Internal pallidal neuronal activity during mild drug-related dyskinesias in Parkinson's Disease: decreased firing rates and altered firing patterns.

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Header: GPi firing during mild drug-induced dyskinesias in PD.
Keywords: Parkinson's disease, globus pallidus internus, apomorphine, dyskinesias, pallidotomy, human.

Number of figures and tables: Eight figures and four tables.
Number of pages: 34

Acknowledgements: Supported by grants from the NINDS: RO1 NS38394, NS40059 (FAL), and NS39933 (PMD). We thank L. Rowland for excellent technical assistance.
Abstract

The neuronal basis of hyperkinetic movement disorders has long been unclear. We now test the hypothesis that changes in the firing pattern of neurons in the globus pallidus internus (GPi) are related to dyskinesias induced by low doses of apomorphine in patients with advanced Parkinson’s disease (PD). During pallidotomy for advanced PD, the activity of single neurons was studied both before and after administration of apomorphine at doses just adequate to induce dyskinesias (21 neurons, 17 patients).

Following the apomorphine injection, these spike trains demonstrated an initial fall in firing from baseline. In 9 neurons, the onset of ‘on’ was simultaneous with that of dyskinesias. In these spike trains, the initial fall in firing rate preceded and was larger than the fall at the onset of ‘on’ with dyskinesias. Among the three neurons in which the onset of ‘on’ occurred before that of dyskinesias, the firing rate did not change at the time of onset of dyskinesias. Following injection of apomorphine, dyskinesias during ‘on’ with dyskinesias often fluctuated between transient periods with dyskinesias and those without. Average firing rates were not different between these two types of transient periods. Transient periods with dyskinesias were characterized by ISI independence, stationary spike trains, and higher variability of interspike intervals (ISIs). A small but significant group of neurons demonstrated recurring ISI patterns during transient periods of ‘on’ with dyskinesias. These results suggest that mild dyskinesias resulting from low doses of apomorphine are related to both low GPi neuronal firing rates and altered firing patterns.
Introduction

Patients with advanced Parkinson’s disease (PD) often respond to levodopa with rapid, random movements which are known as drug-related dyskinesias (43). The current model of basal ganglia function proposes that dyskinesias and other hyperkinetic disorders result from decreased neuronal firing rates in the globus pallidus interna (GPi)(1; 14; 48). This leads to decreased inhibitory outflow from the GPi to the thalamus which may result in increased activity of thalamocortical motor circuits and in dyskinesias (24; 56).

This model is based on studies in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated monkeys which show parkinsonian motor behavior (19; 47), including drug-related dyskinesias (5; 6; 20; 51). High doses of dopamine agonists silence GPi neurons and lead to pronounced dyskinesias (51), as predicted by the current model of basal ganglion function (14). At lower doses firing may or may show decreased rates and altered in firing patterns (5-7; 20).

Intra-operative administration of apomorphine in patients with advanced Parkinson’s disease leads to ‘on’ with dyskinesias, and to GPi firing rates which are lower than those during the ‘off’ state (40; 46). However, decreased GPi neuronal firing rates cannot completely explain drug-related dyskinesias because lesions of GPi both silence these neurons and lead to abolition in drug-related dyskinesias (31; 69). Therefore, changes in measures of GPi activity other than firing rate must account for drug-related dyskinesias.

In patients with PD at the onset of apomorphine induced dyskinesias, the firing of single GPi neurons has been characterized by a change to an irregular pattern (4 neurons, reference (46)), and by no apparent change in the firing pattern (1 neuron, (40)). We now test the hypothesis that GPi neuronal activity is related to the mild transient dyskinesias which are evoked by administration of
low doses of apomorphine to patients with advanced PD and drug-related dyskinesias. The results demonstrate that GPi spike trains during dyskinesias are characterized by both decreased firing rates and altered firing patterns.

Methods.

These studies were carried out in patients with idiopathic Parkinson’s disease and drug-related dyskinesias. Patients who were already scheduled to undergo unilateral pallidotomy were approached for participation in the protocol for this study. The protocol was approved and renewed annually by the Joint Committee on Clinical Investigation at Johns Hopkins University, and by the Institutional Review Board at the National Institute of Neurological Disorders and Stroke. To mimic the effect of levodopa, we used intravenous apomorphine hydrochloride, a nonselective D1/D2-dopamine receptor agonist. Intravenous apomorphine has an immediate (<1 minute) onset of action, and a short (30 minutes) elimination half-life (9; 22; 29; 68). The effect size is identical to that of levodopa, but the response is short-lived.

Preoperatively, the patients’ motor behavior was studied before and after the intravenous administration of apomorphine (9; 22; 22; 29; 68). Intraoperatively, the patients’ motor behavior and GPi neuronal firing were studied before and after administration of the ‘surgical’ dose of apomorphine which was selected during preoperative testing.

Pre-operative studies:

This phase of the study was designed to establish a safe, effective, dyskinesigenic and well-tolerated dose for every patient, thereby limiting risks during the pallidotomy. During the month
preceding surgery, patients were admitted to the NIH Clinical Center (Bethesda, MD) for 2-4 days. Patients were started on domperidone, a peripheral dopamine antagonist, to prevent nausea (9). Regular antiparkinson medications were withheld after 11 p.m. on the day prior to testing until completion of the preoperative evaluations on the following day. Patients then entered an apomorphine dose-finding phase, during which they received an average of 6 intravenous apomorphine injections. Doses were administered at least 2 hours apart to a maximum of 4 per day.

Subjects and evaluators were blinded to the doses given, which ranged from 0.007 to 0.10 mg/kg. Doses were selected by an unblinded safety officer. A randomly inserted placebo injection was given in those cases where there was an unanticipated response at very low apomorphine doses. Vital signs were taken before and several times following each injection. In addition, written comments from all subjective and objective observations were made continuously by the blinded evaluator monitoring the patient (Figure 1D). These comments included the timing of onset of the ‘on’ state (see below), the disappearance of tremor, the appearance of dyskinesias, the response duration, and the occurrence of side-effects.

Formal motor ratings, using selected items of the Unified Parkinson’s Disease Rating Scale (UPDRS) (17), were performed to evaluate parkinsonian symptoms. The modified Abnormal Involuntary Movements Scale (AIMS) was used to rate dyskinesias. These ratings were performed before injection and every 10 minutes after the injection until baseline motor conditions returned (Figure 1). Figure 1 shows results for preoperative testing in one patient. The selected UPDRS-III items included items 20, 22, 23, 26, 29 and 31, describing tremor, rigidity, fingertaps, leg agility, gait, and bradykinesia on a scale from 0 to 4, with a score of 64 indicating maximum impairment (68). Dyskinesias were measured in the head and neck, as well as in the four extremities, on a scale from 0 to 4, where 20 was the maximum dyskinesia score (Figure 1A).
Apomorphine doses were increased by 0.01-0.02 mg/kg until either the maximum allowed dose was reached (6 mg) or side effects occurred. At the end of this phase, an appropriate ‘surgical’ dose for intra-operative use was selected for each subject by the unblinded safety officer, based on the combined criteria of minimal apomorphine-related adverse effects and maximal antiparkinsonian efficacy. The chosen dose was that just adequate to reliably induce dyskinesias, and was always at least 0.01 mg/kg above the lowest effective dose. This “surgical dose” was repeated once, as the first morning dose. Whereas patients were usually injected while sitting in a straight chair to facilitate all aspects of motor ratings, the repeat injection was given with the patient in the supine position, in order to simulate the position in the operating room.

Operative and recording procedures:

The pallidotomy was performed as previously described (35). The GPi was explored with a platinum-iridium electrode etched to a tip of 3-4 µm and coated solder glass to give an impedance of approximately 2.5 MΩ (fabricated in house by LH Rowland). The electrode was advanced toward the GPi as localized by preoperative imaging.

The microelectrode signal was amplified (DAM 80, WPI, Sarasota, Florida, USA), analog filtered (6dB below 300 and above 10,000 Hz) and stored on a video tape recorder (Vetter Model 4000, Petersberg, Pennsylvania, USA). The striatum, GPe and GPi were identified by the features of neuronal activity (13; 41; 70). Responses to sensory stimuli were sought for neurons in the GPe and the GPi during passive movements of upper and lower extremities, both ipsilateral and contralateral to the recording site.
There can be significant difficulties in maintaining single neuron isolation during lengthy human single unit recordings including intervals before and after the administration of apomorphine. Therefore, small movements of the microelectrode were made throughout the recordings from a single neuron to maintain constant size and shape of the action potential, as in our previous studies (see Figures 4 and 5 and references (32; 37; 52)).

The optic tract was identified by light-evoked potentials and microstimulation-evoked phosphenes (≤ 40µA). The internal capsule was identified by microstimulation-evoked muscle twitches. The physiologic map of the locations of the optic tract, internal capsule, and cellular and acellular regions were fitted to the atlas maps to determine the location of the GPi (41; 58; 70). Lesions were made within the part of the GPi where neurons responded to movements of the extremities. Each thermal lesion was made to 70°C with the tip of the electrode 2 mm above the optic tract and to 80°C with the tip 3-4 mm above the optic tract.

Intra-operative studies:

In the operating room, the patient’s motor status was continuously monitored by the neurologist who had performed the pre-operative testing (LVM), and by video recordings of the patient. Prior to apomorphine injection, baseline assessments of rest tremor, rigidity and finger tapping were performed. Tremor was recorded continuously with EMG from wrist flexors and extensors. The patient was instructed to report the moment of turning ‘on’. We interpreted the intra-operative response in terms of the usual response to apomorphine in that patient.

Once initial landmarks had been identified and an appropriate GPi neuron was isolated, the predetermined “surgical” dose of apomorphine was injected intravenously. Close observation and frequent motor testing was performed to document the changes in motor behavior. Patients were
periodically reminded to report when they turned ‘on’. The onset of the apomorphine-evoked ‘on’ state was identified by the improvement in finger tapping, decreased rigidity, and the patients self-report of feeling ‘on’. Based on these combined assessments, motor behavior was categorized as ‘off’, ‘on’ without dyskinesias, and ‘on’ with dyskinesias. Cases in which there was a discrepancy between these assessments of the ‘on’ state are described in the results.

Postoperative analysis:

Postoperatively, neuronal activity was analyzed for all neurons which were well isolated and which had been studied before and after the apomorphine injection. In the case of firing rates the first analysis was carried out across 1 min segments. The action potentials of single neurons were discriminated and digitized at 10 KHz by a standard shape-fitting package (Explorer, Brainwave, Thornton, Colorado, USA) and their times of occurrence were stored at a clock rate of 10 KHz.

The coefficient of variation is a measure of ISI variance which is calculated as standard deviation/mean of the distribution (23; 59). The coefficient of variation does not measure the effect of order of the ISIs within a spike train. Properties which dependent upon ISI order within the spike train include independence of ISIs and stationary firing within a spike train.

Several aspects of random spike train activity were assessed including: the median runs test for stationary spike trains, the serial correlation coefficient for independence of sequential ISIs, and the fit to the Poisson distribution, the most common model of a random spike generator (21; 23; 59). The use of several measure of random firing increased our ability to interpret models for spike train generators suggested by the data.

Stationary behavior of a spike train variable, such as firing rate, was assessed by a median runs test which measures whether fluctuations in the value of that variable over time are different
from that expected at random (4). The spike train was divided into 10 sec sample intervals and the firing rate was calculated for each sample interval. A run was defined as a series of contiguous sample intervals with mean firing rates which were either all above or all below the median firing rate. The number of runs in a spike train was compared with that expected for a spike train with the same number of intervals given a 0.5 probability that any interval will have a value greater than the median. If number of runs in the spike train is significantly different from the expected number then the spike train is nonstationary.

The first serial correlation coefficient is an average measure of the likelihood that adjacent ISIs are jointly greater than or less than the mean ISI. If the coefficient is not significantly different from zero then adjacent ISIs do not vary jointly with each other (independent ISIs)(10; 16; 23; 27). If the coefficient is significantly different from zero then adjacent ISIs vary jointly with each other (nonindependent ISIs). Nonindependent ISIs may have a first serial correlation coefficient that is significantly greater than zero which indicates that adjacent intervals vary directly, i.e. both ISIs are either greater than the mean or less than the mean. Alternately, nonindependent spike trains may have a serial correlation coefficient that is significantly less than zero which indicates that adjacent intervals vary indirectly with each other i.e. one ISI greater than the mean and the other less than the mean.

To test for the presence of a Poisson generator, the ISI histogram was converted to a ISI cumulative density function (CDF). Each CDF bin was the sum of all ISIs in histogram bins to the left of the corresponding bin in the histogram. This sum is divided by the total number of ISIs in the spike train. The presence of this Poisson process was tested by fitting the CDF to an ideal Poisson function of the same mean (K-S statistic in (57)).
The bursty firing of the spike trains was characterized by a technique assessing numbers of action potentials occurring over fixed intervals (28). This technique measures the number of spikes occurring in contiguous intervals of duration equal to the inverse of the mean firing rate; a histogram was then constructed of the number of intervals with different numbers of spikes. The fit of this histogram to a Poisson density function was used to identify spike trains with a Poisson (random) distribution.

*ISI patterns in the spike train:*

To test for the presence of patterned firing in the spike trains we used a recurrence plot approach ((16; 27) cf (11; 12)). The recurrence plot has axes consisting of ordinal numbers of ISIs in the spike train with the first ISI at the origin (16; 23; 27). For example, both axes in Figure 8 (bottom plot) represent ordinal numbers of ISIs in the spike train from the 1 to 250. Therefore, the plot includes a point for each pair of ISI ordinals in the spike train (27). A dot is placed at the point defined by any two ISIs if the pattern of ISIs is similar for the segments of the spike train starting at these two ISI ordinals (see below). For example, the left dot in the line indicated by the arrow in the bottom recurrence plot indicates that the ISI pattern beginning at ISI ordinal number 9 (Y axis) is similar to that for beginning at ISI 194 (X axis).

The similarity of the two segments is calculated from the sum of absolute distances between the corresponding, sequential ISIs in the two segments divided by the first ISI in the first segment. For example, the three corresponding, sequential ISI pairs for the line at the arrow in the bottom recurrence plot of Figure 8 are (9,194), (10, 195) and (11, 196). The two segments are considered to be similar if this value is less than the threshold value, r; the number of ISIs in the segment is indicated by d (16; 23; 27). The threshold values of r < 0.2 and d=3 were selected as in prior
studies (11; 12; 16; 27). The main diagonal is always completely dotted since each segment is identical to itself.

Two similar segments will result in a line of dots parallel to the main diagonal (arrows in Figure 8 - left) since similarity will be found for pairs of corresponding sequential ordinals within that pair of segments. The number of such lines indicates the number of similar patterns in the spike train, and the number of points in the line indicates the number of similar ISIs in a sequence. However, such sequences may occur at random in any spike train. Therefore, we shuffled the data to test whether the number of recurrent patterns was significantly greater than that expected at random for the set of ISIs in the spike train.

The data are shuffled by proceeding through the spike train interval by interval, and swapping pairs of neighboring intervals with a probability of 0.5 (27). This process was repeated five times to generate one shuffled spike train and the associated recurrence plot. One hundred such plots of shuffled spike trains were analyzed to determine the mean and 95% confidence interval (CI) of the number of lines expected from the spike train at random. This approach leads to an estimate of variability which is not dependent upon the assumption of any distribution for the number of lines. In Figure 8 (right panels), the number of lines in the unshuffled and shuffled data are shown as a function of line length. Points in the plot of shuffled data are indicated by the presence of error bars (mean +/- 95% confidence interval). The number of lines occurring in 95% of shuffles of the spike train is a direct measure of the number of lines expected at random given the ISIs in the spike train. If the error bars of the shuffled spike train do not overlap the unshuffled spike train, then there are significant recurrent patterns in the unshuffled spike train to a certainty of P<0.05.

*Crosscorrelation analysis.*
For all neurons, simultaneous EMG signals for flexors and extensors of the wrist and extensors of the elbow were digitized. Standard techniques were used to process these two signals and to compute their raw spectral estimate (4; 23; 39). These raw spectral estimates were averaged across raw frequency estimates to produce the autopower and crosspower. The autopower of a signal is the square of the magnitude of the signal as a function of frequency. The cross-spectrum of the two signals is composed of the magnitude (cross-power spectrum) and phase spectrum. The square of the cross-power is often divided by product of the averages of the two autospectra to produce the coherence. The coherence is used to estimate the probability that the two signals are linearly related, which is to say that one signal could be described as a linear function of the other (4). GPi activity might be related to dyskinetic movements by a model involving sensory transmission to GPi, motor output from GPi or sensorimotor integration, as in a feedback control system or network (8). A detailed treatment of these possibilities is beyond the scope of this study.

**Statistical analysis:**

Student t-tests or ANOVA with post hoc tests with corrections for multiple comparisons (Tukey Honestly Significant Difference - HSD) were used to compare normally distributed parametric data. Parametric data which were not normally distributed were tested by Mann-Whitney or Kruskal-Wallis. Non-parametric data were tested with a Fisher or Chi square test, as appropriate. For all tests, the null hypothesis was rejected for P<0.05 (59). Statistical analysis of multiple periods within the spike train of a single neuron is described in the results section dealing with that data (*Transient periods of ‘on’ with ....*).
Results

Under this protocol, 28 patients received apomorphine preoperatively and 17 patients received apomorphine intra-operatively, as summarized in Table 1. These patients had Parkinson’s disease with long duration (6 to 25 years), with midline motor symptoms indicating advanced disease (Hoehn and Yahr stage 3 or greater), and with drug-related dyskinesias (53). GPi neurons were not recorded in the other eleven patients during surgery because of a preoperative response characterized by either limited efficacy of apomorphine (n=4), violent dyskinesias (n=1), or because of the inability to ‘hold’ GPi neurons during microelectrode recording (n=2), or other practical reasons (n=4).

The “surgical” apomorphine dose for the 17 patients ranged from 0.015 to 0.06 mg/kg (0.044 ± 0.022 mg/kg, mean ± SD). At this individualized dose, the mean improvement in pre-operative UPDRS motor score was -58±16% (range -35 to -91%) (Table 1). All 17 patients reported they were ‘on’ following the selected dose, and all but 3 developed dyskinesias. This dose was not associated with clinically relevant adverse events, such as nausea or lightheadedness. Mild paraesthesias and yawning frequently preceded the benefit, providing additional markers for the imminent onset of dopaminergic motor effects. As anticipated, apomorphine injection induced a characteristic clinical response in each individual subject.

- Place Table 1 about here-

In the operating room, all seventeen patients received their ‘surgical’ dose of apomorphine. Four of seventeen patients received a second apomorphine injection during recording from a second neuron more than 30 minutes after the first injection, and after the first injection had worn off. In total, the spike trains of 21 neurons, (17 patients, 20 trajectories) were studied before and after
apomorphine injections. In these neurons, GPi neuronal activity was studied before (10 to 30 sec) and after the administration of apomorphine (362 ± 192 sec).

**GPI Neuronal Properties: Location and Firing Rates:**

The location of all 21 neurons was within the sensorimotor part of GPi as inferred from the physiologic mapping on a standard atlas map (58)(Methods: Operative Procedures). All neurons were within the borders of GPi in the 21mm lateral plane of the atlas map (see Figure 1 in (34)). These neurons were all within the sensorimotor portion of GPi as demonstrated by study the neuronal RFs before administration of apomorphine. These neurons had either RFs (16 neurons, see Results) or had no RF themselves but were adjacent to neurons which did have RFs. RFs were not studied after the apomorphine injection in order to record as much of the ongoing neuronal activity as possible without the artifacts produced by the bilateral sensory examination.

Prior to the apomorphine injection, all patients were in their typical ‘off’ state. Intravenous apomorphine administration led to a decrease in firing rate from baseline and a change in the subjects’ clinical condition. For each neuron, mean firing rates of recorded neurons were measured in one minute periods. Analysis of average firing rates across these 21 neurons revealed significantly higher rates immediately prior to injection (77.2 ± 6.6/sec) than at the firing rate nadir after the injection (36.5 ± 5.5/sec, p=0.0002, t-test). When normalized to the firing rate during the one minute prior to the injection, the fall in the averaged firing rate occurred 4 minutes after the apomorphine injection.

Analysis within individual neurons demonstrated a significant decrease in firing rate (p<0.05), from the baseline to the nadir after apomorphine administration in 15 out of 21 GPi neurons (maximum decrease 19-100% of baseline, mean 66% ± 25). The 15 neurons with a fall in
firing rate included 9 with simultaneous ‘on’ and dyskinesias and, 6 in neurons for which the onset of dyskinesias followed ‘on’ (see below, Results: *Relationship of dyskinesias to GPi* ...). The initial decrease in firing rate started as early as the first minute after the apomorphine injection (Figure 2A). The nadir in firing rate preceded or was simultaneous with the onset of dyskinesias in all 15 of these neurons, suggesting that a fall in firing rate is a necessary condition for the occurrence of dyskinesias.

In the other 6 out of 21 injections, apomorphine was not effective either in turning the patient ‘on’, or in leading to a decrease in firing rate. In 4 of these 6 injections, the ‘on’ state was not achieved, either subjectively or objectively. During these four injections, 2 of the neurons recorded showed a nonsignificant decrease in the firing rate (77.8 to 47; 38.6 to 25/sec), 1 showed a significant fall in firing rate (70.1±5.7 to 57±6.1, p=0.0139, t-test, neuron 20251), and 1 showed an increase in the firing rate (56.7 to 96). In the 2 remaining injections out of 6, the patients turned ‘on’ without dyskinesias and showed small increases in their finger tapping (15 to 25, and 15 to 20/min)(see below). These 2 injections did not produce decreases in firing rates (50.7/sec to 47, and 56.5 to 92). Apomorphine had produced the ‘on’ state and dyskinesias in all 6 patients during preoperative screening.

*Place Figure 2 about here*

*Relationship of dyskinesias to GPi neuronal spike trains:*

Among the 15 apomorphine injections which turned patients ‘on’ with dyskinesias finger tapping rates increased significantly from 14.3 ± 1.3 to 26.3 ± 2.1/minute (paired t-test, p<0.0001). The latency from injection to ‘on’ with dyskinesias was 127 ± 143 sec. The firing rate is shown as a function of time after apomorphine injection for an individual neuron in Figure 2A. Figure 2B
shows the results for all 15 neurons with firing rates normalized to 2 min before the injection. Firing rates decreased significantly from the baseline firing rate (84.8 ± 8.2, p=0.002, t-test) to immediately after the onset of ‘on’ (49.9 ± 6.4/sec). This figure demonstrates a dramatic fall in mean normalized firing rate from the baseline to the nadir.

- Place Figure 3 about here -

Figure 3A shows the neuronal firing rates of the nine neurons recorded following injections after which patients simultaneously turned ‘on’ and developed dyskinesias. This plot shows average firing rates plotted relative to the onset of ‘on’ with dyskinesias and demonstrates fall in firing rate prior to the gradual fall in firing rate which occurs around the onset of ‘on’ with dyskinesias. For these nine neurons the fall in firing rate between 15 seconds prior to the initial fall in firing rate versus 15 seconds following the initial fall was greater (38 ± 25/sec, P<0.05, paired t-test) than that around the time of ‘on’ with dyskinesias (12 ± 13/sec, mean difference per neuron 23.9 ± 27.2/sec). There was a significant fall in firing rate around the time of onset of ‘on’ with dyskinesias on within neuron analysis of all 9 neurons. The possibility that this fall was due to the onset of ‘on’, or dyskinesias, or both was studied in the six out of fifteen injections for which ‘on’ preceded dyskinesias.

- Place Figure 4 about here -

Among these six neurons, the onset of ‘on’ could not be determined exactly in 1 neuron. In another two, the interval between ‘on’ and appearance of dyskinesias was too short for analysis. Among the remaining three, the firing rate of one dropped considerably around the onset of ‘on’ (Figure 3B) but did not change from before versus after the onset of dyskinesias,(21.1 versus 20.0/sec) (Figure 3C). In the case of another neuron, the patient’s self report of ‘on’, the resolution
of tremor, and faster finger tapping occurred 190 seconds after apomorphine injection (Figure 4B – arrow B). The patient developed dyskinesias 80 seconds later, long before the significant change in firing rate for this neuron (Figure 4B – arrow C).

- Place Figure 5 about here -

A third injection demonstrated end-of-dose dyskinesias both pre-operatively (Figure 5A – 45 min) and intra-operatively (Figure 5B – arrow C). During the pre-operative testing, this patient exhibited brief (20 sec), beginning-of-dose dyskinesias and longer (5 min, Figure 5A), end-of-dose dyskinesias. Intra-operatively, there were only end-of-dose dyskinesias which began while firing rates were low (Figure 5B – arrow C). Firing rates before and immediately after the onset of dyskinesias initially were similar (0.1 vs. 0.2/sec). However, about 3 minutes after the onset of dyskinesias (Figure 5B – arrow C) the firing rates increased significantly while dyskinesias continued until the patient turned off (Figure 5B – arrow D). This neuron demonstrates that end-of-dose dyskinesias can occur as firing rates increase from ‘on’ to ‘off’. In summary, the onset of ‘on’ was associated with a fall in firing rate 2 out of 3 neurons while there was no change in firing rates at the onset of dyskinesias in 3 out of 3 neurons.

Transient periods of ‘on’ with or without dyskinesias

The relationship between neuronal firing rates and motor behavior is also demonstrated by analysis of ‘off’, transient ‘on’ periods with dyskinesias, and transient ‘on’ periods without dyskinesias. These transient periods resulted from the low doses of apomorphine used in this study, as indicated by the 6/21 intraoperative injections did not turn the patients ‘on’. At these doses dyskinesias were mild, and were often discontinuous over the interval of ‘on’ with dyskinesias. To
examine properties of spike trains related to dyskinesias at these doses, we next examined changes in spike trains in transient ‘on’ periods with dyskinesias versus those ‘on’ without dyskinesias and ‘off’ (for example, see reference (40)).

The progression of dyskinesias over these intervals was studied by examination of the intra-operative EMG, video, and audio recordings. An example of a patient with transient periods of dyskinesias during the intervals of ‘on’ without or with dyskinesias is shown by the thin line under the EMG recordings in Figure 6A (neuron 266025). In panel A, transient periods of dyskinesias began approximately 1½ minutes from the beginning of the panel and ended approximately 3 minutes later. The second period of dyskinesias started at 7 minutes prior to the end of the panel. These transient periods were analyzed if they had a duration of greater than 1 minute. Gaps in the record correspond to measurement intervals for finger tapping, as in figures 4 and 5.

The spike trains for the fifteen neurons were studied during injections which clearly turned patients ‘on’ produced dyskinesias. Additionally, we studied two neurons which clearly changed from ‘off’ to ‘on’ without dyskinesias (see above. Results: GPi neuronal … ). Among these neurons, we identified seventeen transient periods of ‘on’ without dyskinesias and twenty-five transient periods of ‘on’ with dyskinesias (Figure 6).

The variability within spike trains for a single neuron in one motor state is indicated (see below) by the large numbers of nonstationary spike trains measured across different periods of one type, and by differences in binary variables between different epochs of one type in one neuron. In order to address this variability, multiple periods within the spike train of a single neuron were classified into periods of ‘off’, ‘on’ with dyskinesias, and ‘on’ without dyskinesias (40). For each period, continuous variables (including average firing rates, etc.) and binary variables were
calculated. To include variability between and within neurons, we used linear and logistic regression models with robust variance estimates (LRM) (15; 61). This technique was used to compare the means of the parametric variables and the odds of the binary variables across the three periods. The robust variance estimates appropriately account for correlation across periods within a single neuron. For a binary variable, such as stationary firing during ‘on’ versus ‘off’ the odds ratio (OR) is a measure of the relative likelihood of stationary firing during ‘on’ versus ‘off’. In this example, the OR was calculated as the proportion with stationary / nonstationary for ‘on’ divided by that for ‘off’.

Firing rates were higher in ‘off’ (76/sec, LRM - Table 2) versus both transient periods without dyskinesias (53/sec) and transient periods with dyskinesias (58/sec). Differences between transient periods without dyskinesias versus transient periods with dyskinesias were not significant, as reported in previous studies measuring transient periods (40).

*Measures of random spike trains: Stationary firing rates, ISI independence, and Poisson Distributions.*

As measured by a median runs test, firing rates were stationary during ‘off’ periods (9/17) less often versus transient periods with dyskinesias (19/25, LRM see Table 2), but not versus transient periods without dyskinesias (14/17). Stationary spike trains were not different between ‘on’ periods without or with dyskinesias. Spike trains can be non-stationary as a result of either more runs or fewer runs than expected at random. Non-stationary spike trains had fewer runs than expected at random for ‘off’ intervals (8/8), transient periods without dyskinesias (2/3), and transient periods with dyskinesias (4/6). Differences between these proportions were not significant
(OR < 1.5, P > 0.16, LRM). Therefore, spike trains during dyskinesias were commonly stationary. When they were nonstationary these spike trains had longer runs above or below the median than expected at random.

The periods described by independent ISIs (serial correlation coefficient) were less common among neurons/periods during ‘off’ (5/17) versus during transient periods with dyskinesias (16/25, table 2), but not versus during transient periods without dyskinesias (11/17). When nonindependent, ISIs were less related indirectly during ‘off’ (0/17), than during ‘on’ with (6/17) or without dyskinesias (6/25), which indicates that during ‘on’ long ISIs were likely to be followed by short, and visa versa.

Poisson distributions described the firing rate less commonly than expected at chance (P < 0.01, Binomial) during the ‘off’ periods (0/17), and the periods of ‘off’ without dyskinesia (1/17), and the transient periods with dyskinesia (1/25). The early latency cumulative distribution functions (Figure 7C, lowest row) were shifted to the right so that the shortest ISIs were longer than that expected by a Poisson distribution. This pattern was seen to a greater extent than expected by chance (P = 0.01, Binomial) during ‘off’ intervals (17/17), ‘on’ without dyskinesia intervals (16/17), and ‘on’ with dyskinesia intervals (24/25).

Therefore, spike trains were often random as measured by stationary firing rates and ISI independence, during ‘off’, and ‘on’ with or without dyskinesias. However, the data were infrequently consistent with the Poisson model which is the most common model of random process. Therefore, we examined other variables bearing on the nature of the spike train generator.

The coefficient of variation (CV, Standard Deviation/Mean) was computed as a basic measure of ISI variance. The CV of ‘Off’ intervals (0.83, Table 2) was significantly less versus that
of ‘on with dyskinesias’ (1.13) but not versus transient periods without dyskinesias (0.98).
Differences between ‘on’ periods with and without dyskinesias were not significant.

Models of generators of the spike train.

The autocorrelogram can be used to characterize processes generating spike trains (23; 54). Across neurons and periods, all autocorrelograms demonstrated an initial post-spike refractory period which is seen at the far left of each autocorrelogram (Figure 7, top and middle row, consistent with reference (49)). This effect is seen in the inset (Figure 7, top row, left column) which shows the post-spike inhibitory peak at an expanded scale. The post-spike inhibition is also indicated by the frequent occurrence of a right shift of the short latency ISI CDF distribution (stepped trace, Figure 7, lowest row) relative to the Poisson distribution (smooth).

At longer lags the autocorrelograms were remarkably flat (Figure 7, upper row) for the ‘off’ intervals (13/17), the transient periods without dyskinesias (13/17), and the transient periods with dyskinesias (22/25). The flat autocorrelation functions indicate ISI durations are evenly distributed which is consistent with the high ISI variance of these spike trains. These functions are also consistent with a model in which the spike generator is independent of feedback or feedforward spike-related processes (54).

- Place Figure 7 about here -

Short latency peaks following the initial inhibition occurred in the autocorrelograms for all three periods for two neurons (156021, 212029), i.e. ‘off’ and transient periods with and without dyskinesias. This peak is seen at the far left of the autocorrelograms in Figure 7, middle row
(neuron 156021). These peaks had maxima of less than twice the mean firing probability at latencies of 5 to 10 ms, and durations of less than 10 ms at half maximum amplitude. A third neuron (22028) had such a short latency peak during the ‘on’ with dyskinesias’ interval only. The autocorrelograms with a short latency peaks may be the result of the shadowing effect by which a neuronal refractory period is followed by a short peak (2).

We next examined the possibility that a bursting generator explained firing of the GPi neurons. By the Kaneoke and Vitek method of burst detection three neurons were found to have bursty firing (28) including was found for neuron 147062 during ‘off’, 20174 during ‘on’ without dyskinesias, and 235028 during ‘on’ with dyskinesias.

We next examined recurrence plots to determine whether the data was consistent with a generator producing patterned firing (16; 27). The recurrence plots in Figure 8 (left) show short lines parallel to the diagonal (see arrows and Methods: Recurrence plots) which indicate recurrent spike train ISI patterns. Recurrent patterns (Figure 8, right) were significantly greater than random when the raw (unshuffled) data was above the 95% confidence interval of the shuffled data (error bars). In the case of four neurons there were significant recurrent patterns as illustrated in Figure 8 (right panels) in the longest transient period of ‘on’ with dyskinesias recorded for that neuron (neurons 156021, 14726, 147062, 178030) but not in periods of ‘off’ or ‘on’ without dyskinesias (Figure 8, right, upper two panels). The spike trains of these four neurons during ‘on’ with dyskinesias demonstrated ISI dependence in 3/4 neurons and with a non-Poisson distribution (4/4 neurons). The presence of significant recurrent patterns with non-independence of ISIs, and a non-Poisson distribution strongly suggest that these spike trains were the result of a pattern generator.
Significant recurrent patterns occurred only during transient periods of ‘on’ with dyskinesias. This consistency of spike trains with recurrent patterns is significantly different from our expectation that these spike trains will occur equally in all three types of intervals, i.e. ‘off’, transient periods of ‘on’ with and without dyskinesias (P=0.01, combinatorial analysis, see (59)). This analysis suggests that spike trains of a subpopulation of neurons during dyskinesias result from a generator which produces patterns of ISI intervals (12; 27).

**RF related neuronal activity:**

Among the 16 neurons with RFs the location of the RF was on the upper extremity (8 neurons), or lower extremity RFs (5 neurons), or both (3). Five neurons had no RFs. None of the binary and parametric variables described above were significantly different between neurons with RFs versus neurons without RFs. RF examinations were not carried out after apomorphine injection to minimize interference with recordings of ongoing neuronal activity.

**Discussion**

The results of this study support our hypothesis that GPi neuronal activity is related to the mild transient dyskinesias which result from low doses of apomorphine in patients with advanced PD. In most patients, ‘on’ and dyskinesias began simultaneously, and were associated with a significant fall in firing rates. In some patients, the onset of dyskinesias followed the onset of ‘on’ and the firing rate did not fall at the onset of dyskinesias. The firing rates during the ‘off’ state were higher than firing rates during transient periods of ‘on’, either without or with dyskinesias. These results suggest that a fall in firing rate is associated with the onset of ‘on’ but not the onset of dyskinesias. Transient periods of ‘on’ with dyskinesias were characterized by stationary firing rates,
independent ISIs, and greater ISI variability. Therefore, decreases in firing rate associated with the ‘on’ state are combined with changes in the firing pattern which may produce transient ‘on’ periods with dyskinesias (65; 71).

Methodological considerations:

The effects of injection of the ‘surgical’ apomorphine dose were measured preoperatively for each patient (9). This approach not only provided an optimal, individual apomorphine dose but it allowed us to predict the intraoperative response. The increased degree of certainty about the clinical effects was useful in the interpretation of less common responses, such as end-of-dose dyskinesias (Figure 5B)(44).

The total number of neurons recorded in this study was limited because we studied the activity of single neurons during both ‘off’ and ‘on’ periods. To our knowledge, very few human GPi neurons have previously been studied in both these periods. This approach substantially reduces the variability associated with study designs in which different groups of neurons form the populations for each period ‘off’, and ‘on’ without or with dyskinesias.

The variability of the data was also decreased by use of a model which accounted for differences within neurons during periods of ‘off’, and ‘on’ with or without dyskinesias. ‘Intermittent’ periods with apomorphine-induced dyskinesias have previously been studied during ‘on’ periods without dyskinesias (40). We have now approached this data with a statistical model which appropriately accounts for correlation across periods within a single neuron, i.e. linear and logistic regression models with robust variance estimates (see Results: Transient periods of ‘on’ with or without dyskinesias).
Previous studies of spike train firing patterns during drug-related dyskinesias in MPTP monkeys have reported decreased random firing patterns by a global measure of random firing (5; 6). Many different properties of a spike train can be characterized as random firing (10). In order to facilitate comparison with the recurrence plot analysis we have measured three of these features including stationary firing in the spike train, ISI independence, and the presence of a Poisson process – the most common model of a random process. In the present results, the independence of ISIs and the stationary spike trains during dyskinesias suggest that a global measure of random firing might be increased after apomorphine, contrary to the results with the global measure used in MPTP monkeys (5; 6). However the number of neurons with Poisson firing distributions was unchanged, suggesting that random firing was unchanged. During periods of ‘on’ with dyskinesias the neurons with recurrent firing patterns were characterized by non-independent ISIs and by a non-Poisson distribution. These characteristics are consistent with non-random patterning of the spike train, but are unlike the majority of our results during dyskinesias. Therefore the present results suggest a complex interaction between random properties of the spike train and dyskinesias.

Apomorphine, GPi activity and dyskinesias:

Our findings at the transition from ‘off’ to ‘on’ are compatible to those in previous studies. Prior to apomorphine administration the neuronal firing rate in GPi during the ‘off’ state was 77/sec, comparable to neuronal activity in patients with PD reported by others (18; 25; 64) (Table 3), and in MPTP monkeys (7; 19) (Table 4). In the MPTP monkeys, firing rates of GPi neurons were higher than those in normal monkey by an average of 20-30% (40).
In contrast to most human studies, the present investigation utilized intravenous apomorphine at doses just adequate to induce dyskinesias (Table 3). This strategy led to 15 injections which produced ‘on’ with dyskinesias and 6 injections (6/21) which did not produce ‘on’ or dyskinesias. This strategy also produced a decrease in GPi firing rate in our dyskinetic PD patients which was smaller than those in a study of PD patients receiving non-dyskinetic, subcutaneous, doses of apomorphine (see Table 3 (40)). This strategy produced a decrease in GPi firing rate which was quantitatively similar to that found in MPTP monkeys after infusion of non-dyskinetic doses of subcutaneous apomorphine or oral levodopa (7; 19)(Tables 3 and 4). Therefore, the present doses of apomorphine were just adequate to produce dyskinesias, consistent with previous studies.

For neurons recorded during simultaneous onset of ‘on’ and dyskinesias after administration of apomorphine, mean firing rates were consistent with previous reports (Table 3). The firing patterns of GPi neurons have previously been examined (4 neurons, 4 patients) during the ‘off’ state and after administration of apomorphine induced dyskinesias (see Table 3 and reference (46)). In that study, firing rates during ‘on’ with dyskinesias were approximately 50% below those during the ‘off’ state, and the spike train was characterized by an irregular pattern. Firing rates during periods of dyskinesias were not different from those during ‘on’ periods without dyskinesias. Similarly, after the transition from ‘off’ to ‘on’ with ‘mild dyskinesias’, a single neuron was reported to have a firing rate of 40% below baseline (60 Hz), apparently without any change in the firing pattern (40).

High doses of apomorphine produced ‘clear dyskinesias’ and a reduction in GPi firing rates, during which neurons were ‘nearly completely silenced’ (Table 4 and reference (20)). This is consistent with another study reporting that ‘firing rates declined profoundly in almost all (GPi) neurons’ during administration of high doses of levodopa (Table 4 and references (51; 56)). In MPTP monkeys, the administration of apomorphine at doses as little as 20µg/kg produced
dyskinesias and relatively small reduction in GPi firing rates (65%) (20). Boraud reported reliable dyskinesias and a 50% reduction in firing rates at low apomorphine doses in MPTP monkeys (Table 4). The present results during mild, transient dyskinesias may represent one end of a continuum, the other end of which is characterized by the intense, constant dykinesias induced at high doses of levodopa or apomorphine (Papa – personal communication) (20; 51).

A relationship between decreasing GPi neuronal firing rates and more severe hyperkinetic movement disorders is consistent with the literature of these disorders (26). A similar relationship between dystonia and GPi neuronal firing rates was found in an individual patient in whom firing rates decreased successively as dystonia became more severe in response to repeated voluntary movements (38). Irregular firing patterns were characterized by ‘low frequency modulation and pauses’ and were more pronounced than in PD and less pronounced than in hemiballismus but did not increase with increasingly severe dystonia (see also reference (26)). Similarly, GPi neuronal firing rates were correlated inversely with severity of dystonia in a population of patients undergoing pallidal surgery for dystonia (60). In that study, irregularity of the spike train was higher than in PD or normal macaques but did not apparently change with the severity of dystonia. Finally, increased low frequency, non-periodic neuronal activity was observed in a GPi recipient nucleus of thalamus, and was correlated with and led EMG during dystonia (36). These results demonstrate that GPi neuronal firing patterns combined with a low firing rate are common features of different hyperkinetic movement disorders.

In the present results, a group of neurons had spike trains with non-independent ISIs, non-Poisson distributions, and recurrent patterns of ISIs (Figure 8). These GPi neurons may be influenced by a generator which produces recurrent patterns of ISIs during transient periods of ‘on’
with dyskinesias (12; 16; 27). Recurrent patterns have been found in the spike trains of both motor and sensory systems in both vertebrates and invertebrates (12).

In sensory systems, different patterns of thalamic spike train activity result from different stimuli (33) and influence the synaptic efficacy of the excitatory thalamo-cortical synapse (66). In addition, different patterns of thalamic or peripheral nerve microstimulation can influence the quality and intensity of the evoked sensation (42; 52). In motor systems patterned spike trains have been found to influence the efficacy of excitatory synapses (67; 72).

Patterned firing in invertebrates has also been shown to influence the efficacy of inhibitory synapses and neuromuscular junctions (55; 72). Therefore, the patterned firing of the neurons in the inhibitory GABAergic projection from GPi to the pallidal recipient zone of the thalamus might exert a pattern dependent effect upon postsynaptic elements in the thalamus. The patterned firing of neurons in GPi may likewise be dependent upon inhibitory mechanisms. Inhibitory inputs to GPi from the striatum produce an ‘IPSP usually followed by rebound firing’ which could account for the patterned firing in the present study (30; 49). These inhibitory inputs may be decreased in parkinsonism, and increased during drug-related dyskinesias (1; 14), which suggests that these inputs should be increased after administration of apomorphine. If that is the case then these inhibitory inputs may produce recurrent patterns characterized by a firing pattern/sequence of a pause followed by a sequence of short ISIs.

Of course, the presence of changes in firing patterns during apomorphine induced dyskinesias does not prove that these changes are responsible for dyskinesias. For example, administration of apomorphine to normal monkeys produces decreases in firing rate and changes in neuronal firing patterns without any behavioral manifestation (5; 6). A relationship between
patterns of GPi neuronal activity and EMG during hyperkinetic disorders has been suggested based upon visual and crosscorrelation analysis of EMG with neuronal activity in the GPi and in a GPi recipient nucleus of the thalamus (see results and references (36; 71)). Evidence for a causal relationship is the striking and long-lasting decrease in dyskinesias and hemiballismus which follows lesions of these neurons during GPi pallidotomy (3; 21; 31). Patterned firing of neurons in GPi may explain other hyperkinetic disorders which are effectively treated by pallidotomy, such as hemiballismus (65; 71).
Figure Legends.

Figure 1: Clinical assessments during preoperative apomorphine dose-finding in a single patient. Dyskinesias, rigidity, bradykinesia, tremor and gait were scored before and every 10 minutes after apomorphine injection. A written log was kept of all clinical changes in between rating time points, as is indicated in frame D. This patient had transient dyskinesias when turning ‘on’ (D) but not at the formal rating time points (A). Only motor scores from the side opposite to the subsequent pallidotomy are shown here.

Figure 2: GPi neuronal firing rates relative to apomorphine injection. A, the effect of apomorphine injection (at time 0) on firing rates in an individual GPI neuron, showing a decrease in firing rate within 30 seconds. The time scale is as indicated. The vertical calibration bar indicates the firing rate. B, the effect of apomorphine injection on the average firing rate (± SD) for the group of 21 neurons normalized to the average baseline firing rate for each neuron.
Figure 3: GPi neuronal firing rates before and after apomorphine injection. A, Average GPi neuronal firing rates relative to onset of ‘on’ for injections (n=9) during which ‘on’ and dyskinesias were simultaneous. B, firing rates before and after the onset of ‘on’ for an individual patient in whom the onset of ‘on’ preceded the onset of dyskinesias. The firing rate drops immediately after the onset of ‘on’. C, Firing rates around the onset of dyskinesias in the same patient as panel B. No decrease in firing rate is seen around the onset of dyskinesias. B and C, firing rates are averaged across twenty 100 ms bins. Error bars ± SD.

Figure 4: Motor scores and GPi neuronal recordings following apomorphine injection in a patient in whom the onset of ‘on’ preceded that of dyskinesias. A, the clinical events during the pre-operative apomorphine injections are indicated as a function of time in the plot. The labels below this plot indicate the events during the first 10 minutes as the patient turned on. B, number of finger taps/10sec (upper) and GPi neuronal firing in the same patient following apomorphine injection (point A). The same neuron was held throughout the recording as indicated by the constant shape of the action potential as shown above the lowest panel. In the operating room, it took 190 seconds before the tremor stopped and the patient felt ‘on’ (point B), slightly longer than during preoperative testing. As was the case preoperatively, this was shortly followed by the occurrence of dyskinesias (point C). No reduction in firing rate was seen until approximately 4 minutes later. After about 13 minutes the firing rate began to increase and the tremor reappeared (point D).
Figure 5: Motor scores and GPi neuronal recordings following apomorphine injection in a patient in whom onset of ‘on’ preceded that of the dyskinesias which occurred at the end of the dosage interval. Conventions as in legend for Figure 4.

Figure 6: EMG recordings over a twelve minute period of ‘on’ time including ‘on’ without dyskinesias and ‘on’ with dyskinesias. The lower trace shows an expended time scale image of the interval indicated by the line below wrist flexor in A. See text.

Figure 7: Examples of autocorrelograms for the firing of neurons in GPi. The upper panel shows an example of a neuron with autocorrelograms having constant post-spike firing probability during ‘off’, ‘on - dyskinesias’ or ‘on + dyskinesias’. The middle panel shows a neuron having a brief post-spike increase in firing probability. The lowest row shows the ISI cumulative distribution for this data (stepwise plot) and for a Poisson process of the same mean as the present data (smooth plot). The data distribution is right shifted with respect to the Poisson of the same mean. The post-spike silent period is seen at the left end of the ISI CDF plots (lowest row) and in the inset in the upper left panel, which is a large scale version of the left end of the same ISI histogram.

Figure 8: Recurrence plots and line length distributions for the spike train of a neuron (178030) during ‘off’, transient periods without and with dyskinesias. See text.
<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Disease duration (yrs)</th>
<th>H &amp; Y (off)</th>
<th>Levodopa equivalent dose (mg/day)</th>
<th>Pre-operative apomorphine Doses (N)</th>
<th>Apomorphine dose selected for surgery (mg/kg)</th>
<th>Pre-operative Improvement with selected dose (%)</th>
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<tr>
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<td>719.9</td>
<td>1.05</td>
<td>0.022</td>
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**Table 1:** Patient characteristics. Pre-operative improvement with the selected apomorphine dose was measured by using elements of the motor subscale of the Unified Parkinson’s Disease Rating Scale. The levodopa equivalent dose was calculated as described in studies of pallidotomy (21).
Table 2: Results of statistical testing of periods of ‘off’, and ‘on’ with and without dyskinesias by linear and logistic regression models with robust variance estimates (LRM).

‘On’ refers to ‘on’ without dyskinesias, dys to ‘on’ with dyskinesias. For the comparison ‘On’ vs ‘Off’ Diff refers to the mean for ‘On’ periods minus the mean for ‘Off’, and odds ratio (OR) for stationary firing refers to the likelihood of stationary firing during ‘on’ relative to that for ‘off’ periods. Var is the measure of variability of the data, and P is the P value, as in descriptions of the LRM test (15; 61).

<table>
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<tr>
<th>Outcome</th>
<th>‘On’ vs. ‘Off’</th>
<th>Dyskinesia vs. Off</th>
<th>Dyskinesia vs On</th>
<th>Diff (var) P</th>
<th>OR (var) P</th>
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<td><strong>Firing Rate</strong></td>
<td></td>
<td></td>
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<td>-23.7 (8.6)</td>
<td>4.15 (3.63)</td>
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<td></td>
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<td>0.041 (0.07)</td>
<td>1.04 (0.104)</td>
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Overall P 0.021
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<th>Reference (Year)</th>
<th>Subjects (N)</th>
<th>GPi Firing Rate (Mean ± SD in sec(^{-1})) (n=number of neurons)</th>
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<th>Parkinsonian State</th>
<th>on after Apo</th>
<th>on with Dyskinesias</th>
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<td>Hutchinson (1997)</td>
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<td>85 ± 19 (population study) (n=75)</td>
<td>34 ± 22 (n=18)</td>
<td>3 subjects developed dyskinesias; rates N/A.</td>
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<td>85 (time course Study) (n=10)</td>
<td>49 (n=10)</td>
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<td>Levy (2001)(40)</td>
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<td>72 ± 2.9 (population study) (n=93)</td>
<td>39 ± 2.5 (n=51)</td>
<td>40.4 ± 5 (n=30), ~ 67 µg/kg, SC. Rate during on – dykinesias = on + ‘intermittent dyskinesias’.</td>
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<td>PD patients (N=5)</td>
<td>N/A</td>
<td>143.8 ± 55.6 (n=5)</td>
<td>54.2 ± 19.2</td>
<td>54.2 ± 19.2 ~43µg/kg SC</td>
<td></td>
</tr>
<tr>
<td>Stefani (1999)(62)</td>
<td>PD patients (N=3)</td>
<td>N/A</td>
<td>65.3 ± 12.2 (n=3)</td>
<td>15.1 ± 5.6 (n=3)</td>
<td>N/A (nondyskinetic dose)</td>
<td></td>
</tr>
<tr>
<td>Stefani (1997)(63)</td>
<td>PD patients (N=2)</td>
<td>N/A</td>
<td>45 ± 9.3 (n=1)</td>
<td>20</td>
<td>N/A (nondyskinetic dose)</td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>PD patients (N=17)</td>
<td>N/A</td>
<td>77.2 ± 6.6 (n=21)</td>
<td>N/A</td>
<td>36.5 ± 5.5 (40 ± 22 µg/kg dyskinetic dose)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Overview of GPi firing rates in patients with PD.
~ indicates that dose/kg estimated from dose assuming a 70 Kg man. SC subcutaneous administration.
### Table 4: Overview of GPi firing rates in non-human primates. ~ estimated dose/weight for monkeys.

<table>
<thead>
<tr>
<th>Name (Year)</th>
<th>Subjects (N)</th>
<th>GPi Firing Rate (sec⁻¹) (n=number of neurons ± SD)</th>
<th>Intact State</th>
<th>Parkinsonian State</th>
<th>on after Levodopa/Apo</th>
<th>on with Dyskinesias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergman (1995)(50)</td>
<td>African green monkey (N=3)</td>
<td>53 (n=175)</td>
<td>76 (n=154) MPTP</td>
<td>N/A</td>
<td>N/A (no dopaminergic agent given)</td>
<td></td>
</tr>
<tr>
<td>Miller (1987)(47)</td>
<td>M Mulatta (N=1)</td>
<td>79.5 (n=51)</td>
<td>91.5 (n=79) MPTP</td>
<td>N/A</td>
<td>N/A (no dopaminergic agent given)</td>
<td></td>
</tr>
<tr>
<td>Filion (1991)(19)</td>
<td>M fascicularis (N=5)</td>
<td>78 ± 26 (n=105)</td>
<td>95 ± 32 (n=166) MPTP</td>
<td>N/A</td>
<td>N/A (no dopaminergic agent given)</td>
<td></td>
</tr>
<tr>
<td>Boraud (1998)(7)</td>
<td>M mulatta (N=2)</td>
<td>80.5 ± 20.7 (n=50)</td>
<td>106 ± 29.4, n=42, MPTP</td>
<td>47.4 ± 23.0 (n=78)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Filion, Tremblay (1991) (2011)</td>
<td>M fascicularis (N=1)</td>
<td>apo ~20 µg/kg no behavioral change, 200 µg/kg chewing &amp; agitation. no 'change in pattern or rate' n=3</td>
<td>N/A</td>
<td>N/A</td>
<td>58% reduction (n=18), apo ~20-40 µg/kg (induction of associated with only a partial decrease of activity in GPi, not with complete silence)</td>
<td></td>
</tr>
<tr>
<td>Papa (1999) (51)</td>
<td>M mulatta (N=4)</td>
<td>N/A</td>
<td>46.3 ± 8.1, (n=14) MPTP &amp; dyskinesogenic oral levodopa 1.25-2 gm/day</td>
<td>26 ± 3.1</td>
<td>7.6 ± 1.5 additional decline when dyskinesias started in response to SC levodopa 125-200 mg on a day when oral levodopa withdrawn.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>46 ± 8.2, (n=10), as above.</td>
<td>27 ± 3.4</td>
<td>N/A (nondyskinetic dose). Low dose SC levodopa 50-100 mg.</td>
<td></td>
</tr>
<tr>
<td>Boraud (2001) (5)</td>
<td>M fascicularis (n=3)</td>
<td>63-67 bursty (n=16) to 40-53 regular post apo</td>
<td>80-83 (n=27)</td>
<td>19-30 (n=7) apo dyskinesias (0.1mg/kg).</td>
<td>7.6 ± 1.5 additional decline when dyskinesias started in response to SC levodopa 125-200 mg on a day when oral levodopa withdrawn.</td>
<td></td>
</tr>
<tr>
<td>Present study PD patients (N=17)</td>
<td>N/A</td>
<td>77.2 ± 6.6 (time course study) (n=21)</td>
<td>N/A</td>
<td>36.5 ± 5.5</td>
<td>(40 ± 22 µg/kg dyskinetic dose)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Overview of GPi firing rates in non-human primates.


61. **Statacorp.** *Stata Statistical Software: Release 9.* College Station, TX: StataCorp LP, 2005.


65. Suarez JI, Metman LV, Reich SG, Dougherty PM, Hallett M and Lenz FA.


A Dyskinesia

B Rigidity

C Bradykinesia

D Tremor

E Gait

F Total Motor Score

(Tremor + Rigidity + Bradykinesia + Gait)

Apomorphine dose (mg/kg, i.v.)
- 0.01
- 0.015
- 0.02
- 0.03

Feels 'on'

Gait improved

Transient LLE dyskinesia

Tremor stopped;
Head pressure;
Finger numbness
A  Firing rates during simultaneous ‘on’ and dyskinesia (9 neurons)

B  Firing rates during ‘on’ without dyskinesia (Neuron 212029)

C  Firing rates during ‘on’ with dyskinesia (Neuron 212029)
A. Preoperative Testing

- Rigidity
- Tremor
- Gait
- Dystonia
- Bradykinesia
- Total Motor Score

B. Intraoperative

A: Apomorphine injection (0.015 mg/kg, i.v.)
B: Patient reported turning “on” and disappearance of tremor (190 sec. after apomorphine injection)
C: First onset of foot dyskinesia (270 sec. after apomorphine injection)
D: Reappearance of tremor (920 sec. after apomorphine injection)
E: Performance of finger tap
**A. Preoperative Testing**

- **Rigidity**
- **Gait**
- **Bradykinesia**
- **Tremor**
- **Dystonia**
- **Total Motor Score**

Feels "sort of drunk"; dizzy brief LUE dyskinesia

LUE dyskinesia (5 sec.)

"off", very stiff, frozen LUE dyskinesia (5 sec.)

**B. Intraoperative**

Finger Tap Counts / 10 sec.

- A: Apomorphine Injection (0.06 mg/kg, i.v.)
- B: Patient is "on" (100 sec. after apomorphine injection)
- C: First Dyskinesia (2100 sec. after apomorphine injection)
- D: Patient is "off" (2400 sec. after apomorphine injection)
- E: Performance of finger tap
‘Off’

Spike Autocorrelation 1
235028 (49 : 149)

‘On - Dyskinesias’

156021 (19 : 174)

‘On + Dyskinesias’

ISI CDF
235028 (49 : 149)
'Off' Recurrence Plot

Line Length Distribution
- Unshuffled
- Shuffled
- Line indicating recurrent pattern

'On - Dyskinesia'

'On + Dyskinesia'