Cortical inhibitory function in cervical dystonia

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All authors report no conflicts of interest.

Abstract

Objective: To assess the specificity of cortical inhibitory deficits in cervical dystonia patients.

Methods: A systematic test battery was developed to assess spatial and temporal aspects of cortical inhibition, in motor and somatosensory systems of the hand. We tested 17 cervical dystonia (CD) patients and 19 controls assessing somatosensory spatial inhibition (grating orientation test, interdigital feedforward subliminal inhibition), somatosensory temporal inhibition (temporal discrimination threshold, feedforward subliminal inhibition), motor spatial inhibition (surround inhibition), and motor temporal inhibition (short interval intracortical inhibition).

Results: A significant deficit in CD was observed in both measures of somatosensory spatial inhibition, with a trend in the same direction in our measure of motor spatial inhibition. We found no significant group differences in temporal inhibition measures. Importantly, statistical comparison of effect sizes across the different measures showed that deficits in tests of spatial inhibition were greater than those in tests of temporal inhibition.

Conclusion: Our results suggest that CD is associated with abnormal function of local inhibitory cortical circuits subserving spatial sensory processing. Importantly, this abnormality relates to the somatotopic representation of an unaffected body part.

Significance:

These results clarify the nature of deficits in cortical inhibitory function in dystonia.

Keywords

Cervical Dystonia, Cortical Inhibition, Sensory Systems, Motor Systems

Highlights

- Dystonia is characterized by deficits in cortical inhibition.

- Patients with cervical dystonia were tested on a battery of spatial and temporal inhibitory tests

- Spatial inhibition was selectively affected in cervical dystonia

Introduction

Dystonia is a hyperkinetic movement disorder characterized by the presence of involuntary muscle contractions producing abnormal twisting movements and/or postures(Albanese et al. , 2013). In primary or *isolated* dystonia(Albanese et al. , 2013), widespread and generalized loss of inhibition within the central nervous system is a key pathological finding(Berardelli et al. , 1998, Hallett, 2011). The inhibitory deficit, however, may not reflect a single underlying neurophysiological mechanism. Rather, *inhibition* may refer to a range of neural mechanisms. Functional somatosensory and motor processing both involve inhibitory cortical circuits, which may be affected in dystonia. In some cases, both spatial and temporal aspects of inhibition can be identified at the level of individual neurons. For example, auto-inhibitory synaptic feedback was hypothesised to ensure time-limited responses to stimuli, and enhance temporal contrast(Douglas et al. , 2009), while networks of local inhibitory interneurons were hypothesised to underlie spatial tuning(Laskin et al. , 1979). Therefore, a clear and comprehensive taxonomy of the various cortical inhibitory circuits, and of functional tasks involving different aspects of inhibitory processing, would permit systematic characterization of dystonic deficits.

To date, abnormalities in different cortical inhibitory functions have been reported for different phenotypes of primary dystonia, including spatial spread of motor inhibition(Sohn et al. , 2004, Beck et al. , 2008); temporal properties of motor inhibition(Ridding et al. , 1995, Hanajima et al. , 1998, Edwards et al. , 2003, Kanovsky et al. , 2003, Quartarone et al. , 2003); somatosensory spatial inhibition(Bara-Jimenez et al. , 2000a, Molloy et al. , 2003, Walsh et al. , 2007, Bradley et al. , 2010); and temporal properties of somatosensory inhibition(Tinazzi et al. , 1999, Bara-Jimenez et al. , 2000b, Scontrini et al. , 2009, Bradley et al. , 2012). However, findings regarding some of these functions have been inconsistent(Rona et al. , 1998, Brighina et al. , 2009, Deik et al. , 2012, Kojovic et al. , 2013, Ferre et al. , 2015). Moreover, few studies have investigated multiple aspects of inhibitory function within the same patient group(Bara-Jimenez et al. , 2000b, Sanger et al. , 2001, Beck et al. , 2008, Bradley et al. , 2010). Therefore, current knowledge does not clarify whether there exists a generalised dystonic deficit in inhibitory processing, common to different functions and phenotypes, or whether the deficit is more specific.

Here we developed a systematic test battery, assessing both spatial and temporal cortical inhibitory processing, in both motor and somatosensory systems. We compared the results of these tests between a cervical dystonia group and healthy controls.

Materials and Methods

Participants

Seventeen patients with cervical dystonia (CD, mean age ± SD= 61.3±8.3; nine female; Table 1) and 19 age-matched controls (CTR, mean age ± SD= 61.1±11.1; 11 female) participated in this study (see Supplementary Materials). Only patients with isolated cervical dystonic symptoms and no dystonic arm posturing or dystonic arm tremor were included in the study. The study was approved by the research ethics committee of University College London Hospitals and adhered to the ethical standards of the Declaration of Helsinki.

Patient	Age (years)/ gender	Disease duration (years)	TWSTRS	Handedness	MMSE
1	54/F	3	33	L	30
2	70/M	15	40	R	30
3	70/F	27	37	R	28
4	45/M	13	38	R	30
5	66/F	7	33	R	30
6	47/M	26	30	R	30
7	68/F	37	40	R	29
8	67/F	22	24	R	28
9	66/M	23	32	R	30
10	55/F	14	32	R	29
11	62/M	22	29	R	30
12	48/M	18	24	L	30
13	64/F	26	25	R	30
14	65/M	7	32	R	30
15	60/M	25	28	R	30
16	68/F	6	56	R	29
17	67/F	16	30	R	30

Table 1. Clinical characteristics of patients with cervical dystonia. TWSTRS = Toronto Western Spasmodic Torticollis Rating Scale; MMST = Mini Mental State Examination; F = Female; M = Male; L = Left; R = Right

Procedure

We used a battery of tests of inhibitory functions in both CD and CTR groups. The tests covered inhibitory functions within both motor and somatosensory systems. In addition, the tests were chosen to focus either on spatial inhibition between adjacent motor/somatosensory fields, or on temporal contrast between events. We had two tests of somatosensory spatial inhibition (grating orientation test: GOT; interdigital feedforward subliminal inhibition: IFSI), two of somatosensory temporal inhibition (temporal discrimination threshold: TDT; feedforward subliminal inhibition: FSI), but only one test of motor spatial inhibition (surround inhibition: SI), and of motor temporal inhibition (short interval intracortical inhibition: SICI). All the tests have been published previously, and their relevance to dystonia has been established, though the IFSI test has not previously been used in the context of dystonia. The grouping of tests is shown in table 2. All tests were performed during a single session. The order of motor and sensory testing was counterbalanced. Within the motor testing session, the order of SI and SICI was further counterbalanced. Within the somatosensory session, a fixed order GOT, TDT, FSI, IFSI was used so that ring electrodes for TDT, FSI and IFSI tests could be placed only once and remain in situ.

	Tests for Spatial Inhibition	**Tests for Temporal Inhibition**
Motor	Surround Inhibition (SI)(Kassavetis et al. , 2014)	Short interval Intracortical Inhibition (SICI)(Kujirai et al. , 1993)
Somatosensory	Grating Orientation Test (GOT)(Van Boven et al. , 1994)	Temporal Discrimination Threshold (TDT)(Tinazzi et al. , 2002)
	Interdigital Feedforward Subliminal Inhibition (IFSI)(Ferre et al. , 2016)	Feedforward Subliminal Inhibition (FSI)(Ferre et al. , 2015)

Table 2. Factorial Design of a test battery for spatial and temporal inhibition in somatosensory and motor function.

Motor inhibitory testing

Transcranial magnetic stimulation (TMS) was used to study inhibitory functions of the motor system. Surround inhibition(Kassavetis et al. , 2014) and SICI(Kujirai et al. , 1993) were tested according to previous established protocols (see Supplementary

Materials). They provided markers of motor spatial and temporal inhibition, respectively. For SI, the dependent variable ratio was between average ADM MEP amplitude during voluntary contraction of the FDI, relative to average MEP amplitude at rest. For SICI, the dependent variable was the ratio of the average MEPs obtained following a conditioning pulse, relative to average of unconditioned MEPs.

Somatosensory inhibitory testing

Somatosensory inhibition was assessed by the GOT(Van Boven et al. , 1994) and the TDT(Tinazzi et al. , 2002), which served as markers of somatosensory spatial and temporal inhibition, respectively (see Supplementary Materials). The dependent variables were the smallest grating ridge width whose orientation could be accurately distinguished (GOT), and the minimum interval between two electrocutaneous stimuli that could be accurately judged as simultaneous or successive (TDT).

Feedforward subliminal inhibition was assessed as further marker of temporal inhibition(Ferre et al. , 2015). Participants performed a somatosensory detection task consisting of four randomly-mixed trial types: 15 trials with shock intensity at threshold delivered on the index finger, 15 trials in which a subliminal shock was delivered 30 ms before the threshold test pulse on the index finger, 15 trials in which only the subliminal shock was presented on the index finger, without a threshold test pulse and 15 stimulus absent trials in which neither subliminal shock nor threshold test pulse were given. Participants made unspeeded verbal responses to report whether or not they felt the shock (see Supplementary Materials).

Interdigital feedforward subliminal inhibition was assessed as a further marker of spatial inhibition(Ferre et al. , 2016). The procedure was identical to the previous test, except that subliminal stimuli were delivered via a second pair of electrodes to the middle finger, while participants detected near-threshold shocks to the index finger.

Both FSI and IFSI tests were analysed using a signal detection approach(Macmillan et al. , 1991) to obtain perceptual estimates of sensitivity (d') and response bias (C) in detecting near-threshold shocks to the index finger (see Supplementary Materials). The key dependent variable was the difference in sensitivity to detect the near-threshold shock between trials with a subliminal conditioning pulse, and trials without.

Results

For our main analyses, we calculated values of the dependent variable of each participant in each test, and inspected the dependent variable distributions across participants. Inspection of the dependent variable distributions suggested two departures from normality: for the SICI ratio in the CD group (Shapiro-Wilk test=0.858, p=0.014), and for the TDT measure in the control group (Shapiro-Wilk test=0.870, p=0.015). Since such departures from normality have only minimal effects on type I and type II errors of t-tests with sample sizes in our range(Sawilowsky et al. , 1992), no adjustment or transformation was made.

We compared the performance of the two groups using independent-samples t-tests for each dependent variable. The overall results are shown in Fig. 1.

Motor inhibitory function

Surround inhibition (SI). We used the ratio of MEPmovement/MEPrest of the ADM as a measure of surround inhibition. We found a trend for a higher value of this dependent variable, i.e., less inhibition, in the CD group compared to the controls (t(25.028)=1.746, p=0.093, Cohen's d=0.599, 95% CI for effect size [−0.083, 1.254], unequal variances assumed). Baseline cortical excitability measures are given in the supplementary analyses section.

Short interval IntraCortical Inhibition (SICI). We used the ratio MEPconditioned/MEPunconditioned as a measure of SICI. No difference in SICI ratio was found between groups t(34)=0.88, p= 0.385, Cohen's d=0.293, 95% CI for effect size [−0.371, 0.945]).

Sensory inhibitory function

Grating Orientation Test (GOT). Direct comparison of GOT thresholds revealed a significant difference between patient and control groups (t(34)= 2.418, p= 0.021, Cohen's *d*=0.807, 95% CI for effect size [0.110, 1.468]). Spatial discrimination threshold was significantly higher in CD patients compared to controls.

Interdigital Feedforward Subliminal Inhibition (IFSI). Comparison between groups of the difference in sensitivity attributable to the subthreshold shock showed reduced interdigital feedforward somatosensory inhibition in patients compared to controls (t(34)=

2.273 p= 0.029, Cohen's *d*= 0.759, 95% CI for effect size [0.066, 1.419]). Data on response bias (C) are given in the supplementary analyses section.

Temporal Discrimination Threshold (TDT). The groups did not differ (t(34)= 0.877, p= 0.386, Cohen's *d*=0.293, 95% CI for effect size [−0.371, 0.944]).

Feedforward Subliminal Inhibition (FSI). Comparison between groups of the difference in sensitivity attributable to the subthreshold shock showed no significant difference between feedforward subliminal inhibition in patients and in controls (t(29.547)= 0.801, p= 0.796, Cohen's *d*= -0.085, 95% CI for effect size [-0.737, 0.572], unequal variances assumed). Data on response bias (C) are given in the supplementary analyses section.

Pattern of group differences across measures

The results for each of the six tests are shown in Fig. 1. Taken as a whole, we found significant dystonic deficits only for the two measures of spatial somatosensory inhibition, namely the GOT and IFSI. However, this finding alone cannot justify a claim of specific deficit, since other measures yielded (non-significant) results in the same direction. Classical statistical tests of specificity are often based on showing an interaction between two independent variables(Nieuwenhuis et al. , 2011). However, our study investigated group differences across several *dependent* variables, requiring a different testing approach. Therefore, we used exact randomisation tests to search for *patterns* of between-group differences across the different measures, following the classification of Table 2. This approach shuffles labels for different dependent measures to quantify the chances of obtaining a distribution of effect sizes across the measures as extreme as the one observed(Ernst, 2004). Importantly, and unlike parametric tests, randomization tests make no assumptions about the distribution of effect sizes. We first listed all 90 possible ways of distributing effect sizes for our six measures across the four classes of inhibition investigated (2x2: somatosensory vs motor x spatial vs temporal; see table 2). Next, to compare sensory vs motor effects for each of the 90 shufflings, we averaged the effect sizes of the two somatosensory measures, then pooled across spatial and temporal measures, and finally compared the mean of the somatosensory effect sizes (observed mean 0.435) to the mean of

the motor effect sizes (observed mean 0.446). 32 of the 90 possible reshufflings of our six measures gave a difference between effect sizes that matched or exceeded this observed difference between our actual somatosensory and motor effect sizes of -0.003. The implied p-value was therefore 32/90=0.356. The null hypothesis of no pathway-specific deficit cannot be rejected.

Likewise, to compare spatial vs temporal effects, we averaged the effect sizes of our two somatosensory measures, then pooled across somatosensory and motor measures, and finally compared the mean of the spatial effect sizes (observed 0.691) to the mean of the temporal effect sizes (observed mean 0.199). This was the most extreme of the 90 possible orderings of our six measures: no other reshuffling gave a difference between pooled effect sizes as extreme as this observed difference of 0.492. This implies a p-value of 1/90=0.011, meeting the conventional threshold for rejecting the null hypothesis of no difference between spatial and temporal tests. We therefore suggest that cervical dystonia involves a selective deficit in spatial inhibitory functions.

Finally, we used the same principle to test for the interaction between the somatosensory vs motor and spatial vs temporal factors, calculated as (somatosensory spatial–somatosensory temporal)-(motor spatial–motor temporal). This interaction corresponded to a 0.374 difference in effect sizes. This was exceeded on 17/90 reshufflings, implying a non-significant p-value of .189 for the interaction.

Discussion

We explored sensory and motor inhibitory functions in CD and healthy controls. We tested a non-affected body part. Therefore, our results cannot merely be a secondary consequence of abnormal posture, but must reflect an underlying deficit in sensorimotor cortical processing more generally. We found a significant deficit in the dystonic group, compared to the control group, in two measures of somatosensory *spatial* inhibition (GOT, IFSI). In contrast, somatosensory *temporal* inhibition measures (TDT, FSI) showed comparable performance between the two groups. We found a weak trend for less SI in patients than in controls, but no difference in the SICI measure of homotopic temporal motor inhibition. These results demonstrate for the first time a specific pattern of inhibitory deficits for sensorimotor inhibitory functions in CD.

Isolating the inhibitory deficit in CD using quantitative measures

Somatosensory abnormalities are a central finding in isolated dystonia(Hallett, 1995, Tinazzi et al. , 2009). However, results have not been uniform within and between phenotypes(Molloy et al. , 2003, Bradley et al. , 2012, Deik et al. , 2012). This heterogeneity may have at least three sources. First, within the group of isolated dystonias, the different clinical presentations might correspond to different pathophysiologies. Second, reports of abnormal somatosensory processing have used a range of different test paradigms, and/or different body parts(Tinazzi et al. , 1999, Bara-Jimenez et al. , 2000b, Tinazzi et al. , 2000, Molloy et al. , 2003, Scontrini et al. , 2009, Tinazzi et al. , 2009, Bradley et al. , 2012). Third, the protocols for testing and analysis can differ between studies using the same measures(Tinazzi et al. , 1999, Bara-Jimenez et al. , 2000b, Molloy et al. , 2003, Bradley et al. , 2012). Here, we obtained six measures assessing multiple aspects of inhibition within the sensory and motor systems. Our results identified dystonic deficits in some inhibitory processes, rather than a general deficit affecting all inhibitory processing. In particular, patients with CD had significantly impaired performance in GOT and IFSI.

Performance on the GOT reflects the spatial precision of somatosensory perception, which is related to the cortical receptive field size. Importantly, cortical receptive field size is suggested to be shaped by lateral inhibition mediated by cortical interneurons(DiCarlo et al. , 1998). These interneurons are primarily GABAergic – intracortical administration of the GABA antagonist bicuculline lead to a rapid increase of receptive field size(Hicks et al. , 1983). Previous studies also found that GOT performance on the hand is abnormal in patients with isolated dystonia, including CD, compared to controls(Bara-Jimenez et al. , 2000a, Molloy et al. , 2003, Walsh et al. , 2007, Bradley et al. , 2010).

Interdigital feedforward subliminal inhibition also reflects the spatial organisation of somatosensation. In the subliminal inhibition paradigm, a weak, unperceived somatosensory prepulse impairs the detection of a subsequent, stronger somatosensory stimulus. The prepulse is known to suppress somatosensory cortical processing(Blankenburg et al. , 2003), and may reflect GABAergic cortico-cortical and/or thalamocortical inhibitory mechanisms. Here, we show that this effect also operates across fingers: a sub-threshold shock on the middle finger impaired detection of a subsequent stimulus on the index finger.

Interestingly, CD patients' abnormal *interdigital* feedforward inhibition on our IFSI test coexisted with normal homotopic feedforward inhibition on our FSI test. That is, the effect of a subliminal shock on subsequent perceptual detection of a stimulus on the *same*

finger did not differ between CD patients and controls(Ferre et al. , 2015). The interdigital version of feedforward inhibition also includes a *spatial* interaction between the subliminal and test shock, in addition to the temporal interaction present in the homotopic (FSI) version of the test. This difference may suggest an interesting feature of cortical pathophysiology in CD: spatial interactions between different somatosensory territories may be more strongly affected than temporal dynamics of inhibitory processing within a single territory. Spatial interactions have been attributed to specific classes of interneurons, while temporal phenomena such as prepulse inhibition may be associated with auto-inhibitory homosynaptic connections of projection neurons(Douglas et al. , 2009).

We found that the TDT test did not differ between CD and controls. This result also suggests a relative sparing of temporal processing within dystonic cortex. However, our finding contrasts with several previous reports of impaired *temporal* discrimination in isolated dystonia, including CD(Tinazzi et al. , 1999, Fiorio et al. , 2007, Scontrini et al. , 2009, Bradley et al. , 2012). The reasons for the contrast between our result and previous studies are unclear. Interestingly, our patients were older compared to previous studies and TDT performance is known to deteriorate with age in healthy volunteers(Hoshiyama et al. , 2004). In fact, one recent study of TDT across the lifespan, using the same electrodes and stimulation protocol as we have, found numerically very similar values for TDT in healthy volunteers within the age that we have studied(Ramos et al. , 2016). Moreover, a recent study, which also investigated TDT performance using an automated paradigm with randomized interstimulus intervals (range 1-200ms), also found no differences between patients with CD and healthy controls upon stimulation of the left index finger (Sadnicka et al. , 2017). Interestingly, impaired temporal discrimination in CD was reported to be more prominent for multisensory (visual-tactile) than unimodal stimuli (Aglioti et al. , 2003). The multisensory aspect might reflect an inhibition across the cortical space separating visual and somatosensory areas.

Motorcortical inhibitory function of unaffected body parts in CD

We measured SICI and SI to examine temporal and spatial properties of motor inhibition between CD and controls. We found a trend towards a difference in SI, and no difference between groups in SICI. Earlier studies in primary dystonia suggested an inhibitory deficit, as revealed by SICI(Ridding et al. , 1995, Edwards et al. , 2003, Quartarone et al. , 2009). Subsequent studies found a distinct additional deficit in SI for FHD(Sohn et al.

, 2004, Beck et al. , 2008, Shin et al. , 2012). We did not replicate the SICI result in our group of CD patients, though we found a modest trend for a deficit in SI. Interestingly, dystonic deficits in SICI were not found in some other studies(Rona et al. , 1998, Stinear et al. , 2004, Brighina et al. , 2009, Kojovic et al. , 2013). One of these studies, found a marked reduction in SICI in the dystonic body parts of patients with *acquired* dystonia(Kojovic et al. , 2013), but no difference between patients with idiopathic isolated focal dystonia and controls. Abnormal SICI was also observed in patients with functional (psychogenic) dystonia(Espay et al. , 2006, Quartarone et al. , 2009). These reports prompt the intriguing hypothesis that altered motor cortical inhibition in focal dystonias could be a consequence of prolonged abnormal posture of a specific body part(Espay et al. , 2006), rather than a direct result of dystonic pathology per se. This hypothesis could explain the absence of any SICI effect in our data, since we studied a non-affected body part, which would lack such history of abnormal posture. On the other hand, the abnormal SICI found in asymptomatic DYT1 gene carriers(Edwards et al. , 2003) clearly suggests that abnormal motor inhibitory mechanisms can co-exist with normal postures. Nevertheless, the mechanisms underlying idiopathic, sporadic, isolated focal dystonias may be quite different from those underlying DYT1 positive dystonia, despite common phenotypic characteristics.

Surround inhibition is thought to reflect a process of spatial inhibition in the motor system. In normal motor control, muscles adjacent to the prime mover for a specific action are actively inhibited during action execution. Thus, in healthy volunteers, excitability of the ADM muscle to TMS is reduced just prior to voluntary contraction of FDI. This relative inhibition was reduced in focal hand dystonia(Sohn et al. , 2004, Beck et al. , 2008), but was recently shown to be normal in small group of patients with CD (n=7)(McDougall et al. , 2015). Here, we found a modest trend towards a deficit in SI in the hand of patients with CD, in the predicted direction. One ready explanation of the variability among these various results relates to the body part tested. Studies of the principally-affected body part found abnormal SI, while our study of a non-affected body part found less compelling evidence. We speculate that deficient surround inhibition may not be a general, widespread feature of the dystonic brain, but may show gradation across the somatotopic map, with the most affected body parts expressing least SI. Similar somatotopic gradients have been reported for inhibition of involuntary tic movements(Ganos et al. , 2015). Larger studies are required to reach a clear conclusion regarding surround inhibition deficits in CD.

Dystonia as a disorder of somatosensory integration of spatial stimuli

The concept of dystonia as primarily a somatosensory, rather than motor, disorder has been advanced before(Hallett, 1995). Critically, our somatosensory and motor tests were broadly comparable, since both focus on well-established, general cortical mechanisms of temporal and spatial inhibition. Using this extensive, theoretically-inspired test battery, we have confirmed the somatosensory component of dystonia pathophysiology. However, our permutation analyses are not consistent with a claim of somatosensory *specificity*. Rather, we found evidence for a deficit in *spatial* aspects of inhibition, covering both somatosensory and motor cortical function. Of course, we cannot exclude the possibility that any spatial inhibitory deficit within the motor system could be a secondary consequence of a primary deficit within the somatosensory system, or vice versa. Indeed, the ordering of effect sizes in our study is consistent with a somatosensory-first-then-motor organisation of the dystonic deficit.

Classical physiological models view the cerebral cortex as an array of functional units termed cortical columns. Neurons in each column have a common function because of their distinct pattern of connectivity, maintained by local GABAergic inhibitory connections with adjacent units(Mountcastle, 1997, Blankenburg et al. , 2003). For example, neurons in the somatosensory cortex have lateral inhibitory connections that sharpen tuning to their peripheral receptive fields. Our GOT measure of spatial resolution relies on this local connectivity within the cortical representation of a single digit, while our IFSI measure relies on similar connectivity between digits. When such local connectivity breaks down, neural activity may spread excessively across the somatotopic map(Buonomano et al. , 1998). We speculate that this spread is of two distinct kinds, perhaps reflecting two specific circuit types within sensorimotor cortex. First, short-range inhibitory connections within healthy somatosensory cortex maintain a somatotopic organisation, characterised by segregation between representations of adjacent skin regions. We suggest that in CD, the degree of segregation is reduced, leading to reduced acuity and interdigit inhibition. Importantly, this deficit is found for unaffected body parts, in this case the hand. Second, the neural connections between somatosensory and motor cortices may also be affected in dystonia. Several physiological studies point to tight coupling between homologous fields in somatosensory and motor cortices(Johansson et al. , 1994) presumably subserved by longer-range corticocortical connections(Rocco-Donovan et al. , 2011). This cortico-cortical connectivity could explain how a deficit that has been viewed as primarily somatosensory deficit(Hallett, 1995), can come to influence motor function also, causing abnormal postures.

Pattern of CD deficits across behavioural measures

We investigated differences between a CD group and a volunteer group on six behavioural measures designed around a factorial combination of different forms of inhibition: somatosensory vs motor, and spatial vs temporal. We used exact permutation testing to assess whether the pattern of effect sizes across measures was consistent with a specific inhibitory deficit associated with one of these factors, or their interaction. These analyses give a statistically rigorous approach to investigating specificity within our test battery. Although we found univariate significant deficits in CD patients' inhibitory functions only for our two tests of spatial somatosensory inhibition (GOT and IFSI), analysis of the overall pattern of deficits across our six measures does not support a claim of a specific deficit for spatial somatosensory inhibition alone. In particular, our SI measure of spatial motor inhibition showed a non-significant trend in the same direction, with CD patients showing less surround inhibition than volunteers. Permutation testing of the distribution of effect sizes across our six measures suggested a significant effect of CD on spatial inhibitory processing in general, as opposed to temporal inhibitory processing. The hypothesis that the spatial deficit was specific to the somatosensory system was not supported. Therefore, we suggest that the dystonic deficit is linked to the spatial pattern of local connectivity that implements lateral inhibition within the sensory and motor cortices. This spatial inhibitory function appears to be reduced in both the somatosensory and motor cortical systems of CD patients.

Study limitations

We have systematically studied sensory and motor inhibition in a *non-affected* body part of a group of CD patients. Hence, we cannot make direct comments on the extent of inhibitory deficits in an affected body part (i.e. neck) or make comparisons between unaffected and affected body parts. Indeed few of the specific measures that we used for testing hand function are available for body parts such as the neck. However, studying the hand in CD patients has allowed us to assess the effects of dystonia on several different sensorimotor inhibitory functions, while excluding possible indirect or compensatory effects resulting from the abnormal dystonic postures themselves.

Also, we cannot exclude an effect of botulinum toxin on inhibitory sensorimotor processing, despite the washout period of at least 12 weeks (Kojovic et al., 2011). However, the maximum of such effect, as for example in the GOT, was reported to occur during the

first 4-6 weeks after the application of botulinum toxin injections and was minimal or absent about 8 weeks after treatment (Walsh et al. , 2007). Importantly, the effect of botulinum toxin is not generalized across the different inhibitory domains. For example, neither SICI nor performance on TDT (tested on the hand) were altered in patients with CD during the course of a single botulinum toxin injection cycle (Kojovic et al. , 2011, Scontrini et al. , 2011).

Our test battery was systematic, following the classification of inhibitory functions shown in table 2, but it could not be exhaustive. For example, under the heading of *Motor temporal inhibition*, we tested only SICI at 2.5 ms conditioning-test intervals. We did not test other SICI intervals, nor did we test LICI, which can also be considered a form of otor temporal inhibition. Thus, we do not exclude the possibility that some deficits in motor temporal inhibition may exist, on other tests. In general, our selection of tests was based on previous reports in the literature, and on prior knowledge of physiological mechanisms of inhibition in the healthy and dystonic cortex.

Our sample size of 17 patients was too small to investigate some hypotheses of scientific interest. Of course, one cannot conclude absence of any deficit from a null result, particularly given the limited statistical power of our study. Importantly, we cannot and do not claim that temporal processing is normal in CD. However, our tests on effect sizes showed that spatial processing deficits in our CD group significantly exceeded their temporal processing deficits. Thus, we claim that spatial processing is particularly affected in CD, but we remain neutral regarding whether temporal processing is affected or not. Nevertheless, our study goes beyond many previous studies in both the number of cases of a single dystonic phenotype, and also in the number and comprehensiveness of the tests. Given potential underlying endophenotypic differences, even within the CD population, we hope that such approaches to testing inhibitory function can be scaled up to larger studies of additional clinical populations in the future.

Acknowledgements
The authors would like to cordially thank the patients and healthy controls for their dedicated participation in the study.

Funding

C.G. was supported by a research grant of the Deutsche Forschungsgemeinschaft (DFG; GA2031/1-1 and GA2031/1-2) and is currently supported by the VolkswagenStiftung (Freigeist). E.R.F. and P.H. were supported by European Union Seventh Framework Programme (EU FP7) project VERE WP1. P.H. was additionally supported by a Professorial Fellowship from ESRC and by ERC Advanced Grant HUMVOL 323943.

A.M. was supported by the CooperInt Program from the University of Verona.

P.K. has nothing to disclose.

J.R. is supported by research grants from the Medical Research Council, the Biotechnology and Biological Sciences Research Council and the British Stroke Association.

K.P.B. receives royalties from publication of the Oxford Specialist Handbook Parkinson's Disease and Other Movement Disorders (Oxford University Press, 2008) and of Marsden's Book of Movement Disorders (Oxford University Press, 2012). He received funding for travel from GlaxoSmithKline, Orion Corporation, Ipsen, and Merz Pharmaceuticals.

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Figure 1. Test battery results.

Results of tests of spatial and temporal inhibition in motor and somatosensory functions in cervical dystonia (CD) patients and controls. SI=surround inhibition; SICI=short interval intracortical inhibition; GOT=grating orientation test; IFSI=interdigital feedforward subliminal inhibition; TDT=temporal discrimination threshold; FSI=Feedforward subliminal inhibition. (*: $p<0.05$)