Neuronal Firing Before and After Burst Discharges in the Monkey Basal Ganglia Is Predictably Patterned in the Normal State and Altered in Parkinsonism

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Wichmann, Thomas and Jesus Soares. Neuronal firing before and after burst discharges in the monkey basal ganglia is predictably patterned in the normal state and altered in parkinsonism. J Neurophysiol 95: 2120–2133, 2006. First published December 21, 2005; doi:10.1152/jn.01013.2005. It is known that burst discharges in basal ganglia neurons are more common in parkinsonism than under normal conditions, but changes in the structure of burst or peri-burst epochs have not been reported. In this study, the temporal structure of bursts and the timing of neuronal discharges that precede or follow them were examined in neuronal spike trains recorded in the subthalamic nucleus (STN) and the external and internal pallidal segment (GPe, GPi) in two awake Rhesus monkeys before and after they were rendered hemiparkinsonian by unilateral intracarotid infusion of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Bursts were detected by the “surprise” method. In the normal state, interspike intervals (ISIs) preceding or following bursts were frequently significantly longer than the average baseline ISI, and their duration was correlated with the burst length (i.e., the number of spikes/burst). Significant correlations were also found in all three structures between the burst length and the duration of interburst intervals. The incidence of burst discharges and the proportion of time spent in bursts increased in GPe, STN, and GPi after MPTP treatment. Burst lengths became more tightly related to preburst ISIs in the STN after MPTP treatment and to postburst ISI duration in all three structures. These results show that bursts in spontaneous GPe, STN, and GPi discharge are often preceded or followed by long ISIs, and that burst length, the length of pre- and postburst ISIs, and the length of interburst intervals are related to one another. Complex changes in these interactions may contribute to abnormal information processing in parkinsonism.

INTRODUCTION

The basal ganglia are a group of interconnected nuclei that participate in larger circuits involving thalamus and cortex. Within these circuits, the striatum and subthalamic nucleus (STN) receive input from thalamus and cortex, whereas the internal pallidal segment (GPi) and the substantia nigra pars reticulata (SNr) are the principal output structures, projecting to thalamus and brain stem. The external pallidal segment (GPe) is part of the intrinsic circuitry of the basal ganglia, linking the striatum with STN and GPi (Albin et al. 1989; Alexander et al. 1990; DeLong 1990; Wichmann and DeLong 2003a). Neuronal activity in these nuclei is strongly influenced by the level of dopamine, particularly in the striatum. Because of the many intrinsic connections between the different basal ganglia nuclei, a loss of striatal dopamine is thought to strongly affect the activity of the other basal ganglia nuclei (Wichmann and DeLong 2003a) and, at the behavioral level, to result in parkinsonism.

One of the characteristic discharge abnormalities in parkinsonism is that neurons in STN, GPe, GPi, and SNr have a strong tendency to fire in bursts, i.e., abruptly occurring sequences of closely spaced action potentials (Bergman et al. 1994; Wichmann et al. 1999). Cells under normal conditions also spontaneously fire in bursts (Beurrier et al. 1999; Bevan et al. 2002a; Overton and Greenfield 1995), particularly in drowsiness or sleep (Gatev and Wichmann 2003; Magill et al. 2000b; Urbain et al. 2000, 2002).

Despite the growing evidence documenting the presence of bursts in the discharge of basal ganglia neurons and their increased incidence in parkinsonism, little is known about their temporal structure or about their relationship to neuronal discharges preceding or following them. Such relationships are known to exist in the hippocampus and other structures (Harris et al. 2001).

In this study, we show that bursts in GPe, STN, and GPi, recorded with extracellular recording methods in vivo in monkeys, are not solitary phenomena, but are components of larger firing patterns with a predictable temporal structure. We also show that the tendency of cells to fire in bursts and the temporal structure of bursts and peri-burst periods undergo substantial changes with parkinsonism, induced by treatment with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). These changes might contribute to the motor dysfunction in parkinsonism.

METHODS

General methods of data collection and analysis

The data used for this analysis are a subset of data from two Rhesus monkeys that were also part of another study (Soares et al. 2004). Activity in GPe, STN, and GPi in these animals was first recorded in the normal state. The animals were rendered parkinsonian by treatment with MPTP, and activity in GPe, STN, and GPi was recorded again. Throughout the experiments, we carefully monitored the state of arousal in these animals and recorded only during periods of wakefulness. After completion of the series of recording sessions, the location of the neurons was verified by histologic analysis.

The electrophysiologic data collected in these experiments were extensively analyzed to characterize burst discharges. Oscillatory neuronal activity was also analyzed to assess how oscillations, which
are known to be prevalent in the basal ganglia in parkinsonism, might be related to the observed bursts.

Animals

The two Rhesus monkeys (Macaca mulatta, 4–5 kg, monkeys H and I) that were used for these studies were housed under conditions of protected contact housing, with free access to standard primate chow, water, and supplemental fruit. Before the recording sessions, the animals were adapted to the laboratory environment and trained to sit in a primate chair and permit handling by the experimenter. During the recording sessions, the animals were awake. All experimental protocols were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, the PHS Policy on the Humane Care and Use of Laboratory Animals, and the American Physiological Society’s Guiding Principles in the Care and Use of Animals. All experiments were approved by the Institutional Animal Care and Use Committee at Emory University.

Surgical procedures

After completion of behavioral conditioning, stainless steel chambers for chronic recording (16 mm ID) were stereotactically positioned over a trephine hole under aseptic conditions and isoflurane inhalation anesthesia (1–3%). In each animal, a chamber directed at the pallidum (GPe, GPi) was placed at an angle of 50° from the vertical in the coronal plane and a chamber aimed at the STN was placed at an angle of 36° from the vertical in the sagittal plane. The chambers were affixed to the skull with dental acrylic. Stainless steel head holders were also embedded into the acrylic cap to permit stabilization of the head during the recording sessions.

Electrophysiology

The neuronal activity in GPe, GPi, and STN was recorded extracellularly with tungsten microelectrodes (Frederick Haer Co., Bowdoinham, ME; impedance 0.5–1.0 MΩ at 1 kHz). The microelectrodes were lowered into the brain with a microdrive (MO-95B, Narishige, Tokyo, Japan). A 20-gauge guide tube was positioned with its tip barely penetrating the surface of the brain to protect the electrodes as they passed through the dura. The guide tube was positioned so that its tip barely penetrated the brain’s surface. The electrical signals were amplified (DAM-80 amplifier, WPI, Sarasota, FL), filtered (400–10,000 Hz; Krohn-Hite, Brockton, MA), displayed on a digital oscilloscope (DL1540, Yokogawa, Tokyo, Japan), and made audible through an audio amplifier. The signal was recorded on tape with a video recording adapter (model 3000A, Vetter, Rebersburg, PA). Neurons in the GPe, STN, and GPi were identified by generally accepted characteristics such as high-frequency discharge with pauses in GPe, tonic high-frequency discharge in GPi, and tonic and regular discharge in an area of high background activity in the STN (DeLong 1971; Wichmann et al. 1994).

Administration of MPTP

After completion of observations in the normal state, the animals received MPTP, injected under general isoflurane anesthesia (1–3%) into the right carotid artery (0.5 mg/kg per injection; monkey H received 2 injections, 36 days apart; monkey I received a single injection). Both animals developed similarly obvious signs of parkinsonism on the side contralateral to the injections (i.e., on the left side of their body), including bradykinesia, rigidity, and flexed limb posture. The post-MPTP experiments started 2 months after the last MPTP injection. Throughout the post-MPTP period of observation, the behavioral state of the animals remained stable, as assessed with biweekly behavioral observations and video recordings of the animal’s behavior in an observation cage. The results of these observations are not immediately relevant to the topic of this study, but are reported in our previous study (Soares et al. 2004).

Histology

At the conclusion of the experiments, the monkeys were killed by induction of deep anesthesia with an overdose of pentobarbital sodium, followed by transcardial perfusion with saline and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). The brains were removed and cryoprotected in a 30% sucrose solution in 0.1 M phosphate buffer. The fixed brain was sectioned in coronal planes (50 μm). One of every four sections was stained with cresyl violet for localization of microelectrode tracks. Some of the remaining sections were used for double labeling studies to visualize tyrosine hydroxylase (TH) for documentation of the loss of dopaminergic terminals in the striatum and dopaminergic cells in the substantia nigra pars compacta (SNc). Details and results of the histologic analysis are documented in our previous publication (Soares et al. 2004) and are not reproduced here.

Data analysis

PRELIMINARY DATA ANALYSIS STEPS. Acceptance criteria for cells to be included in the analysis were based on the locations of the cells, the recording quality, and the length of the record. We included cells in the analysis only if reconstruction of the cell’s location, based on stereotactic information, micromanipulator readings gathered during the recordings, and the results of postmortem histologic analysis, confirmed that they were located within one of the target structures (GPe, GPi, or STN). For inclusion into the analysis, cells also had to be adequately isolated throughout the record, as defined by a signal-to-noise ratio of three or greater. The record of electrical activity also had to be at least 5 min in duration to allow for meaningful interpretation.

The tape-recorded activity was played back into a template-matching spike sorter (Alpha-Omega, Nazareth, Israel), which extracted the timing of spike occurrence. These data were stored as interspike intervals (ISIs). The ISI data were imported into Matlab (MathWorks, Natick, MA) for more detailed analysis. For confirmation of adequate signal isolation, we constructed ISI distribution histograms and verified that a refractory period of ≥2-ms duration was present for each recording that was included. In addition, raster displays of the spontaneous firing of each cell were carefully examined and episodes of stationary discharge were selected. This process was done in a blinded fashion to not bias the study outcome.

BURST DETECTION. Bursts were detected as runs of short ISIs, containing at least three spikes. For burst detection, we used the algorithm of Legendy and Salcman (Fig. 1 and Aldridge and Gilman 1991; Legendy and Salcman 1985). The method calculates the “unlikeliness” (or “surprise”) that a given ISI sequence would be found in
a random stream of ISIs, which are assumed to be Poisson-distributed. The exclusion of doublets follows the convention of other studies (Aldridge and Gilman 1991; Legendy and Salcman 1985; Soares et al. 2004). The original description by Legendy and Salcman (1985) uses a surprise value of 10 to separate bursts from nonburst sequences of short ISIs. Applied to our data, this cut-off value resulted in a large number of obvious bursts that were rejected by the algorithm. We therefore used a lower surprise value (3) in our analysis, which yielded a more realistic detection of bursts, similar to what would be called a burst by human observers (for use of the same algorithm with a similar burst detection criterion, see Aldridge and Gilman 1991). A surprise value of three corresponds to a probability of 0.05 of finding a given sequence of spikes within a random sequence.

CALCULATION OF OVERALL BURST FEATURES. The detected burst segments were characterized in terms of the number of bursts/cell, the average and peak intraburst rates, the average burst length (number of spikes) and duration, and the distribution of burst lengths. In addition, the proportion of spikes in bursts compared with the overall number of spikes in the record, and the proportion of time a cell spent in bursts, was calculated. We also calculated the “background” firing rate of each cell, i.e., the rate that resulted after removal of all burst segments of data from the data stream. Because the immediate preburst and postburst epochs were considered to be potentially influenced by the presence of the burst, we used data segments for the background rate calculation that followed the end of bursts by ≥10 ISIs, and ended 10 ISIs before the onset of the respective next burst. Interburst segments lasting <21 ISIs were therefore excluded from the background rate calculation.

ANALYSIS OF THE TEMPORAL STRUCTURE OF PERI-BURST AND BURST SEGMENTS. As the next step of the analysis, peri-burst and burst segments of data were extracted from the ISI data stream, starting from the 10th ISI before the start of the burst and lasting through the 10th ISI after the end of the burst. Peri-burst segments were analyzed after normalization of the data to the background firing rate of each cell, as defined above. The data shown in the result section suggested that the ISIs immediately preceding or following a burst were longer than the ISIs before or after them, respectively. For formal analysis of this point, we calculated for each burst the ratio of the last ISI before a burst to the ISI immediately preceding it (preburst ratio). Similarly, ratios between the first and second ISI following a burst were also calculated (postburst ratio).

For the analysis of the temporal burst structure, we were interested in comparisons of the ISI lengths with their neighbor ISIs inside of the burst. These data were normalized to the length of the first ISI that belonged to the bursts. To generate diagrams of the burst structure (as shown in Fig. 3 and in the middle column in Fig. 7), we used all of the available burst data for each of the data points shown. For example, the average value for the fifth average ISI after burst onset was calculated based on data segments from all bursts lasting six or more spikes (corresponding to 5 or more ISIs), whereas the sixth average ISI was based on data from bursts lasting seven or more spikes (corresponding to 6 or more ISIs), etc. This analysis strategy implicitly assumes that the relative length of ISIs early in the burst does not significantly differ between bursts of short duration and bursts of long duration. In initial tests, we compared the averages of the initial four ISIs of long bursts (bursts with ≥5 ISIs) with the initial four ISIs of short bursts (bursts with ≤4 ISIs), and did not find significant differences (data not shown).

ANALYSIS OF RELATIONSHIPS BETWEEN BURST LENGTH, THE DURATION OF FLANKING ISIS, AND INTERBURST INTERVALS. Bursts of an individual cell were sorted according to the number of spikes within the burst, and the average duration of the immediate pre- or postburst ISIs found in bursts of different lengths was calculated. We also calculated separate averages for bursts of different lengths of the duration of the intervals between such bursts and the bursts preceding or following them (interburst intervals).

ANALYSIS OF OSCILLATORY DISCHARGE. Oscillatory firing patterns are known to become more prevalent in the parkinsonian state and may represent a significant confounding factor in the analysis of burst discharges and interburst intervals. We therefore evaluated the possibility of an interaction between the results of the burst analyses and parameters describing oscillatory activity. For this analysis, ISI data were binned in 10-ms intervals. The resulting time series was used to generate power spectra (with 512-point FFTs, Hanning windows) with a frequency resolution of 0.2 Hz. Confidence limits for the power spectra were generated, based on the mean and SD of power spectra of 50 randomly shuffled renditions of the original ISI data stream, generated through the same conversion routine. Peaks in power spectra were considered significant if at least three neighboring values were found outside of the mean ± 2 SD lines. This criterion is deliberately more inclusive than criteria for oscillation detection used in our previous studies (resulting in a larger proportion of cells identified as oscillatory), so that the relationship between oscillations and bursting could be detected with greater sensitivity. For the purpose of the data summarized in RESULTS and in Table 4, cells were classified as either oscillatory or nonoscillatory, based on the detection of distinct power spectral peaks above 1 Hz. Details of the occurrence of specific oscillatory frequencies are given in RESULTS. Based on a convention that we have used previously (Soares et al. 2004), the power spectra were integrated in four separate spectral bands (1–3, 3–8, 8–15, and ≥15 Hz), and the spectral contents in each band was normalized to the total power in the spectrum.

In some cases, oscillatory firing patterns may represent oscillatory bursting. The presence of oscillatory bursting was assessed with power spectral analyses, based on the time of occurrence of the first spikes in bursts. These analyses were carried out with the same algorithm described above in the section on power spectra of ISI data. It should be noted that these power spectral analyses are based on fewer “events” (in this case, burst onsets), and are therefore more noisy than the spectra based on the timing of ISIs, so that the estimates of the proportion of cells with oscillatory bursts are more conservative than those of the proportion of oscillatory cells.

STATISTICS. Numeric data were managed with the Access database program (Microsoft). Statistical comparisons were carried out with SPSS using t-test with Bonferroni correction for multiple comparisons. Regressions were calculated in Matlab. For all comparisons, \( P < 0.05 \) was accepted as indicating significant differences.

RESULTS

Database

Neurons were accepted for inclusion if they had been recorded with acceptable isolation quality in GPe, STN, or Gpi for at least 5 min, if their ISI distribution histograms showed an absence of ISIs shorter than 2 ms (thus indicating the presence of a refractory period), and if stationary discharge could be shown by examination of raster diagrams. The average record length was similar among the three structures before and after MPTP treatment (Table 1). For each of the basal ganglia structures under study in each state (normal or MPTP-treated), the neuronal data from both monkeys were pooled, after exclusion of intersubject differences in terms of firing rates and the incidence of burst discharges with ANOVA. For GPe, 44 neurons were available from the normal state and 40 from the
### TABLE 1. Characteristics of neuronal firing

<table>
<thead>
<tr>
<th></th>
<th>GPe (n = 44)</th>
<th>MPTP (n = 40)</th>
<th>STN (n = 15)</th>
<th>MPTP (n = 28)</th>
<th>GPi (n = 33)</th>
<th>MPTP (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of record</td>
<td>461.8 ± 185.5</td>
<td>459.4 ± 172.8</td>
<td>465.8 ± 164.4</td>
<td>516.7 ± 140.4</td>
<td>503.2 ± 155.7</td>
<td>495.2 ± 138.2</td>
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<tr>
<td>Average discharge rate, spikes/s</td>
<td>66.1 ± 19.1</td>
<td>48.5 ± 19.3*</td>
<td>23.2 ± 8.8</td>
<td>37.3 ± 12.0*</td>
<td>73.5 ± 18.1</td>
<td>78.1 ± 17.9</td>
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<tr>
<td>Extra-burst discharge rate, spikes/s</td>
<td>60.2 ± 20.7</td>
<td>40.0 ± 20.5*</td>
<td>16.3 ± 8.8</td>
<td>26.3 ± 10.5*</td>
<td>68.3 ± 20.1</td>
<td>68.1 ± 3.4</td>
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<tr>
<td>1 to 3-Hz oscillations</td>
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<tr>
<td>Integrated power spectrum, %</td>
<td>7.1 ± 3.6</td>
<td>5.9 ± 2.1</td>
<td>6.4 ± 2.0</td>
<td>6.2 ± 3.0</td>
<td>5.8 ± 2.2</td>
<td>5.6 ± 2.7</td>
</tr>
<tr>
<td>Number of oscillating cells</td>
<td>3/44 (6.8%)</td>
<td>0/40 (0.0%)</td>
<td>2/15 (13.3%)</td>
<td>6/28 (21.4%)</td>
<td>8/33 (24.2%)</td>
<td>2 (5.9%)</td>
</tr>
<tr>
<td>Number of cells with oscillatory bursts</td>
<td>1/3 (33.3%)</td>
<td>0/0 (0.0%)</td>
<td>0/2 (0.0%)</td>
<td>0/6 (0.0%)</td>
<td>1/8 (12.5%)</td>
<td>0/2 (0.0%)</td>
</tr>
<tr>
<td>3 to 8-Hz oscillations</td>
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<tr>
<td>Integrated power spectrum, %</td>
<td>8.3 ± 3.2</td>
<td>11.5 ± 4.3*</td>
<td>9.8 ± 2.5</td>
<td>14.4 ± 4.7*</td>
<td>9.8 ± 3.5</td>
<td>13.6 ± 5.7*</td>
</tr>
<tr>
<td>Number of oscillating cells</td>
<td>2/44 (4.5%)</td>
<td>7/40 (17.5%)</td>
<td>0/15 (0.0%)</td>
<td>12/28 (42.9%)</td>
<td>1/33 (3.0%)</td>
<td>18/34 (52.9%)</td>
</tr>
<tr>
<td>Number of cells with oscillatory bursts</td>
<td>0/2 (0.0%)</td>
<td>1/7 (14.3%)</td>
<td>0/0 (0.0%)</td>
<td>1/12 (8.3%)</td>
<td>0/1 (0.0%)</td>
<td>6/18 (33.3%)</td>
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<tr>
<td>8 to 15-Hz oscillations</td>
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<tr>
<td>Integrated power spectrum, %</td>
<td>9.3 ± 2.8</td>
<td>14.0 ± 4.3*</td>
<td>12.7 ± 2.2</td>
<td>18.2 ± 3.9*</td>
<td>10.9 ± 2.2</td>
<td>15.8 ± 7.8*</td>
</tr>
<tr>
<td>Number of oscillating cells</td>
<td>4/44 (9.1%)</td>
<td>11/40 (27.5%)</td>
<td>0/15 (0.0%)</td>
<td>14/28 (50%)</td>
<td>3/33 (9.1%)</td>
<td>17/34 (50.0%)</td>
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<td>Number of cells with oscillatory bursts</td>
<td>2/4 (50%)</td>
<td>0/11 (0.0%)</td>
<td>0/0 (0.0%)</td>
<td>3/14 (21.4%)</td>
<td>0/3 (0.0%)</td>
<td>9/17 (52.9%)</td>
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<tr>
<td>&gt;15-Hz oscillations</td>
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</tr>
<tr>
<td>Integrated power spectrum, %</td>
<td>67.0 ± 10.8</td>
<td>62.1 ± 9.4*</td>
<td>65.1 ± 7.1</td>
<td>56.5 ± 10.5*</td>
<td>68.6 ± 7.6</td>
<td>58.3 ± 12.8*</td>
</tr>
<tr>
<td>Number of oscillating cells</td>
<td>0/44 (0.0%)</td>
<td>1/40 (2.5%)</td>
<td>0/44 (0.0%)</td>
<td>2/28 (7.1%)</td>
<td>1/33 (3.0%)</td>
<td>1/34 (2.9%)</td>
</tr>
<tr>
<td>Number of cells with oscillatory bursts</td>
<td>0/0 (0.0%)</td>
<td>0/1 (0.0%)</td>
<td>0/0 (0.0%)</td>
<td>0/2 (0.0%)</td>
<td>0/1 (0.0%)</td>
<td>0/1 (0.0%)</td>
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</tbody>
</table>

Values are means ± SD. *P < 0.05 MPTP vs. normal state. GPe, external pallidal segment; STN, subthalamic nucleus; GPi, internal pallidal segment; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

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**Characterization of bursts and preburst epochs in cells recorded in the normal state**

**ANALYSIS OF PREBURST EPOCHS.** We observed that in many neurons, the discharge in the preburst period was nonrandom for epochs spanning the length of several ISIs, resulting in complex ISI patterns leading up to bursts. The temporal structure of the preburst epochs were evaluated with the analysis shown in Fig. 2A. Peri-burst data for all bursts of individual cells (regardless of the length of the burst) were aligned to the onset of the respective burst, normalized to the cell’s average background firing rate (as defined in METHODS), and averaged across all bursts. The plots show averaged normalized ISIs from the preburst and early burst phases from GPe, STN, and GPi.

A complex pattern of preburst ISIs was apparent in both pallidal segments. In these structures, ISIs tended to shorten gradually up to the second-to-last preburst ISI. In the STN, the gradual shortening of ISIs was not seen, but the second-to-last ISI appeared to be shorter than previous ones. In all three structures the ISIs immediately preceding the burst were substantially longer than earlier ISIs. The presence of a sequence of ISIs with durations substantially longer than earlier ISIs was confirmed by the analysis of average preburst ratios, which were found to be above one in all structures (Table 2). Preburst ratios were independent of the burst length (data not shown).

We found in many cells that there was a relationship between the duration of the final preburst ISI and the number of spikes in the burst. This relationship was evaluated with regression analysis as shown in Fig. 2B. For the regression analysis to be meaningful, we restricted the analysis to cells in which six or more different burst lengths were found, so that the n numbers in some groups were smaller than the total number of cells. A summary of the results of this analysis is shown in Table 4. There was a significant relationship between

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**Burst detection**

The surprise method of burst detection defines a burst as a series of ISIs that are unexpectedly short, relative to the average firing rate of the cell. Examples of burst detection in the pallidum and in the STN are shown in Fig. 1. Both for high-frequency cells in the pallidum (top) and for cells discharging at lower frequency in the STN (bottom), the method reliably identified bursts. Note also that doublets of rapid firing (indicated by the asterisks in Fig. 1) were excluded, because bursts were defined by the algorithm as sequences of at least three rapid spikes (corresponding to ≥2 ISIs).

With the surprise method of burst detection, the identification of a given series of ISIs as a burst depends on the discharge rate of the neuron. Burst detection can therefore be expected to be dependent on the baseline firing rate of the neuron. This expectation was confirmed by regression analysis (data not shown). Thus in all three structures, the average frequency of the spikes within the burst increased as the neuron’s firing rate increased ($r^2$ values ranged from 0.79 in GPi to 0.84 in STN and GPe; $P < 0.01$ in all cases). Similarly, the peak frequency within a burst increased with increases in the firing rate ($r^2$ values ranged from 0.37 in GPi to 0.56 in GPe; $P < 0.01$). However, the number of bursts per cell, the proportion of spikes in bursts, the number of spikes per burst, the proportion of time occupied by bursts, the burst duration, and the surprise value were not significantly related to the cell’s firing rate.
the ISI preceding a burst and the number of spikes in that burst in 20–27% of cells in GPe, STN, and GPi. In many cells, including the example shown in Fig. 2B, the relationship appeared to be nearly linear for short burst lengths, but became more variable for very long bursts.

**Analysis of Burst Structure.** The average temporal structure of bursts in the normal state is shown in the plots in Fig. 3. In all three structures (particularly in the 2 pallidal segments), the first ISI belonging to the burst was shorter than the following ones, whereas the second ISI in the burst tended to be longer than the first or the subsequent two. In later phases of bursts, the lengths of later ISIs gradually increased. The variability of later ISIs was substantially greater than the variability for earlier ISIs in the sequence, as the number of data points contributing to these averages decreased (see Methods for details of calculation).

**Analysis of Postburst Period.** Postbursts ISI sequences were analyzed with methods similar to those used for the preburst ISIs. The analysis revealed that in most cases bursts are followed by a long-duration ISI (Fig. 4A), followed by a series of shorter ISIs and a gradual return to the cell’s average firing rate. This result was confirmed by the analysis of postburst ratios (Table 2), which were uniformly larger than 1, indicating that the ISI immediately following the burst was longer than the second ISI after the burst. Postburst ratios were independent of the burst length (data not shown).

Linear regression analysis, as shown for an example cell in Fig. 4B, revealed that the duration of the postburst ISIs was in many cells related to the length of the preceding burst. The regression analysis was carried out only on cells for which six or more different burst lengths were found, and the results are summarized in Table 4. In 14–20% of all cells in GPe, GPi, and STN, the number of spikes in a burst was related to the duration of the subsequent first postburst ISI. As mentioned for the analysis of preburst ISIs, the relation between burst length and postburst ISI appeared to be most linear for short burst lengths, but became more variable for very long bursts (Fig. 4B).

The regression analysis showed that, in a small number of cells, there was a correlation between burst length and the durations of both the immediate pre- and postburst ISIs (Table 4).

**Analysis of Interburst Epochs.** We also assessed the possibility that the number of spikes in bursts is related to the duration of the interval between any one burst and the burst immediately before or after it. As before, only cells with six or more different burst lengths were analyzed. We found that the number of spikes in a burst was related to the duration of the interval separating it from the preceding burst in 23–43% of cells (Table 4). Regression analysis between the burst length and the length of time to the next one revealed that this type of relationship is also common, being that 44–69% of cells in GPe, STN, and GPi showed this behavior (Table 4).

The regression analysis showed that the burst length was related to both the preceding and following interburst interval in a smaller number of cells (Table 4).

As shown in Table 4, there was no consistent effect of oscillations on the relationship between burst length and the time separating the burst from the preceding or following burst in the normal state.

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**FIG. 2.** Analysis of preburst intervals in the basal ganglia. A: normalized average interstimulus intervals (ISIs; ± SD) aligned to onset of bursts (ISI #1). GPe data are shown on top, STN data in the middle, and internal pallidal segment cell (GPi) data at the bottom. B: example of the analysis of the relationship between burst length and length of the ISI preceding burst onset in one GPi cell. Figure shows results of regression analysis between the number of ISIs in bursts and length of preburst ISI (in ms).
Burst Analysis in Monkey Basal Ganglia

Table 1. Burst characteristics

<table>
<thead>
<tr>
<th></th>
<th>GPe</th>
<th>STN</th>
<th>GPi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 44)</td>
<td>MPTP (n = 40)</td>
<td>Normal (n = 15)</td>
</tr>
<tr>
<td>Number of bursts/cell</td>
<td>978.5 ± 808.0</td>
<td>1343.4 ± 714.6</td>
<td>948.2 ± 467.6</td>
</tr>
<tr>
<td>Intraburst rate, spikes/s</td>
<td>205.4 ± 60.0</td>
<td>158.9 ± 52.3*</td>
<td>102.0 ± 34.2</td>
</tr>
<tr>
<td>Peak intraburst rate, spikes/s</td>
<td>300.4 ± 97.3</td>
<td>242.7 ± 83.8*</td>
<td>2140.8 ± 85.2</td>
</tr>
<tr>
<td>Burst length, spikes</td>
<td>3.0 ± 0.6</td>
<td>3.3 ± 0.5*</td>
<td>3.7 ± 0.6</td>
</tr>
<tr>
<td>Burst duration, ms</td>
<td>16.3 ± 7.7</td>
<td>23.7 ± 9.4*</td>
<td>41.2 ± 18.0</td>
</tr>
<tr>
<td>Proportion of spikes in bursts, %</td>
<td>11.5 ± 10.0</td>
<td>24.4 ± 14.1*</td>
<td>32.9 ± 10.9</td>
</tr>
<tr>
<td>Proportion of time spent in bursts, %</td>
<td>3.5 ± 2.9</td>
<td>6.9 ± 3.3*</td>
<td>7.2 ± 1.8</td>
</tr>
<tr>
<td>Interburst interval, ms</td>
<td>1027.6 ± 1414.2</td>
<td>384.7 ± 277.4*</td>
<td>542.3 ± 247.0</td>
</tr>
<tr>
<td>Preburst ratio</td>
<td>1.7 ± 0.6</td>
<td>2.2 ± 0.9*</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>Postburst ratio</td>
<td>1.7 ± 0.6</td>
<td>2.5 ± 1.0*</td>
<td>2.9 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 MPTP vs. normal state. See Table 1 for abbreviations.

MPTP-induced changes in firing rates and oscillatory activity. As shown in Table 1, the average discharge rate of cells recorded in GPe was significantly reduced in the MPTP-treated state compared with the normal state, whereas it was significantly increased in the STN. GPi firing rates were slightly increased, but this change was not statistically significant. In all three structures, the results remained the same even after bursts were excluded from the data stream (Table 1; see Methods).

Power spectral analysis showed that, in all three structures, the number of oscillatory cells in the 3- to 8- and 8- to 15-Hz ranges increased with MPTP treatment (Table 1). The predominance of oscillatory frequencies in the 3- to 8- and 8- to 15-Hz bands was also apparent in the analysis of integrated power spectra (Table 1). In all three structures, there were significant increases in the integrated power spectra in both of these spectral bands, whereas the relative proportion of the spectra was reduced in the spectral band above 15 Hz. The emergence of oscillatory bursting is apparent from the analysis of interburst intervals (Table 2), which tended to become less variable after MPTP treatment in all three structures. After MPTP treatment, the average interburst interval was in the 3-to 8-Hz range in STN and GPi (Table 2). Peaks in the power spectra of the intervals between the first spikes in bursts were identified in a subset of oscillatory cells. In GPe and STN, the proportion of oscillatory cells that showed peaks in the 3- to 8- and 8- to 15-Hz ranges of the interburst power spectra was substantially higher in the post-MPTP state than in the normal state (Table 1).

Table 3. Parameters of exponential curve fitting of burst length distribution

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPe</td>
<td>371.5</td>
<td>0.9306</td>
<td>0.9989</td>
</tr>
<tr>
<td>MPTP</td>
<td>195.9</td>
<td>0.7027</td>
<td>0.9983</td>
</tr>
<tr>
<td>STN</td>
<td>184.2</td>
<td>0.6933</td>
<td>0.9966</td>
</tr>
<tr>
<td>MPTP</td>
<td>134.2</td>
<td>0.5736</td>
<td>0.9993</td>
</tr>
<tr>
<td>GPi</td>
<td>625.8</td>
<td>1.1320</td>
<td>0.9992</td>
</tr>
<tr>
<td>MPTP</td>
<td>241.4</td>
<td>0.7851</td>
<td>0.9960</td>
</tr>
</tbody>
</table>

The relationship between the average proportion of bursts (P, in %) and the burst length (BL, in the number of ISIs) was fitted to a 2-parameter exponential decay function (P = a * e^(-x/BL)), See also Fig. 5. See Table 1 for abbreviations.

MPTP-induced changes in global parameters describing burst firing. The results of the analysis of ISIs belonging to bursts (as defined in Methods) are shown in Table 2. As expected, based on the regression analysis mentioned in the section on burst detection, the average and peak intraburst rates paralleled MPTP-induced changes in discharge rates. This finding is likely to be at least in part an artifact of the method of burst detection. However, other findings in the comparison of recordings in the normal and MPTP-treated states likely reflect true changes in bursting activity in GPe, STN, and GPi. Thus the average number of spikes/burst increased in all three structures (Table 2). The change in burst length is shown in greater detail in Fig. 5 with histograms of the distribution of burst lengths in GPe, STN, and GPi, before and after MPTP treatment. In all three structures under either condition, a burst length of three spikes (two ISIs) was the most prevalent, but in each structure, MPTP rendered the occurrence of longer bursts more likely. Before and after MPTP treatment, the burst length distribution plots for GPe, STN, and GPi could be fitted with a two-parameter exponential decay function (P = a * e^(-x/BL), where P is the proportion of bursts as percentage of the total number of bursts, and BL is the length of burst in the number of ISIs; Table 3). Increases in the proportion of spikes appearing in bursts, and the proportion of time occupied by bursts were also seen in all three structures (Fig. 6). As another reflection of the more common occurrence of bursts, the average amount of time between bursts (interburst interval) was significantly shortened in GPe and STN.

Several additional findings are also documented in Table 2. In GPe, the average burst duration and the average surprise value of the detected bursts increased after treatment with MPTP. In the STN, the number of bursts/cell increased significantly, whereas the burst duration decreased. The average surprise value decreased after MPTP, but this was not a statistically significant change. In GPi, the number of bursts per cell and in the average surprise value increased after MPTP. MPTP-induced changes in the temporal structure of preburst epochs. MPTP-induced changes in preburst epochs are shown in the left column in Fig. 7. This figure is organized in a similar manner as Fig. 2. In the MPTP-treated state, the preburst ISI showed greater variability, but in GPe and STN, it was longer on average than in the normal state. MPTP induced a significant increase in the preburst ratio in GPe (Table 2).
Regression analysis revealed that the proportion of cells with a significant relationship between prebursts ISIs and burst length was not changed in the MPTP-treated state in GPe and GPi. However, the proportion of cells in which the ISI preceding the burst predicted the length of the upcoming burst more than doubled in the STN (Table 4). In addition, MPTP induced small changes in the polarity of the correlation. Before MPTP treatment, the regression line had a positive slope (thus, preburst ISIs were likely to be longer before longer bursts) in five of eight GPe cells (62.5%), whereas after MPTP treatment, the relationship was positive in all seven cells observed. In the STN, all 3 cells observed in the normal state showed a positive slope to the regression line, and 11/12 cells (91.7%) showed this behavior after MPTP treatment. In GPi, seven of eight...

**FIG. 3.** Analysis of temporal structure of bursts in the basal ganglia. Figures show average ISIs, normalized to the mean of the 1st burst ISI (spike #1) for all three structures.

**FIG. 4.** Analysis of postburst intervals in the basal ganglia. A: normalized average ISIs (±SD) aligned to the end of bursts (ISI − 1). GPe data are shown on top, STN data in the middle, and GPi data at the bottom. B: example of analysis of relationship between burst length and length of the ISI after burst in the same GPi cell that was used for Fig. 2B. Figure shows results of regression analysis between number of ISIs in bursts and length of postburst ISI (in ms).
(87.5%) cells showed this behavior before MPTP, and all seven cells after treatment.

To detect whether the appearance of long preburst ISIs in the MPTP-treated state simply reflects the presence of oscillatory pause-burst-pause-burst patterns of firing, we tested whether the period corresponding to the dominant frequency associated with the observed oscillations in a given cell was within ±20% of the mean preburst ISI of the same cell. This was the case in only a single cell, an STN neuron that had been recorded from after MPTP treatment. This cell showed a mean preburst ISI of 137.4 ms and a power spectral peak at 9.8 Hz, corresponding to a period of 102.4 ms. A direct relationship between the preburst ISI length and the oscillatory period of the cells therefore appears unlikely. However, Table 2 shows that the average interburst interval in GPe, STN, and GPi after MPTP treatment was between 270 and 390 ms, and thus, partially overlaps with the 3- to 8-Hz power spectral range that showed a significant increase in oscillations after MPTP treatment. The possible interaction between oscillations and bursting is also apparent in Table 4, which shows that, in all three structures, oscillatory cells were more likely than nonoscillatory cells to show a significant correlation between the preburst ISI duration and the length of the subsequent burst in the parkinsonian state. However, the same relationship was also found in some nonoscillatory cells.

MPTP-INDUCED CHANGES IN BURST STRUCTURE. Differences in the burst structure between normal and parkinsonian states were observed in the STN and in GPi (Fig. 7, middle column). In both structures, the average ISI duration, normalized to the first ISI within the burst, were shorter in the MPTP-treated state than in the normal state. This was statistically significant only for the third and fourth ISI within the burst.

MPTP-INDUCED CHANGES OF THE TEMPORAL STRUCTURE OF POSTBURST EPOCHS. As shown in the right column in Fig. 7 and in Table 4, the length of the postburst ISI was longer in the MPTP-treated state than under normal conditions in GPe and STN. MPTP treatment also significantly increased the postburst ratio in both nuclei.

Regression analysis showed that burst length was related to the duration of the subsequent ISI in a larger percentage of cells in all three structures after MPTP treatment than before the treatment (Table 4). The regression lines had a positive slope in one of five cells (20%) in GPe before MPTP treatment and in five of seven cells after MPTP (71.4%). In the
STN, a positive slope was found in one of three cells in the normal state (33.3%), whereas three of seven cells (42.9%) showed this behavior after MPTP. In GPi, all five cells (100.0%) with a significant regression line showed a positive regression slope in the normal state, and seven of eight cells (87.5%) after MPTP treatment. For the postbursts ISIs, this is true in STN and GPe, whereas there was little change in GPi.

**TABLE 4. Interaction between burst length and other parameters (based on regression analysis)**

<table>
<thead>
<tr>
<th>Significant relationship between burst length and . . .</th>
<th>GPe</th>
<th>STN</th>
<th>GPi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>MPTP</td>
<td>Normal</td>
</tr>
<tr>
<td>Preceding ISI</td>
<td>Cells</td>
<td>%</td>
<td>Cells</td>
</tr>
<tr>
<td>Nonoscillatory cells</td>
<td>8/35</td>
<td>22.9</td>
<td>3/15</td>
</tr>
<tr>
<td>Oscillatory cells</td>
<td>5/35</td>
<td>14.3</td>
<td>2/13</td>
</tr>
<tr>
<td>Following ISI</td>
<td>5/35</td>
<td>14.2</td>
<td>3/15</td>
</tr>
<tr>
<td>Oscillatory cells</td>
<td>2/8</td>
<td>25.0</td>
<td>2/15</td>
</tr>
<tr>
<td>Preceding and following ISI</td>
<td>1/35</td>
<td>2.9</td>
<td>1/15</td>
</tr>
<tr>
<td>Nonoscillatory cells</td>
<td>1/27</td>
<td>3.7</td>
<td>0/23</td>
</tr>
<tr>
<td>Oscillatory cells</td>
<td>0/8</td>
<td>0</td>
<td>0/23</td>
</tr>
<tr>
<td>Preceding interburst interval</td>
<td>8/35</td>
<td>22.9</td>
<td>5/15</td>
</tr>
<tr>
<td>Nonoscillatory cells</td>
<td>5/35</td>
<td>14.3</td>
<td>5/15</td>
</tr>
<tr>
<td>Oscillatory cells</td>
<td>2/8</td>
<td>25.0</td>
<td>3/15</td>
</tr>
<tr>
<td>Following interburst interval</td>
<td>22/32</td>
<td>68.8</td>
<td>10/15</td>
</tr>
<tr>
<td>Nonoscillatory cells</td>
<td>14/24</td>
<td>58.3</td>
<td>9/23</td>
</tr>
<tr>
<td>Oscillatory cells</td>
<td>2/8</td>
<td>25.0</td>
<td>0/15</td>
</tr>
</tbody>
</table>

**FIG. 7.** MPTP-induced changes in burst and peri-burst intervals in the basal ganglia. Shown are normalized average ISIs (±SD) aligned to onset of bursts (left column) and to the end of bursts (right column). Burst onset and end occurred at spike 1 and −1, respectively. GPe data are shown on top, STN data in the middle, and GPi data at the bottom. Pre-MPTP data are shown as black circles; post-MPTP data are shown as gray circles. Middle column: average ISIs, normalized to mean of the 1st burst ISI (spike 1) for all 3 structures. *P < 0.05.
The proportion of cells for which the burst length is related to both the preceding and the following ISI was increased in all three structures after treatment with MPTP (Table 4).

To test whether the lengths of the postburst ISIs reflect the presence of oscillatory burst-pause-burst-pause patterns of firing, we analyzed in cells with oscillatory peaks whether the period corresponding to such peaks was within ±20% of the mean postburst ISI. None of the cells in this sample showed this behavior, indicating that it is unlikely that there is any direct relationship between the postburst ISI length and the oscillatory period of cells. As described before for the preburst ISIs, Tables 2 (interburst intervals) and 4 show that oscillatory cells were more likely to show a significant correlation between the postburst ISI duration and the length of the preceding burst.

**ANALYSIS OF INTRABURST EPOCHS.** In GPe and STN, the proportion of cells in which the number of spikes in a burst was related to the duration of the interval separating it from the preceding burst was increased after treatment with MPTP, whereas it was decreased by >50% in GPi (Table 4). In GPe, the relationship was positive in five of eight cells (62.5%) in which the burst length was related to the preceding interburst interval under control conditions. After MPTP, a positive slope of the regression plot was observed for only 4/11 cells (36.4%). In the STN, 4/11 cells showed a positive relationship in the normal state (36.4%) and 3/5 cells (60%) after MPTP-treatment. In GPi, a positive regression slope was found in 5/13 cells (38.5%) in the normal state and in 0/7 cells after MPTP.

After MPTP, the regression analysis of the length of time separating a given burst from the following one against burst length showed that this relationship became weaker in the GPe and STN, whereas it appeared to strengthen in GPi (Table 4). MPTP treatment altered the slopes of the regression lines. A positive slope was found in GPe in only 1/22 cells (4.5%) before MPTP treatment, and in 0/15 cells with a significant regression result after MPTP. None of the STN cells showed a positive regression slope (i.e., in all cells with a significant regression, longer bursts predicted shorter interburst intervals). In GPi, positive slopes were found in 1/12 cells before MPTP (8.3%) and in 2/17 cells thereafter (11.8%).

As observed under normal conditions, regression analysis showed that burst length was correlated to both the preceding and the subsequent interburst interval in a smaller number of cells than the number of cells in which burst length was related to just one of the two interburst intervals (Table 4).

**DISCUSSION**

These results show that bursting is common in spontaneous discharge in GPe, STN, and GPi under physiologic conditions and that in many neurons in these structures, the length of ISIs in the peri-burst epoch and the numbers of spikes within bursts are related. The findings suggest that bursts in spontaneous discharge in GPe, STN, and GPi are not simply abruptly occurring episodes of rapid neuronal firing but are often part of larger temporal structures that include epochs spanning several ISIs before and after the burst. In addition, significant relationships were found between the length of bursts and the intervals separating one burst from another, suggesting that in spontaneously discharging cells in STN, GPe, and GPi, the timing of a given burst may in part be determined by the length or intensity of other bursts in the sequence of ISIs.

The study also confirms that induction of the parkinsonian state results in substantial changes in firing rates and burst incidence in extrastriatal basal ganglia neurons. In GPe, STN, and GPi, bursts became more frequent, and occupied a greater proportion of the neuron’s discharge. Such changes have been described before (Filion and Tremblay 1991; Lee et al. 2001; Soares et al. 2004; Wichmann et al. 1999); however, it has not been clear whether bursting and rate changes are independent from one another. Our results suggest that, although changes in bursting undoubtedly alter the overall firing rate of neurons, rate changes are still evident after removal of bursts from the data stream, indicating that such changes do not merely reflect the increased bursting.

We also found that the proportion of cells in which bursts were linked to the flanking ISIs (particularly the postburst ISI) is increased in parkinsonism, and that complex changes occur in the relationships between the burst length and the length of the pre- and postburst intervals. In addition to rate and oscillatory changes, the increased burst incidence and the altered temporal structure of bursts and peri-burst periods may be mechanisms by which information processing in the basal ganglia is disturbed in the parkinsonian state.

**Technical considerations**

We used an established principle of burst detection, i.e., the surprise method (Legendy and Salcman 1985). This method defines bursts as statistically unlikely phenomena. We carefully adjusted the algorithm’s parameters so that the burst detection matched that of a human observer. Burst identification with this method strongly depends on the average discharge rate of the neuron under study. This complicates the comparison of burstiness between groups of neurons in which the baseline firing rate is different, as was the case in comparisons between cells recorded in the same nuclei before and after treatment with MPTP. We found that some descriptors of bursts (such as the peak or average intraburst frequency) were strongly affected by the neuron’s background firing rate, whereas other parameters, such as the number of bursts per cell, the proportion of spikes within bursts, or the amount of time spent bursting did not correlate with the cell’s background firing rate. The latter parameters are therefore more reliable indicators of a cell’s bursting characteristics, independent of changes in baseline firing rate.

Another technical consideration in this study is the stationarity of discharge. Basal ganglia cells recorded in vivo frequently display slow shifts of their firing patterns (Ruskin et al. 1999; Wichmann et al. 2002b), which complicates the generation of reliable estimates of firing rates or indicators of burst firing. We addressed this problem here by carefully choosing data segments that showed stationary discharge. Cells from which no extended segments of stationary discharge were recorded were excluded from the analysis.

It is also worth reiterating that the animals were in a state of quiet wakefulness throughout all of these recordings. Although we carefully avoided recording during episodes of noticeable drowsiness, phenomena such as shifts in attention or early drowsiness cannot be easily controlled. The behavioral state of the animals would have been better defined under task condi-
tions, but behavioral events in tasks would have occurred too frequently; therefore the lengths of the data segments available for burst analysis would have been too short. The applicability of our analysis of spontaneous basal ganglia activity to other conditions, such as during the performance of movements, is unclear at this time, although one would expect that baseline changes in the characteristics of burst discharges in individual cells would also affect their behavior under task conditions.

Finally, it cannot be ruled out that some of the findings in the parkinsonian state are caused by compensatory effects rather than dopamine depletion itself. Numerous compensatory mechanisms have been documented (see descriptions by Bezard et al. 2000, 2003; Zigmond et al. 1990, 2002). In many parkinsonian animals, the effect of these compensatory phenomena is readily apparent as animals show recovery after induction of initially severe parkinsonism. We recorded the post-MPTP data in our animals after a stabilization period of 3 mo, because, in our experience, the greatest degree of behavioral recovery occurs within the first few weeks after MPTP treatment, and a stable parkinsonian state is reached 2–3 mo after the injections. The issue is further complicated by the fact that we used unilateral rather than bilateral MPTP injections. This was done because unilateral treatment results in a parkinsonian state that is better tolerated by the animals. The unilateral dopamine depletion may induce compensatory changes beyond those seen with bilateral depletion (Vila et al. 2000). It is important to realize, however, that both compensatory changes and predominant unilateral involvement are also a common findings in human Parkinson’s disease. Thus although dopamine depletion itself may not have caused all of the MPTP-related findings reported here, our results may still adequately model the overall changes in bursting activity occurring in the extrastriatal basal ganglia in human parkinsonism.

Many bursts are embedded in sequences of ISIs with a stereotyped temporal structure

The findings in cells recorded in the normal state indicate that bursts in GPe, STN, and GPi are often framed by longer ISIs, as numerically indicated by the pre- and postburst ratios. These ratios were >1 in the normal state in 65–70% of all bursts (as opposed to 50% that would be expected by chance alone; data not shown), resulting in a mean preburst ratio of 1.7–2.5. In addition, at least in GPe and GPi, pre- and postburst ISIs appeared to follow a predictable time-course (as shown in Figs. 2 and 4).

Interestingly, in many cells, the length of a given burst was related to the length of the preburst ISI. The burst length distribution plots depicted in Fig. 5 show that the largest number of bursts were, in fact, short (involving only 3 spikes), so that the described relationship between the burst length and peri-burst epochs is based on findings in a relatively small number of longer bursts.

The finding that there is a relationship between the length of the preburst ISI and the length of the subsequent burst supports the idea that some of the bursts occurring spontaneously in the basal ganglia may represent “rebound” bursting. Rebound bursts after strong hyperpolarization have been described in various basal ganglia structures, including the guinea pig pallidum (the equivalent of GPe in monkeys) (Nambu and Linas 1994) and the STN (Beurrier et al. 1999; Bevan et al. 2000, 2002a; Nakanishi et al. 1987; Overton and Greenfield 1995). The study of the rebound burst-producing interaction between GPe and STN has received the most attention in this field. In vitro recording studies in basal ganglia slice preparations in rodents have shown that the spontaneous discharges of STN neurons are driven by a persistent depolarizing sodium current. Action potentials are followed by afterhyperpolarizations, which are at least in part caused by a potassium current that is activated through calcium entry into the cell associated with the action potential (Bevan and Wilson 1999). Single inhibitory postsynaptic potentials (IPSPs) evoked in STN cells by GPe stimulation reset these spontaneous oscillations, whereas multiple IPSPs may induce sufficient hyperpolarization to activate a rebound depolarization, which may then generate a burst of action potentials in the STN cell. The durations of the bursts varies with the intrinsic rebound properties of the postsynaptic STN neuron (Bevan et al. 2000, 2002a; Stanford 2003). While the initial slice recording studies emphasized the role of GABA<sub>α</sub> receptors in this process, more recent work has shown that rebound bursts in the STN can also be elicited by hyperpolarization of STN cells mediated by GABA<sub>β</sub> receptors. Concomitant activation of postsynaptic GABA<sub>α</sub> and GABA<sub>β</sub> receptors was shown to result in more intense bursting (Hallworth and Bevan 2005).

It is therefore perhaps not surprising that long-lasting hyperpolarizations, resulting in long preburst ISIs, would be followed by intense bursting. Rebound bursting has also been discussed recently as a mechanism that explains the appearance of synchronized oscillatory bursts in GPe and STN cells in a basal ganglia co-culture environment (Bevan et al. 2000, 2002b; Plenz and Kitai 1999). Burst output from the STN, because of its comparatively distributed innervation of GPe (Hazrati and Parent 1992; Parent and Hazrati 1995) and other network features, may result in increased entrainment of GPe cells (Bevan et al. 2002b; Plenz and Kitai 1999) and may produce synchronized oscillatory bursts in the GPe–STN network. In our study, the proportion of cells with a relationship between preburst ISIs and burst length was greater among oscillatory cells, although such relationships were also present in cells classified as nonoscillatory (Table 4).

The relationship between bursts and the first postburst ISI is less well explained, but further supports the view that bursts are not isolated clusters of short ISIs, but may represent in many cases components of ISI patterns starting with a long preburst interval, and ending after a long postburst interval.

It is also possible that firing patterns in which bursts are flanked by particularly long ISIs or by sequences of predictably changing ISI length are not generated through mechanisms intrinsic to the basal ganglia, but are superimposed on the firing of basal ganglia neurons by afferent inputs, such as those originating in cortex. Although previous experiments in awake monkeys showed that lesions of areas 4 and 6 in the precentral cortex did not change the intensity of bursts in GPe or GPi (Aldridge and Gilman 1991), more recent experiments have suggested that cortical activity may, in fact, profoundly impact bursting in the basal ganglia (Beurrier et al. 1999; Gatev and Wichmann 2003; Magill et al. 2000a,b, 2004; Wichmann et al. 2002a). This is particularly well documented for the subthalamic nucleus but may also apply to GPe and GPi, because both receive substantial inputs from the STN, which, at least to
some extent, determines their firing patterns (see also Hamada and DeLong 1992). Recent experiments in which cortical areas were stimulated while activity in the basal ganglia was recorded have shown that momentary cortical activation can result in excitation–inhibition sequences of activity in the basal ganglia because of the fact that a given cortical site may be linked to a basal ganglia site through different pathways with different latencies and different polarity (Kita et al. 2004; Nambu et al. 2000). Such response sequences are also known to occur frequently under conditions in which animals are assessed while performing behavioral tasks (Brothier et al. 1991; Crutcher and DeLong 1984; Gdowski et al. 2001; Georganopoulos et al. 1983; Hamada et al. 1990; Wichmann and Kliem 2004; Wichmann et al. 1994). It is conceivable that more subtle or discrete cortical activity may trigger predictable sequences of long and short ISIs in the basal ganglia even under “spontaneous” conditions as in our study.

**MPTP changes in bursting**

Our results confirm that MPTP treatment enhances bursting in GPe, GPi, and STN (Bergman et al. 1994; Boraud et al. 1998; Filion and Tremblay 1991; Lee et al. 2001; Wichmann and DeLong 2003b; Wichmann et al. 1999). Changes in the temporal structure of bursts themselves were minor, but the relationship between the burst length and the preceding and following ISIs changed with MPTP treatment, particularly in oscillatory cells. The last preburst ISI and first postburst ISI were lengthened in GPe and STN. Burst lengths became more tightly related to the preburst ISI in the STN and the postburst ISI in all three structures.

If bursts occur more frequently and are more often flanked by abnormally long ISIs, the cell is spending overall more time in a relatively predictable state that may interfere with the reliable transmission of information through the basal ganglia. Other changes in the physiology of the basal ganglia also support the possibility that the basal ganglia–thalamocortical network becomes more predictable in parkinsonism. For instance, low-frequency oscillations (Bergman et al. 1998; Brown 2003; Brown et al. 2001, 2002; Dostrovsky and Bergman 2004; Goldberg et al. 2002; Karmon and Bergman 1993; Levy et al. 2002; Nini et al. 1995; Wichmann et al. 1999; Williams et al. 2002) as well as enhanced interneuronal synchronization in parkinsonism (Goldberg et al. 2002; Heimer et al. 2002; Morris et al. 2005; Raz et al. 1996, 2001), may indicate a less perturbable state of the basal ganglia–thalamocortical system. Thus at the level of individual basal ganglia cells, oscillatory activity in the parkinsonian state usually indicates that these cells are a subgroup of cells in the basal ganglia that are particularly strongly affected by MPTP treatment (see also Bergman et al. 1994).

The effects of MPTP on the relationship between burst length and the interburst intervals before or after the burst were very complex. In GPe and STN, the relationship between the burst length and the preceding interburst interval became stronger, whereas the relationship to the interburst interval after the burst appeared to weaken. The opposite changes were seen in GPi. It is uncertain how interburst intervals and burst length are related and how the MPTP-induced changes in the relationship between these parameters arise, but our data suggest that these changes are not directly related to evolving oscillatory activity in parkinsonism. In general, a change in the relationship between interburst intervals and burst lengths further supports the idea that information processing in the basal ganglia themselves or in portions of the basal ganglia–thalamocortical circuits that feed into the basal ganglia is disturbed in parkinsonism.

Many of the changes in pre- and postburst ISIs and interburst intervals seem to affect GPi discharge differently than GPe or STN discharge. This cannot be explained by differences in MPTP-induced rate changes alone, because GPe and STN show reciprocal rate changes, and the rate change observed in GPi was not statistically significant in our sample. Our findings add to the impression that burst discharges in GPe and STN are closely linked, as has been suggested by previous experiments in rodents (Bevan et al. 2000, 2002a; Hallworth and Bevan 2005; Plenz and Kitai 1999), but also suggest that abnormalities in the “indirect” basal ganglia pathway, to which GPe and STN belong, do not necessarily translate directly into similar abnormalities in GPi.

In summary, our results show the presence of predictable patterns of ISIs before and after bursts in the normal state. This study also documents that bursts become more frequent and that their temporal structure changes in parkinsonism. Both abnormalities may contribute to abnormal processing in the basal ganglia–thalamocortical circuits in this disorder.

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