Secondary and primary dystonia: pathophysiological differences

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Primary dystonia is thought to be a disorder of the basal ganglia because the symptoms resemble those of patients who have anatomical lesions in the same regions of the brain (secondary dystonia). However, these two groups of patients respond differently to therapy suggesting differences in pathophysiological mechanisms. Pathophysiological deficits in primary dystonia are well characterized and include reduced inhibition at many levels of the motor system and increased plasticity, while emerging evidence suggests additional cerebellar deficits. We compared electrophysiological features of primary and secondary dystonia, using transcranial magnetic stimulation of motor cortex and eye blink classical conditioning paradigm, to test whether dystonia symptoms share the same underlying mechanism. Eleven patients with hemidystonia caused by basal ganglia or thalamic lesions were tested over both hemispheres, corresponding to affected and non-affected side and compared with 10 patients with primary segmental dystonia with arm involvement and 10 healthy participants of similar age. We measured resting motor threshold, active motor threshold, input/output curve, short interval intracortical inhibition and cortical silent period. Plasticity was probed using an excitatory paired associative stimulation protocol. In secondary dystonia cerebellar-dependent conditioning was measured using delayed eye blink classical conditioning paradigm and results were compared with the data of patients with primary dystonia obtained previously. We found no difference in motor thresholds, input/output curves or cortical silent period between patients with secondary and primary dystonia or healthy controls. In secondary dystonia short interval intracortical inhibition was reduced on the affected side, whereas it was normal on the non-affected side. Patients with secondary dystonia had a normal response to the plasticity protocol on both the affected and non-affected side and normal eye blink classical conditioning that was not different from healthy participants. In contrast, patients with primary dystonia showed increased cortical plasticity and reduced eye blink classical conditioning. Normal motor cortex plasticity in secondary dystonia demonstrates that abnormally enhanced cortical plasticity is not required for clinical expression of dystonia, and normal eye blink conditioning suggests an absence of functional cerebellar involvement in this form of dystonia. Reduced short interval intracortical inhibition on the side of the lesion may result from abnormal basal ganglia output or may be a consequence of maintaining an abnormal dystonic posture. Dystonia appears to be a motor symptom that can reflect different pathophysiological states triggered by a variety of insults.
Introduction

Dystonia is a hyperkinetic movement disorder characterized by sustained muscle contraction leading to twisting, repetitive movements and abnormal postures of affected body parts (Fahn, 1988). In the absence of any pathological cause, Marsden et al. (1985) initially proposed that primary dystonia was a basal ganglia disease on the basis that the symptoms closely resembled those of some patients with identified lesions of the basal ganglia or their output pathways (now classified as secondary dystonia). The implication was that similarity of symptoms was caused by a similar underlying pathophysiology. However, primary and secondary dystonias differ in their response to treatment (Neychev et al., 2011); in addition there is emerging evidence that a cerebellar deficit may contribute to symptoms of primary dystonia (Sadnicka et al., 2012). Given the aetiological and clinical heterogeneity of dystonia, the aim of the present study was to test whether primary and secondary forms share a similar pathophysiological mechanism.

Most electrophysiological and neuroimaging studies in dystonia have been conducted on patients with primary dystonia as this is the most common form of the condition (Bressman, 2004). A consistent finding is loss of inhibition at different levels of the CNS, including spinal cord, brainstem and motor cortex (Berardelli et al., 1985; Nakashima et al., 1989; Ridding et al., 1995a). Recent evidence from human studies suggests that abnormally enhanced synaptic plasticity is also an important factor in pathophysiology of primary dystonias (Peterson et al., 2010; Quartarone and Pisani, 2011). Patients with primary focal and primary generalized dystonia have an enhanced response to different plasticity protocols that probe long-term potentiation-like and long-term depression-like synaptic plasticity in motor cortex (Quartarone et al., 2003, 2008; Edwards et al., 2006; Weise et al., 2006; Gilio et al., 2007) or brainstem circuits (Quartarone et al., 2006a). Finally, a range of recent evidence from structural and functional imaging suggests that the cerebellum has some role in primary dystonia. Thus voxel-based morphometric studies have found grey matter changes in the cerebellum of patients with focal dystonias (Draganski et al., 2003; Delmaire et al., 2007; Obermann et al., 2007) whereas functional MRI has revealed changes in movement-related activity (Odergren et al., 1998; Carbon and Edelberg, 2009) and metabolic profile (Hutchinson et al., 2000). A finding of reduced eye blink classical conditioning in focal dystonias provides electrophysiological evidence of functional cerebellar involvement in primary dystonia (Teo et al., 2009).

Although there are some reports that patients with secondary dystonia may share similar abnormalities in inhibitory networks of the motor system to those observed in primary dystonia (Nakashima et al., 1989; Trompetto et al., 2012), there is no information about plasticity or cerebellar function in this group of individuals. The aim of the present study was to provide a more comprehensive comparison of the underlying pathophysiology in primary and secondary dystonias. The results show that there are distinct differences in physiology, implying that the clinical syndrome of dystonia has more than one physiological phenotype. This would be consistent with the fact that dystonia can have many different causes and can respond quite differently to treatment (Neychev et al., 2011). The conclusion is that dystonia represents one (of many) possible stable state(s) into which the motor system can be pushed through a variety of insults.

Materials and methods

Participants

We studied 11 patients with secondary dystonia caused by structural brain lesion (five males and six females, mean age 45.8 years, range 28–68, Table 1), 10 patients with primary segmental dystonia (four males and six females, mean age 46.7 years, range 31–67, Table 2) and 10 age-matched healthy participants (five males and five females, mean age 48.7 years, range 27–67). Patients with secondary dystonia were included if they had (i) unilateral distribution of dystonia; (ii) discrete lesion in the basal ganglia and/or thalamus contralateral to the clinically involved side on MRI or CT; and (iii) no significant pyramidal involvement or hemisensory loss, as assessed by the Ashworth Scale and National Institutes of Health Stroke Scale. All patients were clinically examined and videotaped. Three patients with secondary dystonia had resting dystonia with fixed postures (Patients 1 and 2 fixed dystonia at leg, Patient 5 fixed dystonia at arm), the other eight patients had mobile dystonia at rest, worsened by action. All patients with primary dystonia had segmental dystonia with unilateral arm involvement visible at rest or on maintaining outstretched arm posture. Clinical disease severity was assessed with Burke-Fahn-Marsden scale. All patients treated with botulinum toxin were injected at least 15 weeks before participating in the study. One of the patients with secondary dystonia (Patient 5) underwent unilateral thalamotomy 20 years earlier, with only transient improvement of symptoms. At the time of the study, none of the participants were on any medications that could affect the measurements performed. All participants were right-handed. Eye blink classical conditioning testing was performed on patients with secondary dystonia and, for convenience their data on eye blink classical conditioning were compared with the data of patients with primary dystonia [seven males, six females, mean age 63.7 ± 3.4 (SEM) and healthy participants (six males, five females, mean age 61 ± 4.5 (SEM)], obtained using the same experimental protocol (Teo et al., 2009). Written informed consent was obtained from all participants and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

Electromyographic recordings

EMG recordings were made from the abductor pollicis brevis and adductor digiti minimi muscles on the side contralateral to stimulated cortex with Ag-AgCl surface electrodes using a belly-tendon montage. EMG signals were amplified (×1000) and band-pass filtered.
Table 1 Clinical and demographic characteristics of patients with secondary dystonia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)/gender</th>
<th>Age at dystonia onset (years)</th>
<th>Disease duration (years)</th>
<th>Distribution of dystonia</th>
<th>Cause</th>
<th>Lesion on MRI</th>
<th>BFM score</th>
<th>NIHSS score</th>
<th>Duration of BT treatment (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38/F</td>
<td>28</td>
<td>10</td>
<td>L hemidystonia; fixed at foot, mobile at arm</td>
<td>Ischaemic stroke</td>
<td>R pallidum</td>
<td>9</td>
<td>0</td>
<td>2 (Discontinued in past 4 years)</td>
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<tr>
<td>2</td>
<td>63/F</td>
<td>32</td>
<td>29</td>
<td>L hemidystonia; fixed at foot, mobile at arm</td>
<td>Ischaemic stroke</td>
<td>R striatum</td>
<td>21</td>
<td>2</td>
<td>Not treated</td>
</tr>
<tr>
<td>3</td>
<td>40/M</td>
<td>2</td>
<td>38</td>
<td>R hemidystonia; mobile</td>
<td>Perinatal HII</td>
<td>L thalamus</td>
<td>28</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>68/M</td>
<td>55</td>
<td>13</td>
<td>R hemidystonia; mobile</td>
<td>Ischaemic stroke</td>
<td>L thalamus</td>
<td>18</td>
<td>1</td>
<td>Not treated</td>
</tr>
<tr>
<td>5</td>
<td>63/F</td>
<td>2</td>
<td>61</td>
<td>L hemidystonia; fixed at arm, mobile at leg</td>
<td>Perinatal HII</td>
<td>R lentiform nc.</td>
<td>27</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>38/M</td>
<td>6</td>
<td>32</td>
<td>L hemidystonia; mobile</td>
<td>Ischaemic stroke</td>
<td>R lentiform nc.</td>
<td>16</td>
<td>2</td>
<td>Not treated</td>
</tr>
<tr>
<td>7</td>
<td>28/M</td>
<td>2</td>
<td>26</td>
<td>L arm; mobile</td>
<td>Perinatal HII</td>
<td>R lentiform nc.</td>
<td>19</td>
<td>3</td>
<td>6</td>
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<tr>
<td>8</td>
<td>36/F</td>
<td>3</td>
<td>33</td>
<td>L hemidystonia; mobile</td>
<td>Encephalitis</td>
<td>R lentiform nc.</td>
<td>27.5</td>
<td>1</td>
<td>7</td>
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<tr>
<td>9</td>
<td>48/F</td>
<td>1</td>
<td>47</td>
<td>L hemidystonia; mobile</td>
<td>Perinatal HII</td>
<td>R lentiform nc.</td>
<td>21.5</td>
<td>0</td>
<td>18</td>
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<tr>
<td>10</td>
<td>42/M</td>
<td>18</td>
<td>24</td>
<td>R hemidystonia; mobile</td>
<td>Ischaemic stroke</td>
<td>L striatum</td>
<td>26</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>40/F</td>
<td>1</td>
<td>39</td>
<td>R hemidystonia; mobile</td>
<td>Perinatal HII</td>
<td>L lentiform nc.</td>
<td>13</td>
<td>0</td>
<td>Not treated</td>
</tr>
<tr>
<td>Average</td>
<td>± SE</td>
<td>45.8 ± 13.8</td>
<td>13.6 ± 4.1</td>
<td>32 ± 9.6</td>
<td></td>
<td></td>
<td>20.5 ± 6.2</td>
<td>1.3 ± 0.4</td>
<td>5.7 ± 1.7</td>
</tr>
</tbody>
</table>

BFM = Burke-Fahn-Marsden dystonia score; NIHSS = National Institute of Stroke Scale score; BT = botulinum toxin; F = female; M = male; HII = hypoxic-ischaemic injury; nc = nucleus; R = right; L = left.

Table 2 Clinical and demographic characteristics of patients with primary dystonia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)/gender</th>
<th>Age of dystonia onset (years)</th>
<th>Disease duration (years)</th>
<th>Distribution of dystonia and characteristics</th>
<th>BFM score</th>
<th>Duration of BT treatment (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39/F</td>
<td>29</td>
<td>10</td>
<td>CD and R arm dystonia</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>63/F</td>
<td>59</td>
<td>4</td>
<td>BSP, CD and R arm dystonia</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>29/F</td>
<td>23</td>
<td>6</td>
<td>CD and R arm dystonia (started as graphospasm)</td>
<td>9</td>
<td>Not treated</td>
</tr>
<tr>
<td>4</td>
<td>44/M</td>
<td>40</td>
<td>4</td>
<td>CD and L arm dystonia</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>31/M</td>
<td>24</td>
<td>7</td>
<td>CD and R hand dystonia, started as graphospasm</td>
<td>8</td>
<td>Not treated</td>
</tr>
<tr>
<td>6</td>
<td>53/F</td>
<td>47</td>
<td>6</td>
<td>Laryngeal dystonia, CD and L arm dystonia</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>40/F</td>
<td>32</td>
<td>8</td>
<td>CD and R arm dystonia</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>67/M</td>
<td>20</td>
<td>47</td>
<td>Laryngeal dystonia and L arm dystonia, L dystonic tremor</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>50/M</td>
<td>43</td>
<td>7</td>
<td>CD and R hand dystonia</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>51/M</td>
<td>29</td>
<td>10</td>
<td>BSP, CD and R arm dystonia</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Average</td>
<td>± SE</td>
<td>46.7 ± 14.8</td>
<td>34.6 ± 10.9</td>
<td>10.9 ± 3.4</td>
<td>12.2 ± 39</td>
<td>5.3 ± 1.7</td>
</tr>
</tbody>
</table>

BFM = Burke-Fahn-Marsden dystonia score; BT = botulinum toxin; F = female; M = male; BSP = blepharospasm; CD = cervical dystonia; R = right; L = left.
Transcranial magnetic stimulation

Single and paired pulse TMS of the primary motor cortex was applied using Magstim 200\textsuperscript{2} magnetic stimulators with a monophasic current waveform (Magstim Company). The magnetic stimulators were connected to a standard figure-of-eight coil with mean loop diameter of 9 cm. The intersection of the coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of $\sim 45^\circ$ to the sagittal plane in order to generate a posterior–anterior current in the brain (Kaneko et al., 1996; Di Lazzaro et al., 2004). The 'hot spot' was defined as the optimal scalp position for eliciting motor-evoked potentials (MEPs) of maximal amplitude in the contralateral abductor pollicis brevis muscle.

Corticospinal excitability

The resting motor threshold and active motor threshold were determined according to standard definitions (Rossini et al., 1994). Single MEPs were recorded using a stimulus intensity adjusted to produce MEP amplitude of $\sim 1\text{ mV}$ in the relaxed abductor pollicis brevis muscle (1 mV MEPs) and this intensity was kept constant for assessment of 1 mV MEPs through the experiment. For assessment of 1 mV MEP, at each time point (before paired associative stimulation (PAS) and 0, 15 and 30 min after PAS) 20 MEPs were collected. Input-output curves were assessed by recording four MEPs at each of the 10 intensities of stimulation, increasing in 10% steps from 80–170% of resting motor threshold.

Intracortical excitability

Short-latency intracortical inhibition (SICI) was recorded at rest using a standard paired-pulse paradigm (Kujirai et al., 1993). Intensity of conditioning stimulus was set at 80% active motor threshold, whereas the intensity of the test stimulus was adjusted to elicit an MEP of $\sim 1\text{ mV}$. SICI was probed at an interstimulus interval of 2 ms. Forty MEPs were collected: 20 with conditioning stimulus and 20 with test stimulus alone. For assessment of cortical silent period, 10 single TMS pulses were applied at an intensity of 120% resting motor threshold while participants performed a constant contraction of abductor pollicis brevis at 20% of their maximum voluntary contraction, assisted by visual feedback. Cortical silent period was measured manually from the onset of MEPs to the re-emergence of sustained EMG activity.

Paired associative stimulation

PAS consisted of 200 electrical stimuli to the median nerve at the wrist paired with TMS stimuli over the hot spot for the abductor pollicis brevis muscle, given at the rate 0.25 Hz (Ziemann et al., 2004). Each TMS stimulus was preceded by an electrical conditioning stimulus at an interstimulus interval of 25 ms. Electrical stimulation was applied through a bipolar electrode, with the cathode positioned proximally. The electrical stimuli were constant current square wave pulses with a pulse width of 200 $\mu$s. Intensity of electrical stimulus was 300% of the perceptual threshold, while TMS intensity was adjusted to 1 mV MEP intensity. Subjects were instructed to look at their stimulated hand and count the peripheral electrical stimuli they perceived in order to ensure comparable attention levels between sessions.

Eye blink classical conditioning

The eye blink classical conditioning protocol was delivered as detailed elsewhere (Teo et al., 2009). In brief, an electrical stimulus was applied through a bipolar electrode, with the cathode positioned proximally. The electrical stimuli were constant current square wave pulses with a pulse width of 200 $\mu$s, i.e. unconditioning stimulus, and were delivered to the right supraorbital nerve at an intensity adjusted to obtain stable R2 responses ($\sim 4$–6 times the sensory threshold). Electrical supraorbital nerve stimulus was preceded by a tone, i.e. the conditioning stimulus, produced by a tone generator and presented bilaterally to the subject through binaural headphones at an intensity 50–70 dB above the individual hearing threshold. Conditioning stimulus intensity was kept constant during the experiment. The conditioning stimulus inconsistently produced an acoustic startle response (alpha blink) occurring within 200 ms after conditioning stimulus onset. Repeated pairs of conditioning stimulus and unconditioning stimulus at 400 ms intervals induced conditioned eye blink response (conditioned response) to appear with onsets within 200 ms after unconditioning stimulus. Eye blink classical conditioning sessions consisted of seven blocks: six acquisition blocks (each block contained 11 trials: nine trials of conditioning stimulus–unconditioning stimulus pairs, the 10th trial was unconditioning stimulus only and trial 11th was conditioning stimulus only) followed by one extinction block (11 trials of conditioning stimulus only). For measurement of eye blink classical conditioning, the conditioned responses were counted manually. EMG bursts were regarded as 'alpha blinks' if their amplitude exceeded 50$\mu$V and if latency was $< 200$ ms after the conditioning stimulus. EMG bursts were regarded as conditioned responses if latency was $> 200$ ms after the conditioning stimulus but before the unconditioning stimulus. For the conditioning stimulus only trials, EMG bursts occurring 200–600 ms after the conditioning stimulus were considered conditioned response.

Experimental design

Patients with secondary dystonia were tested on both hemispheres, corresponding to the affected and non-affected side in two different TMS sessions, separated by at least 1 week. The order of the tested hemisphere (affected versus unaffected) was balanced between subjects. Patients with primary dystonia were tested on the hemisphere corresponding to the affected side only, since previous studies showed that in primary dystonia abnormalities in TMS measures are present in affected and unaffected parts of the body (Quarterone et al., 2008). Healthy participants were tested on the dominant hemisphere only (Ridding and Flavel, 2006). In each session we began with baseline assessments of resting motor threshold, active motor threshold and 1 mV MEP, input-output curve, SICI and cortical silent period. We then delivered PAS as described above and assessed the effect of this conditioning protocol on corticospinal excitability (resting motor threshold, active motor threshold, and 1 mV MEPs in abductor pollicis brevis and adductor digiti minimi muscles) and cortical silent period at
three different time points: 0, 15 min and 30 min after PAS. In addition, patients with secondary dystonia underwent a third session for eye blink classical conditioning testing.

Statistical analysis

Distribution of data was assessed using Shapiro–Wilks test of normality. Greenhouse-Geisser method was used where necessary to correct for non-sphericity. For parametric tests (ANOVA) post hoc Tukey tests were used to further analyse significant main effects or interactions. The significance was preset at \( P < 0.05 \).

To test for age differences between patients with secondary dystonia, patients with primary dystonia and healthy controls we used one-way ANOVA with Group as a between subject factor. The differences in disease duration, Burke-Fahn-Marsden scores and duration of botulinum toxin treatment between patients with secondary and primary dystonia were assessed with Mann-Whitney U-test.

The primary aim of this study was to compare the TMS parameters between affected side in patients with secondary dystonia and patients with primary dystonia and healthy participants. Resting motor threshold, active motor threshold, EMG root mean square amplitude, SICI and cortical silent period were compared between groups using ANOVAs with a factor Group (three levels: secondary dystonia-affected side, primary dystonia and healthy participants) as a between-subject factor. For analysis of SICI, conditioned MEP amplitudes were averaged, normalized to average unconditioned MEP amplitudes and entered into ANOVA with the factor Group as between-subjects factor. Input-output curves were compared between groups in ANOVA with the factor Group and the factor Stimulus intensity (10 levels of stimulator output intensity ranging from 80–170% of resting motor threshold intensity) as a within-subjects factor. For analysis of PAS effect on 1 mV MEP amplitude, MEP amplitudes at each time point were averaged, normalized to baseline MEPs and entered into ANOVA with factor Group as between-subjects factor and factors Muscle (two levels: abductor pollicis brevis and adductor digiti minimi muscle) and Time point (three levels: 0 min, 15 min and 30 min after PAS) as within-subjects factors. The effect of PAS on resting motor threshold and active motor threshold and cortical silent period was evaluated using separate ANOVAs with factors Group as a between-subject factor and Time point (three levels: normalized resting motor threshold or active motor threshold or cortical silent period at 0 min, 15 min and 30 min after PAS). As a secondary analysis, we assessed how TMS measures compared between the affected and non-affected side in patients with secondary dystonia, using repeated measures ANOVA or paired sample t-test. For eye blink classical conditioning, the percentage of conditioned responses over different blocks did not follow the normal distribution, therefore non-parametric tests were used. We first compared the number of overall conditioned responses (for all blocks) in each group using Kruskal-Wallis ANOVA. The differences in the number of conditioned responses in each block between groups were then assessed by Mann-Whitney U test. Finally, for each group we used Friedman ANOVA to test if there was a conditioning of eye blink responses across blocks.

Possible correlations between clinical and demographic data (disease duration, Burke-Fahn-Marsden score, duration of botulinum toxin injection treatment) and TMS measures (SICI, averaged PAS response) or eye blink classical conditioning measures (the average percentage of conditioned responses per block) were evaluated with the Spearman correlation analysis.

The significance was preset at \( P < 0.05 \). Unless otherwise stated, data are given as mean ± standard error of the mean (SEM).

Results

Transcranial magnetic stimulation

There was no difference in age between patients with secondary and primary dystonia and healthy participants. As expected, the Burke-Fahn-Marsden score was higher in patients with secondary compared to primary dystonia (\( z = -2.9; P = 0.004 \)) and the disease duration was longer (\( z = -3.14; P = 0.002 \)). No difference was found in the duration of botulinum toxin treatments between dystonia groups (\( z = -0.72; P = 0.93 \)).

Corticospinal excitability

At baseline, no significant difference was found in resting motor threshold, active motor threshold, 1 mV MEPs TMS intensity or EMG root mean square amplitude between patients with secondary and primary dystonia and healthy participants or between affected and non-affected sides in patients with secondary dystonia (Table 3).

As expected, a repeated measures ANOVA showed a significant effect of Stimulus intensity \( [F(9, 207) = 28.9; P < 0.001] \) on the input-output relationship, due to an increase of MEP size with increasing intensity, whereas there was no effect of the factor Group and no Group × Stimulus intensity interaction. The side comparison in secondary dystonia, also revealed a significant effect of Stimulus intensity \( [F(9, 36) = 13.6; P < 0.001] \), whereas the main factor Side and the interaction Side × Stimulus intensity were both non-significant. These results indicate that there was no difference in baseline corticospinal excitability between patients with secondary dystonia and primary dystonia and healthy participants or between the affected and non-affected side in patients with secondary dystonia (Fig. 1A and B).

Short-interval intracortical inhibition

ANOVA revealed a significant effect of the factor Group \( [F(2, 27) = 5.11; P = 0.01] \), due to less SICI in patients with secondary dystonia compared with healthy participants \( (P = 0.01) \), whereas there was no difference between primary and secondary dystonia or between primary dystonia and healthy participants. When the affected side was compared with the non-affected side in secondary dystonia, a paired-sample t-test revealed that there was less SICI \( (P = 0.02) \) on the more affected side (Fig. 2A).

Cortical silent period

ANOVA revealed no difference in cortical silent period between patients with secondary dystonia, primary dystonia and healthy participants. A paired-sample t-test showed no difference in cortical silent period between the affected and non-affected side in secondary dystonia (Fig. 2B).

Paired associative stimulation

An ANOVA revealed a significant effect of Group \( [F(2, 28) = 12; P < 0.001] \), due to a larger response to PAS in patients with
primary dystonia compared to both patients with secondary dystonia \((P < 0.001)\) and healthy participants \((P < 0.001)\), whereas there was no difference between patients with secondary dystonia and healthy participants. Factors Muscle and Time point were not significant as were all two-way and three-way interactions, indicating that the PAS response was higher in primary dystonia at all three time points after PAS in both abductor pollicis brevis and adductor digiti minimi muscles (Figs 3A and B and 4). When the affected side was compared to the non-affected side in secondary dystonia, an ANOVA revealed a significant effect of the factor Muscle \([F(1, 9) = 8.7; \ P = 0.02]\), due to a larger response to PAS in abductor pollicis brevis compared with adductor digiti minimi. Factors Side and Time point were not significant, as were the interactions between main factors, indicating that there was no difference in the PAS response between the affected and non-affected side in secondary dystonia and that there was no spread of the PAS effect to the adductor digiti minimi muscle on either side (Fig. 3C).

There was no difference in of the effect of PAS on resting motor threshold or active motor threshold or cortical silent period in patients with secondary dystonia (both sides), patients with primary dystonia or healthy participants.

**Eye blink classical conditioning**

ANOVA revealed a significant difference in age between the groups \([F(2, 32) = 5.9; \ P = 0.006]\), because our patients with secondary dystonia were younger than the primary dystonia control subjects \((P = 0.007)\) and healthy participants \((P = 0.03)\).

For the eye blink classical conditioning data, a Kruskal-Wallis ANOVA revealed a significant effect of the factor Group

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**Table 3** Baseline corticospinal excitability and statistics

<table>
<thead>
<tr>
<th></th>
<th>Secondary dystonia non-affected side</th>
<th>Secondary dystonia affected side</th>
<th>Primary dystonia</th>
<th>Healthy participants</th>
<th>Statistics b</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMT %</td>
<td>51.45</td>
<td>53.8</td>
<td>48.4</td>
<td>51.3</td>
<td>(F(2,28) = 0.93)</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>(3.09)</td>
<td>(3.4)</td>
<td>(1.45)</td>
<td>(1.44)</td>
<td>(P = 0.4)</td>
</tr>
<tr>
<td>AMT %</td>
<td>38.6</td>
<td>39</td>
<td>37.6</td>
<td>40</td>
<td>(F(2,28) = 0.22)</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>(2.32)</td>
<td>(1.74)</td>
<td>(1.4)</td>
<td>(1.3)</td>
<td>(P = 0.8)</td>
</tr>
<tr>
<td>1 mv MEP %</td>
<td>57.2</td>
<td>64.1</td>
<td>59.2</td>
<td>62.8</td>
<td>(F(2,28) = 0.85)</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>(2.6)</td>
<td>(3.8)</td>
<td>(2.0)</td>
<td>(1.56)</td>
<td>(P = 0.44)</td>
</tr>
<tr>
<td>RMS amplitude (mV)</td>
<td>0.02</td>
<td>0.022</td>
<td>0.02</td>
<td>0.019</td>
<td>(F(2,28) = 0.80)</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>(0.001)</td>
<td>(0.0013)</td>
<td>(0.0011)</td>
<td>(0.0009)</td>
<td>(P = 0.46)</td>
</tr>
</tbody>
</table>

RMT = resting motor threshold; AMT = active motor threshold; RMS = root mean square.

*a* paired sample t-test comparing non-affected to affected side in patients with secondary dystonia.

*b* One-way ANOVA comparing secondary dystonia (more affected side) patients versus primary dystonia patients versus healthy participants.

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**Figure 1** Input/output curves. The mean MEP amplitude (± SEM) is given on the y-axis against the stimulus intensity given on the x-axis (as a percentage of resting motor threshold stimulus intensity). (A) The input-output curves in patients with secondary dystonia, patients with primary dystonia and healthy participants are not significantly different. (B) There is no difference in input-output curves between the affected and non-affected side in patients with secondary dystonia. RMT = resting motor threshold.
Figure 3  PAS effect on corticospinal excitability, as measured by change in 1 mV MEP amplitude in abductor pollicis brevis and adductor digiti minimi muscle. (A) In the abductor pollicis brevis (APB) muscle, patients with primary dystonia have a higher response to PAS at all three time points (i.e. 0 min, 15 min and 30 min after PAS) compared with patients with secondary dystonia and healthy participants (**P ≤ 0.01). There is no difference in PAS response between patients with secondary dystonia and healthy participants. Averaged MEP amplitudes at each time point after PAS is normalized to baseline averaged MEP (before PAS) and given on the y-axis; time is given on the x-axis. (B) Patients with primary dystonia have a spread of PAS effect in non-median innervated adductor digiti minimi (ADM) muscle (**P ≤ 0.01), that is not present in patients with secondary dystonia or healthy participants. (C) There is no difference in PAS response between the affected and non-affected side in patients with secondary dystonia. On both the affected and non-affected side, PAS response is larger in abductor pollicis brevis compared to adductor digiti minimi muscle (*P ≤ 0.05). PAS response is expressed as an averaged response for three time points after PAS (0, 15, 30 min) and normalized to baseline MEPs.
[χ² = 10.2; P = 0.006]. Post hoc Mann-Whitney-U tests showed that this was due to more conditioned responses in secondary compared to primary dystonia in Blocks 2–6 and more conditioned responses in healthy participants compared to patients with primary dystonia (Blocks 4–6) (see Table 4 for statistics). There was however no difference between patients with secondary dystonia and healthy participants. We further confirmed with Friedman ANOVA that the number of conditioned responses increased over blocks in both patients with secondary dystonia [χ² = 22.4; P < 0.001] and healthy participants [χ² = 22.9; P < 0.001], but not in patients with primary dystonia [χ² = 3.53; P = 0.6] (Fig. 5).

We found no significant correlation between clinical and demographic data and TMS or eye blink classical conditioning measures in our patients.

### Discussion

The main findings of the present study are (i) the response to PAS in patients with secondary dystonia is no different to that in healthy participants, in contrast to the enhanced response in patients with primary dystonia; (ii) patients with secondary dystonia have reduced SICI, on the side of the lesion only; and (iii) eye blink classical conditioning is worse in patients with primary than in those with secondary dystonia.

### Differences in paired associative stimulation induced plasticity between secondary and primary dystonia

The enhanced response to PAS that we found in our patients with primary dystonia is in line with several previous studies using a variety of plasticity-testing protocols (Quartarone et al., 2003; Edwards et al., 2006; Weise et al., 2006). It was, however, surprising to find that the response to PAS was normal in secondary dystonia. This is unlikely to be due to differences in baseline corticospinal excitability, as the input-output curves and motor thresholds were the same in all three groups that we studied. Nor is it likely to be a result of the longer duration and more severe dystonic symptoms in the patients with secondary dystonia. Although the present study only examined cases of primary segmental dystonia, previous investigations from this laboratory have found enhanced responses to experimental plasticity protocols even in patients with primary generalized dystonia, whose symptoms began in childhood (Edwards et al., 2006) and were so severe as to require bilateral pallidal deep brain stimulation (Ruge et al., 2011). In addition, there was no correlation between disease duration and the response to PAS in our patients with secondary dystonia.

We have reported previously (Kojovic et al., 2011) that botulinum toxin treatment can transiently reduce the response to PAS in patients with primary dystonia, which then returns to the level present before botulinum toxin injection after a few months. Since all the patients in the present study were investigated at least 15 weeks after their last injection, this acute effect of botulinum toxin is unlikely to have influenced the present results. Nevertheless, it is difficult to speculate on whether there might have been possible chronic effects of botulinum toxin on motor cortex plasticity, as this has not been previously investigated. Several of the patients with secondary dystonia had been treated for many years and it is possible that this could have permanently reduced their PAS response and skewed the group data even though there was no difference in mean duration of treatment in the primary and secondary cases. This seems unlikely to have been the case as there was no correlation between duration of botulinum toxin treatment and the response to PAS protocol.

In the absence of other explanations, we suggest that enhanced motor cortex plasticity is an inherent, genetically determined trait (endophenotype) specific for primary dystonia that predisposes some individuals to develop dystonia. As suggested by Quartarone et al. (2006b) this may result in an excessive tendency to form associations between sensory input and motor output, leading to dystonia, particularly under circumstances involving frequent repetition of specific movements. The fact that sensorimotor plasticity is normal not only in secondary but also in psychogenic dystonia (Quartarone et al., 2009) further confirms that abnormally enhanced plasticity is an endophenotypic trait specific to primary dystonia.

Secondary dystonia is believed to be related to functional changes in sensorimotor circuits after brain injury (Burke et al., 1980), but the exact mechanism underlying the changes and the anatomical regions in which they occur are not well understood. Diffusion tensor imaging and functional MRI studies in patients with subcortical strokes suggest that no significant reorganization occurs in the ipsilesional primary motor cortex per se, but rather within the white matter tracts (Fries et al., 1993; Jang, 2007) with a contribution of the corticospinal tract from the unaffected hemisphere (Jankowska and Edgley, 2006). Thus, it may be that the

### Table 4 Statistics of eye-blink classical conditioning (Mann-Whitney U-tests)

<table>
<thead>
<tr>
<th></th>
<th>Block 1</th>
<th>Block 2</th>
<th>Block 3</th>
<th>Block 4</th>
<th>Block 5</th>
<th>Block 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary dystonia versus primary dystonia</td>
<td>Z = –0.90</td>
<td>Z = –2.05</td>
<td>Z = –2.54</td>
<td>Z = –3.19</td>
<td>Z = –2.93</td>
<td>Z = –0.62</td>
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<tr>
<td>Healthy participants</td>
<td>P = 0.4</td>
<td>P = 0.04</td>
<td>P = 0.01</td>
<td>P = 0.001</td>
<td>P = 0.003</td>
<td>P = 0.009</td>
</tr>
<tr>
<td>Secondary dystonia versus healthy participants</td>
<td>Z = –0.74</td>
<td>Z = –1.77</td>
<td>Z = –1.15</td>
<td>Z = –0.76</td>
<td>Z = –0.36</td>
<td>Z = –0.96</td>
</tr>
<tr>
<td>Healthy participants</td>
<td>P = 0.5</td>
<td>P = 0.08</td>
<td>P = 0.2</td>
<td>P = 0.5</td>
<td>P = 0.7</td>
<td>P = 0.3</td>
</tr>
<tr>
<td>Primary dystonia versus healthy participants</td>
<td>Z = –1.71</td>
<td>Z = –0.27</td>
<td>Z = –1.18</td>
<td>Z = –2.49</td>
<td>Z = –2.74</td>
<td>Z = –2.41</td>
</tr>
<tr>
<td>Healthy participants</td>
<td>P = 0.09</td>
<td>P = 0.8</td>
<td>P = 0.2</td>
<td>P = 0.01</td>
<td>P = 0.006</td>
<td>P = 0.02</td>
</tr>
</tbody>
</table>
principal pathological processes spare the function of ipsilesional primary motor cortex. In a PET activation study Ceballos-Baumann et al. (1995) showed that the pattern of primary motor cortex activity differs between patients with acquired hemidystonia and idiopathic torsion dystonia. Similarly, a combined functional MRI and diffusion tensor imaging study on a patient with hemidystonia caused by a penetrating injury of caudate and lentiform nucleus showed that there was no significant functional reorganization in the primary motor cortex after injury (Werring et al., 1998). This would be consistent with the normal response to PAS in our patients.

Eye blink classical conditioning and its possible relation to paired associative stimulation response in dystonia

Eye blink classical conditioning, as used in human studies, is a form of predictive learning that lesion studies have shown to depend on the integrity of the olivo-cerebellar circuit (Gerwig et al., 2007). Indeed, in healthy individuals, continuous theta burst stimulation over cerebellum, which is thought to interfere with function in cerebellar circuits, abolishes eye blink classical conditioning (Hoffland et al., 2012). Previously we had found that eye blink classical conditioning was markedly reduced—compared with healthy volunteers—in patients with primary focal hand and/or cervical dystonia and had speculated that this was further evidence in favour of a cerebellar involvement in primary dystonias (Teo et al., 2009). In the present study, patients with secondary dystonia showed preserved eye blink classical conditioning that did not differ from healthy control subjects. Eye blink classical conditioning decreases with age (Finkbiner and Woodruff-Pak, 1991; Bellebaum and Daum, 2004) and therefore the age difference between compared groups could have been a confounding factor. However, even though our patients with secondary dystonia were younger than both the healthy control subjects and patients with primary dystonia, their eye blink classical conditioning was similar to healthy control subjects and superior to eye blink classical conditioning in primary dystonia. Therefore, younger age is unlikely to be a reason for apparently normal eye blink classical conditioning in our patients with secondary dystonia. The implication of our findings is that the pathophysiology of secondary dystonia is more localized than that of primary dystonia.

Although eye blink classical conditioning and PAS are usually thought to test quite different circuits in different parts of the brain, there may be some connection between the two that could potentially link the present results in primary and secondary dystonias. Recent work has shown that the response to some PAS protocols is modulated by inputs from the cerebellum; thus a disordered cerebellum could potentially lead to abnormal PAS (Hamada et al., 2012, Poppa et al., 2013). In healthy volunteers, the effect of a PAS25 protocol (that is, with an interval of 25 ms between median nerve and TMS pulse) is reduced or abolished by concurrent and/or direct current stimulation over the cerebellum or by preconditioning with excitatory intermittent theta burst stimulation (Hamada et al., 2012, Poppa et al., 2013); in contrast, preconditioning the cerebellum with continuous theta burst stimulation enhanced PAS (Poppa et al., 2013). Thus, the effect of motor cortex PAS25 depends on the functional state of cerebellar output.

From the data outlined above, the combination of enhanced response to PAS25 and decreased eye blink classical conditioning in primary dystonia is similar to what occurs with cerebellar continuous theta burst stimulation in healthy volunteers: eye blink classical conditioning is reduced and PAS25 plasticity increased. The conclusion is that a cerebellar disorder in patients with primary dystonia may be related to their abnormal response to PAS. However, this is unlikely to be the whole story. The response to PAS21.5 (that is, PAS with a 21.5 ms interval between stimuli) is unaffected by cerebellar direct current stimulation (Hamada et al., 2012) in healthy participants yet it is still enhanced in primary dystonias (Weise et al., 2006), suggesting that there is an intrinsic disorder of cortical plasticity in addition to any secondary influence from a disordered cerebellum.

The role of reduced intracortical inhibition in dystonia

The final finding of our study is that patients with secondary dystonia had reduced SICI on the affected side. This is in line with a recent finding of reduced SICI in patients with dystonia caused by lentiform nucleus lesions (Trompetto et al., 2012). The present data showed only a non-significant trend toward reduced SICI in patients with primary dystonia, in contrast with reduced SICI reported in some previous studies (Ridding et al., 1995a; Edwards et al., 2003; Quartarone et al., 2003). However, others have found SICI to be normal in primary dystonia (Rona et al., 1998; Stinear and Byblow, 2004; Brighina et al., 2009). This inconsistency probably reflects the large between-subject variability of intracortical inhibition that is present even in healthy subjects (Wassermann, 2002), as well as methodological differences between studies (conditioning stimulus intensity, unconditioned MEP amplitude, interstimulus intervals, rest versus active condition of the target muscle). The pathophysiological significance of reduced intracortical inhibition in dystonia remains obscure (Berardelli et al., 2008) and there is still no uniform hypothesis to account for reduced SICI in all forms of dystonia. Reduced SICI is not specific for dystonia and is found in other basal ganglia diseases, including Parkinson’s disease and Tourette’s syndrome (Ridding et al., 1995b; Ziemann et al., 1997). Therefore, a loss of intracortical inhibition may be regarded as a non-specific maladaptive change within the motor cortex, caused by chronic disorganized basal ganglia output. Our finding would fit with this hypothesis, as SICI was only abnormal on the clinically affected side of our patients with secondary dystonia. Alternatively, reduced SICI may arise as a consequence of maintaining an abnormal dystonic posture that could have triggered cortical reorganization through aberrant afferent input (Espay et al., 2006). This hypothesis is nevertheless insufficient to explain the reduced SICI in non-affected body parts in primary focal dystonia (Sommers et al., 2002), or in non-manifesting DYT1 mutation carriers (Edwards et al., 2003). Considering the pathophysiological importance of reduced SICI in different types of dystonia, the
conclusion is that reduced intracortical inhibition must co-exist with other abnormalities, to cause clinical expression of dystonia: for example, increased plasticity and/or abnormal cerebellar function in primary dystonia (Quartarone et al., 2003), psychogenic factors in non-organic dystonia (Espay et al., 2006; Quartarone et al., 2009) or injury to basal ganglia and its connections in secondary dystonias (Trompetto et al., 2012).

There was no significant difference in cortical silent period duration between groups, although there was a tendency toward a shortening of the cortical silent period on the affected side in both secondary and primary dystonia, compared with control subjects. The literature on cortical silent period in dystonia has been less consistent than for SICI, with studies reporting normal cortical silent period (Stinear and Byblow, 2005) or reduced cortical silent period (Chen et al., 1997) or an abnormality was restricted to a specific task (Tinazzi et al., 2005). SICI and the cortical silent period are thought to depend on GABA-A and GABA-B cortical interneurons, respectively and therefore could be differentially affected by disease (Werhahn et al., 1999; Di Lazzaro et al., 2006; Hallett, 2011). This might explain the abnormal SICI and normal cortical silent period in our patients with secondary dystonia. Trompetto et al. (2012) suggested that the cortical silent period is reduced in secondary dystonia when the lesion is restricted to striatum, while it might be normal if the lesion involves pallidum or thalamus. We did not find the duration of cortical silent period to be related to the anatomical site of the lesion.

Limitations of the study

We acknowledge the limitations of our study. Our sample of patients with secondary dystonia is heterogeneous regarding aetiology and anatomical site of the lesion. Although it is possible that different lesions could have different functional effects on motor cortex plasticity, we believe this is unlikely given the similar response to PAS among all patients with secondary dystonia, including the lack of spread into the non-target adductor digiti minimi muscle (Fig. 4). Another limitation of our data is the long interval between the brain injury and TMS study. With the present design, we cannot exclude the possibility that motor cortex plasticity was affected at the time of emergence of dystonia, and then over time has reverted to normal. This issue could be addressed in a prospective study that would need to include a large number of patients, given that only a small proportion of patients with subcortical lesions will go on to develop dystonia.

Conclusion

We have demonstrated that primary and secondary dystonia do not share the same pattern of electrophysiological abnormalities. In secondary dystonia caused by structural brain lesions, the response to PAS is normal and therefore enhanced sensorimotor cortical plasticity is not required for clinical expression of dystonia. Our data also suggest that enhanced cortical plasticity does not reflect a functional change arising secondary to dystonic activity, but rather represents a specific pathophysiological trait of primary dystonia. Cerebellar function as measured by eye blink classical conditioning is not affected in secondary dystonia, indicating that functional involvement of the cerebellum is not a universal feature of dystonia. The present study however does not resolve the ongoing debate as to whether changes in cerebellar activity in primary dystonia are (i) compensatory; (ii) an epiphenomenon occurring secondary to abnormal activity elsewhere within the sensorimotor network; or (iii) are a primary part of the pathophysiology of dystonia (Teo et al., 2009; Sadnicka et al., 2012). The compensation hypothesis is based on the idea that cerebellar hyperactivity in functional brain imaging of patients with primary dystonia can compensate for abnormalities in motor cortical plasticity. It is supported to some extent by the fact that in healthy subjects alterations of cerebellar activity using transcranial direct current stimulation reduce responsiveness to a subsequent PAS protocol (Hamada et al., 2012). In primary dystonia this compensatory activity may have

![Figure 4](http://brain.oxfordjournals.org/)

**Figure 4** Averaged PAS response in individual participants. For each participant, PAS response is expressed as an averaged MEP amplitude for three time points after PAS (i.e. 0 min, 15 min and 30 min after PAS) and plotted on the y-axis. For secondary dystonia, data refer to the affected side. APB = abductor pollicis brevis; ADM = adductor digiti minimi.
deleterious effects on sensitive tests of cerebellar function, such as eye-blink conditioning, even though clinical signs of cerebellar dysfunction are absent. This would fit with the present finding that since there are no abnormalities in motor cortical plasticity in secondary dystonia, there is no need for compensatory cerebellar activity, and thus eye-blink conditioning is normal. Nevertheless, the data also could fit into the alternative hypothesis that cerebellar abnormalities are an intrinsic feature of primary dystonia, since they are absent in secondary cases.

Our findings may give some insight into why the stimulation-based therapeutic interventions that are thought to interfere with motor cortex plasticity, such as repetitive TMS and deep brain stimulation, might not be as useful in patients with secondary as in primary dystonia (Andrews et al., 2010; Vidalhiet et al., 2012). Further exploration of differences in pathophysiological mechanisms in different types of dystonias may have implications in selecting the most appropriate treatment among different alternatives and also for developing new therapeutic strategies.

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**References**


