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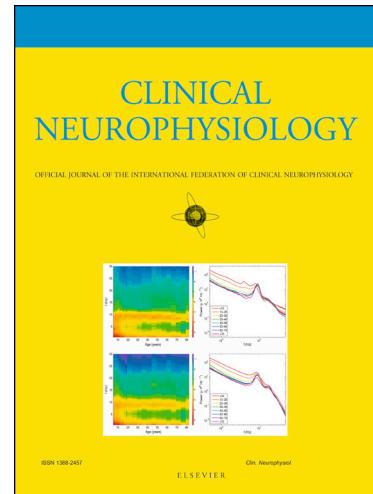
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Dystonic neck muscles show a shift in relative autospectral power during isometric contractions

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Keywords: cervical dystonia; EMG; autospectrum; isometric contraction; cumulative distribution function.

Highlights

- Dystonic muscles displayed increased 3-10 Hz power with reduced 10-30 Hz power.
- Similar shifts were detected in head forces and moments in cervical dystonia patients.
- We associate these shifts with prokinetic sensorimotor control.

ABSTRACT

Objective: To identify effects of a deviant motor drive in the autospectral power of dystonic muscles during voluntary contraction in cervical dystonia patients.

Methods: Submaximal (20%) isometric head-neck tasks were performed with the head fixed, measuring surface EMG of the sternocleidomastoid, splenius capitis and semispinalis capitis in CD patients and controls. Autospectral power of muscle activity, and head forces was analyzed using cumulative distribution functions (CDF). A downward shift between the theta/low alpha-band (3-10 Hz) and the high alpha/beta-band (10-30 Hz) was detected using the CDF_{10} , defined as the cumulative power from 3-10 Hz relative to power from 3-30 Hz.

Results: CDF_{10} was increased in dystonic muscles compared to controls and patient muscles unaffected by dystonia, due to a 3-10 Hz power increase and a 10-30 Hz decrease. CDF_{10} also increased in patient head forces.

Conclusions: Submaximal isometric contractions with the head fixed provided a well-defined test condition minimizing effects of reflexive feedback and tremor. We associate shifts in autospectral power with prokinetic sensorimotor control.

Significance: Analysis of autospectral power in isometric tasks with the head fixed is a promising approach in research and diagnostics of cervical dystonia.

1 Introduction

Idiopathic cervical dystonia (CD) is a neurological movement disorder characterized by involuntary neck muscle contractions (Fahn, 1988). The pathophysiology of CD remains unclear, although functional and morphometric changes have been found in several brain areas, such as the cerebral cortex (Draganski et al., 2003, Egger et al., 2007, Obermann et al., 2007, de Vries et al., 2012), superior colliculus (Holmes et al., 2012), thalamus (Krauss et al., 1999, Chang et al., 2002, Butterworth et al., 2003, Kupsch et al., 2006, Vidailhet et al., 2007), and cerebellum (LeDoux and Brady, 2003, Neychev et al., 2008, Sadnicka et al., 2012, Prudente et al., 2013). Current evidence points towards differences in the neuronal circuitry of multiple areas in the brain (Berardelli et al., 1998, Lehéricy et al., 2013). In particular, power in local field potentials (LFP) of the Globus Pallidus internus (GPi) shows an increase around 4-10 Hz and a reduction around 11-30 Hz (beta-band) in generalized and cervical dystonia patients (Silberstein et al., 2003). Such beta-band brain rhythms are thought to contribute to sensorimotor control opposing the execution of movement (Pfurtscheller et al., 1996, Engel and Fries, 2010), i.e. contribute antikinetically, and frequencies above 60 Hz (gamma-band) are considered prokinetic (Engel and Fries, 2010). It is hypothesized that aberrance in these rhythms may relate to the hyperkinesia in CD patients (Weinberger et al., 2012). Indeed, excessive 3-10 Hz (Chen et al., 2006, Sharott et al., 2008) and reduced 8-20 Hz (Liu et al., 2008) pallidal activity may correlate with dystonic tremor (Deuschl et al., 1998).

Muscle (electromyography) frequency spectra have been used to identify specific movement disorders. For example, in patients with writer's cramp, increased 11-12 Hz intermuscular coherence between limb muscles has been found in comparison to controls mimicking similar muscle activation patterns. This indicates aberrance in synchronization of presynaptic inputs (Farmer et al., 1998). In limb dystonia, autospectral EMG of muscles with clinical signs of dystonia exhibits less relative power above 70 Hz than healthy subjects (Go et al., 2014).

Symptomatic limb dystonia patients carrying the DYT1 gene show a 4-7 Hz peak in the autospectrum of affected limb muscles, which is absent in asymptomatic carriers, patients with fixed dystonia, and controls (Grosse et al., 2004). In CD, differences between autospectral EMG of dystonic and healthy muscles have been found, where peaks around 10-12 Hz were absent in dystonic splenius capitis (SPL) muscles (Tijssen et al., 2000, Tijssen et al., 2002). Similar experiments with a larger patient and control group only partially confirmed these results (Nijmeijer et al., 2014). Within patients, autospectral peaks between 8 and 14 Hz were absent in 7 out of 9 affected SPL muscles but also in 3 out of 10 unaffected SPL muscles, providing a limited discriminative power. These observations, however, were made in seated subjects with their head free while applying forces to a handheld device. Considering that 2 out of 10 patients demonstrated an obvious tremor (Nijmeijer et al., 2014), reflexive feedback in reaction to dystonic and/or tremulous movement during these head free conditions may have caused healthy muscle activity providing misleading results.

The aim of the present study was to determine whether differences in the autospectral EMG could be observed between muscles clinically diagnosed to be dystonic and healthy muscles when head movement was eliminated through isometric fixation of the head and torso. Head fixation and head force feedback provided a standardized task with precise contraction instructions in direction and amplitude. It also minimized reflex contributions to muscle activity since reflexive stabilization of the head and neck was not needed, thus allowing observed differences between patients and controls to be attributed to a central rather than peripheral origin. We hypothesized that the dystonic muscles would show a similar, but more consistent, shift in power of the EMG spectra during isometric contractions as compared to head free conditions. More specifically, we predicted there would be a relative drop in the high alpha/ beta-band (10-30 Hz) (Tijssen et al., 2000, Tijssen et al., 2002) and an increase in theta/low alpha-band (3-10 Hz) (Deuschl et al., 1998, Liu et al., 2008). In addition, by fixating

subjects to an overhead load cell, we extended our analysis to examine changes in the net motor output (forces and moments) during neck muscle contraction tasks. We hypothesized that a 3-10 Hz increase and a 10-30 Hz decrease would also be visible in the measured forces and moments of CD patients during isometric muscle contractions.

2 Materials and methods

This paper presents partial results of a series of three experiments performed on the same group of CD patients. 1) Head free tasks at the Amsterdam Medical Center with EMG spectral analysis in isometric tasks (see introduction) and intermuscular coherence analysis (Nijmeijer et al., 2014). 2) Isometric tasks with the head fixed at Delft University of Technology, where performance and muscle coordination were reported in de Bruijn et al. (2015) and where the current paper presents spectral analysis of muscle activity and head forces and moments. 3) Dynamic stabilization tasks on a motion platform at Delft University of Technology (Forbes et al., 2017).

2.1 Experimental

Experiments were carried out with ethical approval from the Delft University of Technology and the Amsterdam Medical Center, in accordance with the Declaration of Helsinki. All participants gave written informed consent.

2.1.1 Subjects

Ten CD patients (5 males, age 56 ± 11 years) and ten age matched controls (4 males, age 55 ± 14 years) participated in the experiments at the Delft University of Technology. The severity of the disorder for each patient was quantified using the TSUI (Tsui et al., 1985) and TWSTR (Consky and Lang, 1994) scales. All patients were under the treatment of botulinum toxin (BoNT) and to minimize effects of treatment the experiments were performed at least three months after the last BoNT injection. In the analyses, muscles that were identified during

clinical assessment for the purposes of BoNT treatment were considered to be dystonic. This allowed for grouped comparisons between dystonic and unaffected patient muscles. Dystonic muscles varied per patient and are listed in Table 1.

2.1.2 Isometric task

Subjects were seated and wore a tightly fitted cushioned helmet that was fixed in an isometric device (de Bruijn et al., 2015). Visual feedback of the generated force magnitude and direction (in force tasks) and twist moment magnitude (in moment tasks) was presented through a custom made interface (de Bruijn et al., 2015) to improve the reliability of submaximal contraction tasks (Burnett et al., 2007).

Head forces and moments were measured by an overhead six axis load cell (MC3-6-500, AMTI Inc., Watertown, USA). Force and moment signals were passed through an analogue low-pass filter (2nd order, critically damped at 1024 Hz) and sampled at 2000 Hz. Surface EMG was recorded bilaterally with paired unipolar micro electrodes (2 mm sintered discs with shielded carbon cable, TMS International BV, Oldenzaal, The Netherlands) placed 2 cm apart along the muscle fibers and a ground electrode was placed on the ulna. The skin was shaved if necessary and cleansed with rubbing alcohol, a conductive gel was applied, and adhesive tape was used to avoid tension on the electrodes (Hermens et al., 2000). Subjects then applied muscle contractions to ensure correct electrode placement and eliminate crosstalk. Electrodes were adjusted if activity of adjacent muscles was detected. The sternocleidomastoid (SCM), splenius capitis (SPL), and semispinalis capitis (SS) muscles were recorded with a sample rate of 2000 Hz.

2.1.3 Tremor assessment

Several patients demonstrated indications of tremulous movement during clinical prescreening prior to participating in this study. A short experiment was therefore conducted

to assess levels of dystonic tremor (Deuschl et al., 1998) by fixing subjects in a chair while the head was free to move. Three-dimensional kinematic data of the head was recorded with six markers at 200 Hz using an Oqus 6-camera motion capture system (Qualisys AB, Gothenburg, Sweden), following methods of a previous study (Forbes et al., 2013).

2.1.4 Task instruction

The experimental protocol was explained to the subjects before the start of the experiment. In the isometric experiment, subjects were asked to remain seated upright with their hands on their laps. Once secured, subjects performed a number of practice runs to familiarize themselves with the device and the nature of the visual feedback. Subjects first applied isometric maximum voluntary muscle contractions (MVC) using their neck muscles to generate force in eight horizontal directions at 45° intervals, and moments in left and right twist. Subjects then performed sub-maximal (20%) voluntary contractions (sub-MVC) in these same directions. MVC trials were repeated three times lasting five seconds each and sub-MVC tasks were repeated two times lasting ten seconds each. Subjects were given a minimum of ten seconds rest between subsequent trials.

In the experiment with the head free to assess tremor, patients were asked to remain seated, close their eyes, and allow the dystonia to dictate head posture. Controls were asked to mimic and hold the dystonic posture of a matched patient with their eyes closed. The experiment consisted of two repetitions each lasting 100 seconds.

2.2 Spectral analysis

2.2.1 Isometric analysis

MVC strength was assessed for the eight horizontal and two twist directions. MVC forces and moments were estimated using a moving average with an interval of 1000 ms and selecting the highest value out of the three repetitions. The level of force or moment to be generated

during sub-MVC tasks was defined as 20% of the MVC. Isometric performance and minimal and mean EMG in submaximal (20%) and maximal contraction have been published in a previous paper (de Bruijn et al., 2015). This paper presents frequency domain analysis aiming to disclose spectral changes in the EMG of dystonic muscles and patient head forces.

Because beta-band peaks in EMG are observed specifically during submaximal contractions (Conway et al., 1995, Brown, 2000), the frequency content of horizontal head forces, twist moments, and muscle EMG data is presented in particular for the sub-MVC tasks (see section on influence of BoNT in the discussion). The longer duration of the sub-MVC task relative to the MVC task also allowed for a more robust spectral analysis. We analyzed each muscle during directional tasks where the muscle was expected to be most active according to its preferred direction of activation as reported in the literature (Gabriel et al., 2004). This included one horizontal force and one twist moment task for each muscle. The SCM was analyzed during flexion (forward) force and contralateral twist moment tasks, and the SPL and SS were analyzed during extension (rearward) force and ipsilateral twist moment tasks. The first two seconds of each trial were discarded and repetitions were concatenated, resulting in 16 seconds of data for analysis per task. The raw bipolar EMG was notch filtered at 50 Hz, high pass filtered at 20 Hz (4th order zero-phase Butterworth) and full wave rectified (De Luca et al., 2010). Data was then sectioned into segments of two seconds, over which autospectra were calculated and averaged over the segments resulting in a frequency resolution of 0.5 Hz.

The autospectrum (0.5-999 Hz) of all muscles was first assessed using conventional methods (Tijssen et al., 2000, Tijssen et al., 2002), i.e. by evaluating power in frequency ranges (bins) relevant to CD and healthy motor control as reported in the literature. Excessive autospectral power between 3-10 Hz (Sharott et al., 2008) as well as decreased power above 10 Hz (Tijssen et al., 2000) have both been related to dystonic muscle activity. It must be noted that the former was primarily focused on autospectral power in pallidal local field potentials (LFP)

and LFP-EMG coherence, but an increased 3-10 Hz autospectral EMG was also observed. In controls on the other hand, corticomuscular (antikinetic) beta-band activity has been identified as high as ~30 Hz (Conway et al., 1995, Engel and Fries, 2010). The low (30-60 Hz) and high gamma band (60-90 Hz) have also been related to prokinetic movements in CD (Liu et al., 2008, Tsang et al., 2012, Weinberger et al., 2012). Hence, frequency bins were defined as 0.5-3, 3-10, 10-30, 30-60, 60-90, 90-150, and 150-999 Hz. No specific effects were expected above 90 Hz, so higher frequencies were arbitrarily sectioned at 150 Hz. Each EMG signal was normalized to its total autospectral power, and within each frequency bin the power was summed.

Patients were expected to show an autospectral power increase between 3-10 Hz and a decrease between 10-30 Hz, as motivated in the introduction and above. To further evaluate the expected shift in EMG power to lower frequencies within the 3-30 Hz range, the normalized cumulative distribution function (CDF) was calculated. CDF provides a qualitative measure for the relative distribution of autospectral power. The CDF was normalized to be zero at 3 Hz and one at 30 Hz, such that the CDF at 10 Hz (CDF_{10}) signified the fraction of power present in the lower (3-10 Hz) band compared to the full (3-30 Hz) band. Thus, CDF_{10} quantified shifts in spectral power with a single measure. The 3 Hz initial frequency was chosen as the lowest frequency where dystonic activity has been reported in neck muscle recordings (Deuschl et al., 1998, Chen et al., 2006, Liu et al., 2008, Sharott et al., 2008). The 30 Hz final frequency was chosen to align with the highest frequency in the beta-band (11-30 Hz), where peaks in this range are thought to originate from sensorimotor brainwaves opposing the execution of movement (Pfurtscheller et al., 1996, Engel and Fries, 2010). Decreased activity in the beta-band visible in surface EMG of dystonic muscles is believed to have origins in disturbed sensorimotor control (Tijssen et al., 2000, Tijssen et al., 2002, Silberstein et al., 2003).

The cross-over frequency (10 Hz) was placed between the frequency range where increased autospectral dystonic muscle activity was observed (3-10 Hz) and the beta-band (11-30 Hz).

Because Renshaw cells have been shown to inhibit EMG activity specifically around 10 Hz to improve physiological tremor (Williams and Baker, 2009), we argue that 10 Hz provides a good location to separate the low and high frequency ranges. To investigate whether changes in the EMG spectrum could also be detected in the head forces and moments, the autospectrum, CDF and CDF_{10} were calculated for horizontal forces in the force task, and for the twist moments in the moment task.

2.2.2 Tremor assessment

Kinematic data of the head's axial rotation was evaluated with the head free to move. The 200 seconds of motion data were sectioned into segments of four seconds, and autospectra were calculated and averaged over the segments, resulting in a frequency resolution of 0.25 Hz.

2.3 Group comparison and statistical measures

Force and moment results were compared between control and patient groups. EMG was divided into three groups: 1) control muscles as measured from healthy control subjects, 2) dystonic patient muscles – selected as patient muscles that were diagnosed as dystonic and hence treated with BoNT (see Table 1), and 3) patient unaffected muscles – selected as patient muscles not being treated with BoNT and regarded as unaffected by CD. The authors acknowledge the fact that incorrect categorization of patient muscles may have been possible and will address this in the discussion. For each group, EMG data from all subjects were analyzed for right and left muscles separately, for bilateral muscles combined (pooled), and for all muscles combined (pooled). The pooled muscle sets consisted of 60 control muscles (20 SCM, 20 SPL, 20 SS), 28 patient dystonic muscles (10 SCM, 11 SPL, 7 SS), and 31 patient unaffected muscles (10 SCM, 8 SPL, 13 SS). Pooling thus enabled statistical comparisons between groups for the six individual left and right muscles, for three bilateral

muscle groups, and for a single group containing all muscles where larger pools allowed for more statistical power.

All group comparisons were made using a nonparametric Linear Mixed Model, where group and gender were set as fixed factors for all groups. When comparing forces and moments, the task direction (eight horizontal and two twist directions) was given as an additional fixed factor. T-statistics and the degrees-of-freedom (df) were presented with significance levels below 0.05. To account for multiple significance testing the p-values were adjusted using the Benjamini-Hochberg False-Discovery-Rate (FDR) (Benjamini and Hochberg, 2000).

Finally, to assess the possible effect of tremor on our results, the CDF₁₀ of all pooled muscles and the forces and moments were recalculated after removal of tremulous patients (see Results).

3 Results

3.1 Muscle responses

The frequency bin analysis showed the expected relative shift in power from 10-30 Hz to 3-10 Hz in patient dystonic muscles (

Figure 1). Patient dystonic muscles showed significantly more power in the 0.5-3 Hz (mean=0.047, SD=0.026, $t(80)=-2.56$, $p=0.03$), 3-10 Hz (mean=0.069, SD=0.027, $t(80)=-2.35$, $p=0.03$), and the 60-90 Hz high gamma bins (mean=0.13, SD=0.040, $t(80)=-3.6$, $p=0.03$) as compared to controls (respectively mean=0.035, SD=0.011; mean=0.057, SD=0.020; mean=0.099, SD=0.030). In addition, dystonic muscles demonstrated reduced power between 10-30 Hz (mean=0.14, SD=0.10) compared to controls (mean=0.17, SD=0.07, $t(80)=2.0$, $p=0.04$). This reduced power is also seen in Figure 2 top. The same trend was observed when comparing patient dystonic and patient unaffected muscles, but effects were not significant for the tested frequency bins.

Elevated CDF_{10} for patient dystonic muscles compared to control muscles (see Table 2 and Figure 2 bottom) confirmed the relative shift in power from 10-30 Hz to 3-10 Hz ($t(158)=-7.86$, $p<0.01$). The elevated CDF_{10} in dystonic muscles seemed to be caused by excessive power at low frequencies between 4-10 Hz (sharp increase in the CDF plot), and a lack of power around 10-20 Hz where we see a sharp increase in controls, but not in patient dystonic muscles. A clear trend could be observed in the CDF_{10} of individual and grouped muscles, where CDF_{10} of patient dystonic muscles was always higher than control and patient unaffected muscles. Although few differences between patient dystonic and patient unaffected muscles were statistically significant (Table 2), we did observe a significant increase in patient dystonic muscle CDF_{10} when all muscles were pooled ($t(117)=-4.07$, $p<0.01$).

Figs 1 & 2 near this point

3.2 Force and moment responses

CDF curves of forces and moments (twist moments shown in Figure 3 bottom) showed a similar relative power shift in patients from 10-30 Hz to 3-10 Hz as observed in muscles. Patients had approximately 22% less power between 10-30 Hz than controls and patient CDF₁₀ was significantly higher than controls ($t(188) = -4.3$, $p < 0.01$). Specifically, controls appeared to have considerably more power around ~13 Hz (Figure 3 top), represented by a sharp increase in the CDF plot at that frequency. The same trends were observed in horizontal loading directions (forces) as in twist (moments) and there was no significant difference in CDF₁₀ between directions.

Fig 3 near here

3.3 Dystonic tremor

In the additional experiment with the head unsupported, two out of ten patients (patients 2 and 8) were found to present peaks in head motion at 4.5 and 4 Hz respectively, which were thought to be caused by a dystonic tremor (Figure 4 and Table 1). In addition, patient 7 showed excessive head motion relative to all other subjects without the presence of a clear autospectral peak, which was clinically assessed to be caused by a jerking motion rather than tremor. To verify if the observed group differences were not merely a result of the tremor or jerk observed in these patients, between-group significant differences in CDF₁₀ were recalculated after exclusion of these three patients. CDF₁₀ was not affected by this exclusion and the CDF₁₀ of all pooled patient dystonic muscles (last row Table 2) remained significantly higher than control and patient unaffected muscles.

Fig 4 near here

4 Discussion

This study demonstrated a shift in autospectral power from 10-30 Hz to 3-10 Hz in CD patients during submaximal isometric contractions. This shift was visible in the EMG of dystonic muscles as well as in isometric (head) forces and moments generated by patients.

4.1 Discriminative power

This study presents a method to discriminate dystonic and unaffected muscles. The isometric setup with the head fixed minimized effects of reflexive feedback and tremor, compared to previous studies in which force tasks were performed with the head free against a hand-held device. This study reinforces results from our previous study performed on the same CD patients with the head free (Nijmeijer et al., 2014). In the previous study, effects were significant only for SPL and only between dystonic muscles and healthy controls. The current study shows significant effects for all tested neck muscles (SPL, SS, SCM) when comparing dystonic muscles to controls which were also significant when comparing dystonic to unaffected patient muscles for SPL. Additionally, the current study uniquely shows significant effects in the autospectra of head forces and moments.

Effects were most clear and most significant in the CDF₁₀, which was defined as the fraction of power present in the lower (3-10 Hz) band compared to the full (3-30 Hz) band. The autospectral power distribution (

Figure 1) supports our hypothesis of a downward autospectral shift in dystonic compared to control muscles since we observe both a power increase in the lower band (3-10 Hz) and a decrease in the upper band (10-30 Hz).

Thus, we conclude that submaximal isometric head tests with the head fixed, using EMG spectral analysis is a promising method to identify dystonic muscles, and to study aberrant control in CD. However, it shall be noted that while significant effects were found in

relatively small groups for all muscles studied, the methods presented are not sufficiently discriminative to reliably identify dystonic muscles in individual patients. It is promising that the proposed method provides coherent and significant results with non-invasive surface EMG, but intramuscular EMG may be even more discriminative as it is less likely to pick up adjacent muscle activity. This will be investigated in follow up studies.

4.2 Influence of BoNT

The patients that took part in this experiment had previously been treated with BoNT. Because the prevalence of botulinum naive patients was low in the geographical region where this study was performed the choice was made to use patients that had not been treated with BoNT for at least three months. However, BoNT likely still has some effect after this period (Kim et al., 2010). The EMG amplitude of a muscle appears to decrease after it is injected with BoNT (Dressler and Rothwell, 2000), as is the case for the turns per cycle (Fuglsang-Frederiksen et al., 1998, Erdal et al., 1999) and muscle fiber conduction velocity (Lange et al., 2007). The latter is thought to reduce the median frequency of autospectral EMG (Lowery et al., 2002). A reduced median frequency of autospectral EMG was observed during 70% MVC in muscles injected with botulinum toxin compared to muscles given a placebo (Kim et al., 2010). To address the influence of BoNT on our results, the median frequency of autospectral EMG was calculated over the 0-500 Hz spectrum (rather than between 3-30 Hz), following Lowery et al. (2002). No differences were observed in the full-bandwidth median frequency between clinically defined dystonic and unaffected muscles 20%-MVC (dystonic: 75.5 ± 5.5 Hz, unaffected: 76.8 ± 4.5 Hz). Lowery found a median frequency of 80.2 ± 9.4 Hz in the unfatigued brachioradialis muscle during 30% MVC. In the current paper we present results for 20% sub-MVC. We subsequently repeated the analyses performed in this article during MVC, arguing that the above mentioned decreases due to BoNT would be most clearly visible during maximal contractions. During MVC, no differences were found between patients and

controls in any of the CDF measures, nor in the autospectral frequency bins in the range of interest for this article (3-30 Hz). It is therefore highly unlikely that the results described in this study are caused by influences of BoNT. Nevertheless, the effects of the toxin are complex and future studies with untreated patients will be necessary to provide complete certainty.

4.3 Hypothesis behind pathophysiology

The elevated power between 3-10 Hz and reduced power between 10-30 Hz in dystonic muscles concurs with other studies, where increases in the theta-band (Sharott et al., 2008) and decreases in the low-beta band (Tijssen et al., 2000, Tijssen et al., 2002) have been reported in the autospectral EMG of dystonic muscles. The autospectral EMG of dystonic muscles showed increased power between 3-10 Hz and 60-90 Hz and decreased power between 10-30 Hz. We tentatively relate this difference between dystonic and non-dystonic muscles to a similar change in distribution observed when comparing EMG of healthy muscles during an isometric task (as in this experiment) to EMG during (planned) execution of motion. Beta-band (11-30 Hz) brain rhythms are thought to contribute antikinetically to sensorimotor control, while alpha-band (3-8 Hz) and gamma (60-90 Hz) bands are prokinetic (Pfurtscheller et al., 1996, Engel and Fries, 2010). In the brain, movement related rhythms occurring in these frequency bands have been found by measuring local field potentials (LFP) of deep brain areas (Silberstein et al., 2003, Liu et al., 2008). High amplitude LFP's in the beta-band (~11-30 Hz) are considered to be elevated when a constant submaximal motor command is desired or maintained (Engel and Fries, 2010, Brittain and Brown, 2014), while gamma-band (60-90 Hz) LFP's are high during (intended) motion (Brown, 2003, Kuhn et al., 2008, Jenkinson and Brown, 2011, Brittain and Brown, 2014). Increased prokinetic gamma frequencies (Liu et al., 2008, Weinberger et al., 2012) and reduced antikinetic beta-band frequencies (Silberstein et al., 2003, Kuhn et al., 2008, Liu et al., 2008) have been found in

CD patients in the brain. Although observations have been made relating brain rhythms to EMG (Conway et al., 1995), their relationship is complex. Nevertheless, it is an inviting thought that the autospectral changes observed in dystonic muscles may be related to prokinetic brain rhythms that are picked up in the EMG of dystonic muscles and even concur with spectral changes in forces and moments measured at the head.

4.4 Study limitations

A possible confounding factor in our results is that CD patients can be more prone to muscle fatigue due to the involuntary activity of dystonic muscles (Fahn, 1988). In fatigued muscles, an increase in muscle twitch force together with a decrease in discharge rate of the motor units occurs to maintain a constant force (De Luca et al., 1996, Carpentier et al., 2001). Motor unit firing rates are found not to drop any lower than 11 Hz (Carpentier et al., 2001) during fatiguing muscle contractions at 25% MVC lasting on average eight minutes. Considering that our task consisted of 20% MVC lasting only ten seconds, it is unlikely that motor discharge rates dropped below 10 Hz, at which point autospectral peaks related to the rate of motor unit discharge would have influenced the CDF₁₀ measure. We therefore do not expect fatigue to have had a significant influence on our findings.

Another limitation to this study is that there is currently no gold standard for the identification of dystonic muscles. We relied on clinical experience and subjective assessments of a neurologist, who treated most patients for several years, improving the identification of dystonic muscles as the treatments progressed. However it remains possible that some muscles were incorrectly categorized. Presumably, misidentified muscles would make it more difficult to discriminate between patient dystonic and patient unaffected muscles. However, despite the low number of subjects and the possibility of incorrectly categorized muscles, a clear discrimination between patients and controls was found.

5 Conclusions

This study has demonstrated a shift in autospectral power to lower frequencies in dystonic SCM, SPL, and SS muscles, as well as head forces and moments in CD patients during submaximal isometric voluntary contractions. The simultaneous 3-10 Hz increase and beta-band 10-30 Hz decrease in dystonic muscles may be related to more prominent prokinetic sensorimotor control. This study is novel in its ability to discriminate dystonic muscles with non-invasive surface EMG and discriminate CD patients using head forces. Both for EMG and head forces the CDF₁₀ proved to be a robust measure capturing the autospectral shift. The current results show significant differences at a group level. Further research including intramuscular EMG can show whether CDF₁₀ can contribute to a more reliable diagnosis of individual dystonic muscles.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Captions

Figure 1. Normalized autospectrum of pooled muscles divided into bins. Box plots indicate median, first and third quartile, and dotted lines show a 9-91% point span. The shaded area denotes the 3-30 Hz frequency band discussed in detail in this article. Significant increases in dystonic muscles compared to controls are seen at low frequencies and in the gamma range (60-90 Hz), and in the high alpha, beta range (10-30 Hz).

Figure 2. EMG during sub-MVC in the horizontal and twist task. (a) Shaded areas in the autospectrum (in microvolts) indicate one standard error of the mean. (b) The CDF curves (bottom) are consistently higher in dystonic muscles at 10 Hz, signifying a relative decrease in 10-30 Hz power (see Table 2 for significance). Asterisks indicate individual CDF_{10} values.

Figure 3. Twist moment frequency response during a 20%MVC twist task. (a) The autospectrum (in Nm) seems to show a lack of power in the beta-band in patients. (b) The Cumulative Distribution Function (bottom) confirms this as it is significantly higher in patients at 10 Hz. The same trends were observed in horizontal forces.

Figure 4. Autospectrum of head twist while the head was free. Patients 2 and 8 were considered to have dystonic tremor and patient 7 was considered to have jerk.

Tables

Table 1: Patient characteristics. The TSUI and TWSTR scales indicate the severity of the disorder. The TSUI observes the amplitude and duration of sustained movements and tremors with a maximum score of 25. The TWSTR adds the subject's severity of Torticollis (0-35), disability (0-30), and pain (0-20) and has a maximum score of 85. High values always indicate an increased severity. The dystonic sternocleidomastoid (SCM), splenius capitis (SPL), and semispinalis capitis (SS) are given per patient based on treatment.

Patient number, gender, age			information on the disorder				
			TSUI	TWSTR	Duration (years)	Rotation ^a	
			Left	Right		tremor or jerk ^b	muscles treated with BoNT
1	m	38	7	16	4.5	2	SCM-L, SPL-R
2	f	63	11	30.1	4	2	SCM-L, SPL-L, SPL-R
3	f	56	9	41.75	4	3	SCM-L, SPL-R
4	m	46	8	23	11	1	SCM-L, SCM-R
5	f	74	12	24	25	3	SPL-L, SS-L, SCM-R
6	m	45	16	22	4	3	SCM-L, SS-L, SPL-R
7	f	63	17	31	6	2	SPL-L, SCM-R, SS-R
8	f	60	15	24	10	2	SPL-L, SS-L, SPL-R, SS-R
9	m	56	7	17	13	1	SCM-L, SPL-R
10	m	61	13	29.75	2	3	SCM-L, SPL-L, SS-L ^c

^a Rotation (head twist): (-) no deviation, (1) <15°, (2) 15°-30°, (3) >30°.

^b Observed in the head-free experiment to assess tremor – see Figure 4.

^c Removed as electrode had come loose during testing.

BoNT: botulinum toxin; TWSTR: Toronto Western Spasmodic Torticollis Rating Scale; SCM-L: left sternocleidomastoid; SPL-L: left splenius capitis; SS-L: left semispinalis capitis; SCM-R: right sternocleidomastoid; SPL-R: right splenius capitis; SS-R: right semispinalis capitis.

Table 2: Muscle CDF₁₀. High values indicate a redistribution of autospectral power from 10-30 Hz to 3-10 Hz.

Muscle		CDF ₁₀ median (S.D.)			Pairwise comparisons	
		Controls	Patients		Control vs. dystonic.	Unaffected vs. dystonic
			Dystonic	Unaffected		
SCM	right	0.19 (0.04)	0.25 (0.04)	0.21 (0.03)	t(24)=-5.4 p<0.01	t(18)=-2.9 p=0.02
	left	0.22 (0.03)	0.23 (0.06)	0.22 (0.04)	t(28)=-3.0 p<0.01	t(18)=-0.3 p=ns
	right & left	0.20 (0.04)	0.25 (0.05)	0.22 (0.03)	t(54)=-5.6 p<0.01	t(38)=-1.8 p=ns
SPL	right	0.18 (0.03)	0.23 (0.05)	0.20 (0.03)	t(29)=-3.2 p=0.02	t(18)=-1.5 p=ns
	left	0.19 (0.05)	0.28 (0.05)	0.20 (0.03)	t(23)=-2.8 p=0.02	t(17)=-2.9 p=0.02
	right & left	0.19 (0.04)	0.24 (0.05)	0.20 (0.03)	t(54)=-4.3 p<0.01	t(37)=-3.1 p=0.01
SS	right	0.18 (0.05)	0.24 (0.03)	0.20 (0.04)	t(23)=-3.1 p<0.01	t(18)=-1.8 p=ns
	left	0.19 (0.06)	0.24 (0.03)	0.21 (0.07)	t(21)=-2.4 p=0.03	t(18)=-0.9 p=ns
	right & left	0.19 (0.05)	0.24 (0.03)	0.20 (0.06)	t(46)=-3.9 p<0.01	t(38)=-1.8 p=ns
All muscles		0.19 (0.04)	0.24 (0.05)	0.20 (0.04)	t(158)=-7.86 p<0.01	t(117)=-4.1 p<0.01
All muscles, excluding tremor patients 2,7,8		0.19 (0.04)	0.23 (0.04)	0.20 (0.04)	t(144)=-8.1 p<0.01	t(81)=-3.2 p<0.01

CDF₁₀: Cumulative Distribution Function at 10 Hz; S.D.: standard deviation; SCM: sternocleidomastoid; SPL: splenius capitis; SS: semispinalis capitis; ns: not significant.

