Neuronal Responses to Passive Movement in the Globus Pallidus Internus in Primary Dystonia

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Abstract

Abnormal sensory processing has been implicated in the pathophysiology of primary dystonia. In the globus pallidus internus (GPi), the primary output structure of the basal ganglia, many neurons respond to sensory (proprioceptive) stimulation. Here, we have characterized GPi neuronal responses to passive movement of the contralateral limbs in 22 patients with primary dystonia undergoing microelectrode recording for placement of deep brain stimulator leads. We plotted coordinates of cells responding to limb movement in a common space. We observed distinct representations of leg and arm movement localized to the dorsal and ventral part of the posterior GPi, respectively. Comparing patients with generalized dystonia versus patients with segmental craniocervical dystonia, there was no difference in the volumes or separations of leg and arm related territories. In contrast to parkinsonism, only a small minority of units were responsive to movement across multiple joints. Abnormally increased directional selectivity was found in units responding to dystonic limbs compared to non-dystonic limbs. Some affected GPi neurons therefore appear to have altered proprioceptive tuning for movement direction. There is an apparent preservation of GPi somatotopic organization in dystonia, in comparison with prior studies of GPi somatotopic organization in nonhuman primates and humans with Parkinson’s disease.
Introduction

Dystonia is a movement disorder characterized by slow involuntary muscle contractions, producing abnormal, sometimes painful, movements or postures. Current theories attribute dystonia to a dysfunction of the basal ganglia-motor cortex circuitry (Hallett 1998; Mink 1993). The primary output nucleus of the basal ganglia, the globus pallidus internus (GPi), is an important structure for the expression of dystonic symptoms. Alteration of the activity of GPi neurons by pallidotomy or deep brain stimulation alleviates dystonia symptoms in many patients (Kupsch et al. 2006; Ondo et al. 1998; Vidailhet et al. 2005). Abnormalities in spontaneous GPi neuronal discharge have been documented with respect to discharge rate, bursting behavior, and oscillatory activity (Lenz et al. 1998; Merello et al. 2004; Silberstein et al. 2003; Starr et al. 2005; Tang et al. 2007; Vitek et al. 1999; Zhuang et al. 2004), although there remains controversy regarding spontaneous GPi discharge rates in dystonia (Hutchison et al. 2003).

In neurologically intact non-human primates, there is a population of neurons in the posterior region of the GPi that respond to proprioceptive stimuli such as rotations of a joint or taps of a tendon or muscle (Baron et al. 2002; DeLong 1971; DeLong et al. 1985; Turner and Anderson 1997). In most studies, the locations of cells with proprioceptive responses show a somatotopic organization, with leg related cells located dorsal and medial to arm related cells (Baron et al. 2002; DeLong et al. 1985), though not all studies have found this (Filion et al. 1988). A similar relationship between arm-related
and leg-related territories also been shown in the GPi of humans undergoing microelectrode guided surgery for Parkinson’s disease (Guridi et al. 1999; Vitek et al. 1998).

Many lines of evidence implicate abnormal sensory inputs or abnormal spatial organization of cells responsive to sensory inputs in the pathophysiology of dystonia. Increased size of receptive fields has been found in somatosensory cortex in patients with focal unilateral hand dystonia (Bara-Jimenez et al. 1998; Garraux et al. 2004), and in the ventrocaudal and ventralis intermedius thalamic nuclei in patients with generalized dystonia (Lenz and Byl 1999; Lenz et al. 1999). These findings suggest that the distributions of movement-related cells in the GPi might be altered in size or location for dystonic body regions compared to normal regions. In a single case of pallidal surgery for segmental arm dystonia, Lenz et al (Lenz et al. 1998) reported a larger than expected region responding to passive arm movements.

Perception of joint movement is impaired in dystonia (Grunewald et al. 1997; Putzki et al. 2006), and central processing of paired proprioceptive inputs shows a lack of normal inhibitory interactions (Tinazzi et al. 2000). In a different movement disorder, parkinsonism, there is known to be an alteration in the proprioceptive responsiveness of GPi neurons, with an increase in the proportion of multi-joint responses (Filion et al. 1988; Vitek et al. 1998). In primary dystonia, however, GPi neuronal discharge in response to proprioceptive stimulation has not been extensively studied (Hutchison et al. 2003; Lenz et al. 1998).

Here, we investigated the modulation of GPi neuronal discharge by passive joint manipulation, in 22 patients with primary dystonia undergoing microelectrode guided
placement of deep brain stimulator leads in the awake state. We studied the somatotopic
organization of sensory-responsive neurons and characterized directional selectivity by
comparing neuronal responses to limb movements in opposing directions (e.g., flexion
versus extension). Physiologic measures were compared for patients with normal limb
function (segmental craniocervical dystonia) versus patients with arm and leg
involvement (generalized dystonia). We tested the following hypotheses: (1) Generalized
dystonia is associated with abnormally enlarged representations of affected limbs in the
GPi, compared with segmental craniocervical dystonia, and (2) Neurons responding to
passive movement in dystonic limbs show alterations in the timing or amplitude of
movement-related changes in discharge rate, compared with neurons responding to
passive movement in nondystonic limbs.

Methods

Experimental subjects and clinical characterization

The study population consisted of patients >15 years old undergoing unilateral or
bilateral microelectrode-guided placement of deep brain stimulators into the GPi for the
treatment of primary segmental or generalized dystonia. The study was approved by the
University of California, San Francisco, institutional review board for human research,
and all patients signed informed consent for the study prior to participation. A
quantitative measure of dystonia severity was determined by a movement disorders
neurologist (JLO) in the month prior to surgery, using a standard clinical rating scale, the
Burke Fahn Marsden Dystonia Rating System (BFMDRS) for motor function (Burke et al. 1985). The BFMDRS is a 120-point scale used to rate the severity of dystonia in nine body regions, taking into account both the severity and frequency of dystonic movements. A higher score reflects greater severity. Patients were grouped into two categories: Segmental craniocervical dystonia (patients with individual limb BFMDRS scores of zero, but with a nonzero score for neck muscles or neck plus facial muscles, and generalized dystonia (patients with nonzero BFMDRS scores for at least two limbs, with or without trunk/neck/face involvement).

Surgical procedure and microelectrode recording

Surgical technique has been previously detailed (Starr et al. 2006). Briefly, subjects’ heads were fixed in a stereotactic headframe and magnetic resonance imaging (MRI) of the brain was obtained on the morning of surgery to directly visualize the boundaries of the GPi. Images were imported into a stereotactic surgical planning software package (Framelink version 4.1; Medtronic-SNT, Boulder, CO). The initial anatomic target was selected as a point at the base of the posterior part of the internal pallidum, immediately dorsal to the lateral border of the optic tract. Typical initial target coordinates in mm with respect to the midcommissural point were: Lateral=20, vertical = -5, anteroposterior = 2. Trajectories were planned to these targets in an approximately parasagittal plane, beginning near the coronal suture.

All patients underwent micro-electrode recording (MER) in the awake state. Because of the inability of most dystonic patients to remain still for frame placement or
stereotactic MR imaging, these initial steps were performed under propofol-induced sedation. Propofol was then stopped at least 30 minutes prior to the start of pallidal recording, since propofol is known to affect pallidal neuronal discharge (Hutchison et al. 2003). All patients were alert and oriented at the start of microelectrode recording. Some patients had dystonic spasms even in the absence of attempted voluntary movement.

During the surgical procedure, rigid fixation of the stereotactic headframe to the operating table prevented excessive head movement.

The initial MER trajectory was directed at the stereotactic target, and 1-3 serial MER penetrations were made per hemisphere in parallel trajectories separated by 2-3 mm. Single-unit recordings were obtained with glass-coated platinum/iridium microelectrodes (impedance 0.4-1.0 Mohm at 1000Hz, Microprobe, Inc., Gaithersburg, MD or FHC Inc, Brunswick ME). Recordings were bandpass filtered (300 Hz-5 kHz), amplified, and played on an audio monitor and oscilloscope using the Guideline System 3000 (FHC, Inc.). Microelectrodes were advanced into the brain using a motorized microdrive (FHC, Inc).

The general responsiveness of GPi neurons to limb movements was assessed on-line, during intra-operative recording. In order to screen a large number of cells for movement related activity, investigator-imposed or “passive” movements of contralateral limbs were conducted for every well-isolated cell encountered after entry of the microelectrode into the GPi. Although only a subset of cell recordings were digitized for further quantitative analysis (see below under “Quantitative analysis of perimovement discharge”), all cells included in the analyses described below were well-isolated as indicated by consistent action potential shapes and the presence of a refractory period.
Well-isolated cells were encountered approximately every 0.3-0.5 mm. High-velocity, repetitive alternating movements in opposing directions were generated by the investigator at the shoulder, elbow, wrist, hip, knee, and ankle joints for each tested unit. Patients were instructed neither to assist nor resist the investigator's manipulations. Although the examiner attempted to generate discrete movements of individual joints, the inherent biomechanical linkages in the limbs prevented absolute isolation of the movements. Ipsilateral responses were not systematically examined, due to the time constraints imposed by human surgery as well as previous findings in humans with PD that ipsilateral pallidal responses to passive manipulation are less frequent than contralateral responses (Lozano et al. 1996; Vitek et al. 1998). The proprioceptive responsiveness of a neuron and its assignment to movements of one or more joints was determined by concurrence between the examiner and one or more operating room staff based on audiovisual assessments of the response. Responses were assessed in an unblinded manner. Responsive neurons, their assigned joint movements and their locations were noted. For cells responding to more than one joint, each relevant joint was noted.

Nuclear localizations of recorded cells were assigned as follows: cells encountered between the internal medullary lamina (lamina pallidi medialis in the Schaltenbrand and Wahren human brain atlas (Schaltenbrand and Wahren 1977)) and the optic tract were considered internal pallidal cells; those recorded between the striatum and the internal medullary lamina were considered external pallidal cells; and those near the presumed GPe-GPi border, on a track where a definite white matter boundary was not identified, were excluded from formal analysis because of their uncertain location. The
location of cells with respect to the corticospinal or corticobulbar tracts was not systematically assessed, since identification of these tracts by microstimulation-induced muscle activation was possible only in a minority of cases. The location and discharge characteristics of cells along each microelectrode track were plotted manually on graph paper. Scaled track reconstructions were then superimposed on drawings of parasagittal slices from the Schaltenbrand and Wahren human brain atlas (Schaltenbrand and Wahren 1977) for visual confirmation of nuclear identity (GPi or GPe), as described by Lozano et al. (Lozano et al. 1996) and Vitek et al. (Vitek et al. 1998).

**Determination of coordinates for movement-related cells**

Postoperative MR imaging on the day following surgery was used to determine the final lead position at high resolution (volumetrically acquired gradient echo imaging at 1.5 mm slice thickness) (Starr et al. 2006). The coordinates of the distal tip of the stimulator and of the entry point were measured with respect to the mid-commissural point and the angulation of the lead with respect the axial plane passing through the commissures was calculated trigonometrically (Framelink 4.1 software, Medtronic Inc). The lead tip location on axial MRI was used as a “reference point” to reconstruct, post-hoc, the AP, lateral and vertical coordinates of cells on each microelectrode trajectory, with respect to the midcommissural point, as has been previously described (Rodriguez-Oroz et al. 2001; Theodosopoulos et al. 2003). Adjustments were made for the varying approach angles of MER tracks between subjects, using the angulation of the DBS electrode with respect to the axial plane passing through the commissures.
Both inter- and intra-subject analyses of cell locations were conducted to assess somatotopic organization in the GPi. Coordinates from all subjects were pooled to obtain the largest sampling of joint representations. The mean XYZ coordinates of the pooled arm-response and pooled leg responsive cells were determined, and the distance D between their mean coordinates calculated using the formula $D = \sqrt{(x_2-x_1)^2 + (y_2-y_1)^2 + (z_2-z_1)^2}$. The volume of the ellipsoid representing the locations of the central 25% and central 50% of upper- and lower-limb responsive neurons was determined using principal components analysis and the F-distribution. The orientation of the ellipsoid axes in XYZ space was determined by a principal components analysis, which identified the three orthogonal axes that accounted for the greatest variance of an XYZ dataset. The ellipsoid was then scaled in size to include the central 25% and 50% of the XYZ dataset based on the assumption that the distribution of the dataset approximated an F-distribution (Ghilani and Wolf 2006). The volume of the ellipsoid was calculated using the standard formula $V = \frac{4\pi(a*b*c)}{3}$, where a, b and c are lengths of the three ellipsoid axes. A bootstrap method was then used to estimate the 95% confidence limits of the volume determination, using 1000 random sub-samplings of the grouped XYZ coordinates (DiCiccio and Efron 1996). Bootstrapping is a well-established method used to estimate the distribution of a population-derived measure by: i) extracting many subsamples from the population; ii) calculating many estimates of the measure from those subsamples (e.g., estimates of volume); and then iii) calculating standard error across the many estimates. Bootstrapping was chosen for the present application because the method avoids assumptions of normality and because it remains accurate for smaller population sizes (N<20) compared with alternative approaches. Comparisons were made between
all generalized dystonia patients versus the segmental craniocervical dystonia patients, as well as between a more homogeneous subset of generalized dystonia patients, those positive for the DYT1 mutation, versus the segmental craniocervical dystonia patients.

Quantitative analysis of peri-movement discharge

A subset of neurons that showed a reproducible response to passive movement in the initial multi-joint screening was selected for quantitative investigation, focusing only on movement of the joint that produced the maximal modulation of discharge on the initial screen. This subset consisted of those cells for which single unit isolation could be maintained for at least one minute following the initial qualitative screening. To record the timing of onset of each movement, a triaxial accelerometer (FHC, Inc.) was strapped to the limb distal to the joint being moved. During passive contralateral limb movement across a single joint, unit discharge was digitized (20 kHz sampling rate) simultaneously with the voltage output of the accelerometer summed electronically across the three axes. Each passive movement (alternating between flexion and extension or internal and external rotation) was followed by 3-5 seconds of immobility and repeated 6-13 times.

Action potentials were discriminated off-line by cluster cutting in principal components space (Plexon off line sorter, Plexon Inc, Dallas, Tx). Neuronal data were analyzed for discharge rate only if at least six movement trials in each direction were successfully completed. The onsets of investigator-imposed joint movements were detected by manual inspection of individual accelerometer traces. The time of initial deflection in acceleration from baseline was taken as the onset of movement (Figure 1).
Movements in opposing directions were distinguished easily because they were performed strictly in alternating order.

Accelerometry was used only to define the timing of onset of movement (as shown in Figure 1), not to characterize direction of movement. For each cell tested, there were only two directions, about a single joint. For flexion/extension pairs, movement occurred in the same plane but in opposite directions. For ball-and-socket joints (hip and shoulder) particular care was taken by the examiner to ensure that flexion and extension movements took place in the same plane given the larger degree of freedom at these joints. For internal rotation/external rotation pairs, care was taken to rotate the joint about the same axis, but in opposite directions for opposing movements. Peri-stimulus mean spike density functions (SDFs; i.e., 1 msec resolution spike delta functions smoothed with a 50 ms sigma Gaussian (Szucs 1998)) were averaged across trials aligned on movement onsets. The degree of spike-train smoothing was guided by an empirical estimation of the properties of the underlying signals of interest (i.e., the smoothness of movement-related changes in firing) (Kass et al. 2005). Representative neuronal data from the current dataset were inspected after application of gaussians with sigmas ranging from 10 ms to 100 ms. Smoothing with a 50 ms sigma gaussian, combined with the statistical test described below, was found to reliably capture peri-movement changes in firing that were consistent across repetitions of the same movement.

Statistically significant increases or decreases in neuronal activity were determined by comparison with the mean and variance of the SDF in a pre-stimulus baseline period. With movement onset defined as time=0, the pre-stimulus baseline period was from -1.5 to -0.5, and the analysis period lasted 1500 msec from -0.5 to 1.0
sec. A significant response was defined as a deviation from the mean baseline firing rate that exceeded three times standard deviation (3xSD) of the baseline for at least 50 msec. The beginning of the response corresponds to the first millisecond of the SDF whose value falls outside of the 3xSD threshold. This threshold provided an omnibus sensitivity of p<0.05 after correcting for multiple comparisons (actual p=0.05/30 comparisons), where the number of independent comparisons was estimated from the duration of the analysis period divided by the degree of smoothing (i.e., 1500 msec/50 msec).

For neurons that showed a significant change in firing in response to at least one of the two possible movement directions, the directional selectivity of the neuron was parameterized using a directional selectivity index (DSI) (Suarez et al. 1995):

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DSI = |1 - (NP/P)|,
\]

where NP equals the mean change in firing rate from baseline in the non-preferred direction and P equals the mean change in firing rate in the preferred direction. The preferred direction is defined as the direction which elicits the largest change in the mean firing rate (either positive or negative) during the period of significant change from baseline. By this convention, DSI= 0 means that the cell response to movement was equal for both directions and therefore not directionally selective. DSI = 1 means that the cell response (either increase or decrease in firing) only occurred in one movement direction. DSI>1 means that the cell demonstrated an increase for one movement direction and a decrease for the opposing movement direction. Cells were considered directionally selective if the DSI was greater than 0.50.
Results

Study population

Data from 22 patients with primary dystonia were analyzed. Patient characteristics are given in Table 1. Seventeen patients were categorized as having generalized dystonia due to nonzero BFMDRS scores in at least two limbs (see methods), and five had segmental craniocervical dystonia without limb involvement (BFMDRS scores of zero for all limbs). Ten of the generalized dystonia patients had juvenile-onset dystonia, including six patients who were positive for the DYT1 mutation (DYT1+) and four patients who were negative (DYT1-). All patients with segmental craniocervical dystonia had adult onset forms and none were positive for the DYT1 mutation.

Medications at the time of surgery are given in Table 2.

Proportions of movement related cells and multi-joint responses

During the initial intraoperative screen of responses to passive movement based on audiovisual monitoring, 374 well-isolated single GPi neurons were tested in 18 patients. Responses to passive movement of contralateral limb joints were found in 153 units (41% of all units tested). On average, eight movement responsive units were found per patient. (In four of the 22 patients in this study, less extensive microelectrode mapping was done, and these four contributed data only to the directional selectivity analysis described below.) Multiple joint responses were observed in 19 units (13% of
movement responsive units), and in all cases occurred within a limb (no cells were responsive to both upper and lower limb movements). The proportion of units with multi-joint representation was not statistically different between dystonic limbs (10%) and non-dystonic limbs (14%) (P=0.43, Chi-test).

Somatotopic organization of movement-related cells

A gross impression of the somatotopic organization of sensorimotor GPi in dystonia was gained by observation of the order in which cells were encountered along single micro-electrode recording tracks. Tracks were made in approximately parasagittal planes at a 60 degree angle from the intercommissural line. Of 68 MER tracks, 40% had only arm-responsive units and 31% had only leg-responsive units. On tracks with both arm- and leg-responsive units (N=20), leg-responsive units were encountered before arm-responsive units in 79% of tracks, whereas the converse was only found in 21%. Thus, leg responses predominated in the more dorsal recordings and arm responses in the more ventral recordings.

The mean X, Y, and Z coordinates for all cells responsive to a specific limb and joint, pooled from all subjects, are provided in Table 3, and plotted in 2-dimensional plots in Figure 2. When analyzed by each coordinate plane, differences between positions of leg- versus arm-responsive neurons were significant in the X (mediolateral) and Z (dorsoventral)-planes (P=0.04 and <0.0001, respectively, t-test), but not in the Y (anteroposterior)-plane (P=0.35, t-test). Overall, leg-responsive neurons were more dorsal and slightly more medial than arm-responsive neurons. Proximal joint responses were
more common than distal joint responses (Table 3). Furthermore, a general proximal to distal joint organization was discerned in the dorsoventral dimension (Figure 2).

Arm and leg representations in generalized versus craniocervical dystonia

To better characterize the size and degree of overlap of arm-related and leg-related motor territories in generalized versus segmental craniocervical dystonia, we generated probability ellipsoids for the spatial distributions of arm-related and leg-related cells, centered on their mean coordinates (Figure 3). The volumes of the ellipsoids, and the confidence limits on the volumes (determined by bootstrap methods, see Methods section) are given in Table 4. The volume of the probability ellipsoid for both upper and lower extremity responsive-units was not different in patients with generalized dystonia compared to segmental craniocervical dystonia. In order to control for variability in cell distributions that may be introduced by pooling heterogeneous patient groups, we also compared limb-related probability ellipsoids for six patients with DYT1+ generalized dystonia with the five patients with segmental craniocervical dystonia. This showed again that there was no significant difference between arm and leg volumes. This was true for both the central 25% and the central 50% of cells.

The mean scalar distances between the centers of arm and leg territories for these pooled cell distributions did not differ between generalized dystonia (all subjects) and craniocervical dystonia. The separation between the mean upper and lower limb representations were calculated for each individual hemisphere (for those GPis where both limb representations were found, N=20 hemispheres), and the distances were then
pooled. The mean distance was 2.69mm (SD = 1.46). No difference in distance was observed between patients with craniocervical versus generalized dystonia (P=0.97, t-test, craniocervical: 2.61±1.27mm, generalized: 2.72±1.56mm, mean ± SD).

**Directional selectivity of movement related cells**

Forty-nine units from 14 patients with a robust response to movement on the initial subjective screening were selected for further quantitative analysis of movement in opposite directions about a single joint. Seven units were removed from directional selectivity analysis since they did not in fact exhibit significant changes in mean firing rate from baseline with movement, when analyzed quantitatively. Twenty-seven cells showed changes during movement in limbs that were dystonic, and 15 cells corresponded to non-dystonic limbs (BFMDRS limb motor score of 0 for the relevant limb). The mean baseline (spontaneous) firing rate for all 42 units was 64.4 spikes/sec (±18.5 SD).

Peri-movement spike density functions were generated from an average of 10.1 repetitions (range 6-13) of a limb movement. Examples of various response patterns are shown in Figure 4. The most common response type was an increase in firing to both directions of movement (N=22). A selective increase to only one movement direction was found in six units and a selective decrease in four units (DSI=1). Eight units had reciprocal increase and decrease responses for opposing directions of movement (e.g., Fig. 4C, DSI>1). Only two tested units had decreases for both directions of movement. For movement directions eliciting an increase in firing rate, in a small number of cases (17%), an initial increase was followed by a smaller decrease in firing. In these cases,
only the major component of the rate change (the increase) was used to calculate the DSI. Overall, 23 units were deemed directionally selective with DSI>0.5.

Pooling the firing rate changes with movement across all 42 neurons, the mean change in firing rate was +32.0 spikes/sec (± 15.1 SD; absolute values of increase and decrease in firing rate). The mean onset and offset times with respect to the initial accelerometer deflection were +119 msec (± 170 SD) and +385 msec (± 180 SD), respectively. The mean change in spike rate for each neuron was plotted to illustrate the overall prevalence of response increases versus decreases related to both preferred and non-preferred directions for limb movements (Figure 5).

*Passive movement responses in dystonic versus non-dystonic limbs*

The DSI and other parameters of cell discharge were compared to the BFMDRS for the specific limb tested (Table 5). Cells corresponding to affected limbs (BFMDRS>0) possessed a higher DSI compared to non-affected limbs (BFMDRS=0) (Table 5, unpaired t-test, P< 0.05; DSI for affected limbs= 0.88±0.14; for non-affected limbs=0.53±0.13, mean ± SD). Pearson’s correlation analysis also showed a significant association between DSI and individual joint BFMDRS (r=0.352; Fisher’s r to z, P=0.02;). There was trend toward lower baseline discharge rate for neurons modulated by movement in dystonic limbs versus non-dystonic limbs (Table 5) but this was not significant.

Ten of 14 units (72%) that had a decrease response to at least one phase of movement corresponded to dystonic limbs. Accordingly, the presence of a decrease
response was associated with higher limb BFMDRS score (t-test, P=0.03, mean BFMDRS for response decrease=6.5; for lack of response decrease =4.8).

Dystonia subtypes were also analyzed for associated changes in directional selectivity. Overall, there was no significant difference in directional selectivity between patients with juvenile-onset DYT1- and DYT1+ generalized dystonia, and adult-onset idiopathic generalized and segmental craniocervical dystonia (Figure 6, ANOVA, P=0.81). DSI did not correlate with the change in either the total or the contralateral limb BFMDRS scores at one year after surgery (compared with baseline preoperative scores) (total BFMDRS: r=0.144, P=0.36; contralateral limb BFMDRS: r= 0.038, P=0.81).

Discussion

We investigated the properties of movement-responsive Gpi neurons in 22 humans with primary dystonia undergoing implantation of deep brain stimulators in the awake state. Based on responses to passive contralateral limb movements, somatotopic organization and neuronal directional selectivity were studied. We found no evidence for reorganization in the spatial organization of movement related cells. We did find an increase in directional selectivity and a larger proportion of spike rate decreases in response to proprioceptive stimulation, in cells related to dystonic limbs compared to cells related to nondystonic limbs.

There are a number of pathways by which proprioceptive information could be transmitted to Gpi neurons. In the best documented pathway in primates, peripheral
Proprioceptive signals carried within the dorsal column/medial lemniscus system are transmitted to the “propioceptive shell” of the ventroposterolateral thalamus (Vitek et al. 1994). Proprioceptive information is then transmitted to primary somatosensory cortex, from there to sensorimotor striatum and subsequently to Gpi via either direct or indirect pathways. Proprioceptive information might also access the basal ganglia via other pathways, including a disynaptic pathway from deep cerebellar nuclei (via the thalamus) to striatal cells originating the indirect pathway (Hoshi et al. 2005), cortical projections conveyed via STN to the Gpi (Nambu et al. 2002), direct connections of the thalamic centromedian nucleus to the sensorimotor striatum (Smith et al. 2004), or via the pedunculopontine nucleus (which receives lemniscal sensory input, and projects to STN and Gpi) (Pahapill and Lozano 2000).

**Multi-joint proprioceptive responses are uncommon in dystonia**

We found a relatively low proportion of multi-joint responses (13%) in dystonia patients. This is consistent with the only other study of human dystonia that addressed multi-joint responses (Hutchison et al. 2003), and contrasts with the findings in human PD and nonhuman primate MPTP-induced parkinsonism, in which a high proportion of units (32-77%) respond to movement about more than one joint (Filion et al. 1988; Taha et al. 1996; Vitek et al. 1999). The proportion of multi-joint responsive units did not differ between dystonic limbs and non-dystonic limbs. Of note, in the normal nonhuman primate, few or no Gpi cells respond to multiple joints movements (Baron et al. 2002;
DeLong et al. 1985; Filion et al. 1988). The presence of some multi-joint responses in dystonia may therefore be abnormal. Overall, however, our findings indicate that loss of joint specificity in the basal ganglia, a key feature of parkinsonism, is much less prominent in dystonia. In parkinsonism, the presence of multi-joint responses may be a manifestation of abnormal synchronized activity in pallidal neuronal subcircuits that function independently in the normal state (Nini et al. 1995). It is not known if GPi neuronal activity has abnormal synchronization in dystonia.

**Somatotopic organization of limb proprioceptive responsive cells in GPi is grossly preserved in human dystonia**

With respect to proprioceptive responses in GPi, we found distinct representations of the contralateral arm and leg, and of individual joints within limbs. Arm-responsive units were clustered inferiorly and slightly lateral relative to leg-responsive units. Our results are consistent with, and extend, previous descriptions of the functional organization of the GPi, in both nonhuman primates (Baron et al. 2002; DeLong et al. 1985) and in humans with Parkinsons’s disease (Guridi et al. 1999; Vitek et al. 1998). Thus, our somatotopic map of GPi in dystonia does not show evidence for gross distortion of the body map, and in fact reveals a dorsal to ventral organization of proximal-distal responses within a limb that prior studies have not shown.

Two studies in primary dystonia patients have investigated somatopic organization of GPi on the basis of maximal symptomatic improvements produced by
DBS electrodes placed in various parts of the GPi (Tisch et al. 2007; Vayssiere et al. 2004). Both authors found greater improvements in arm function from contacts placed more ventrally and posteriorly, which was not the case with leg improvement. The spatial separation of arm related and leg related improvement is consistent with the finding that arm related cells are relatively ventral to leg related cells in dystonia patients, although we did not find a significant difference in the distribution of arm versus leg cells in the anteroposterior axis.

Volumes of distributions of proprioceptive cells

In prior studies of dystonia, abnormal alterations in the size and overlap of sensory representations of body parts have been found in several brain regions. Enlarged finger representations have been documented in the primary somatosensory cortex in patients with focal hand dystonia (Bara-Jimenez et al. 1998; Elbert et al. 1998) and in a nonhuman primate model of focal dystonia (Byl et al. 1996). A functional MRI study showed an abnormal body map for active movement in the putamen in focal hand dystonia (Delmaire et al. 2005) Lenz et al. (Lenz and Byl 1999; Lenz et al. 1999) found enlarged receptive fields in the cutaneous sensory area and in the cerebellar receiving area of the thalamus in dystonia patients when compared to control patients undergoing similar procedures for essential tremor or chronic pain.

Here, however, analysis of limb representational volumes did not demonstrate marked alterations in the size or overlap of limb representations in generalized dystonia, compared with limb representations in patients with craniocervical dystonia (whose limbs
were neurologically normal). Thus, any loss of specificity of sensory function at the
cortical level appears to not be fed forward through the basal ganglia. This could be
explained by the high selectivity of corticostriatal (Turner and DeLong 2000) and striato-
pallidal (Crutcher and Alexander 1990) projection neurons, which may reflect a general
information segregating function hypothesized for BG circuits (Bar-Gad et al. 2003).
The present results suggest that any information segregating function performed by basal
ganglia circuits remains intact in dystonia.

There are other possible explanations for the discrepancy between cortex and
pallidum with respect to limb representation changes in dystonia. Several prior studies of
abnormal sensory representations in dystonia focused on cutaneous sensory responses,
whose cortical or thalamic organization may respond differently to disease states than
proprioception. Finally, since we only studied responses to passive movements, we did
not rule out the possibility that expanded body representations would be found for active
responses.

*Directional selectivity and inhibitory responses to proprioceptive feedback are increased
in dystonic body parts*

In our study, a positive correlation was found between the degree of directional
selectivity and disease severity as measured by the BFMDRS. This finding was initially
unexpected based on reports of abnormalities in proprioception and kinesthesia in
dystonia patients (Grunewald et al. 1997; Putzki et al. 2006; Tinazzi et al. 2000). Further
analysis, however, showed that there was a greater prevalence of decrease responses (inhibition of firing) in units responding to affected limbs that probably accounts for the greater directional selectivity of these cells.

The greater prevalence of decrease responses in GPi is consistent with abnormalities in cortical activation previously described in dystonia. If an excessive proportion of GPi neurons decrease their firing rate phasically in response to movement, the thalamocortical pathway should be activated. Consistent with this hypothesis, functional imaging in patients with generalized dystonia has shown excessive movement-related activation in the supplementary motor area, a target of the thalamocortical projection that is under tonic inhibition by GPi (Eidelberg 1998).

Pitfalls in interpretation

Data for this study were pooled from many subjects with varying genetic influences, as some were positive for the DYT1 mutation but many were not. Both juvenile onset and adult onset forms were included, and symptom duration varied. It is not known whether the various types and ages of onset of dystonia studied here actually represent the same disorder at the level of pallidal physiology (Defazio et al. 2007). In performing invasive microelectrode recordings in humans, however, it is impossible to extensively sample GPi physiology within single subjects. Thus, the common approach to achieve statistically significant comparisons is to pool data across subjects. We have
attempted to limit the heterogeneity of the subject pool by excluding patients with secondary and tardive dystonias.

In studies of invasive recordings in humans, it is not possible to have a completely normal control group. We attempted to circumvent this problem by comparing physiological responses in dystonic limbs with limbs clinically unaffected by dystonia. However, abnormal brain physiology may be present in brain regions that correspond to clinically unaffected areas (Berardelli et al. 1998; Defazio et al. 2007). In focal hand dystonia, abnormalities related to the sensory system are primarily present in the representation of the hand and not of other body parts (Fiorio et al. 2006), but in cervical dystonia, limbs may yet have abnormal sensory representations in spite of normal function on clinical testing (Fiorio et al. 2007). Thus, our data might show more pronounced effects of dystonia if they were compared against a true “normal” control group. An additional difficulty, as in many physiological studies, is that it is unclear whether neuronal properties we observed are causally related to, or simply a result of, the presence of dystonia (Berardelli et al. 1998).

Hutchison et al (Hutchison et al. 2003) have shown pronounced effects of propofol sedation on basal ganglia discharge. Lingering effects of propofol sedation given prior to neuronal recording could have affected movement related responses, in spite of starting the recording after at least a 30 minute sedative free interval. Three half-lives are used to characterize the pharmacokinetic elimination of propofol, but the longest half-life (approximately 12 hours) is only relevant at very low plasma concentrations, in the range of 0.01 mgrams/ml (Fechner et al. 2004). This is well below the levels shown to influence neuronal recording in laboratory animals (Fechner et al. 2004). GPi neuronal discharge
rates recorded in this study are consistent with those reported in other studies of unsedated dystonia patients (Hutchison et al. 2003; Merello et al. 2004; Tang et al. 2007).

The technique of extracellular single unit recording biases the sample towards larger cells, and would miss cells that may have had very low spontaneous discharge rates. The cells recorded are likely to be those giving rise to the pallidothalamic projection (via the lenticular fasciculus or the ansa lenticularis), since these have the largest cell bodies in GPi (Parent et al. 2001).

Conclusions

We have studied responses to passive movement in G Pi neurons in patients with primary dystonia. We found no evidence for gross alteration in the body map within G Pi, in comparison with published data for nonhuman primates and patients with Parkinson’s disease. We found no significant difference in the sizes of arm and leg territories in comparing generalized with isolated craniocervical dystonia. A low proportion of cells responded to movements of multiple joints. The directional selectivity of neurons, and the proportion of cells whose discharge decreased in response to one phase of movement, were greater in limbs affected by dystonia than in limbs that were not. These findings suggest that abnormal body map representations, reported previously for sensorimotor cortex and putamen in various forms of dystonia, are not present at the level of the internal pallidum. The presence of decrease-type responses to proprioceptive stimulation in dystonic limbs is consistent with movement-related over-activity in thalamocortical pathways.
Acknowledgments:

We would like to thank Paul House, M.D. for his advice on data collection techniques for cell locations, and Karl Sillay, M.D., Sho Shimamoto, and Alec Glass, M.D. for their helpful comments on the manuscript.

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Disclosures:

None
REFERENCES


# TABLES

## Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th>Dystonia type</th>
<th>Age at Surgery (years)</th>
<th>Duration of Symptoms (years)</th>
<th>BFMDRS Motor Scores</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Cranial-Cranio cervical</td>
<td>Arm</td>
<td>Leg</td>
</tr>
<tr>
<td>All subjects (N=22)</td>
<td>42.3 ± 18</td>
<td>15.3 ± 9.7</td>
<td>52.0 ± 20.7</td>
<td>12.3 ± 9.8</td>
<td>8.52 ± 9</td>
<td>8.08 ± 11</td>
</tr>
<tr>
<td>Generalized, juvenile-onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DYT1+ (N=6)</td>
<td>19.0 ± 4.6</td>
<td>12.0 ± 7.1</td>
<td>52.1 ± 14</td>
<td>5.8 ± 3.8</td>
<td>17.0 ± 7.0</td>
<td>18.0 ± 9.8</td>
</tr>
<tr>
<td>DYT1- (N=4)</td>
<td>37.0 ± 11</td>
<td>26.0 ± 5.9</td>
<td>59.2 ± 30</td>
<td>9.4 ± 6.3</td>
<td>15.0 ± 9.0</td>
<td>10.0 ± 15</td>
</tr>
<tr>
<td>Generalized, adult-onset (N=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segmental Craniocervical (all adult onset) (N=5)</td>
<td>49 ± 8.9</td>
<td>9.7 ± 5.4</td>
<td>27.9 ± 7.4</td>
<td>11 ± 4.2</td>
<td>5.0 ± 5.0</td>
<td>5.3 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>62 ± 8.2</td>
<td>18.0 ± 13.0</td>
<td>25.2 ± 17</td>
<td>23.0 ± 13.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as mean± standard deviation

BFMDRS= Burke-Fahn-Marsden Dystonia Rating System

(Cranio cervical score is sum of points assigned to eyes, face, mouth, neck, and speech/swallow. Arm and leg scores are sum of bilateral limb scores.)
Table 2. Preoperative medications taken by study subjects

<table>
<thead>
<tr>
<th>Dystonia subtype</th>
<th>Case #</th>
<th>Pre-Operative Medications</th>
<th>(Dose-total mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalized, juvenile onset, DYT1 positive</td>
<td>1</td>
<td>Trihexyphenidyl 8, clonazepam 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Trihexyphenidyl 7, diazepam100, Baclofen PO 80, carbamazepine 700</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Trihexyphenidyl 3, Baclofen IT 600</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Baclofen PO 60 and Baclofen IT 1485</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Trihexyphenidyl 2</td>
<td></td>
</tr>
<tr>
<td>Generalized, Juvenile onset, DYT1 negative</td>
<td>7</td>
<td>Baclofen PO 20, clonazepam 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Clonazepam 2, trihexyphenidyl 4, botox</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Trihexyphenidyl 35, clorazepate 25, baclofen PO 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Gabapentin 2400</td>
<td></td>
</tr>
<tr>
<td>Generalized, adult onset</td>
<td>11</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Lorazepam 8, Hydrocortone 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Baclofen 60, gabapentin900, propranolol 240</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Clonazepam 3.75, -Venlafaxine 150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Ethopropazine300, alprazolam1.5, sertraline100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Venlafaxine 187.5, trazadone150, clonazapam 2.5</td>
<td></td>
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<tr>
<td></td>
<td>17</td>
<td>Clonazepam 1.5, diazepam 5</td>
<td></td>
</tr>
<tr>
<td>Segmental craniocervical (all adult onset)</td>
<td>18</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Botox A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Trihexyphenidyl 6, gabapentin 600, baclofen PO 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>Clonazepam 1.5, baclofen PO 60</td>
<td></td>
</tr>
</tbody>
</table>

Botox injections are listed for patients who received them within 3 months prior to surgery.
### Table 3. Coordinates of Passive Movement-Responsive Pallidal Neurons

<table>
<thead>
<tr>
<th></th>
<th>X (mm)</th>
<th>Y (mm)</th>
<th>Z (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N mean ± SD</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>All</td>
<td>153</td>
<td>19.67 ± 1.6</td>
<td>3.09 ± 2.4</td>
</tr>
<tr>
<td>Upper limb†</td>
<td>67</td>
<td>19.89 ± 1.7</td>
<td>2.90 ± 2.1</td>
</tr>
<tr>
<td>Shoulder</td>
<td>22</td>
<td>19.61 ± 1.6</td>
<td>3.02 ± 1.6</td>
</tr>
<tr>
<td>Elbow</td>
<td>27</td>
<td>20.07 ± 1.6</td>
<td>3.02 ± 2.4</td>
</tr>
<tr>
<td>Wrist</td>
<td>11</td>
<td>20.26 ± 2.2</td>
<td>2.93 ± 2.1</td>
</tr>
<tr>
<td>Lower limb†</td>
<td>86</td>
<td>19.5 ± 1.58</td>
<td>3.23 ± 2.6</td>
</tr>
<tr>
<td>Hip</td>
<td>44</td>
<td>19.67 ± 1.41</td>
<td>3.04 ± 2.6</td>
</tr>
<tr>
<td>Knee</td>
<td>25</td>
<td>19.34 ± 2.07</td>
<td>2.69 ± 2.7</td>
</tr>
<tr>
<td>Ankle</td>
<td>5</td>
<td>19.59 ± 1.14</td>
<td>5.03 ± 0.6</td>
</tr>
</tbody>
</table>

* Positive values by convention are lateral, anterior, and superior relative to the AC-PC midpoint for the X, Y, and Z dimensions, respectively.

† Total for each limb includes cells responsive to more than one joint.

Comparison between upper and lower limb coordinates (Unpaired t-test)

<p>| P-value | 0.039 | 0.348 | &lt;0.0001 |</p>
<table>
<thead>
<tr>
<th></th>
<th>Central 25%</th>
<th>Central 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume (mm³)</td>
<td>C.I. (mm³)</td>
</tr>
<tr>
<td>All Generalized Dystonia (N=13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>41.4</td>
<td>32.3-56.8</td>
</tr>
<tr>
<td>Arm</td>
<td>29.3</td>
<td>22.4-47.5</td>
</tr>
<tr>
<td>DYT1+ Generalized Dystonia Subset (N=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>18.0</td>
<td>13.6-30.7</td>
</tr>
<tr>
<td>Arm</td>
<td>27.5</td>
<td>18.5-48.7</td>
</tr>
<tr>
<td>Craniocervical Dystonia (N=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td>21.3</td>
<td>15.5-35.3</td>
</tr>
<tr>
<td>Arm</td>
<td>37.9</td>
<td>25.6-68.9</td>
</tr>
</tbody>
</table>
Table 5. Comparison of GPi Neurophysiologic Properties in Units Responding to Affected and Non-Affected Limbs in Dystonia

<table>
<thead>
<tr>
<th>Property</th>
<th>Total Dystonia</th>
<th>Non-Dystonic Limbs</th>
<th>Dystonic Limbs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=42</td>
<td>N=17</td>
<td>N=25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline (spikes/sec)</td>
<td>64.4 ± 18.5</td>
<td>69.1 ± 17</td>
<td>61.2 ± 19</td>
<td>0.201</td>
</tr>
<tr>
<td>Response magnitude (spikes/sec)</td>
<td>32.0 ± 15.1</td>
<td>34.0 ± 16.5</td>
<td>30.8 ± 14.1</td>
<td>0.573</td>
</tr>
<tr>
<td>Onset (msec)</td>
<td>0.119 ± 0.17</td>
<td>0.063 ± 0.12</td>
<td>0.156 ± 0.17</td>
<td>0.141</td>
</tr>
<tr>
<td>Duration (msec)</td>
<td>0.262 ± 0.18</td>
<td>0.238 ± 0.13</td>
<td>0.276 ± 0.204</td>
<td>0.571</td>
</tr>
<tr>
<td>DSI</td>
<td>0.76 ± 0.11</td>
<td>0.53 ± 0.13</td>
<td>0.88 ± 0.15</td>
<td>0.048</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Examples of modulation of GPi neuronal discharge during passive limb manipulation, from three different subjects. In each example, the upper trace is the spike train recorded during a brief passive movement about a single joint, and the lower trace is the accelerometer signal marking the onset of movement. Vertical arrows indicate the times considered to be the start of the movement. A, increase in discharge during shoulder internal rotation. B, increase in discharge during hip flexion. C, decrease in discharge during hip flexion.

Figure 2. Spatial distribution of joint-specific responses in dystonia. Two-dimensional plots of the mean coordinates for pallidal units responding to each tested joint. Coordinates are in mm with respect to the midcommissural point. The positive direction is anterior, superior, and lateral. The inset for each plot shows tracings of pallidal boundaries drawn from sections of the Schaltenbrand and Wahren human brain atlas (Schaltenbrand and Wahren 1977). The asterisk shows the reference location of the mean coordinates for all movement responsive cells on the GPi tracings and joint plots, to illustrate the area of the GPi that was sampled.

Figure 3. Two- and three-dimensional plots of cell distributions, and corresponding probability ellipsoids, for limb responsive neurons in DYT1+ generalized (A) and craniocervical (B) dystonia patients. The ellipsoid volumes represent the central 25% of the cell distribution. Arm is shaded light gray; leg is shaded dark gray. No significant
difference was found between limb representational volumes in these two patient populations (See Table 4).

Figure 4. Four representative pallidal neuronal responses to passive limb movement. For each example, neuronal responses for reciprocal movements are shown in left and right columns. For each neuron, the top panel shows a mean spike density function centered at the initial deflection in limb acceleration (time = 0 sec, horizontal dotted lines = thresholds for significant changes in mean discharge rate). The bottom panel shows a raster display of neuronal responses to each individual trial. A. Wrist joint: flexion (left) and extension (right) resulted in nearly equivalent firing increases (Directional Selectivity Index (DSI)=0.24). B. Shoulder joint: abduction (left) evoked a brisk increase response, but abduction (right) resulted in no appreciable change in firing (DSI=1.0). C. Hip joint: flexion (left) evoked an increase response while extension (right) suppressed pallidal firing (DSI=1.97). D. Shoulder joint: both abduction (left) and adduction (right) evoked a reduction in firing (DSI=0.28)

Figure 5. Distribution of mean spike rate changes for preferred and non-preferred directions of movement. Open circles represent neurons responding to movement in nondystonic limbs, while open triangles represent neurons responding to movement in dystonic limbs. Neurons represented in the lower right and upper left quadrants were the most directionally selective with DSI>1.
Figure 6. Directional selectivity of pallidal units in different dystonia subtypes. No significant difference was found in units recorded from patients with generalized juvenile onset DYT 1 positive (JUV DYT1+), generalized juvenile onset DYT negative DYT1- (JUV DYT1-), generalized adult-onset idiopathic (Adult Idio), and craniocervical dystonia (ANOVA, P=0.81, mean ±s.e.m.).
Figure 3.

194x172mm (600 x 600 DPI)
Figure 4.

172x179mm (600 x 600 DPI)
Figure 5.
Figure 6.