain stimulation electrodes in STN and 6 patients undergoing a pallidotomy. Doses of APO (2.5–8 mg) were sufficient to produce an on state, but not intended to induce dyskinetic movements. Following baseline recordings from a single neuron, APO was administered and the activity of the neuron followed for an average of 15 min. The spontaneous discharge of neurons encountered before (n = 309), during (n = 146, 10–60 min), and after the effect of APO had waned (n = 127, >60 min) was also sampled, and the response to passive joint movements was noted. In both nuclei, APO increased the overall proportion of spikes in burst discharges (as detected with Poisson “surprise” analysis), and a greater proportion of cells with an irregular discharge pattern was observed. APO significantly decreased the overall firing rates of GPi neurons (P < 0.01), but there was no change in the overall firing rate of neurons in the STN (P = 0.68). However, the mean firing rates of STN neurons during APO-induced movements (choreic or dystonic dyskinesias) that occurred in four patients were significantly lower than off-period baseline values (P < 0.05). Concurrent with a reduction in limb tremor, the percentage of cells with tremor-related activity (TCs) was found to be significantly reduced from 19 to 6% in the STN and 14 to 0% in the GPi following APO administration. APO also decreased the firing rate of STN TCs (P < 0.05). During the off state, more than 15% of neurons tested (STN = 93, GPi = 63) responded to passive movement of two or more joints. After APO, this proportion decreased significantly to 7% of STN cells and 4% of GPi cells (STN = 28, GPi = 26). These findings suggest that the APO-induced amelioration of parkinsonian symptoms is not solely due to a decrease in overall activity in the GPi or STN as predicted by the current model of basal ganglia function in PD.

INTRODUCTION

The characteristic loss of dopaminergic innervation of the striatum in animal models of Parkinson’s disease (PD) has been associated with an excessive tonic inhibition of thalamic motor and brain stem nuclei (DeLong 1990) by the globus pallidus internus (GPi) (Filion and Tremblay 1991; Miller and DeLong 1987). The administration of apomorphine (APO), a nonselective dopamine receptor agonist, decreases the mean spontaneous discharge of the GPi in monkeys rendered parkinsonian with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Filion et al. 1991) and in patients with PD (Hutchison et al. 1997a; Merello et al. 1999). This decrease occurs concurrently with the improvement in bradykinesia within the time course of the behavioral on period associated with the medication. An APO-induced decrease of GPi activity in PD patients is hypothesized to result from two separate striatal influences, 1) driving of the direct GABAergic striatopallidal pathway and 2) inhibition of the glutamatergic drive from the subthalamic nucleus (STN) to the GPi (Bergman et al. 1994; Miller and DeLong 1987). The latter influence is believed to be due to stimulation of striatal dopamine D2 receptors resulting in disinhibition of the GABAergic globus pallidus externus (GPe) projections to the STN (Albin et al. 1989). An increase in GPe activity following APO administration has been reported in both MPTP-treated monkeys (Filion et al. 1991) and PD patients (Hutchison et al. 1997a). APO has also been shown to decrease the firing rates of STN neurons of rats with 6-hydroxydopamine–induced lesions of the nigrostriatal pathway (Kreiss et al. 1997). However, the effect of APO on the STN in parkinsonian monkeys or humans is not known.

The amelioration of parkinsonian symptoms following lesions of the GPi or STN is consistent with the view that both these nuclei are hyperactive in parkinsonian monkeys and patients with PD (Aziz et al. 1991; Bergman et al. 1990; Gill and Heywood 1997; Lang et al. 1997; Lozano et al. 1995; Obeso et al. 1997). However, many of the changes in the activity of neurons of the GPi and STN that occur following the depletion of striatal dopamine represent changes in the pattern of neuronal discharge in addition to changes in the overall firing rate. In MPTP-treated monkeys, increased bursting activity is observed in STN (Bergman et al. 1994) and GPi (Filion and Tremblay 1991; Wichmann et al. 1999). Oscillatory activity that is time locked to the parkinsonian tremor (i.e., tremor activity) is also observed in both nuclei of MPTP.
monkeys (Bergman et al. 1994; Nini et al. 1995) and PD patients (Hutchison et al. 1997b; Rodriguez et al. 1998). GPi neurons in parkinsonian animals also have exaggerated responses and multi-limb receptive fields (Filion et al. 1988). Since these changes occur in response to decreased striatal dopamine, it is possible that the antiparkinsonian effect of APO may be related to a reversal of these pathological patterns and rates of activity in the GPi and STN. The aim of the present study was to test the hypothesis that changes in neuronal firing patterns and receptive field responses of the STN and GPi occur concurrently with observed improvements in the clinical features of PD patients due to the administration of APO.

M E T H O D S

Patient groups and procedures

STN PATIENT GROUP. Studies of the STN were performed in 13 individuals undergoing microelectrode-guided placement of deep brain stimulation (DBS) electrodes for the treatment of the symptoms of PD. The group consisted of seven females and six males and at the time of operation had a mean age of 62.8 yr (range, 46–76 yr). The median OFF Hoehn and Yahr rating of the time of operation had a mean age of 62.8 yr (range, 46–76 yr). The APO was titrated to establish a dose sufficient to produce an ON state, but not characterized. All STN patients were challenged preoperatively with confirmed that there were no differences in the neuronal discharge comparison between these two individuals and the other STN patients drilled, and only data from cells recorded at least 30 min after propofol had completely worn off were included. A subsequent comparison between these two individuals and the other STN patients confirmed that there were no differences in the neuronal discharge characteristics. All STN patients were challenged preoperatively with APO to establish a dose sufficient to produce an ON state, but not produce dyskinetic movements. The average dosage was 4.7 ± 0.8 mg. All patients were premedicated with domperidone (Motilium, Janssen), a peripherally acting dopamine receptor antagonist to minimize undesirable side effects (nausea, vomiting, and hypotension). To rate the clinical effect of APO, patients underwent a preoperative partial Unified Parkinson’s Disease Rating Scale motor assessment (UPDRS; Part III) during the practically defined OFF state (12–14 h after last medication) and during an APO-induced ON state. Motor scores were determined by summing UPDRS items 18–31 (i.e., greatest possible score is 108).

The use of microelectrode recording to localize DBS electrode placement in the STN has previously been described in detail (Hutchison et al. 1998). Briefly, parasagittal trajectories at either 10.5 or 12 mm from the midline passed through the thalamic reticular nucleus and/or anterior thalamus, zona incerta, STN, and the substantia nigra pars reticulata. Single-unit microelectrode recording and stimulation mapping allowed the identification of physiological landmarks and cell localization. Exploration of the neuronal activity was carried out in the presumed motor portions of the STN. The main characteristics that were identified to localize the motor portion of the STN were neurons with tremor-related activity, neurons that responded to passive or active movements, and microstimulation effects such as tremor reduction or arrest. The anterior-posterior limits of the STN were delimited by regions with sparse neuronal activity and a reduced background noise compared with that observed in the STN. Single units were recorded using Parylene-C–coated tungsten microelectrodes with an exposed tip size of 15–25 μm. Microelectrode tips were plated with gold and platinum to reduce the impedance to about 0.2–0.4 MΩ at 1 kHz. Signals were amplified and filtered using Axon Instruments equipment (GS3000 system, Axon Instruments, Foster City, CA). All neuronal data were recorded simultaneously with wrist flexor/extensor electromyographic and accelerometer to monitor movement. Data were stored on an analog videotape by a digital recorder (model S-100, Instrutech, Port Washington, NY) and analyzed off-line with a similar setup. Single-unit event times were discriminated using a dual window discriminator (DDIS-1, BAK Electronics, Mount Airy, MD) and a storage oscilloscope in spike-trigger mode.

All patients were studied during the stereotaxic surgery 12–14 h after the last oral dose of levodopa and were considered to be in the OFF state (i.e., nonmedicated). The time course of the effects of APO was evaluated intra-operatively by a trained clinician (E. Sime). Patients were asked to report when they started to feel the effects of APO. The time and duration of limb tremor or the occurrence of drug-induced dyskinetic or dystonic movements were also noted. In each patient, a stable and well-isolated single unit was found, and APO was administered subcutaneously following 2–3 min of stable recording. These neurons were recorded for as long a period as possible, usually until there was an objective effect of the medication (tremor reduction, dyskinesias) and patients felt a subjective beneficial effect of medication. However, in some cases stable recordings from single neurons were lost after a shorter period of time. Thus other neurons sampled before and after APO administration were included in a population study if they were well isolated (i.e., a high signal-to-noise ratio of the action potential vs. the background noise) and stable. The median sampling time of each neuron was 28.5 s (n = 611, minimum = 20 s), and the median number of discriminated action potentials per neuron was 1,100. Patients were always at rest when tonic activity was sampled.

GPi PATIENT GROUP. The recording procedures for the GPi patients have been previously described (Lozano et al. 1996). The patients in the GPi study were those undergoing pallidotomy. This group consisted of six patients in whom APO-induced changes in population firing rates were previously reported (Hutchison et al. 1997a). In that study, GPi neurons were sampled from the pre-sumed posteroventral portion of the GPi, an area corresponding to the location of the subsequent pallidotomy. Further analysis of changes in firing patterns, receptive fields, and tremor cells are now reported here. These six patients received an average of 3.7 ± 0.6 mg of APO during their pallidotomy. For this group of patients, the median sampling time of each neuron was 30.1 s (n = 261, minimum = 28 s), and the median number of discriminated action potentials per neuron was 1,880.

The indications for pallidotomy were akinetic-rigid parkinsonian syndromes with fluctuating ON-OFF periods and drug-induced dyskinesias (Lozano et al. 1996). Similarly, the indications for STN DBS are bilateral limbic and axial manifestations of Parkinson’s disease, medication-refractory motor fluctuations, and levodopa-induced dyskinesias (Kumar et al. 1998), thus it is unlikely that the two patient groups differ significantly with respect to their symptoms.

Data analysis

All neuronal data were subdivided into three groups. The “pre-APO time” block group included all cells sampled before the administration of APO, the “APO period” time block group included all cells sampled between 10 and 60 min after the administration of APO, and the “post-APO” time block group comprised all cells sampled at 60 min or more following APO administration. This breakdown followed the time course of GPi and GPc cells following APO administration used in previous studies (Hutchison et al. 1997a; Merello et al. 1999) and matched the time course of the clinical effects in the preoperative APO trials of the 13 STN patients. It has also been reported that APO is rapidly absorbed following subcutaneous injection, with peak levels achieved within 5–10 min in most patients (Gancher 1995). Tonic spontaneous single-unit activity included in this study was collected with the patient at rest and without any passive joint manipulation or voluntary movements. Both “aperiodic” and “periodic” bursting discharge in addition to average neuronal discharge (firing rate = FR) were characterized.
Bursting discharge was quantified using two different burst measures. 1) The Poisson “surprise” method of burst detection as described by Legendy and Salcman (1985) was employed to detect burst discharges with a Poisson surprise value of >5. The proportion of spikes in burst discharges compared with the total number of spikes sampled for each cell was determined before and after APO. This gave a measure of the “burstiness” of each neuron since irregularly discharging neurons will have a greater proportion of spikes that participate in bursts when compared with regularly discharging neurons. 2) The spike discharge pattern was also characterized as being “regular,” “random,” or “irregular” by comparison to a Poisson process. The method employed here is a simplified version of a burst analysis scheme presented by Kaneko and Vitek (1996). Briefly, the discharge density was determined by calculating the number of spikes in an interval equal to the reciprocal of the mean firing rate. The number of occurrences of no spikes, one spike, two spikes (and so on) in each time interval was then counted, and a discharge density histogram was constructed. This discharge density histogram represents the probability distribution of the neuron’s discharge density and can easily be compared with a discharge density of a Poisson process with a mean of 1 by using a $\chi^2$ goodness-of-fit test. If the neuronal discharge pattern was random, its discharge density distribution would be statistically similar to that of a Poisson process. If the neuron’s discharge pattern was significantly nonrandom, then two possibilities existed. Either the spikes occurred in a regular discharge pattern where the probability of finding one spike per time segment was high, or the spikes occurred in an irregular discharge pattern where the probability of finding no spikes or many spikes per time segment was high. Since a Poisson process with a mean of one has a variance equal to one, the former case represents a significantly non-Poisson discharge density distribution with a variance of less than one, and the latter case represents a significantly non-Poisson discharge density distribution with a variance of greater than one. This method was a convenient way to characterize regular, random, and irregular spike discharge patterns between groups of neurons since the patterns of neuronal discharge between two cells with different mean tonic activity could be directly compared.

Bursting discharge was defined as periodic if bursts were rhythmic (i.e., a roughly constant period of time between each burst). Periodic bursting discharge was quantified using both time (autocorrelation) and frequency (spectral) domain analysis. Autocorrelation analysis was used to detect and grade periodic neuronal activity that could resemble oscillatory discharge yet might be more intermittent in nature. Autocorrelation histograms of spike trains were plotted for 1.5 s (150 bins, 10 ms each) and 200-ms (200 bins, 1 ms each) intervals and quantified to the units of rate (spikes/s) (Ables 1982). The latter histograms were smoothed over 5-ms bins using a raised cosine bell. Autocorrelation distributions were used to index the strength of the periodic activity. The strength of the oscillation was graded according to standard examples given by Karmon and Bergman for oscillating cells in MPTP-treated nonhuman primates (Karmon and Bergman 1993). For example, only tremor cells (TCs; cells that oscillate within the frequency range 3–8 Hz, close to the frequency of the limb tremor) with a strength of five or greater were considered for further analysis. High-frequency (>10 Hz) periodic oscillatory activity was statistically evaluated by locating at least two successive peaks (not including the initial peak that is related to bursting discharge) within the first 100 ms of the autocorrelation function (constructed from 200 bins, 1 ms each). Peaks were considered significant if they were found outside the area defined by the mean ± 2 SD of the 100- to 200-ms time interval. The frequency of oscillation was then determined by calculating the reciprocal of the peak-to-peak time of two successive peaks. Spectral analysis was used to quantify long-duration oscillatory activity. Neural discharge was converted into waveform data that were used in subsequent fast Fourier transform (FFT) analysis. The event channels were converted to waveform data by integration of spike times over 5 ms (i.e., digitization rate of 200 Hz) and smoothed over a period of 10 ms using a raised cosine bell function. Standard spectral techniques resulted in 256 spectral estimates between 0 and 100 Hz, thereby yielding a frequency resolution of 0.39 Hz (Spike2 software, Cambridge Electronic Design, Cambridge, UK). Spectral peaks were considered significant only if they had a signal-to-noise ratio of >4. Noise was taken as the average of all spectral estimates between 0.8 and 30 Hz.

Only cells that had a stable baseline and were clearly distinguishable from the background noise were chosen for receptive field (RF) mapping. The time spent investigating the somatosensory RF properties of a neuron was considerably longer than the time spent recording tonic activity when the patient was at rest. RF mapping took on the order of 3–5 min per cell and was carried out following tonic activity sampling. Passive movements were performed in a vigorous manner about a single joint. A positive response was indicated by a phasic excitation or inhibition of neural discharge that was robust to repeated trials. Contra- lateral and ipsilateral wrist, elbow, and shoulder (and sometimes ankle) manipulation were assessed. In this study, we did not check for knee and hip responses because brisk movements of the entire leg often resulted in slight movement artifacts in the microelectrode recording or loss of the neuronal recording. The responses of all of the cells tested to passive limb manipulation were divided into the following categories: no response (no detectable RFs), single joint, double joint, and multiple joint responses (more than 2 joints or bilateral responses).

Statistical analyses of the effect of APO on populations of neurons were carried out using ANOVA followed by an All Pairwise Multiple Comparison Procedure (Student-Newman-Keuls) for normally distributed data. In cases of nonnormality, data were compared using an ANOVA on ranks procedure (Kruskal-Wallis) followed by an All Pairwise Multiple Comparison Procedure (Dunn’s Method). Clinical scores were compared using the Wilcoxon signed-rank test. Differences in proportions of cells in different categories were evaluated using $\chi^2$ tests. Statistical significance was assigned at $P < 0.05$ (i.e., $\alpha = 0.05$). Unless indicated otherwise, all errors in this study are reported as means ± SE.

**RESULTS**

**Clinical effects of APO**

The preoperative beneficial effect of APO was similar in both patient groups. In the STN group of 13 patients, APO significantly improved the group median UPDRS motor scores from 44.5 to 15.5 ($P < 0.01$). In the GPI group of six patients, APO significantly improved the group median UPDRS motor scores from 46.0 to 16.5 ($P < 0.01$).

**STN GROUP.** All of the 13 patients in the STN group were given an intra-operative amount of APO that was intended to produce an ON state without dyskinesia (determined from the preoperative APO trials). However, in three patients this dose was insufficient, and a supplemental APO injection was administered ($\frac{1}{2}$ the original dose). Eleven patients reported feeling ON at a mean time of 18.9 ± 9.0 (SD) min after the APO injection (the 2nd injection for the 3 patients receiving a supplemental dose). Eleven patients reported feeling ON at a mean time of 18.9 ± 9.0 (SD) min after the APO injection (the 2nd injection for the 3 patients receiving a supplemental dose). Conditions in the operating room such as the stress and anxiety for the patient as well as a prolonged drug holiday likely led to the fact that some patients displayed APO-induced dyskinetic movements and others required higher doses than preoperative amounts. The two patients that did not report feeling ON were not given a supplemental dose of APO since they displayed drug-induced movements (dystonic dyskinesias). In total, there were four patients who became...
dyskinetic. In all cases, dyskinetic (choreic or dystonic) movements were mild, intermittent, and involved low-amplitude movements. Limb tremor was significantly reduced or abolished at 20 ± 5 (SD) min in six of the nine patients that had limb tremor at the beginning of surgery.

**GPi GROUP.** Following the intra-operative administration of APO, two patients reported that they were becoming ON at 6 and 11 min, but they did not display APO-induced involuntary movements. At the start of the procedure, three patients had a pronounced limb tremor, and the administration of APO was found to abolish limb tremor and to produce dyskinesias. The remaining patient did not report feeling ON, yet this patient developed mild drug-induced dyskinesias.

**Single-unit studies**

**STN.** A single neuron was continuously recorded before and following the administration of APO in 10 patients. These neurons were recorded for at least 9 min and a mean time of 14.7 ± 4.6 (SD) min after APO administration. Figure 1A (left) demonstrates that there were varied changes in the firing rates of 10 STN neurons following APO administration. The top right and bottom right panels of Fig. 1A show the percent change versus baseline values of the firing rates and the proportion of spikes in bursts, respectively. Decreases in the average firing rate of >30% with a concurrent increase in the proportion of spikes in bursts were observed in four neurons; three of these patients reported feeling the effects of the medication during the period of the recordings. A firing rate histogram of one of these neurons (indicated by the thick dashed line) is displayed in the left panel of Fig. 1B, and examples of the pattern of firing of this neuron are shown in the right panel. This patient became mildly dyskinetic during 14–43 min post-APO. Dyskinesias also appeared during the period of recording in one other case. In both cases firing rates were more than 30% below the pre-APO value at the time the dyskinesias appeared (indicated with X in Fig. 1A).

Changes in tonic firing rate were also associated with the emergence of tremor-related activity and concurrent limb tremor in one cell. Figure 1C shows the changes in the autocorrelogram (calculated over nonoverlapping 2-min time segments) of the STN neuron that, following an initial increase, was seen to decrease its firing rate by 40% (marked by the thick dotted line in A). The patient developed prominent bilateral tremor in the upper extremities shortly after the administration of APO. This is seen in some patients and has been termed “beginning-of-dose deterioration” (Merello and Lees 1992). The development of bilateral tremor matched the time course of the increase in tonic firing rate and the appearance of 4-Hz oscillatory neural discharge. Furthermore, a subsequent decrease in firing rate was accompanied by a decrease in the oscillatory neural activity and limb tremor.

**GPI.** Three GPi neurons were recorded for at least 10 min following the administration of APO. The change in firing rates (top) and the corresponding change in the proportion of spikes in bursts (bottom) of these neurons are shown in Fig. 2. All three patients felt the effects of the medication during this time (time of occurrence indicated by black dots), which corresponded to a reduction from the pre-APO baseline firing rates (mean decrease ~30%). These decreases in firing rate were associated with increases in the proportion of spikes in bursts. One patient became mildly dyskinetic after a 40% decrease in the firing rate of the single cell ( – – – – , ×). The cell had a discharge rate of ~60 spikes/s during episodes of mild dyskinesia. In another patient (○○○), there was a marked reduction in spontaneous discharge that coincided with a cessation of ongoing limb tremor at 5 min after the APO dosing (†) and the appearance of dyskinetic movements 4 min after the end of the single-unit recording.

**Population analysis; firing rates and pattern**

**STN.** There were 216, 95, and 74 cells examined during the pre-APO period, APO period, and post-APO time period, respectively (see METHODS). The distribution of the average neuronal firing rates and the discharge firing patterns of STN neurons are shown in Fig. 3A. The means of the firing rate distributions were not significantly different from the baseline value of 37.1 ± 1.1 Hz during the APO period or post-APO period (P = 0.68, ANOVA on ranks; Fig. 3A, left). Note also that there was no change in the shape of the population firing rate distribution. However, there was an increase in the proportion of neurons that discharged in an irregular or random manner (*P < 0.05, χ² = 4.75, n = 311). These changes in discharge firing patterns were accompanied by an increase in the overall proportion of spikes in bursts from 20.2 ± 0.8% to 25.6 ± 1.6% during the APO period (†P < 0.01, ANOVA on ranks) and remained elevated at 23.8 ± 1.5% during the post-APO period (Fig. 3A, right). In four of the patients with APO-induced dyskinesias, there was a significant decrease from the baseline mean firing rate of 38.3 ± 1.7 Hz (n = 63) to 28.1 ± 1.8 Hz (n = 25; P < 0.05, ANOVA on ranks), and the proportion of spikes in bursts increased from 19.8 ± 1.3% to 23.8 ± 1.8% (P < 0.05, ANOVA on ranks) for cells recorded during the time period when these movements were present.

**GPI.** The distributions of the average neuronal firing rates and the discharge firing patterns of GPi neurons are shown in Fig. 3B. There was a significant decrease of the average neuronal firing rate to 39 ± 2.5 Hz (n = 51) during the APO period compared with both pre-APO (72 ± 2.9 Hz, n = 93) and post-APO periods (82 ± 4.1 Hz, n = 53; †P < 0.01, ANOVA on ranks; Fig. 3B, left). The proportion of neurons that discharged in an irregular or random pattern significantly increased during the APO period (*P < 0.01, χ² = 16.2, n = 197). The proportion of spikes in bursts was also increased from 16.2 ± 1.2% to 26.1 ± 2.4% during the APO period (†P < 0.01, ANOVA on ranks). In contrast to the STN group, there was no difference in the change of the mean firing rates from pre-APO values between the patients with and without dyskinesias. In addition, the mean firing rates of cells recorded in the four patients during time periods with dyskinetic movements (n = 30, 40.5 ± 5.0 Hz) to those recorded in the two patients without dyskinesias during the APO period (n = 18, 39.6 ± 5.2 Hz) were not different (P = 0.45, ANOVA on ranks). There was no difference in the proportion of spikes in bursts between the two groups (P = 0.40, ANOVA on ranks).

**Population analysis; oscillatory cells**

**STN.** Before the administration of APO, 19% (42/216) of all STN neurons examined displayed significant tremor-related activity. These cells were found in 10 patients, 9 of whom had
limb tremor at the beginning of the surgery. TCs were observed during episodes with limb tremor and also during episodes with no noticeable limb tremor. An example of a typical autocorrelogram and power spectrum for an STN TC is given in Fig. 4A. The average overall spontaneous discharge (FR) of these cells was $43.5 \pm 2.2$ Hz. Oscillatory frequencies other than within the limb tremor frequency range were also observed. There were 18 neurons that displayed a high-frequency oscillatory component that was between 10 and 30 Hz. An example is shown in Fig. 4B. The average FR of these high-frequency oscillatory cells was $43.6 \pm 4.7$ Hz. Neurons with high-frequency oscillatory components were located in areas containing TCs; 12/18 high-frequency oscillatory cells were located within 1 mm of TCs. In addition, six of the TCs were also
observed to have a high-frequency oscillatory component (>10 Hz). An example of an STN neuron with multiple frequency oscillatory behavior is shown in Fig. 4C. Both TCs and high-frequency oscillatory cells were found to have significantly higher firing rates than nonoscillatory cells (34.6 ± 1.2 Hz, n = 156; P < 0.01, t-test). The distribution of oscillatory frequencies of STN neurons sampled during pre-APO period, APO period, and post-APO period is shown in Fig. 5A.

Following the administration of APO, the proportion of TCs encountered was significantly reduced from 19 to 6.3% (6/95 neurons sampled in 11 patients; P < 0.01, χ² = 9.34, n = 311). In the six patients in whom APO significantly reduced or abolished limb tremor (49 neurons sampled), only one TC and eight neurons with high-frequency oscillatory activity were encountered, and they were all in one patient (see Fig. 5A, middle left). The mean firing rate of the six TCs was 24.6 ± 5.0 Hz and was significantly lower than the mean firing rate of TCs before the administration of APO (P < 0.05, ANOVA). Neurons with high-frequency oscillatory activity had similar firing rates during APO to those encountered before APO dosing (43.2 ± 5.3 Hz). APO did not affect the firing rates of non-oscillatory STN cells (n = 76), but these cells did have a greater proportion of spikes in bursts when compared with pre-APO period values (n = 156; P < 0.01, t-test).

During the post-APO period, the proportion of the TCs (14/74) was the same as that found before the administration of APO (P = 0.95, χ² = 0.34, n = 290). These TCs were found in six patients (limb tremor was abolished and no TCs were found during the APO period in 3 of these). The mean firing rate of the TCs was 44.0 ± 5.9 Hz, which was significantly higher than that found during the APO period and the same as the mean TC firing rate of the pre-APO period (P < 0.05, ANOVA).

GPi. Before the administration of APO, 14% (13/93) of all GPi neurons examined displayed tremor-related activity. These cells were located in four patients (3 of whom had a pronounced limb tremor at the start of the procedure). The average firing rate of the TCs was 84.5 ± 7.3 Hz and was significantly higher than the firing rate of the nonoscillatory GPi neurons in these four patients (65.3 ± 3.1 Hz, n = 54 cells, P < 0.01). There were no GPi TCs encountered that also had a high-frequency oscillation component (see Fig. 5B). Only two GPi neurons with a high-frequency oscillation (>10 Hz oscillation) were encountered in all six patients. The distribution of oscillatory frequencies of GPi neurons sampled during pre-APO period, APO period, and post-APO period is shown in Fig. 5B. There were no TCs encountered in the GPi during the APO period (51 neurons examined in the 6 patients). During the post-APO period 22.6% (12/53) of the neurons were TCs. These neurons were located in the same four patients who displayed TC activity before the administration of APO. The firing rate of the 12 TCs was 96.0 ± 8.9 Hz. This was significantly higher than the firing rate of nonoscillatory cells found in this group of four patients (78.8 ± 4.5 Hz, n = 37, P < 0.05).

Population analysis; receptive fields

STN. The proportion of neurons that responded to movements about one, two, or more joints or had no detectable RFs (i.e., no response) are displayed in Fig. 6A. The administration of APO was found to decrease the proportion of cells that had a response to limb movement from 56 to 25% and decrease the proportion that responded to two joints from 13 to 7% (P < 0.05, χ² = 8.31, n = 150). There were no neurons with multiple or bilateral RFs found during the APO period. During the post-APO period, the proportion of cells with no detectable RFs decreased to 52% (n = 14). The relative proportions of single RFs to double and multiple RFs did not significantly change in either the APO period or the post-APO period (P = 0.71, χ² = 0.66, n = 74). When the average firing rates, bursting, or oscillatory patterns were compared between groups of cells that had no response, single, double, or multiple RFs, no significant differences were evident, and APO did not preferentially affect any of these groups.

GPi. The proportions of neurons that had no detectable RF and those that responded to movements about one, two, or more joints or had no detectable RFs (i.e., no response) are displayed in Fig. 6B. The administration of APO decreased the proportion of cells that responded to limb movement from 54 to 20% (P < 0.01, χ² = 9.43, n = 102). APO decreased the proportion of cells that responded to passive movement of two joints from 10 to 4%. During the APO period, there were no neurons with a multiple or bilateral RF encountered. The relative proportions of single RFs to double and multiple RFs did not significantly change during this period or during the post-APO period (P = 0.11, χ² = 4.50, n = 46). There were no significant differences between firing rates, bursting, or oscillatory behavior of cells that did not have a detectable RF and those that did have detectable RFs nor was
there any significant differential effects of APO on the neuronal activity of these groups.

**DISCUSSION**

This study demonstrates that the administration of APO in patients with PD causes marked changes in the firing pattern, firing rate, and responses to passive limb movement in the GPi and STN. An APO-induced reduction in mean spontaneous activity occurred in the GPi at both the single-cell and population level and is consistent with the effects of dopaminergic medication in MPTP-treated monkey studies (Boraud et al. 1998; Filion et al. 1991) and the prediction of the current model of the pathophysiology of the basal ganglia in PD (Albin et al. 1989; DeLong 1990). This is the first demonstration of the effects of APO in the primate STN. According to the current model of the basal ganglia, systemic administration of APO is predicted to decrease the mean firing rates of STN neurons (Bergman et al. 1994; Miller and DeLong 1987). However, evidence supporting this prediction is unclear. APO has been shown to decrease the spontaneous discharge of STN neurons in 6-OHDA–treated rats (Kreiss et al. 1997), but contrary to predictions APO increases the firing rates of STN neurons in intact rats (Kreiss et al. 1997). In the present study, both increases and decreases were observed in the mean firing rates of single STN neurons following APO administration. It is unlikely that this was simply due to the fact that the cells were monitored for only a short period of time. In five STN cells monitored for 15 min or more, no mean overall increase or decrease was observed, although the patients started to experience the effects of APO during the recording period (Fig. 1A). In addition, in contrast to GPi, there was no significant difference in the overall mean firing rate of STN neurons recorded in the APO period when compared with pre-APO values (\( \chi^2 = 4.75, n = 311 \)). APO also significantly increased the proportion of spikes in bursts in STN neurons when compared with pre-APO values (\( \chi^2 = 16.2, n = 197 \)) and the proportion of spikes in bursts of GPi neurons (\( \chi^2 = 0.01, \text{ANOVA on ranks} \)).

The variability in the effect of APO on the firing rates of single STN neurons observed in this study may be due to sampling of functionally different neurons that have different connectivity and sensitivity to APO. Heterogeneity in the func-

![Graphs showing the effects of APO on populations of neurons in the STN and GPi.](http://jn.physiology.org/)

**FIG. 3.** Graphs and plots showing the effects of APO on populations of neurons in the (A) STN and (B) GPi. Population firing rate distributions are shown in the leftmost panels (10-Hz bins). The downward arrows in these graphs represent the means of the corresponding distributions. Numbers in parentheses at the bottom of the left set of vertical bars (i.e., proportion of random, irregular, and regular discharges) indicate the number of cells within each group. Data from the same cells were used to construct the firing rate distributions and the right bar graphs. A: there was no significant difference in the mean firing rate of neurons in the STN due to APO administration, but the proportion of cells with a regular discharge pattern decreased during the APO period (\( \chi^2 = 0.05, \chi^2 = 1.25, \text{ANOVA on ranks} \)). APO also significantly increased the proportion of spikes in bursts in STN neurons when compared with pre-APO values (\( \chi^2 = 0.01, \text{ANOVA on ranks} \)). B: APO reduced the mean firing rate of GPi neurons from pre-APO values and post-APO means (\( \chi^2 = 0.01, \text{ANOVA on ranks} \)). APO significantly increased the proportion of neurons with irregular or random firing patterns (\( \chi^2 = 0.01, \text{ANOVA on ranks} \)).
tion of STN neurons is suggested by the diversity of brain regions that provide afferent input to the STN. As well as input from GPe, the STN receives massive input from the cerebral cortex (Canteras et al. 1990; Carpenter et al. 1981). The STN forms reciprocal projections with the pedunculopontine nucleus (Hammond et al. 1983; Lavoie and Parent 1994) and receives input from the parafascicular nucleus of the thalamus (Mouroux et al. 1995; Orieux et al. 2000). In a recent study by Orieux et al. (2000), it was demonstrated that STN hyperactivity in the 6-hydroxydopamine–treated rat model of PD is due in part to an increase of excitatory input arising from both these nuclei. Dopaminergic agonists can also influence STN activity by acting on dopaminergic cells projecting to the STN (Campbell et al. 1985; Descarries et al. 1987; Francois et al. 2000; Hassani et al. 1997; Meibach and Katzman 1979) and at dopamine receptors within the STN itself (Bouthenet et al. 1987; Campbell et al. 1985; Hassani and Feger 1999; Kreiss et al. 1996; Mehta et al. 2000).

In the four of six GPi patients presenting mild dyskinesias, the mean activity of the GPi was not significantly lower than in those GPi patients without dyskinesias. It is, however, possible that the neurons in this sample were not related to the area of the body in which the dyskinesias occurred. A recent study by Papa et al. (1999) found that GPi neurons in MPTP-treated monkeys were nearly silenced during episodes of drug-induced dyskinesias. However, the dyskinesias were produced by administration of oral carbidopa/levodopa doses that were at least twice as high as those used to reverse parkinsonian disability.

**FIG. 4.** Examples of oscillatory activity in STN neurons (A–C) with 3 types of periodic oscillatory activity before the administration of APO. Each row shows a smoothed autocorrelogram (time base of 750 ms and binwidth is 1 ms) of the neuronal discharge, power spectrum of the spike signal (0.39-Hz resolution), and an inter-spike interval histogram for a single neuron (1-ms bins). A: an STN neuron with tremor-related activity. Patient was tremulous at this time. A strong single peak at $\sim 4$ Hz is evident in the power spectrum. B: STN neuron with high-frequency oscillatory activity centered around 16 Hz. The interstimulus interval (ISI) histogram is single peaked and nearly symmetrical, suggesting that the oscillatory pattern is not due to intermittent bursting activity. C: STN neuron with multiple frequency oscillatory behavior. The power spectrum gives one peak at 4 Hz and another at $\sim 18$ Hz. Dashed horizontal lines in autocorrelograms are the average discharge rate. The number at the bottom right corner is the number of events used to construct the autocorrelogram. The signal-to-noise ratio of peaks in the power spectrum is indicated by the number above each peak. The arrows in the inter-spike interval histograms indicate the median inter-spike interval.

**FIG. 5.** Distribution of oscillation frequencies of cells in the STN (A) and GPi (B). Total number of cells sampled is given in top left of each panel. Cells with tremor activity or a high-frequency oscillatory component are represented by gray squares. Cells with tremor activity and a high-frequency component are represented by white squares (i.e., both frequencies are denoted so that each cell is represented by 2 squares). The squares with a dot represent a group of cells all recorded in 1 patient during the ON period (see RESULTS).
without producing dyskinesias. It has been reported that MPTP-treated monkeys or patients with PD can develop APO-induced dyskinesias even when the firing rates of GPi neurons are not dramatically reduced (Filion et al. 1991; Merello et al. 1999). In contrast to GPi, patients with on period dyskinesias did have a lower mean firing rate of STN neurons than in patients without these movements. This finding supports observations that indicate an involvement of the indirect pathway (GPe-STN-GPi) in the pathogenesis of dyskinesias (Crossman 1990). Further evidence is provided by Hutchison et al. (1997a), who reported an increase in the firing rate of a single GPe neuron coincident with the occurrence of dyskinesias in a PD patient. The contribution of suppressed STN activity to the induction of dyskinesias is also consistent with local inactivation studies of the STN in MPTP monkeys (Wichmann et al. 1994) and humans (our unpublished observations). Dyskinesias can also be induced by disinhibition of the GPe by the GABA agonist bicuculline, which should lead to decreases in STN activity (Matsumura et al. 1995).

Although APO did not change the mean firing rates of STN neurons, it nevertheless may have contributed to the amelioration of parkinsonian symptoms by “normalizing” the pattern of neuronal discharge. Likewise, in addition to decreasing the firing rates of GPi neurons, the alterations in firing pattern produced by APO might have contributed to therapeutic effects of the drug. Indeed, it has been suggested that increased irregularity of discharge and bursting in the GPi and STN contribute to the development of the disorders of movement seen in PD (Bergman et al. 1994; Filion and Tremblay 1991; Miller and DeLong 1987; Wichmann et al. 1999). Specifically, these changes in firing patterns involve an increase in the proportion of 1) aperiodic bursting cells and 2) oscillatory cells or periodic bursting cells such as STN/GPi TCs. Although neurons displaying oscillatory “bursting” activity can be regarded as a special case of aperiodic bursting cells, it is important to compare the effect of APO on both types of discharge patterning because these phenomena may occur with different mechanisms and may have different physiological meanings (Kaneoke and Vitek 1996).

This study demonstrated that bursting was increased in both the STN and GPi during the period of time in which APO reduced the patient’s parkinsonism, in contradiction to the notion that increased bursting contributes to the parkinsonian pathophysiology. However, the results from previous studies examining the effect of dopaminergic medication in the GPi of parkinsonian monkeys or patients with PD are unclear. In MPTP-treated monkeys, Filion et al. (1991) reported that APO administration was associated with a more regular firing pattern in the GPi, even when APO induced only a moderate decrease in firing rate but still produced a dyskinetic state. This is in contrast to Boraud et al. (1998), who found no significant decrease in the number of GPi bursting cells with L-DOPA administration in MPTP-treated monkeys. In agreement with our results, Merello et al. (1999) reported that previously high-frequency tonic discharge neurons in the GPi displayed reduced tonic firing rates and burst-like discharges during APO-induced dyskinesias in patients with PD. In the present study, we demonstrated that in the STN and GPi, there is an increase in bursting during the APO period even when the contribution of oscillatory cells (i.e., periodic “bursting” cells) is excluded. That is, APO increased the amount of aperiodic bursting. However, the functional significance of the change in bursting is beyond the scope of this study. Elucidating the role of APO on bursting activity will likely require more powerful methodology such as simultaneous recording techniques that can assess the degree of correlation of spikes between basal ganglia neurons and the effect of dopaminergic medication (Bergman et al. 1998).

Our demonstration of tremor-related activity is consistent with previous reports of TCs in both the GPi and STN in off period PD patients (Hurtado et al. 1999; Hutchison et al. 1997b; Magarinos-Ascone et al. 2000; Rodriguez et al. 1998). The mean firing rates of TCs in both the STN and the GPi before APO administration were significantly greater than the mean firing rates of neurons with no oscillatory components, and this is similar to the findings of previous studies in PD patients (Hutchison et al. 1994; Levy et al. 2000) and in MPTP-treated monkeys (Bergman et al. 1994). These findings suggest that excessive neuronal activity in these nuclei might promote tremorgenesis. This study is the first documentation of APO-induced changes in neuronal tremor activity in any part of the basal ganglia of MPTP-treated primates or PD patients. Interestingly, APO-related changes in mean firing rates of STN neurons were closely associated with changes in tremor-related
activity (see Fig. 1C). Changes in the proportion of cells displaying tremor activity in both the STN and GPi were quite similar; during the APO period, the proportion of TCs significantly decreased along with a reduction in limb tremor. If the tremor-related activity of these neurons is due to tremor-related peripheral afferent inputs, then the reduction is simply due to the APO-induced reduction in tremor. However, while TCs were encountered in patients during episodes with limb tremor, TCs were also observed during episodes with no noticeable limb tremor and in one STN patient without detectable limb tremor. These observations support the notion that tremor oscillations in the basal ganglia may be present but may not result in observable limb tremor (Wichmann et al. 1994). A possible explanation is that, although tremor activity is present, it is not synchronized to a great enough extent to produce coordinated muscle activity (Bergman et al. 1998).

This study has shown that a significant number of STN neurons have high-frequency oscillatory activity and that some neurons concurrently display a tremor frequency component. We have also reported high-frequency oscillations of STN neurons in a separate group of six PD patients with predominant limb tremor (Levy et al. 2000), and similar observations have been reported in the STN and GPi of tremulous MPTP-treated monkeys (Bergman et al. 1994; Raz et al. 2000). In the present study, many neurons with high-frequency oscillations were found in regions that contained TCs, and, apart from a single patient who displayed high-frequency oscillations (and 1 TC) in the ON state, the incidence of these neurons following APO administration was reduced along with the proportion of TCs. Yet unlike TCs, the firing rate of neurons with high-frequency oscillations was not changed due to APO, suggesting that tremor oscillations and high-frequency oscillations might be due to separate mechanisms. It is also conceivable that the incidence of neurons with high-frequency oscillatory activity was reduced if these hyperactive neurons could not faithfully transmit high-frequency oscillations once their firing rate became suppressed due to the action of APO. We have recently demonstrated using simultaneous recording techniques that in the STN, pairs of neurons with high-frequency oscillatory activity consistently display a strong in-phase synchronization while pairs of neurons displaying tremor activity have a variable phase relationship (Levy et al. 2000). A possible hypothesis is that synchronous high-frequency oscillatory activity might be due to glutamatergic cortical input (Nambu et al. 1996), most notably 15- to 30-Hz beta oscillations (i.e., “idling rhythms”) from the primary motor cortex (Brown and Marsden 1999). The presence of this synchronous input at the level of the STN might then promote tremor activity in the basal ganglia especially during periods of rest. Dopaminergic therapy could act to reduce cortical influences by reducing the off-period hyperactive discharge of cells displaying tremor-related activity.

Several studies have noted that following MPTP treatment and the emergence of parkinsonian symptoms, GPi neuronal responses to passive limb movement increase in magnitude and lose specificity (Filion et al. 1988), and GPi and STN neuronal responses to elbow torque application increase (Bergman et al. 1994; Boraud et al. 2000). Although we did not determine the effect of APO on somatosensory responses from the leg (and therefore underestimated the number of “leg”-responsive neurons), the results for the upper extremities are nonetheless highly relevant because of the anti-parkinsonian effect of APO on these body regions (as demonstrated with the preoperative effect of APO on UPDRS motor scores). We found that before the administration of APO, many neurons in both the STN and GPi responded to movements about two or more joints. Following APO dosing, there was a decrease in multi-limb responses in both the STN and GPi. These data support the hypothesis that a loss of RF specificity is a characteristic of off period parkinsonism. Another possibility is that APO administration resulted in a diminished response to afferent input, thereby decreasing the percentage of neurons that had single RFs in addition to those with multiple RFs.

Recent electrophysiological studies have suggested that dopamine replacement therapy does more than simply decrease the overall spontaneous activity of the STN and GPi in animal models of PD (Allers et al. 2000; Boraud et al. 1998) and in patients with PD (Brown et al. 2001). Using cross-correlation techniques, it has been shown that a hallmark of parkinsonism is the breakdown of independent firing of pallidal neurons (Nini et al. 1995). It has been proposed by Bergman et al. (1998) that dopamine can also serve to facilitate the independent action of striato-pallidal circuits and that the loss of striatal dopamine leads to synchronized activity within the basal ganglia. Our results concerning the APO-induced changes in tremor activity and somatosensory receptive fields are consistent with this hypothesis and provide further evidence for extension of the current model (DeLong 1990), which does not explain the emergence of tremor activity or loss of specificity to passive joint movement, to include changes in neuronal firing patterns in addition to mean firing rates.

We thank E. Halkett and Dr. Y. J. Kim for assistance. We gratefully acknowledge Axon Instruments for providing the electrophysiological equipment used in this study. A. M. Lozano is a Medical Research Council of Canada clinician scientist.

Funding for this study was provided by the Parkinson’s Foundation of Canada, Canadian Institutes of Health Research, and a Center of Excellence Award from the National Parkinson Foundation.

REFERENCES


NEURON RESPONSE TO APOMORPHINE IN PARKINSON'S DISEASE


