Spontaneous Pallidal Neuronal Activity in Human Dystonia: Comparison With Parkinson’s Disease and Normal Macaque

Philip A. Starr,1,4 Geoff M. Rau,1 Valerie Davis,1 William J. Marks, Jr.,2,4 Jill L. Ostrem,2,4 Donn Simmons,1 Nadja Lindsey,3 and Robert S. Turner1,4

1Departments of Neurological Surgery, 2Neurology, and 3Nursing, University of California, San Francisco; and 4Parkinson’s Disease Research, Education, and Clinical Center at the San Francisco Veterans Affairs Medical Center, San Francisco, California

Submitted 17 September 2004; accepted in final form 3 February 2005

Starr, Philip A., Geoff M. Rau, Valerie Davis, William J. Marks, Jr., Jill L. Ostrem, Donn Simmons, Nadja Lindsey, and Robert S. Turner. Spontaneous pallidal neuronal activity in human dystonia: comparison with Parkinson’s disease and normal macaque. J Neurophysiol 93: 3165–3176, 2005. First published February 9, 2005; doi:10.1152/jn.00971.2004. Dystonia is a movement disorder defined by sustained muscle contractions, causing twisting and repetitive movements and abnormal postures. To understand the abnormalities in pallidal discharge in dystonia, we have analyzed the spontaneous activity of 453 neurons sampled from the internal or external pallidum (GPi or GPe) of 22 patients with dystonia, 140 neurons from 11 patients with Parkinson’s disease (PD), and 157 neurons from two normal non-human primates (NHPs; Macaca mulatta). All recordings were performed without systemic sedation. Mean GPi discharge rate in dystonia was 55.3 ± 1.3 (SE) Hz. This was significantly lower than in the normal NHPs (82.5 ± 2.5 Hz) and lower than in PD patients (95.2 ± 2.3 Hz). Mean GPe discharge rate in dystonia (54.0 ± 1.9 Hz) was lower than in the normal NHPs (69.7 ± 3.3 Hz) and was indistinguishable from that in PD patients (56.6 ± 3.5 Hz). Mean GPi discharge rate was inversely correlated with dystonia severity. GPi showed increased oscillatory activity in the 2- to 10-Hz range and increased bursting activity in both dystonia and PD as compared with the normal NHPs. Because the abnormalities in discharge patterns were similar in dystonia compared with PD, we suggest that bursting and oscillatory activity superimposed on a high background discharge rate are associated with parkinsonism, whereas similar bursting and oscillations superimposed on a lower discharge rate are associated with dystonia. Our findings are most consistent with a model of dystonia pathophysiology in which the two striatal cell populations contributing to the direct and indirect intrinsic pathways of the basal ganglia both have increased spontaneous activity.

INTRODUCTION

Dystonia is a movement disorder defined by sustained muscle contractions, causing twisting and repetitive movements and abnormal postures. The pathophysiology of dystonia is incompletely understood, but it is thought to involve the loop circuit from sensorimotor cortices (SMC) through parts of the basal ganglia and ventrolateral thalamus and back to cortex (Marsden et al. 1985; Vitek et al. 1999). The globus pallidus internus (GPI) occupies a critical position in this circuit, being the major output structure of the basal ganglia “motor” territory (Alexander et al. 1990). Decreases and increases in discharge of the GABAergic neurons of GPI are believed to facilitate and suppress, respectively, the activity of recipient thalamocortical circuits and, eventually, muscle activity (Wichmann and DeLong 1998). Hypo- and hyperkinetic movement disorders have been modeled as imbalances in the suppressive or facilitatory effects of pallidal output (Bergman et al. 1990; Mink 2003; Wichmann and DeLong 1998).

The resurgence of microelectrode-guided basal ganglia surgery for movement disorders affords the opportunity to study pallidal electrophysiology in a variety of disease states. Pallidal single-cell discharge characteristics are best documented in Parkinson’s disease (PD). PD in the off-medication state has been found to be associated with excessive and abnormally patterned neuronal activity in the motor territory of the GPi (Hutchison 1998; Levy et al. 2001; Magnin et al. 2000; Sterio et al. 1994).

Several groups have described single-unit microelectrode recordings in dystonic humans in the awake state, but unresolved questions remain. Four reports, describing data collected from a total of 16 patients reported that the mean spontaneous GPi discharge rate is abnormally low in dystonia (Lenz et al. 1998; Merello et al. 2004; Sanghera et al. 2003; Vitek et al. 1999). In contrast, Hutchison et al. (2003) showed that in seven dystonic patients, GPi discharge was not reduced and was in fact similar to the hyperactive pallidal discharge of Parkinson’s disease. Given the heterogeneity in the type and severity of dystonia represented in prior series, analysis of a larger number of cases, controlling for disease type and severity, is needed to resolve the discrepancies.

Vitek et al. (Vitek 2002; Vitek et al. 1999) have focused on patterns of discharge in the dystonic GPi, proposing that intermittent bursts of high-frequency discharge are a prominent feature of dystonia. It is unclear, however, if bursts in single-unit discharge occur in a periodic manner and, if so, in what frequency range. Abnormal oscillatory activity in specific frequency bands has been proposed to be a fundamental feature of disordered basal ganglia function (Bergman et al. 1994; Brown 2003; Ruskin et al. 2002; Silberstein et al. 2003). This motivates a closer investigation of oscillatory activity in single-unit data in dystonia.

When the dystonic state is studied in isolation, it is difficult to determine which of the electrophysiologic characteristics are actually abnormal and which abnormalities are unique to the dystonic state. The optimal control group would be normal humans, but single-unit recording in normal humans cannot be performed due to the invasiveness of the technique. Of note,
many similarities have been found between GPi recording obtained from non-human primates (NHPs) in the parkinsonian state (Filion and Tremblay 1991; Miller and DelLong 1987; Wichmann et al. 1999) and those from humans with PD (Hutchison 1998; Levy et al. 2001; Magnin et al. 2000; Sterio et al. 1994). This suggests that pallidial physiology of the NHP will provide a reasonable approximation to what would be observed in the normal human. A direct comparison of GPi discharge in human dystonia with that of the normal NHP, using similar methodology, however, has not been performed previously.

To clarify the nature of abnormal basal ganglia output in dystonia, we have analyzed the spontaneous activity of 453 pallidal neurons sampled from 22 patients with dystonia, 140 neurons from 11 patients with Parkinson’s disease, and 157 neurons from two normal NHPs (Macaca mulatta). We used identical recording and analysis methods for all subjects. Recordings were obtained without systemic anesthetics or sedatives.

METHODS

Patient population

Single-unit recording in GPi and GPe was performed in awake patients undergoing physiologic mapping for placement of DBS electrodes. Of 28 dystonia patients mapped between 1999 and 2004, 22 were included in this study. All subjects gave informed consent according to a protocol approved by the Institutional Review Board. Patients were excluded from this study if physiological mapping had to be performed with general anesthesia or intravenous sedation, or if recordings from fewer than five units from GPi or GPe were amenable to analysis. A quantitative measure of dystonia severity was obtained in the month prior to surgery, using a standard clinical rating scale, the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) (Burke et al. 1985), by a movement disorders neurologist (W. J. Marks, Jr or J. L. Ostrem). All patients underwent magnetic resonance imaging (MRI) of the brain prior to surgery, and all juvenile-onset dystonia patients had genetic testing for the presence of a mutation at the DYT-1 locus (Ozelius et al. 1997). We also studied 11 patients undergoing pallidal DBS implantation for PD, who were rated preoperatively with the UPDRS.

Surgical procedures and data recording

HUMAN. Procedures for pallidal localization and electrophysiology were similar to those documented in recent publications (Lozano et al. 1996; Starr 2003; Starr et al. 2004; Vitek et al. 1998). Most patients required sedation with propofol for placement of the stereotactic headframe and stereotactic MRI, due to involuntary muscle spasms. All patients, including PD patients, received propofol for placement of a foley catheter immediately prior to surgery and for the surgical incision. In all cases, propofol was stopped ≥30 min prior to the start of pallidal recording, and the total dose of propofol given did not exceed 200 mg. All patients were alert and oriented at the start of microelectrode recording. Patients were instructed to make no voluntary movements during the recording, although most patients with dystonia at rest did experience spontaneous dystonic spasms during the recording.

For a subset of patients, four channels of surface electromyographic (EMG) data were collected (DelSys, Boston, MA) at 1000× gain, filter band-pass of 100–800 Hz, and sampling rate of 20 kHz. For patients with generalized dystonia, EMG data were recorded from the contralateral biceps, triceps, rectus femoris, and hamstrings. For patients with cervical dystonia, EMG data were recorded from the contralateral biceps, triceps, trapezius, and sternocleidomastoid. Summed triaxial acceleration was recorded from the contralateral wrist. These signals were used to document any spontaneous movement that occurred during the recording period as well as for statistical correlation with neuronal activity.

Single-unit discharge was recorded with glass-coated platinum/iridium microelectrodes, impedance 0.4–1.0 MΩ at 1000 Hz (Microprobe, Gaithersburg, MD, or FHC, Brunswick, ME). Recordings were filtered (300 Hz to 5 kHz), amplified, played on an audio monitor, and digitized (20-kHz sampling rate) using the Guideline System 3000 (Axon Instruments, Foster City, CA, now distributed by FHC). Microelectrodes were advanced into the brain using a motorized microdrive (Axon Instruments clinical micropositioner or FHC microdrive).

In a typical surgical case, three to four parallel parasagittal microelectrode penetrations were made serially through GPe and GPi on each side, separated by 2–3 mm, in one to two parasagittal planes. All except two patients were operated bilaterally, and neuronal data collected from both hemispheres were pooled. The optic tract (OT) was detected by light-evoked action potential discharge at the pallidal base. Cells were recorded at approximately every 300–800 μm along each trajectory. Somatosensory examination was performed during all recordings to detect cells responsive to movement, so as to determine whether the region recorded was within the motor territory of the relevant nucleus. Neuronal activity was collected for a minimum of 20 s. Prior to recording, subjects were asked to remain as still as possible during these periods of recording. The location and discharge characteristics of cells along each microelectrode track were plotted on scaled drawings, noting also the locations of white matter laminae and the OT. The tracks were superimposed on drawings of parasagittal sections from the Schaltenbrand and Warren human brain atlas, according to a visual judgment of “best fit” of the tracks to the atlas. Cells encountered between the internal medullary lamina and the optic tract were considered internal pallidal cells, whereas those between the striatum and the internal medullary lamina were considered external pallidal cells. Cells near the presumed GPe-GPi border, on a track where a definite white matter boundary was not identified, were excluded from analysis due to their uncertain localization.

Surgical procedures and data recording

NHP. Two rhesus macaques (male, 10 kg and female, 6 kg) were surgically prepared for recording using aseptic surgery under isoflurane inhalation anesthesia. All aspects of animal care were in accord with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals,” and all procedures were approved by the UCSF Institutional Animal Care and Use Committee. A cylindrical stainless steel chamber (18 mm ID) was implanted with stereotactic guidance over a burr hole allowing access to nuclei of the posterior basal ganglia [centered on Horsley-Clark anterior 10, lateral 20, depth 20 (Winters et al. 1969)]. The chamber was oriented parallel to the coronal plane at an angle of 35° from vertical. The chamber was fixed to the skull with bone screws and dental acrylic. Bolts were embedded in the acrylic to allow fixation of the head during recording sessions.

After a minimum interval of 1 mo after placement of recording chambers, serial microelectrode penetrations through the globus pallidus were performed along parallel coronally oriented trajectories separated by 1.0 mm. No sedative agents were used for the recording sessions. Microelectrodes were identical to those used in the human. The electrodes were advanced into the brain using a hydraulic microdrive (MO-95, Narishige International, Tokyo, Japan). Neuronal signals were amplified (10,000×), filtered (150-Hz to 8-kHz band-pass), and digitized (40 kHz) using one channel of a Multichannel Acquisition Processor (Plexon, Dallas, TX). A 2-min record of continuous data was collected for each neuron while the animal rested quietly in a sound- and light-shielded chamber. Neurons were then examined for
responses to experimenter-imposed manipulations of leg and arm joints, the trunk, and face.

Analysis of spontaneous activity

Digitized spike trains were imported into off-line spike sorting software (Plexon) for discrimination of single populations of action potentials by principal components analysis. This software generated a record of spike times (subsequently reduced to millisecond accuracy) for each action potential waveform detected. The interspike intervals (ISIs) between successive spike times were used to evaluate stationarity of discharge, to calculate parameters of the ISI distribution, to construct autocorrelograms, and to evaluate the data stream for the occurrence of bursting or irregularity in discharge (see following text). Analyses were performed in Labview and Matlab programming environments.

Neuronal data were included in this study only if action potentials could be discriminated with a high degree of certainty, if the complete record of ISIs fulfilled statistical criteria for stationarity of discharge [as tested off-line with the runs-test (Tuckwell 1988)], if the number of recorded action potentials >800, and if the spontaneous activity of the neuron was recorded for ≥20 s (human) or 60 s (NHP). [For the units that met these criteria, the mean stationarity was 0.37 ± 0.17 (±SD).]

NONOSCILLATORY BURSTING. The data were submitted to a variety of pattern detection algorithms to assess nonoscillatory bursting. To facilitate comparison of our data with other publications on discharge abnormalities in movement disorders, three methods for burst detection described in other studies were used here: the “L,” statistic, after Kaneoke and Vitek (1996) and Goldberg et al. (2002); the “burst index” (Hutchison et al. 2003), defined as the mean ISI divided by the modal value; and the Poisson “surprise” method of Legendy and Salcman (Legendy and Salcman 1985; Wichmann et al. 1999). In this latter method, bursts in the discharge stream are defined as segments of data with a Poisson surprise value of >10. The minimum number of spikes that can constitute a burst in this method was four. The resulting data were tabulated as the proportion of ISIs within bursts compared with the total number of ISIs in the entire data stream.

OSCILLATORY ACTIVITY. Autocorrelograms were used to detect oscillatory activity. Autocorrelation functions were calculated from the ISI data for lags of −500 to +500 ms, low-pass filtered (100-Hz cutoff, Remez FIR method), and plotted for visual inspection. The functions were statistically evaluated following methods modified slightly from Raz et al. (1996). First, the central trough associated with the refractory period of a cell was removed from the autocorrelation to reduce high-frequency noise. The mean of the autocorrelogram was subtracted and the autocorrelogram was resampled to 5-ms bins so as to improve the resolution of low-frequency oscillations. A power spectrum was computed from the rebinned autocorrelogram (±500 ms in 5-ms bins), providing a 0.4-Hz resolution of frequencies. The fast Fourier transform for this analysis was calculated using nonoverlapping segments of 512 data points and a boxcar window of the same length. The activity of a cell was considered to have a significant oscillatory component if the power spectrum had one or more significant peaks between 2 and 30 Hz. A peak in the spectrum was considered significant if either of the following two criteria were met: 1) peak signal-to-noise ratio (SNR) exceeded 7 SD. Peak SNR was computed as the difference of peak power and mean power (2–30 Hz) divided by SD of the entire power spectrum (0–100 Hz). 2) Oscillation index (OI) of the peak exceeded 10%. Peak OI reflected the area under a spectral peak and above mean power (2–30 Hz) divided by the total power under the spectrum. These thresholds for statistical significance of SNR and OI were established empirically according to a Monte Carlo analysis to avoid assumptions about the population statistics of those measures (Tsau and Chen 1989). In this analysis, SNR and OI measurement were computed for 10,000 synthetic “random” spike trains generated assuming Poisson firing statistics and a mean firing rate of 50 Hz. The thresholds cited above were found to reject false positives within these synthetic spike trains with 95% accuracy.

Analysis of spike-EMG coherence

For data sets that included EMG and accelerometer recordings, the EMG data were rectified, smoothed (100-Hz low-pass, zero phase lag Remez FIR method), and sub-sampled to a 200-Hz sampling rate. A spike density function (SDF) was constructed from the neuron’s spike train as the sum of Gaussian functions (unit area, 10-ms variance) centered on individual spike times. (For a detailed justification and description of this method, see Szucs 1998). The SDF was then low-pass filtered (100-Hz cut-off) and sub-sampled to a 200-Hz sampling rate. Cross-correlograms and coherence functions were computed for all SDF–EMG or SDF–acceleration combinations according to the methods of Halliday et al. (1995). Instances of significant coherent oscillation in neuronal activity and EMG/acceleration were detected as peaks in the coherence function <30 Hz that had a raw probability of P < 0.0002. This threshold was determined to reject false positives in 95% of cases in Monte Carlo analyses of identically processed random synthetic data.

Hypothesis testing

Hypothesis testing was performed using the SPSS statistical package (SPSS, Chicago, IL). We tested the hypothesis that mean spontaneous pallidal discharge parameters in patients with dystonia, patients with PD, and normal NHPs are different, using the independent sample t-test (for continuous data) or the χ² test (for categorical data). We tested the hypothesis that mean pallidal discharge rate in dystonia correlates with dystonia severity using Spearman’s rho. Difference in severity and discharge rate between subtypes of dystonia was tested using the Kruskall Wallis exact test, two sided.

RESULTS

Patient characteristics

Characteristics of the 22 dystonia patients (labeled cases A–V) are presented in Table 1. Because dystonia is a heterogeneous disorder, different etiologies were represented in this series: idiopathic dystonia (14 cases), tardive dystonia (3 cases), and secondary dystonia (3 cases). Two cases were classified as unknown etiology because the patients were adopted at ages 2 and 5 with a movement disorder already present, normal MRI scans of the brain, and an unknown prior medical history. In these two cases, secondary dystonia from perinatal or early childhood brain injury cannot be ruled out. The three patients with secondary dystonia had definite structural brain abnormalities on MRI, as follows: bilateral parietal encephalomalacia (case R, posttraumatic dystonia), T2 hyperintensity in the lentiform nuclei (case S, pantothenate-kinase associated neurodegeneration), and a focal left posterior thalamic T1 hypointensity (case T, poststroke dystonia). All other patients had normal brain MRIs. The idiopathic dystonias were further characterized as juvenile onset, positive for the DTY-1 mutation (Ozelius et al. 1997) (4 cases); juvenile onset, negative for the DYT-1 mutation (4 cases); or adult onset (6 cases).

Two patients (cases E and T) had a visible dystonic tremor during surgery (Svetel et al. 2004). Three cases (A, F, and O) had minimal dystonia at rest but had action-induced dystonia that produced severe disability, including inability to use the arms for any skilled activities (case A) and inability to walk (case O). Clinical characteristics of the 11 PD patients were as...
follows: age at surgery = 59 ± 8 (SD) yr; duration of symptoms = 15 ± 5 yr; baseline UPDRS motor subscore in the off medication state = 43 ± 8, daily levodopa dosage = 980 ± 390 mg. All PD patients manifested prominent bradkinesia during surgery, whereas none manifested obvious dystonia. Only one of the PD patients had tremor during surgery.

**Pallidal discharge rate in dystonia, normal NHPs, and PD**

Summary data, in which all units recorded in each condition (dystonia, normal NHPs, and PD) are pooled, are shown in Table 2. Mean GPi discharge rate in dystonia was lower than that in the normal NHPs and in Parkinson’s disease patients (P < 0.001 for both comparisons, 2-sample independent t-test). Mean GPe discharge rate in dystonia was lower than in the normal NHP, but it was statistically indistinguishable from that in PD. (The infrequently recorded, low-frequency GPe bursting cells were deliberately excluded from the analysis.)

GPi and GPe discharge rates for the individual cases of dystonia, compared with NHPs and patients with PD, are illustrated in boxplots in Fig. 1. All dystonic patients had lower...
account for the differences between groups in neuronal firing. Recorded during the last half (sessions were statistically indistinguishable from those recorded during the first half of those microelectrode mapping was relatively long (3 h). The mean GPi discharge rates recorded during the first half of those sessions were statistically indistinguishable from those recorded during the last half (P = 0.72, independent sample t-test), reinforcing the view that anesthetic dosing cannot account for the differences between groups in neuronal firing.

median GPi discharge rates than all of the PD patients. Median discharge rates in dystonia were generally lower than those in the normal NHPs, but several dystonic patients (cases C, H, and M–O) had median discharge rates in the same range as those of the normal NHPs.

To better understand the variability in GPi discharge rates between dystonia patients, mean GPi discharge rates for all cases of dystonia were plotted as a function of dystonia severity and etiology (Fig. 2). Statistical comparison of group means for dystonia subtypes is provided in Table 3. GPi discharge rates were found to be significantly higher for patients with primary dystonia (57.8 ± 1.5 Hz) and tardive dystonia (54.6 ± 3.1 Hz) versus those with secondary dystonia (34.0 ± 3.5 Hz; Kruskall Wallis exact test, 2-sided, P = 0.05). For patients with primary dystonia, there was a significant inverse correlation of GPi neuronal discharge rate with the severity of dystonic symptoms (P = 0.028, Spearman’s rho), and this correlation accounted for 33% of the variability in discharge rate. GPi discharge rate was not correlated with dystonia severity or type.

Pallidal discharge rate as a function of time elapsed from administration of sedative agents

All patients in both disease groups received propofol for sedation during stereotactic frame placement and MRI that was halted ≥30 min prior to the start of recording. The NHPs received no anesthetic in the days prior to recording. To determine if discharge rates in patients were affected by lingering effects of the anesthetic agent, we examined neurons recorded in a subset of cases where the total duration of microelectrode mapping was relatively long (>3 h). The mean GPi discharge rates recorded during the first half of those sessions were statistically indistinguishable from those recorded during the last half (P = 0.72, independent sample t-test), reinforcing the view that anesthetic dosing cannot account for the differences between groups in neuronal firing.

Table 2. Spontaneous pallidal discharge parameters in dystonia, normal NHPs, and PD

<table>
<thead>
<tr>
<th></th>
<th>GPi</th>
<th>GPe*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>No. of units</td>
<td>302</td>
<td>96</td>
</tr>
<tr>
<td>Mean rate, Hz</td>
<td>55.3+/-1.3</td>
<td>82.5+/-2.5</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001†</td>
<td>P &lt; 0.001‡</td>
</tr>
<tr>
<td>Mean proportion of burst discharges (Legendy method)</td>
<td>0.026+/-0.001</td>
<td>0.017+/-0.002</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001†</td>
<td>P &lt; 0.001‡</td>
</tr>
<tr>
<td>Mean L statistic</td>
<td>5.9+/-0.08</td>
<td>5.1+/-0.08</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001†</td>
<td>P &lt; 0.001‡</td>
</tr>
<tr>
<td>Mean burst index of normalized ISI distribution</td>
<td>3.7+/-0.1</td>
<td>2.3+/-0.2</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001†</td>
<td>P &lt; 0.001‡</td>
</tr>
<tr>
<td>Proportion of units with significant oscillations</td>
<td>0.282 (85/302)</td>
<td>0.114 (11/96)</td>
</tr>
<tr>
<td>(χ² = 14.1, P &lt; 0.0001)†</td>
<td>(χ² = 3.1, P &lt; 0.001)†</td>
<td></td>
</tr>
<tr>
<td>Mean frequency of significant oscillations, Hz</td>
<td>6.0+/-0.5</td>
<td>4.8+/-1.1</td>
</tr>
<tr>
<td></td>
<td>6.2+/-1.4</td>
<td>7.2+/-1.5</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>4.1+/-0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. GPi and GPe, internal and external pallidum; PD, Parkinson’s disease; ISI, interspike interval. *GPe burster cells not included in this analysis; †indicates a value that was significantly different from that for normal macaque by t-test; ‡indicates a value that was significantly different between dystonia and PD by t-test.

Mediation of bursting or irregular discharge

Group means for the various measures of discharge pattern in dystonia, normal NHPs, and PD are given in Table 2. In GPi, all three measures of bursting and discharge irregularity (the L statistic, burst index, and proportion of burst discharges by the “Poisson surprise” method) were significantly higher in dystonia than in the normal NHPs (P < 0.001, independent sample t-test). The parkinsonian GPi had a similarly high proportion of burst discharges by the Poisson surprise method, and this was also significantly different from the normal NHPs (P < 0.001). It is worth noting that both L statistic and Poisson surprise statistics were found to be sensitive to GPi firing rates, the former negatively and the latter positively correlated (P < 0.001 Spearman’s rho; for separate comparisons within the dystonic, NHP, and PD datasets). The increased prevalence of burst discharges in the dystonic GPi, however, was found independent of which burst statistic was used. GPi bursting was not consistently different between dystonia subtypes (Table 3), although one measure of bursting, (Poisson surprise method) did show a higher proportion of burst discharges in tardive dystonia compared with primary dystonias.

Bursting discharge in GPe cells did not differ greatly between dystonia and normal NHP. Two of the measures of bursting (the L statistic and the proportion of burst discharges by the Poisson surprise method) were statistically indistinguishable. The third measure, the burst index, was significantly higher (indicating greater burstiness) for dystonia, at the threshold of significance, P = 0.05 (Table 2). The group bursting in GPe did not differ between dystonia subtypes.

Raster diagrams showing the pattern of discharge of GPi cells exhibiting notably abnormal behavior are shown in Fig. 3. Because increased bursting was a feature of both dystonia and PD, we performed a more detailed comparison of intra-burst properties (detected by the Poisson surprise method)
between these two conditions. The mean frequency within the burst (206 ± 3 Hz for dystonia and 236 ± 4 Hz for PD) were higher in PD than in dystonia (independent sample t-test, \( P < 0.001 \)), although the quantitative difference was small and may reflect the difference in the underlying discharge rate. The mean burst duration, number of spikes per burst, and maximum frequency within the bursts did not differ.

**Oscillatory neuronal activity**

An example of a Gpi unit with oscillatory discharge in dystonia is shown in Fig. 4, A (neuronal recording) and B (frequency spectrum of the autocorrelogram). Group statistics for oscillatory activity are provided in Table 2 (bottom 2 rows). In the dystonic state, 85 of 302 Gpi neurons and 20 of 151 Gpe neurons had oscillatory activity according to autocorrelation analysis. In PD, 18 of 101 Gpi neurons and 7 of 39 Gpe neurons were oscillatory. For both nuclei, the proportion of neurons with oscillatory activity was significantly higher in dystonia than in the normal NHPs. For both nuclei in all conditions, the mean frequency of oscillations was between 3 and 8 Hz, and the distribution of frequencies was unimodal (shown for Gpi in Fig. 4D, left). Thus the only significant difference in single unit discharge between PD and dystonia was in mean Gpi discharge rate, not in oscillatory activity.
Correlations of involuntary muscle activity with neuronal discharge

A total of 169 GPi neurons in 15 dystonic patients and 72 GPi neurons in 8 PD patients were recorded simultaneously with four channels of EMG. In patients with dystonic spasms or tremor at rest, EMG recording from the affected muscle groups showed involuntary activity at rest. For 45 (27%) GPi cells in dystonia there was at least one significant peak in the SDF-EMG coherence function for at least one of the EMG channels. For cells showing SDF-EMG coherence, the mean number of significant peaks was 1.4. For 11 (15%) GPi cells in PD, there was at least one significant peak, and for these, the mean number of significant peaks was 1.9.

Figure 4C shows the SDF-EMG coherence function for the neuronal discharge and EMG in A. The distribution of SDF-EMG coherence frequencies in GPi for dystonia and PD are shown in Fig. 4D (right). The distribution is bimodal in both cases, with peaks in the 2- to 10-Hz range and in the 20- to 30-Hz range. For the dystonic GPi, the median frequency of coherence was 16.4 Hz (range, 0.4–29 Hz), whereas for the parkinsonian GPi the median was 19.1 (range 0.7–29). In both disease states, there were approximately equal numbers of SDF-EMG pairs with positive phase delays as there were pairs with negative phase delays.

Of the 85 GPi units in dystonia that manifested oscillatory activity (by autocorrelation analysis), only 5 of the units showed significant coherence with at least one of the recorded EMG channels at the same frequency (The unit in Fig. 4, A–C, is 1 such unit). This was in spite of the fact that EMGs were recorded from the most affected muscle groups. Four of the five units were in the two patients with overt dystonic tremor present during surgery (cases E and T). Of the 18 GPi units in PD patients with oscillatory activity, only 1 unit showed EMG coherence at the same frequency, and this was in the one patient with tremor during surgery. Figure 4D shows that the frequency distributions of neuronal oscillations are dissimilar to the distributions of SDF-EMG coherence. Thus for the

TABLE 3. Spontaneous discharge parameters in subtypes of dystonia standard error of the mean

<table>
<thead>
<tr>
<th></th>
<th>GPi</th>
<th></th>
<th>GPe*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary Dystonia</td>
<td>Secondary Dystonia</td>
<td>Primary Dystonia</td>
<td>Secondary Dystonia</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>16</td>
<td>3</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>No. of units</td>
<td>225</td>
<td>50</td>
<td>99</td>
<td>23</td>
</tr>
<tr>
<td>Mean rate, Hz</td>
<td>57.8 +/- 1.4</td>
<td>54.6 +/- 3.1</td>
<td>34.0 +/- 3.5</td>
<td>53.4 +/- 2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean proportion of burst discharges</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Legendy method)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.024 +/- 0.001</td>
<td>0.032 +/- 0.003</td>
<td>0.022 +/- 0.003</td>
<td>0.020 +/- 0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean L statistic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.8 +/- 0.1</td>
<td>6.0 +/- 0.18</td>
<td>6.6 +/- 0.25</td>
<td>5.3 +/- 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean burst index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>of normalized ISI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.4 +/- 0.13</td>
<td>3.6 +/- 0.2</td>
<td>6.0 +/- 0.9</td>
<td>2.6 +/- 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proportion of units with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>significant oscillations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.257 (58/225)</td>
<td>0.360 (18/50)</td>
<td>0.333 (9/27) Silence</td>
<td>0.091 (9/99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(chi^2 = 3.9, P &gt;</td>
<td>(chi^2 = 1.8, P &gt; 0.4, vs. Primary dystonia)</td>
<td>0.087 (2/23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1, vs. Primary dystonia)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean frequency of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>significant oscillations, Hz</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.8 +/- 0.5</td>
<td>5.4 +/- 0.8</td>
<td>9.1 +/- 2.1</td>
<td>10.2 +/- 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *GPe burster cells were excluded from this analysis; †denotes significant difference from primary dystonia, by t-test.
majority of units in the majority of patients, there was no consistent relationship between the presence or absence of oscillatory neuronal discharge and the presence or absence of SDF-EMG coherence, and where both were present, no consistent relationship in their frequencies was found.

For GPe, the smaller number of units with coherent SDF-EMG oscillations in (15 in dystonia, 0 in PD) precluded detailed analysis. The median frequency of coherence was 13.5 Hz (range, 2–30 Hz) for dystonia.

DISCUSSION

The major findings of this study were that mean spontaneous GPi discharge rate in human dystonia was lower than that of the normal NHP and that in primary dystonia, it was inversely correlated with dystonia severity. Mean GPi discharge in PD was higher than in the normal NHP. In both dystonia and PD, nonoscillatory bursting and oscillatory activity were increased compared with the normal NHP. Oscillations occurred predominantly in the 2- to 10-Hz range, were seen in all dystonia subtypes, and, in most cases, were not associated with tremor.

Comparisons with prior studies of GPi discharge rate in dystonia

A handful of studies have been published on pallidal neuronal physiology in awake humans with dystonia (23 cases total) (Hutchison et al. 2003; Lenz et al. 1998; Merello et al. 2004; Sanghera et al. 2003; Vitek et al. 1999). These series focused primarily on spontaneous discharge rates. Four studies of 16 total awake patients did show a reduced GPi discharge rate in the majority of dystonia cases, but this was not true for the seven cases presented by Hutchison et al. (2003). This discrepancy might be accounted for by variations in symptom severity of the dystonic patients studied. In the study of Hutchison et al. (2003), five patients had BFMDRS severity scores reported, and two of these were <15. In our series, such patients had higher pallidal discharge rates, indistinguishable from the normal NHPs and only 10–20 Hz lower than in PD. We also found that patients with secondary dystonias associated with abnormal MRI findings had lower GPi discharge rates than patients with primary or tardive dystonia of similar severity. Thus heterogeneity in dystonia subtype could also account for variability in published physiological data.

The finding that symptom severity influences GPi discharge rate is supported by Lenz et al. (1998), who showed in a single case that pallidal discharge rate decreased as the severity of dystonic spasms increased. Reduced pallidal outflow in dystonia is also consistent with studies in a rodent model of genetic dystonia, the dt-sz hamster, in which spontaneous discharge in the entopendular nucleus (rodent homologue of GPi) was reduced in the dystonic state compared with the normal state (Bennay et al. 2001).

Implications for alterations in the direct and indirect pathways

Models of the contribution of the basal ganglia to the control of movement have emphasized two major intrinsic pathways linking the basal ganglia input (striatum) to the basal ganglia outputs, GPi and SNr. A variety of derangements in these pathways have been proposed as the basis for dystonia (Hallett 1998; Perlmutter et al. 1997; Vitek 2002). In the model of Vitek, striatal inputs to both pallidal segments are hyperactive. GPe then becomes hypoactive, and the STN becomes hyperactive. The GPi is subjected to conflicting influences from the direct and indirect pathways, but the net result is suppression of GPi discharge due to a greater influence of the direct pathway. Because we found that both GPe and GPi have reduced activity in dystonia, our data are most consistent with this model. Our results suggest that abnormal basal ganglia discharge rates are present at rest as well as during movement because the rate abnormalities were present in two of three patients who did not have involuntary muscle activity at rest in the operating room. Hyperactivity in striatal projection neurons that innervate both GPe and GPi could account for simultaneous reductions in firing rates in both pallidal segments (Parent et al. 1995).

Oscillations and bursts in pallidal discharge are prominent features of dystonia

Abnormal oscillations in pallidal single-unit activity have been well documented in PD (Hurtado et al. 1999; Hutchison et al. 1997; Levy et al. 2001, 2002) and in animal models of dystonia.
In this paper, we show that oscillations in single-unit activity in both pallidal segments are also a prominent feature of dystonia, and occur predominantly in the 2- to 10-Hz range. Oscillatory pallidal activity in human dystonia has previously been documented in local field potential (LFP) recordings (Silberstein et al. 2003), which reflect synchronized activity across many neurons. LFP oscillations occurred in the same 2- to 10-Hz frequency range as the single-unit oscillations reported here and thus may reflect the same underlying signal.

The presence of tremor can lead to neuronal oscillations in motor areas of basal ganglia nuclei via proprioceptive sensory feedback. It is unlikely that the oscillatory activity we recorded in dystonia was due exclusively to sensory feedback from joint or muscle because it was present in those patients who did not have dystonic tremor or spontaneous muscle spasms while at rest (cases A, F, and O). Furthermore, even in patients with dystonic spasms at rest, only a small percentage of oscillatory neurons in dystonia showed coherent EMG activity at the same frequency. Thus 2- to 10-Hz single-unit pallidal oscillations in
dystonia probably reflect an abnormal “idling rhythm” rather than a sensory response to abnormal spontaneous movements (Brown 2003). Bursting is a type of temporal ordering of short and long ISIs such that short ISIs tend to occur in clumps (Ruskin et al. 2002). Bursting may be oscillatory or nonoscillatory. GPI bursting in human dystonia has been reported previously (Hutchison et al. 2003; Merello et al. 2004; Vitek et al. 1999). However, descriptions of discharge pattern are difficult to interpret in the absence of comparison data from nondystonic subjects, and only one study (Hutchison et al. 2003) provided a quantitative statistical measure of bursting. Here, we show using multiple statistical measures of bursting activity that both dystonia and PD are characterized by increased bursting activity in comparison to the NHP. While oscillatory units contained a higher proportion of burst discharges than nonoscillatory units, pallidal bursting in both dystonia and PD was not exclusively limited to oscillatory cells. That bursting is a prominent feature of dystonia is also supported by studies in the dt-sz hamster model of dystonia in which bursting in the entopendular nucleus was observed only during the dystonic phase of the animal’s ontogeny (Gernert et al. 2002). The finding of pallidal neuronal synchrony in dystonia (Silberstein et al. 2003) and in PD (Hurtado et al. 1999; Levy et al. 2002; Silberstein et al. 2003) suggests a potential mechanism for GPI bursting in movement disorders. If the GPe “pauser” cells providing convergent input onto an individual GPI cell produced synchronized pauses, then GPI bursts would result from the brief periods of excessive disinhibition. Investigation of this possibility will require simultaneous recording from multiple neurons.

Neuronal correlates of dystonia in relation to other movement disorders

We found that the abnormal bursting and oscillatory activity seen in dystonia is remarkably similar to that of PD patients with predominant bradykinesia and rigidity but that the two conditions differed in mean GPI discharge rates. Similarities in the discharge pattern abnormalities between dystonia and PD is not surprising given that dystonia can occur as a symptom of PD, and it also occurs transiently in the MPTP-treated NHP prior to the appearance of parkinsonian bradykinesia and rigidity (Perlmuter et al. 1997). Unlike discharge rate, statistical measures of bursting and oscillation did not correlate with disease severity, arguing that the observed firing pattern abnormalities do not “encode” dystonia. These observations suggest that abnormally patterned activity in the GPI superimposed on a low discharge rate produces dystonia, whereas similar abnormalities superimposed on a higher mean rate produce bradykinesia. In support of this concept, Hashimoto et al. (2001) measured GPI discharge rate in a PD patient during a dystonic phase and a nondystonic phase and showed a much lower discharge rate in the dystonic phase.

Like idiopathic dystonia, levodopa-induced dyskinesias in PD are associated with reduced GPI firing rates and abnormal bursting discharge (Levy et al. 2000; Merello et al. 1999), yet these two conditions are phenotypically distinct. Differences in interneuronal synchronization, which was not studied here, might provide the physiologic correlate for this distinction. In addition to lowering mean GPI discharge rate, levodopa treatment in PD reduces neuronal synchronization (Brown et al. 2001; Silberstein et al. 2003). Thus dyskinesias occurring at peak levodopa dosage in PD are probably the result of unsynchronized, low-frequency pallidal activity. Our findings, taken together with those of Silberstein et al. (2003), suggest that if pallidal outflow is both decreased and synchronized, dystonia rather than dyskinesia may result.

Other pallidal abnormalities could be important in dystonia. Enlarged neuronal receptive fields have been observed in the thalamus (Lenz et al. 1999) and cortex (Sanger et al. 2001) in human dystonia, and in the cortex in a primate model of dystonia (Byl et al. 1996). There are conflicting data on whether or not this is the case in the dystonic GPI (Hutchison et al. 2003; Lenz et al. 1998), and we did not systematically examine receptive field size of movement-related cells.

Pitfalls in interpretation

In this study, discharge rate and pattern in dystonia and PD were defined as “abnormal” in relation to single-unit discharge in the normal Rhesus macaque, collected and analyzed by identical methods. The use of a different species as a control group could be seen as problematic, but single-unit data from normal humans cannot be obtained. Cross-species comparisons of pallidal physiology in the parkinsonian state, however, support the similarity of pallidal physiology between humans and NHPs. The discharge rates of GPI and STN neurons are very similar for NHPs in the parkinsonian state and for humans with PD (Hutchison 1998; Hutchison et al. 1998; Sterio et al. 1994). In addition, the prevalence of burst discharges observed here in the GPI in PD is nearly identical to that reported previously for parkinsonian NHPs using the same statistical analysis methods (Wichmann et al. 1999). Given the similarity of pallidal physiology in NHPs and humans in the parkinsonian state, it is reasonable to assume this would also be the case for the normal state.

Due to the fact that MER in humans was performed along nearly parasagittal trajectories, the part of the GPe explored was not in the center of the motor territory, which is dorsolateral (Francois et al. 2004; Hedreen and DeLong 1991) and not immediately dorsal, to the GPI. Thus the GPI physiology we studied in the human may not fully reflect abnormalities that could be present in a more central region of the motor subterritory. This difficulty does not affect the GPI recordings because the trajectory was designed to traverse only the posterior (motor) GPI and because cells responsive to passive joint movements were found throughout the regions of GPI explored in each case.

Sampling bias may have affected our results. We attempted to minimize this by collecting data from dystonia and PD patients, as well as NHPs, using similar methodology. Finally, lingering effects of propofol sedation given prior to neuronal recording could have suppressed pallidal discharge rates, in spite of starting the recording after at least a 30-min sedative-free interval. Three half-lives are used to characterize the pharmacokinetic elimination of propofol, but the longest half-life (~12 h) is only relevant at very low plasma concentrations, in the range of 0.01 mg/ml (Fechner et al. 2004). This is well below the levels shown to influence neuronal recording in laboratory animals (Fechner et al. 2004). Several results argue against an effect of propofol in this study. The mean GPI
discharge rate for PD patients was elevated (close to 100 Hz), despite having received similar doses of propofol in a similar time frame as the dystonia patients. In addition, one would expect a residual propofol effect to gradually decrease during a recording period lasting several hours, whereas we found no difference in discharge rates for neurons recorded early in the case versus late in the case.

Conclusions

Human dystonia is associated with abnormal oscillatory activity and abnormal bursting activity in comparison with the normal NHP. Severe dystonia is also associated with reduced mean GPi discharge rate in contrast to the rigid-akinetic form of PD, which is associated with an increased mean rate. Discrepancies in prior studies of pallidal physiology in dystonia probably reflect heterogeneity in disease severity and subtype. Differences in mean discharge rate and/or neuronal synchrony may determine whether abnormally patterned pallidal activity is manifested as dystonia, parkinsonism, or dyskinesia. Our data are consistent with a model in which both direct and indirect basal ganglia intrinsic pathways are overactive.

Acknowledgments

M. Mori contributed to early data analysis for this project. S. Heath contributed to data collection.

Grants

This work was funded by National Institute of Neurological Disorders and Stroke Grants K08 NS-002201 to P. A. Starr and NS-39146 to R. S. Turner.

References


Miller WC and DeLong MR. Altered tonic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of parkinson-


