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Thalamic Post-Inhibitory Bursting Occurs in Patients with Organic Dystonia more often than Controls

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Abstract

We now test the hypothesis that post-inhibitory bursting in the human pallidal receiving nucleus of the thalamus (ventral oral) mediates inhibitory pallido-thalamic transmission during dystonia. We have compared thalamic single neuron activity in nine patients with organic dystonia to that in a patient with psychogenic dystonia (Psyd) and in healthy waking monkeys.

In organic dystonia, EMG power is commonly concentrated at the lowest frequency of the smoothed autopower spectrum (0.39Hz). Therefore, segments of spike trains with a signal-to-noise ratio ≥ 2 at 0.39 Hz were termed dystonia frequency (DF) segments, which occurred more commonly during dystonia related to movement. Those with a SNR < 2 were termed non-dystonia frequency (nDF) segments, which are associated with spontaneous dystonia. We concentrated on nDF activity since neuronal activity in our controls was measured at rest. Neuronal spike trains were categorized into those with post-inhibitory bursts (G, grouped), with single spikes (NG, non-grouped), or with both single spikes and bursts (I, intermediate). nDF spike trains in ventral oral had more G category firing in dystonia than in controls. The burst rate and the pre-burst silent period in nDF firing of organic dystonia were consistently greater than those of both the monkeys and the patient with Psyd. The distribution of the pre-burst silent period was bimodal with a longer mode of approximately GABA_B (gamma amino butyric acid receptor - type b) duration.

These results demonstrate distinct differences of post-inhibitory bursting in organic dystonia versus controls. The presence of inhibitory events consistent with GABA_B duration suggests interventions for treatment of dystonia.

Keywords

dystonia; thalamus; single neuron analysis; low threshold spike bursts; thalamotomy

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1. INTRODUCTION

Dystonia is a movement disorder characterized by sustained muscle contractions leading to twisting repetitive movements and abnormal postures (Fahn, 1988), and by abnormal activity in the forebrain (Hallett, 1998; Hallett, 2011; Mink, 2003; Rothwell, 2007). Previous studies have shown thalamic activity characteristic of patients with dystonia versus controls with chronic pain (Lenz et al., 1999) or psychogenic dystonia (Psyd)(Kobayashi et al., 2011). In particular, spike trains in patients with dystonia often showed spectral peaks at dystonia frequency (0.39 Hz) with a signal-to-noise ratio (SNR) ≥ 2 (DF, dystonia frequency activity)(Lenz et al., 1999).

The current model of basal ganglia function suggests that this thalamic activity arises from inputs to the thalamus from the internal segment of the globus pallidus (pallidum) and is transmitted to the periphery via thalamo-cortical connections (Albin et al., 1989; DeLong, 1990; Mink, 1998; Vitek, 2002). The pallido-thalamic connection is mediated through an inhibitory, GABAergic synapse from pallidum upon the pallidal receiving zone of the thalamus, which includes monkey VLa (ventral lateral anterior) and human Vo (ventral oral) (Anderson and Turner, 1991; DeVito and Anderson, 1982; Hirai and Jones, 1989; Lenz et al., 1999; Percheron et al., 1993; Schaltenbrand and Walker, 1982). Modeling studies have suggested that pallidal firing will reliably influence single spike firing in the pallidal receiving zone of thalamus only when the thalamic firing rate is greater than 50/sec (Smith and Sherman, 2002), which is greater than that observed in Vo (Kim et al., 2009; Ohara et al., 2007). This finding has led to the suggestion that cortico-thalamic excitatory inputs are the main drivers of firing in the pallidal receiving zone (Anderson and Turner, 1991).

A different mechanism of this inhibitory connection has been suggested by studies in the brain of songbirds (Farries et al., 2005; Luo and Perkel, 1999; Perkel et al., 2002). This mechanism proposes that the inhibitory input from the bird brain basal ganglia-like structure to the thalamus-like structure leads to post-inhibitory bursting (low threshold spike or LTS bursts) which encodes motor behavior, i.e. the bird song. Low threshold spike (LTS) burst firing occurs in waking human Vo (Ohara et al., 2007) and is significantly altered by cognitive tasks (Kim et al., 2009), which suggests that LTS bursting in humans is part of the thalamic response to input from the pallidum. In turn, abnormalities of LTS bursting might influence thalamo-cortical connectivity in movement disorders, such as dystonia.

Thalamic LTS results in a characteristic pattern of thalamic neuronal firing (see section 4.5.1)(Ramcharan et al., 2000b; Roy et al., 1984; Steriade et al., 1997). We now test the hypothesis that abnormal LTS bursting in the human pallidal receiving nucleus Vo mediates inhibitory pallido-thalamic transmission during dystonia. Thalamic neuronal signals were recorded during thalamic stereotactic surgical procedures for the treatment of dystonia, and experimental thalamic recordings in healthy waking monkeys. The results show that post-inhibitory LTS bursting is preferentially found during non-dystonia (nDF) firing among Vo neurons in patients with organic dystonia versus a patient with Psyd, and versus neurons in VLa in healthy monkeys (Kobayashi et al., 2011).

2. RESULTS

The present studies were carried out in a population of ten patients with dystonia who underwent a thalamotomy under local anesthetic between 1985 and 1990 at either the Toronto General Hospital or the Johns Hopkins Hospital. At that time, thalamotomy was an accepted surgical procedure for the treatment of dystonia (Andrew et al., 1983; Cardoso et al., 1995; Tasker et al., 1988; Tolosa and Marti, 1997). The present thalamic data in dystonia

is unique; it is unlikely that it can be collected again because thalamic procedures are no longer performed for the treatment of dystonia (Anderson and Lenz, 2006; Ozelius et al., 2011).

Clinical features of these patients have previously been described in detail (Kobayashi et al., 2011; Lang, 1995; Lenz et al., 1999) and are listed in Supplementary File 1. One of the ten patients (patient G) was diagnosed as Psyd after two microelectrode-guided thalamotomies for the treatment of presumed organic dystonia. The other nine cases were diagnosed with organic dystonia.

Previous publications describe results in these ten patients in terms of spectral analysis of spike train and EMG activity, and neuronal somatic sensory activity (Lenz et al., 1999). As controls in the present study, we used the data recorded in the patient with Psyd (Kobayashi et al., 2011) and the data recorded in two monkeys, neither of which has previously been published. There has been no analysis of thalamic LTS burst firing in dystonia in these previous reports.

Our patient with Psyd, at the time of the first surgery, was a thirty-six year old woman with a two year history of dystonia involving left oro-mandibular structures and her left upper and lower extremity, as previously described (Kobayashi et al., 2011; Lang, 1995). There were substantial differences in the EMG sampling between this patient (wrist flexors and biceps) and the nine patients with organic dystonia, who were operated later (flexors and extensors of the wrist and elbow). Spectral analysis and thalamic sensory activity in the patient with Psyd as compared to organic dystonia patients has been published (Kobayashi et al., 2011; Lang, 1995).

2.1. Dystonia Frequency (DF) versus non-Dystonia Frequency (nDF)

In a previous paper, segments of thalamic and EMG activities with a SNR of greater than or equal to 2 in the lowest frequency (0.39 Hz) of the smoothed spectrum were more common in patients with dystonia than in controls operated for the treatment of chronic pain (Lenz et al., 1999). In that paper and in this one segments of spike trains having this characteristic were termed DF segments, while those with a SNR < 2 at a frequency of 0.39 Hz were termed nDF.

Figure 1 shows an example of an approximately 50 sec recording of a spike train, presented as ten contiguous 5.12 sec intervals. As explained below (*Methods: Spectral analysis*), signals were analyzed in five 10.24 sec contiguous segments. The 10.24 sec segments in Figure 1 were analyzed in the spectral domain and classified sequentially as DF, DF, nDF, nDF, and nDF, as labeled to the left of each pair of 5.12 sec lines, which make up a 10.24 sec segment. Examination of this figure suggests that firing rates appear to be higher and burst rates lower in the DF versus nDF intervals.

Neuronal spike trains composed of both nDF segments and DF segments were commonly found in the pallidal (22/56, 39%) and in the cerebellar receiving nuclei (42/89, 47%). Therefore, single neurons often switched between nDF and DF segments during the course of recordings from a single neuron, so that our analysis of these segments reflects dynamic features of the spike trains.

2.2. Analysis of LTS Bursting Activity: n versus n+1 plots

As a bias-free measure of neuronal bursting activity, we constructed plots of the ISI before an action potential in a spike train (n, horizontal axis) versus the ISI after (n+1, vertical axis), designated as a n versus n+1 plot (Figure 2C)(Kim et al., 2009). This plot is a graphic

display of the characteristics of the spike train overall, as reflected by clusters of points in the plot, see below.

Raw neuronal activity from the thalamus is shown in Figure 2A, while discriminated spike trains and n versus $n+1$ plots are shown in Figures 2B and 2C, for neurons 1 through 4, as labeled. Alpha-numerical labels above the four plots in Figure 2C indicate the neuronal identifier and the firing pattern of the segment (DF or nDF). Thalamic LTS bursting activity was identified and analyzed using segments of spike trains, with numbers and classifications of segments, as detailed in Table 1.

Neuronal spike trains composed of bursts (grouped - G firing category) are indicated in the plot of n versus $n + 1$ by the cluster at the lower right of Figure 2C4. This cluster indicates a long ISI (approximately 300 to 400 ms) followed by a short ISI (< 6 ms), the defining characteristic of the first spike in an LTS burst. The cluster at the upper left indicates a short ISI ($<$ approximately 10 ms) followed by a long ISI (approximately 100 ms), characteristic of the final ISI in an LTS burst.

Figure 2C4 is a G firing category spike train with a small lower left cluster and a larger upper right cluster, a pattern consistent with doublets of action potentials comprising the LTS (Figure 2A). In comparison, the large left lower cluster and smaller right upper cluster in Figure 2C3 indicate a spike train with many bursts having more than 2 ISIs per burst while the small upper right cluster indicates that there are few spikes between bursts.

Neuronal spike trains composed almost exclusively of single spiking activity with relatively constant firing rates and with infrequent LTS (Figure 2B1) were classified into the non-grouped (NG) firing category. The n versus $n+1$ plot of a neuron in the NG firing category demonstrates a single central cluster of ISIs, as shown in Figure 2C1.

Intermediate (I) spike trains (Figure 2B2) are composed of both single spikes and LTS bursts which result in a central cluster as well as a peripheral cluster located in the lower right of the n versus $n+1$ plot, i.e. post-inhibitory LTS bursts.

As a test of the reliability of visual classification of neuronal spike train categories, the Calinski statistic was used; this technique estimates the number of clusters (k) for a given spike train (see previous analysis (Kim et al., 2009; Schwarz, 1978)). In none of the spike trains tested was the visual classification (G firing category $n=6$, I $n=4$, NG $n=4$) changed by cluster analysis, so that this cluster analysis validated the results of visual classification (see (Kim et al., 2009)).

2.3. nDF and DF Firing in Relationship to EMG, and Behavioral Condition

Our prior studies have demonstrated that patients with dystonia have significantly more DF thalamic activity than do 'control' patients with chronic pain (Lenz et al., 1999), or with psychogenic dystonia (Psyd (Kobayashi et al., 2011)). In the present analysis of data segments, DF activity was more likely to occur during the task condition (pointing movement) versus the spontaneous condition (arm at rest) (64/160 vs 39/139, $P=0.030$, Chi Square) and conversely nDF activity was more likely to occur in the spontaneous condition.

We next tested the Spike $\times$ EMG coherence at dystonia frequency among neurons ($n=53$) recorded during both nDF and DF segments firing patterns in patients with dystonia. Spike $\times$ EMG coherence at dystonia frequency (0.39 Hz) for DF segments (0.25, 0.11 – 0.49 median, 5% - 95%ile) was significantly greater than nDF segments (0.18, 0.11 – 0.29, $P=0.01$, Paired Wilcoxon). Post hoc testing revealed that coherence was higher for DF versus nDF among wrist flexors (0.26, 0.07 - 0.49 vs 0.16, 0.05 – 0.32, $P=0.01$) and extensors (0.26, 0.09 – 0.62

vs 0.18, 0.06 – 0.31, $P=0.01$). Although spike $\times$ EMG coherence was lower overall, segments with nDF activity commonly had coherence which indicated a significant spike $\times$ EMG cross-correlation for EMG channels and segments (18.4% of segments), and were significantly associated with the spontaneous condition.

Thalamic DF spike train firing was more likely to occur during the pointing task, and to be coherent with EMG signals. However, during the spontaneous task condition thalamic firing was associated with higher spike $\times$ EMG coherence only in the case of wrist extensors (0.22, 0.014 – 0.48 vs 0.18, 0.0035 – 0.67). This suggests that thalamic DF activity may be involved preferentially in the mechanism of dystonia related by active movement, while nDF activity is related to dystonia which occurs spontaneously. nDF firing was also more likely to be observed in controls with Psyd and in healthy monkeys, which were also studied during the spontaneous task. Neither of the present controls or the controls with pain had segments with DF activity. Therefore, we concentrated upon the analysis of nDF, which is associated with spontaneous dystonia.

2.4. Firing Categories of DF versus nDF Neuronal Activity

Table 1 (upper three rows) shows proportions of spike trains in the G, NG and I firing categories as a function of nDF activity among populations of patients with organic dystonia, and monkey and Psyd controls. None of the neurons in either the Vo of the patient with Psyd or the VLa of the monkeys had DF firing, while nDF firing patterns were found in both controls. Therefore, only nDF activity was compared between organic dystonia versus monkeys and the patient with Psyd.

nDF activity in Vo of organic dystonia patients was characterized by many more G spike trains and fewer NG spike trains (Figure 2C1, and Table 1 – upper three rows) than nDF in both the patient with Psyd (Vo) and healthy monkeys (VLa). nDF firing occurred in all populations but was significantly associated with the G category only in organic dystonia, so that nDF firing sometimes results from frequent, intense inhibitory events in organic dystonia but not in controls. Differences in proportions of firing category between the two control groups were not significant for any of the three categories ($P>0.24$ for all categories, Fisher).

2.5. Distribution of LTS Burst parameters

Human thalamic spike trains are composed of LTS bursts characterized by parameters which are often composed of more than one distribution (Kim et al., 2009). Therefore, we used the technique of the Bayesian Information Criterion (BIC) to identify whether the distribution of each of these parameters consists of more than one component (Farries et al., 2005; Kim et al., 2009; Luo and Perkel, 1999).

We considered the possibility that burst parameters were described by a single distribution (1 component model), a mixture of two distributions (2 component model), a mixture of 3 distributions (three component model), and so on. For any two estimated models, the one with the highest value of the BIC is identified as the optimal number of components contributing to the histogram. The analysis provides the estimated mean and standard deviation for each identified component. For distributions with two components, these measures indicate the probability that a single observation belongs to either the first group or the second group.

Among dystonia patients the burst rate and preburst interspike interval (PBISI) parameters both showed two components. The PBISI in Vo showed two peaks, the lower peak with PBISIs of (76 ± 10 ms: Mean $\pm$ Standard Deviation) and the higher peak with PBISIs of (194 ± 27). These PBISI peaks are possibly consistent with the activity of gamma amino

butyric acid receptors types a and b (GABA_A and GABA_B) (Soltesz, Lightowler, Leresche, and Crunelli, 1989). In addition, there were two peaks for Burst Percentage in Vo with lower (3.8 ± 3.4) and higher values (45 ± 24)

2.7. LTS firing of Organic Dystonia versus Monkeys and the Patient with Psyd

The burst rates in controls were significantly greater than zero, by nucleus, in controls including monkey VL_a (0.14 ± 0.017 / sec, mean $\pm$ SEM), and the patient with Psyd Vo (0.20 ± 0.016) (Ohara, Taghva, Kim, and Lenz, 2007a). This significant post-inhibitory LTS burst firing in controls suggests that LTS bursting is a part of normal inhibitory pallido-thalamic transmission.

The spike train and burst characteristics (see *Methods: Analysis of Bursting*) were then analyzed for differences between dystonia nDF versus nDF of the control populations, by a linear and logistic regression model with robust variance estimates (Dingle et al., 2002; Statacorp, 2005). There were no DF segments in monkeys or in the patient with Psyd so that only nDF could be compared between dystonia versus controls. In Table 1 (lower three rows) values in the different rows represent the estimated difference in the mean nDF outcome values of the dystonic patients versus that of each control separately. All standard errors are adjusted for the correlation observed within a given cell, and the p-value is for testing whether there is a significant difference in the means across these two groups.

The burst rate in nDF of organic dystonia in the pallidal receiving zone was significantly higher versus nDF in monkeys and the patient with Psyd. This is consistent with the higher numbers of G spike trains and lower numbers of NG spike trains among nDF versus nDF segments for both controls (see Table 1, upper three rows). Segments with nDF activity had lower firing rates for Vo neurons in dystonia versus VL_a in the monkeys. The PBISI of nDF segments in dystonia was consistently longer versus nDF in both monkeys and the patient with Psyd (Table 1, lower 3 rows). Therefore, in the pallidal receiving zone the frequency of LTS bursting was substantially higher in dystonia versus both controls, and the pre-burst inhibition was substantially longer.

3. DISCUSSION

We have tested the hypothesis that abnormal post-inhibitory (LTS) bursting in nucleus Vo mediates inhibitory pallido-thalamic transmission during dystonia. The results show that significant LTS burst firing occurs in VL_a of healthy monkeys (Section 2.7), which is consistent with prior studies (Martinez-Conde et al., 2002; Ramcharan et al., 2000a). In addition, LTS burst rates were significantly greater than zero in the Psyd control (Section 2.7). These findings suggest that post-inhibitory LTS bursting is a component of inhibitory pallido-thalamic transmission in healthy primates, which was suggested by studies in songbirds (Farries et al., 2005; Luo and Perkel, 1999; Perkel et al., 2002).

In nDF segments for neurons in Vo, the G firing category occurs in patients with organic dystonia, but in neither control group (Table 1, row 1). Therefore, the present results demonstrate that LTS bursting is an important component of neuronal activity in Vo of patients with organic dystonia. It is unlikely that this connection could be mediated through inhibitory modulation of single spike firing rate of neurons in Vo (Smith and Sherman, 2002).

A basic assumption of this study is that thalamic activity in dystonia can be characterized in terms of DF and nDF firing patterns (Lenz et al., 1999; Kobayashi et al., 2011). Cells with nDF activity were frequently correlated with EMG activity overall and were associated with

the spontaneous condition. Therefore, nDF firing patterns may be related to spontaneous dystonic movements.

Stimulation of Vim (ventral intermediate) and Vo can increase dystonia (Figures 9 and 10 in (Lenz et al., 1999)) and lesions of Vim and Vo can decrease dystonia (Figure 11 in (Lenz et al., 1999))(Andrew et al., 1983; Cardoso et al., 1995; Tasker et al., 1988; Tolosa and Marti, 1997). These results support the suggestion that LTS burst firing in Vo neurons play an important role in dystonia.

3.2. Methodological Concerns

We adopted the spike burst template (50-06-16, see *Methods: Analysis of Burst*), which has been used to identify LTS bursting in studies of a monkey thalamic slice (Ramcharan et al., 2000a). The utility of this template in identifying LTS bursting has been demonstrated in awake primates (Kim et al., 2009; Ramcharan et al., 2000a; Ramcharan et al., 2005). This burst template was used in the present study because there are no human thalamic intracellular studies to validate a human burst template.

It is possible that these extracellular recordings and analyses will identify bursts, which are not associated with underlying LTS, although the burst fits the template. Since we cannot identify this potential source of error without intracellular recordings it will be reflected in the variance of the statistical analyses.

The number of controls in the sample for this study is small, which includes one patient with Psyd and two healthy monkeys. Studies of intracranial recordings in behaving monkeys often include only two monkeys (Darbin et al., 2006; Kakei et al., 1999; Mink and Thach, 1991), and have been used previously as a control for human pallidal activity during dystonia (Starr et al., 2005). The small numbers of monkeys studied is offset by the high resolution of such studies.

Among the present controls the patient with Psyd was a unique human control since this patient was undergoing non-organic movements which were similar to those of organic dystonia (Lang, 1995; Lenz et al., 1999)(Kobayashi et al., 2011). If the controls were patients undergoing surgery for a different movement disorder (e.g. Essential Tremor) then the abnormal activity in the control group might illuminate the pathology of Essential Tremor more than that of dystonia (Hua and Lenz, 2005). Finally, the comparisons are consistent between dystonia versus monkeys and versus the patient with Psyd (Table 1 - lower three rows – Burst rate and PBISI). This suggests that thalamic activity in dystonia is different from rest (monkeys) and from the non-organic dystonic movements (the patient with Psyd). These findings lead to the conclusion that thalamic activity is associated with dystonic movements in organic dystonia. However, it must be noted that the small numbers of controls limit the extent to which the results can be generalized.

In the present results, the monkey controls were studied during the spontaneous condition and displayed nDF firing patterns, like patients with organic dystonia and Psyd. Therefore, we concentrated on the analysis of nDF segments, which are associated with spontaneous dystonia. The consistency of the results between Vo of patients with dystonia versus VLa of monkey and Vo of human Psyd controls demonstrates that the neuronal LTS firing during spontaneous dystonia is different from diverse control groups. In view of these results the consistency in the proportions of firing categories between the two control groups supports the suggestion that thalamic activity in dystonia is different from controls.

3.3 LTS Bursting

Parametric analysis of burst rates demonstrates that significant LTS bursting occurs in the pallidal relay nucleus of controls, although these rates are lower than in dystonia ((Ohara, Taghva, Kim, and Lenz, 2007b) and Section 2.7). In Vo, nDF segments in dystonia versus in controls are characterized by significantly larger numbers of G spike trains. No G spike trains were observed in either control group (Table 1). nDF firing in patients with organic dystonia versus nDF firing in controls is characterized by higher LTS burst rates, lower firing rates, and by longer pre-burst inhibitions in Vo and VL_a (PBISI, Table 1, lower 3 rows). These results suggest the presence of increased inhibitory input to Vo by mechanisms including increased numbers and duration of inhibitory events, which may be mediated by transmission through GABA_B receptors (Section 2.5, and Table 1, lower three rows) (Sherman and Guillery, 2001; Soltesz et al., 1989). This explanation is consistent with the BIC analysis of LTS burst parameters, which indicates that there are two separate distributions of the PBISI. The longer of these two may be consistent with the duration of GABA_B mediated conductances. Therefore, LTS bursting may be mediated through GABA_B receptors during nDF activity in spontaneous dystonia.

This activity-dependent change in inhibitory processes between GABA_A and GABA_B could be the result of activity patterns in pathways innervating a neuron like the irregular firing of neurons in patients with dystonia (Bal et al., 1995; Sherman and Guillery, 2001). These changes may also be due to alterations in the distribution of GABA receptors on the neuronal membrane (Sherman and Guillery, 2001; Soltesz et al., 1989). Alternately, prolonged hyperpolarization may result from inhibition-evoked phosphorylation of T channels, or from the characteristics of particular T channels (Pape et al., 2004; Perez-Reyes, 2003). These differences in parameters of LTS may suggest new medical approaches to the treatment of dystonia based upon the pharmacology of GABA receptors (Agid et al., 2007; Mohler et al., 2008; Rudolph and Mohler, 2006) or LTS channels (Porcello et al., 2003; Waxman, 2007).

A striking finding in this analysis is the presence of G firing only in patients with dystonia, and LTS bursting occurs more frequently in patients with dystonia than in either control group (Table 1). Although the occurrence of this firing suggests greater inhibition of the thalamus it also leads to intense bursting. Furthermore, LTS bursting may increase transmission of thalamic (burst) activity to the cortex (Swadlow et al., 2002). In the somatic sensory system, LTS bursting is related to greater synaptic efficacy and greater transmission of thalamic inputs to cortex (Beierlein et al., 2002; Hallett, 2011; Swadlow and Gusev, 2001). Therefore, thalamic burst firing recorded in Vo may produce larger cortical responses than single spikes, and may be correlated with EMG activity during organic dystonia (Lenz et al., 1999). In this way, burst firing of neurons in Vo during nDF or DF activity could lead to increased cortical activity in dystonia, and to dystonia itself (Asanuma et al., 2005; Ozeliuss et al., 2011).

4. METHODS

We will describe methods for human and monkey studies of both the pallidal, and the cerebellar relay nuclei (Hirai and Jones, 1989). We have previously reported, in detail, all the methods for human studies including surgical, recording, spectral and ISI analysis techniques (Kim et al., 2009; Lenz et al., 1999). The protocol was reviewed and approved annually by the Institutional Review Board of the Johns Hopkins University. All patients signed an informed consent for these studies.

4.1 Human Operative Procedures

Observations were made during the physiological exploration preceding either stereotactic thalamotomy for the treatment of dystonia or the implantation of deep brain stimulating electrodes for the treatment of chronic pain (Hua et al., 2001). During the first stage, the Leksell stereotactic frame was applied and the coordinates of the anterior commissure - posterior commissure (AC-PC) line were determined.

Neuronal activity in the thalamus was recorded with a high impedance microelectrode (Lenz et al., 1988a). The first electrode trajectory was directed toward the human principal somatic sensory nucleus (Vc, ventral caudal), the most reliable landmark for the exploration. Subsequently, the region anterior to Vc, including the human thalamic cerebellar relay nuclei (Vim, Ventral intermediate), and Vo was mapped by analysis of both single neuron activity and effects of micro-stimulation (Lenz et al., 1999). The location of Vim and Vop was determined by moving the anterior border of Vc on the atlas map to the location of the most anterior cell in the region where cells responded to cutaneous sensory stimulation (Lenz, Kwan, Martin, Tasker, Dostrovsky, and Lenz, 1994; Schaltenbrand and Walker, 1982).

During neuronal recordings, we examined the spontaneous firing pattern, the relationship of spontaneous activity to dystonia, and the neuronal activity during somatic sensory stimulation and active movement. Cutaneous sensory cells responded to touch or pressure to the skin. Deep sensory cells responded to joint movement or squeezing of the subcutaneous structures but did not respond to squeezing of skin deformed by these stimuli. Care was taken to isolate movements to single joints.

The lesion site in patients with dystonia was located anterior to Vc where the majority of cells responded to cutaneous stimulation (Lenz et al., 1998; Lenz and Byl, 1999). The lesion sites were chosen in regions where cells displayed activity related to dystonic movements and where micro-stimulation evoked changes in the dystonic movements. Lesions were made by a standard technique, as previously described (Lenz et al., 1995; Lenz et al., 1999).

4.2 Human Behavioral Tasks

Dystonic movements occurred spontaneously with the upper limb at rest in all ten patients (spontaneous condition) and increased when the patients elevated their arm (pointing or task condition). Patients were seated in a reclining position with the back tilted 20° above the horizontal. Patients were asked to point toward a corner of the room (arm elevated). This action flexed (45°) and abducted the shoulder (45°) and extended the elbow, wrist, metacarpo-phalangeal, and inter-phalangeal joints (approximately 160°). Neuronal activity was assessed for 20 to 60 sec with the arm in this position.

Standard techniques were used to record surface EMG activity in wrist flexors, wrist extensors, biceps, and triceps (Hua and Lenz, 2005; Lenz et al., 1999). Neuronal and EMG signals were recorded on a multiple channel tape recorder (Model 4000, Vetter Co., Rebersburg, PA). An audio channel recorded an ongoing description of the procedure, and another channel recorded the signal from a foot pedal indicating the duration of somatic sensory stimuli.

Tape recorded neuronal and EMG activities were examined offline, as described below (Spectral Analysis)(Lenz et al., 1999). The present report focuses on single neuron activity recorded in the region where cells exhibited activity related to active or passive movements of the upper extremity. Action potentials were discriminated by action potential amplitudes occurring within two separate windows separated by an adjustable delay, using a window discriminator (Lenz and Byl, 1999; Lenz et al., 1999).

4.4 Monkey Studies

All surgical procedures, behavioral protocols and data collection procedures have been described previously (Miocinovic et al., 2007), and were approved by the Institutional Animal Care and Use Committee at the University of Minnesota and complied with United States Public Health Service policy on the humane care and use of laboratory animals.

4.4.1 Surgical Procedures—Two adult rhesus monkeys (*Macaca mulatta*; 05M009 - female, 5.2 kg; 03M004 - male, 8 kg) were used in this study. In brief, CT and MRI images of the animal were merged using the Cicerone software package to facilitate surgical planning and chamber localization. With the precise stereotaxic coordinates for the monkey predetermined using Cicerone, the recording hardware was implanted in an aseptic surgical procedure under isoflurane anesthesia with the head held in a primate stereotaxic instrument. For each chamber, a craniotomy was performed to expose the dura. The recording chamber was then implanted over the craniotomy site and secured into place using dental acrylic. One chamber was targeted for recording of the motor thalamus (Hirai and Jones, 1989; Vitek et al., 1996). A construct of titanium screws recording chamber and head stabilization receptacle was bonded together with dental acrylic. Post-operative prophylactic antibiotics were given preoperatively and continued postoperatively for 10 days.

4.4.2 Single Unit, Multi-channel Neuronal Recording—The locations of the thalamic nuclei were identified by mapping the activity of single neurons during a series of penetrations using dual microelectrodes (Alpha Lab, AlphaOmega, Israel). Micro-stimulation was used to assist in the classification of recorded units following published guidelines (Lenz et al., 1988a; Lenz et al., 1999). During recording sessions, the monkey was seated in a primate chair with the head restrained. Epoxylite-insulated tungsten microelectrodes (impedance 1.0 – 3.0 M Ω at 1 KHz, FHC Inc., Bowdoin, ME) were advanced using a multi-channel microdrive system (Alpha Lab, AlphaOmega, Israel) attached to the recording chamber.

The recording chamber was oriented such that the microelectrode penetrations were made in the parasagittal plane, regardless of the recording chamber used. This approach allowed recordings from neurons within specific thalamic subnuclei, using dual penetrations through the recording chamber. As the microelectrodes were advanced, neuronal activity was monitored continuously for spontaneous firing or as the monkey was examined by the researcher to determine neuronal receptive fields. An electrophysiological map of the area around thalamus was compiled by classifying neuron activity and was used to determine nuclear boundaries by techniques similar to those used during human functional neurosurgery (see above and (Kobayashi et al., 2011; Lenz et al., 1999)). All recordings were done with the monkey in an awake state.

4.4.3. Data Processing—Signals from each microelectrode were amplified and filtered using standard techniques (Alpha Lab software, Alpha Omega, Israel). The filtered analog signals were sampled and stored on a computer hard disk for later offline analysis. Neuronal activity was discriminated and analyzed using the same techniques as the human data (described below).

4.5 Data Analysis

The same techniques for parametric burst, spectral, and statistical analysis were used in both humans and monkeys. A graphical analysis technique for classifying LTS bursting activity, based upon n versus $n+1$, plots, is described in section 2.2. Individual LTS bursts in the spike train were identified by criteria used in studies of waking cynomolgous monkeys (Ramcharan et al., 2000a; Ramcharan et al., 2000b). The presence of LTS associated with

these bursts has been confirmed by intracellular studies in thalamic slices from the same species (Ramcharan et al., 2000a).

4.5.1 Analysis of Bursts—The criteria to select the LTS bursts from the spike train were as follows: 1) the inter-spike interval (ISI) preceding the first action potential in the burst had a duration > 50 ms, 2) the ISI following the first action potential in the burst had a duration of < 6 ms, and 3) all following action potentials are considered as part of the burst if their ISI increased by no more than 2 ms for each succeeding action potential, up to maximum ISI of 16 ms. We have repeatedly described, validated and illustrated the identification of LTS bursts by these criteria (Kim et al., 2009; Lee et al., 2005; Lenz et al., 1989).

All bursts meeting these criteria, including bursts composed of two action potentials, were included in the burst rate (BR); the percentage of action potentials occurring in bursts was measured by the burst percentage (BP). We also measured the firing rate (FR). As an indicator of the duration of the inhibitory event preceding a burst, we measured the pre-burst ISI (PBISI).

4.5.2. Spectral Analysis—We have repeatedly described and illustrated the methods of spectral analysis used in this report (Hua and Lenz, 2005; Lenz et al., 1988b; Lenz et al., 1999; Lenz et al., 2002). Briefly, the data were analyzed in the spectral domain since this analysis can produce quantitative information about the correlation between signals, which can be interpreted statistically (Glaser and Ruchkin, 1976; Lenz et al., 1988b; Lenz et al., 1999; Oppenheim and Schafer, 1975). Since data recorded in the earlier studies, carried out in Toronto, were divided into contiguous 10.24 sec segments all data was similarly segmented for the statistical analysis and spectral quantities were averaged across segments. We had data segments which were long enough to be analyzed by a standard spectral analysis technique, rather than by a multi-taper technique which would have been required for shorter segments (Percival and Walden, 1993).

The spike train was converted into an equivalent analog signal by use of the French-Holden algorithm (French and Holden, 1971). The EMG signal was first band-pass filtered to eliminate movement artifact, and then full-wave rectified and low-pass filtered at 20 Hz to produce a signal known as the demodulated EMG (Elble and Randall, 1976; Lenz et al., 1988b).

Standard techniques were used to take raw spectral estimates of the neuronal and EMG signals (Jenkins and Watts, 1968; Lenz et al., 1988b; Oppenheim and Schafer, 1975). These raw spectral estimates were averaged to produce the smoothed autopower (or autospectrum) and crosspower (or crossspectrum) to produce the lowest frequency estimate at 0.39 Hz with successively higher estimators every 0.78 Hz. The autopower is an estimator of the magnitude of a signal as a function of frequency, specifically calculated as the root mean square of the Fourier transform of the signal. The SNR is the autopower at a given frequency divided by the average spectral autopower, and indicates the extent to which power in the signal is concentrated at that frequency. A SNR > 2 at some frequency indicated that power in that signal was concentrated at that frequency to a significant degree (Jenkins and Watts, 1968). The crossspectrum of the spike and EMG signals was composed of the crossspectral power and the phase spectra.

The square of the crosspower divided by the product of the averages of the two autospectra produced the coherence, which is an estimator of the likelihood that one signal could be described as a linear function of the other. The probability such a relationship can be tested by statistical analysis of the coherence function.

4.5.3 Statistical Analysis—Our most basic parametric measures of the spike train were compared between DF versus nDF across nuclei by an ANOVA, with post hoc testing including a correction for multiple comparisons (Tukey honestly significant difference, HSD) (Snedecor and Cochran, 1967).

A more general model accounted for differences between segments of DF versus nDF segments within neurons recorded in different nuclei of patients with dystonia versus two control groups. This statistical model was a linear and logistic regression model with robust variance estimates which appropriately accounts for correlation across segments within a single neuron (Dingle et al., 2002; Statacorp, 2005).

Specific questions with parametric data that were not normally distributed were tested by Mann-Whitney or Kruskal-Wallis, and descriptive statistics were expressed as the median and 5 - 95%ile of the distribution (Confidence interval of the median, CI). Paired analysis was carried out using the non-parametric Wilcoxon matched pair test. Differences between proportions were tested with a Chi-square or Fisher test, as appropriate. The null hypothesis was rejected at $P < 0.05$ in all statistical tests.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Do post-inhibitory (LTS) bursts mediate pallidothalamic transmission in dystonia?

Bursts were more common in dystonia than psychogenic dystonia and monkeys (controls)

Spike trains with LTS bursting were common in dystonia but absent in controls

Pre-burst silent periods in dystonia were greater than those of controls

The duration of pre-burst inhibition suggests therapeutic interventions in dystonia

Figure 1. Thalamic spike train incorporating segments DF and nDF activity

A. Spike train in a patient with organic dystonia is shown by the times of occurrence of the discriminated spikes (upper trace) indicated by the vertical ticks for action potentials of the raw spike train (middle trace). The lower trace (left) shows a raw recording of a spike burst (doublet) and the shape of the discriminated action potentials (right). Alpha-numerics in the upper left are the cell identifier, and the diagnosis of the patient. **B.** Shows the spike train over an approximately 50 second interval with the occurrence of spike bursts shown by dots above the spike train. The data is divided into ten 5.12 sec recordings, where each pair formed a 10.24 sec segment, which was classified as DF (dystonia frequency) or nDF (non-

dystonia frequency) as indicated to the left of each pair of 5.12 sec segments. These 10.24 sec segments were classified sequentially as DF, DF, nDF, nDF, and nDF, as labeled.

Figure 2. Examples of spike trains and n versus $n+1$ plots

A. Spike train is shown as the raw microelectrode signal on the right; shape of the discriminated action potential is on the left. **B.** Discriminated spike trains with LTS bursts of action potentials (see section 4.5.1) are indicated by dots above the discriminated spike train. **C.** The number of the n versus $n+1$ plots (1 to 4) were calculated from the spike trains of the same number shown in B. Neuronal spike trains included those with post-inhibitory bursts (G, grouped), with single spikes (NG, non-grouped), or with both single spikes and bursts (I, intermediate) as described in section 2.2.

Table 1

Neuronal firing characteristics of the different groups of subjects. The upper three rows show the proportions of neurons with different categories of firing patterns, G – grouped, NG – non-grouped, and I - intermediate. The P values refer to the difference between the proportions of category type for nDF dystonia versus the corresponding control.

Subject Group/ Variable	nDF Dystonia	nDF Psychogenic Dystonia Control	nDF Healthy Monkey Control
Proportion of G firing	8/26 (31%)	0/34, P=0.0006	0/16, P=0.016
Proportion of NG firing	9/26 (35%)	26/34 (76%), P=0.0016	15/16 (94%), P=0.0003
Proportion of I firing	9/26 (35%)	8/34 (24%), P=1	1/16, P=0.061
Firing rate nDF dystonia minus control		-5.58 ± 2.21 , P=0.04	-2.8 ± 1.9 , P=0.16
Burst rate nDF dystonia minus control		0.54 ± 0.07 , P=0.0001	0.59 ± 0.07 , P=0.0001
PBISI nDF dystonia minus control		86 ± 18 , P=0.009	71 ± 18 , P=0.01

The lower three rows show firing characteristics for spike trains recorded in human Vo for dystonia nDF minus the patient with Psyd (Vo), and minus Monkey (VL), and minus the patient with Psyd (Vo). The results include the estimated difference (standard deviation of the difference) and significance of the difference. PBISI is the abbreviation for pre-burst interspike interval.