Somatosensory Mismatch Negativity is abnormal in Cervical Dystonia

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Abstract

Objective: Previous electrophysiological and psychophysical tests have suggested that somatosensory integration is abnormal in dystonia. Here, we hypothesised that this abnormality could relate to a more general deficit in pre-attentive error/deviant detection in patients with dystonia. We therefore tested patients with dystonia and healthy subjects using a mismatch negativity paradigm (MMN), where evoked potentials generated in response to a standard repeated stimulus are subtracted from the responses to a rare “odd ball” stimulus.

Methods: We assessed MMN for somatosensory and auditory stimuli in patients with cervical dystonia and healthy age matched controls.

Results: There was a reduction in peak amplitude of somatosensory MMN in cervical dystonia. However, auditory MMN was normal in this cohort of patients.

Conclusion: These results suggest that pre-attentive error/deviant detection, specifically in the somatosensory domain, is abnormal in dystonia. This could underlie some previously reported electrophysiological and psychophysical abnormalities of somatosensory integration in dystonia.

Key words
Mismatch negativity (MMN); Dystonia; Somatosensory integration.
Introduction

Dystonia is characterized by abnormal postures of the affected body part (Marsden, 1976). This motor dysfunction is the visible part of dystonia, but there is a significant body of evidence suggesting that failures in sensorimotor integration and pure sensory abnormalities are also of relevance in the pathophysiology of dystonia (Odergren et al., 1996; Hallett, 1995). In this regard, we have recently showed that gating or suppression of sensory evoked potentials (SEPs) around the onset of a voluntary movement is abnormal in focal dystonia (Macerollo et al., 2016). This phenomenon is called sensory attenuation (Rushton et al., 1981), which is an important component of voluntary movements relating to ‘top down’ suppression of afferents via the motor cortex (Seki et al., 2012). Macerollo et al. (2016) found that N20 and N30 were not attenuated at movement onset in patients with dystonia and this differed from healthy controls. In addition, Murase et al. found an abnormal loss of SEP suppression whilst patients with writer’s cramp were preparing a movement and were waiting for a cue to move but no abnormality during movement (Murase et al., 2000).

It is unknown how such sensory abnormalities relate to the pathophysiology of dystonia. In addition, it is possible that rather diverse sensory/sensorimotor integration abnormalities might be related to fewer, more fundamental deficits. For example, we have recently suggested that temporal discrimination abnormalities might relate to a more general deficit in criterion setting rather than abnormal perception of millisecond timing. Here we were interested in another general sensory phenomenon: the involuntary biasing of attention to an an unpredictable change in a sensory sequence of signals: mismatch negativity (MMN). This is a negative component of the event related potential (ERP) (Garrido et al., 2009) occurring at about 150-250ms following a stimulus (Sams et al., 1985) and is calculated by subtracting the ERP from
a standard repeated stimulus from that produced by a rare “oddball” stimulus (Naatanen et al., 2007). Error or deviation detection which this task probes is likely to be of fundamental evolutionary importance (Garrido et al., 2009). There are an enormous number of stimuli competing for our limited conscious resources at any one time. It would thus seem highly beneficial to have a system that is at a pre-attentive stage capable of detecting salient change in the environment and biasing attentional focus towards this change (Garrido et al., 2009; Todd et al., 2012).

MMN has been most studied in the auditory domain. It has been proposed that auditory MMN (aMMN) originates from a neuronal network involving connections between the superior temporal gyrus and the inferior and medial frontal gyrus (Friston et al, 2005; Garrido et al., 2007, 2009 (Friston et al. 2003; Garrido et al. 2008).

MMN has been also studied in the somatosensory domain using vibrotactile sensation (Alho et al. 1992; Kekoni et al. 1997; Shinozaki et al. 1998; Akatsuka et al. 2005; Spackman et al. 2007, 2010; Butler et al. 2011, 2012). Vibrotactile stimulus was used at different durations of or different frequencies for standard and oddball stimuli (Kekoni et al. 1997; Spackman et al. 2007, 2010; Butler et al. 2011). Other protocols used electrical stimulus delivered to the index finger or little finger (Akatsuka et al. 2005; Restuccia et al. 2007, 2009). However, the anatomical network involved in the production of sensory MMN (sMMN) remains poorly defined.

Here, we tested the hypothesis that patients with cervical dystonia would have abnormal (reduced) somatosensory MMN. We expected that this deficit would be restricted to somatosensory MMN, and therefore that auditory MMN would be normal in dystonia.
Materials and Methods

Participants

Eighteen patients with adult-onset isolated cervical dystonia were recruited. All patients were treated regularly with botulinum toxin (Dysport®) injections and the last injection was at least 3 months prior to the study. Patient details are summarised in Table 1. Eighteen healthy age and gender matched controls were also examined (Patient: 6 men and 12 women, mean age 58.8 ±11.7 years; Control: 7 men and 11 women, mean age 55.2 ± 10.9 years). A neurological examination was performed on all participants and was negative for significant cognitive problems, sensory signs or hearing loss. All participants were right handed and gave their written informed consent. The experiments conformed to the standards set by the Declaration of Helsinki and were carried out with approval of the local ethics committee.

MMN Study

Auditory and somatosensory MMN were assessed in each subject. The order of somatosensory and auditory MMN assessment was counter-balanced across subjects and groups. Both types of MMN were examined in a single block of 500 trials with an inter-stimulus interval of 1000ms. There was a 2 minute break between each block.

Somatosensory MMN

Vibratory stimuli were delivered via an electromagnetic mechanical stimulator (Ling Dynamics System) with a 3-cm-diameter circular probe grasped in the palm of the right hand. The probe was positioned orthogonally to, and under slight pressure, against the palm of the right hand. Stimulation was applied at an amplitude of 0.2–0.5 mm and a frequency of 70Hz (Kassavetis
et al., 2012). All subjects wore earphones to prevent auditory evoked potentials from the noise of the vibrator. The standard stimulus was XXX, and the oddball stimulus was xxxx, delivered pseudorandomly in a block of 500 trials with an interstimulus interval of 1000ms. The standard stimulus was delivered in 80% of trials and the oddball stimulus in 20% of trials.

**Auditory MMN**

Auditory stimuli were delivered via a single speaker placed 0.5m in front of subjects. In order to ensure that the stimuli were clearly audible, the intensity was set at 65 dB which was considerably above the auditory threshold of all subjects. The experiment consisted of one block of 500 trials with an interstimulus interval of 1000ms. Auditory stimuli at a frequency 333Hz of two different durations (30 ms and 150 ms) were delivered pseudorandomly. The standard stimulus was delivered in 80% of trials and the oddball stimulus in 20% of trials.

**EEG recordings and processing**

Patients with continuous tremor/spasm of the neck or the score of head tremor more than 2 (Table 1) during the visit were not recruited. Subjects were asked to sit in a comfortable position in a high-backed chair to reduce neck muscle contraction. Pre-selected video with no sound was played during the experiment with the monitor placed 0.5 m away from the subjects. Thirty-two Ag/AgCl scalp electrodes (Fp1, Fpz,Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FCz, FC2, FC6, M1, T7, C3, Cz, C4, T8, M2, CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8, O1, Oz,O2) placed according to the 10-20 system were used for the electroencephalogram (EEG) recording. Electrode impedance was kept below 5 kΩ. During recording, the sampling rate was set at 512 Hz, and data were filtered online with a 0.3-100 Hz band-pass filter. After recording, the data were band-pass filtered at 1-30 Hz. The M1 reference was used for online recording and average reference was used for offline analysis. Epochs of -50 to 500 ms were extracted using EEGLab V.11 software (http://sccn.ucsd.edu/eeglab/). Baseline correction was applied with
respect to a time window 50ms prior to stimulus onset. Epochs with voltages exceeding 100 µV were automatically rejected in order to exclude blinks, eye movements and muscle artefacts. EEG sweeps were averaged per individual and the MMN was calculated by subtraction of deviants from standard ERPs.

Data analysis

Data were analysed using SPSS (version 20.0). Averaged mismatch negativity waveforms were compared between the patients and the healthy groups for auditory and somatosensory oddball stimuli.

Mismatch negativity measures

MMN was defined as the peak negativity to deviant stimuli occurring within the 150-250 ms latency range in both oddball types, in line with previous MMN studies (Naatanen et al., 2007).

Our statistical analyses proceeded in two steps. First, to identify the electrode with maximal amplitude effects in MMN and to test for differences in scalp distribution between group and oddball type conditions, multivariate repeated measures analyses of variance were performed on 9 leads (F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4) (Hammerer et al., 2012; Chen JC et al., 2014a&b). Data were normalized across the 9 leads and 18 subjects separately for 4 conditions (control sMMN, control aMMN, patient sMMN, and patient aMMN) for this first step in order to equate amplitude differences between conditions which might distort distribution effects (McCarthy et al., 1985). The within-subject factors: laterality (3 levels: left, medial, right), anterior-posterior (3 levels: frontal, central, parietal) and oddball stimulations (somatosensory and auditory) and between-subject factor: groups (2 levels: cervical dystonia and healthy) were performed. This resulted in a four-way repeated measures GLM for localization.
(Group*oddball type*anterior-posterior*laterality) on normalized data. Given that we were most interested in testing for oddball condition differences with different groups in scalp distributions, we focused on interactions of oddball condition and groups type with the laterality*anterior-posterior interaction. A follow-up 3-way repeated measures GLM in each oddball condition with normalized data was later tested to locate the maximal effect of the MMN.

Having identified the electrode with the maximal effect of the MMN, we then assessed in a second step oddball stimulation and group effects on non-normalized data in a two-way repeated measures GLM (Group*oddball type). In these analyses, we focused only on the maximal effect electrode from the localization analyses.

Finally, follow-up pairwise comparisons were run to assess the effect within levels of the group factor (cervical dystonia and healthy). Only effects with effect sizes > 0.35 (based on the intraclass correlation coefficient: \( \rho_I \)) were considered for follow-up analyses to avoid reporting non-essential effects. Greenhouse-Geisser corrected results were reported when assumptions of sphericity were not met. The peak latency of MMN was later tested using the electrode selected by the peak amplitude. With the method as used for amplitude analysis, a two-way repeated measures GLM on un-normalized data for Group effects, oddball type effects and interaction effect was run.

*Evoked potentials to standard stimuli*

We also examined the possibility that differences in the MMN could be due to differences caused by a general alteration of standard ERPs and not by deviant detection (Umbricht et al., 2000; Umbricht et al., 2002; Korostenskaja et al., 2007). Therefore, we analysed the N60 and
P150 components of the ERP to the standard somatosensory stimulus (Akatsuka et al., 2005; Spackman et al., 2010) as well as N1 and P2 components of the ERP to the standard auditory stimulus.

The N1 component was defined as the most negative peak occurring in the 50-150 ms after stimulus onset and P2 as the most positive peak in the 150-250 ms. The N60 component was defined as the most negative peak in the 0-100 ms window and P150 as the most positive peak in the 100-200 ms window (Umbricht et al., 2002; Chen JC et al., 2014a&b). The statistical analysis was the same as MMN analysis.

**Results**

No differences were found between the two study groups (patients and controls) for age (p = 0.42) and gender (p = 0.74).

*Mismatch negativity*

The number of accepted trials was comparable between somatosensory and auditory stimuli and between the patient and control groups. Somatosensory MMN and auditory MMN were observed visually after both somatosensory and auditory deviants. Figure 1A shows the grand average of sMMN and aMMN in each group at the maximal effect electrodes.

In a first step, a four-way repeated measures GLM for localization (Group*oddball type *anterior-posterior*laterality) on normalized data was conducted to examine the electrodes with the largest MMN effects across oddball types for later tests of the group effects. We saw an oddball type*anterior-posterior*laterality interaction effect (F (4,136) = 27.8, p < 0.00, ρl = 0.91) but not group*oddball type*anterior-posterior*laterality interaction effect (F (4,136) =
0.5, p < 0.72). These results indicated different distribution across two oddball types (somatosensory vs. auditory), but no group difference in distribution.

A follow-up 3-way repeated measures GLM of normalized data was run to test the maximal effect electrodes of sMMN and aMMN separately. In line with previous studies (Teo et al., 2009; Odergren et al., 1998) we observed the largest sMMN effect at the C3 electrode (anterior-posterior*laterality interaction; F (2.7, 91.3) = 27.3, p < 0.00, \( \rho_I = 0.88 \)) and the largest aMMN effect at the F4 electrode (anterior-posterior*laterality interaction; F (3.1, 103.8) = 3.3, p < 0.02, \( \rho_I = 0.37 \)). As can be seen in Figure 1B, neither the distribution of the sMMN nor aMMN differed across groups (group*anterior-posterior*laterality interaction; F (2.7, 91.3) = 1.1, p = 0.37; F (3.1, 103.8) = 1.5, p = 0.21).

Accordingly, in the next step we focused on the C3 electrode for sMMN and F4 electrode for aMMN for further 2-way repeated measures GLM of normalized data to assess group, oddball type main effects and interaction effects. A significant main effect of oddball type (F (1, 34) = 21.5, p < 0.00, \( \rho_I = 0.91 \)) (Table 2) indicated larger amplitude MMN in the sMMN condition compared to the aMMN condition (Fig. 1A).

A significant group*oddball type interaction effect was observed (F (1, 34) = 4.5, p = 0.04, \( \rho_I = 0.63 \)). A follow up independent t-test for sMMN data, showed a smaller sMMN amplitude in dystonic patients compared to controls (mean difference control-dystonia: -1.0 \( \mu \)V ± 0.3, p < 0.00, t = -3.1). However the amplitude of aMMN did not differ between groups (mean difference control-dystonia: -0.2 \( \mu \)V ± 0.2, p = 0.24, t = -1.2) (Fig. 1C).

The latency of MMN showed neither oddball type, group main effect nor oddball type*group interaction effect is significant (two-way repeated measures ANOVA, oddball type: F (1, 34) = 0.3 p = 0.62; group: F (1, 34) = 0.5, p = 0.49; oddball type*group: F (1, 34) = 2.7, p = 0.112) (Table 2) (Fig. 1D).
Evoked potentials to standard stimuli

No significant group*oddball type interaction effects or group main effect were observed between N60 amplitudes and N1 amplitudes, N60 latencies and N1 latencies, P150 amplitudes and P2 amplitudes or as P150 latencies and P2 latencies. These data therefore argued against an alteration of standard sensory processing in our cervical dystonia patients.

Table 2 shows the peak amplitudes and latencies of N1 and P2 to standard tones in the aMMN condition and the peak amplitudes and latencies of N60 and P150 to standard vibratory stimuli in sMMN for the maximal effect electrode.

With the same method described above for MMN analysis, we first conducted a four-way repeated measures GLM for localization (Group*oddball type*anterior-posterior*laterality) on normalized data to examine the electrodes with the largest N60 and N1 effects across conditions for later tests of the group effects.

We observed a significant interaction effect of oddball*anterior-posterior*laterality (F (3.4, 105.2) = 30.4 p < 0.00, ρI = 0.88) but not group*oddball*anterior-posterior*laterality (F (4, 136) = 0.2 p < 0.93). A further 3-way repeated measures GLM of normalized data was run to test the maximal effect electrodes of N60 and N1 separately. The largest N60 amplitude was at the Fz electrode (F (3.1, 104.4) = 33.9, p < 0.00, ρI = 0.89), and the largest N1 amplitude was at the Cz electrode (anterior-posterior*laterality interaction; F (2.6, 89.8) = 4.1 p < 0.01 ρI = 0.47). We focused on these electrodes in a two-way repeated measures GLM to assess the oddball type and group effects in amplitude and latency separately. There was a significant main effect of oddball type in amplitude (F (1, 34) = 306.5 p < 0.00, ρI = 0.99) and in latency
(F (1, 34) = 173.2, p < 0.00, \rho I = 0.99) which indicated significant difference in amplitude and latency (as expected) between N60 and N1.

P2 and P150 amplitudes and latencies were tested in the same way. We observed a significant interaction effect of oddball*anterior-posterior*laterality (F (4, 136) = 5.6, p < 0.00, \rho I = 0.46) but not group*oddball*anterior-posterior*laterality (F (4, 136) = 0.4, p = 0.80). A further 3-way repeated measures GLM of normalized data was run to test the maximal effect electrodes of sMMN and aMMN separately.

The largest P150 effect was at the C3 electrode (anterior-posterior*laterality interaction; F (2.9, 98.6) = 6.2 p < 0.00, \rho I = 0.57), and largest P2 was at the Cz electrode (F (2.9, 97.4) = 33.9 p < 0.00, \rho I = 0.52). Two-way repeated measures GLM on the maximal effect electrode to assess the oddball type and group effects in amplitude and latency separately showed a significant oddball type main effect in amplitude (F (1, 34) = 13.5 p < 0.00, \rho I = 0.86) but not in latency (F (1, 34) = 0.6, p = 0.45) which indicated significant difference in amplitudes but not in latencies between P150 and P2.

**Discussion**

Here we demonstrate that pre-attentive somatosensory error/deviant detection is abnormal in patients with cervical dystonia. This appears to be a specific abnormality in the somatosensory domain as we did not find a similar abnormality in auditory MMN.

Furthermore, the different result on aMMN, which did not show abnormalities compared to the healthy subjects, confirms that the pathological signal processing is related just to the somatosensory system.
While the mechanism of and structures responsible for the generation of auditory MMN have been extensively studied, there has been less exploration of the mechanism and anatomy of somatosensory MMN. For auditory MMN, it has been proposed that short-term glutamatergic plasticity is responsible for adaptation/habituation to the standard stimulus (Umbricht et al., 2000). This adaptation is proposed to be linked to generation of a predictive model of expected input which, when faced with an unexpected “oddball” stimulus, produces a change in evoked potential (Garrido et al., 2009). Thus, auditory MMN is proposed to rely on a network of linked structures including the primary auditory cortex, superior temporal gyrus, and inferior frontal cortex (Garrido et al., 2007).

sMMN is much more rarely studied, and consequentially, models to explain the neuronal network generating this phenomenon are not well defined. However, the dissociation in our dystonic subjects between normal aMMN and abnormal sMMN is consistent with previous reports that there are different networks that generate these two phenomena (Molholm et al. 2005; Restuccia et al. 2007; Spackman et al. 2010; Butler et al. 2011). Our somatosensory MMN data, in accordance with other published studies (Spackman et al., 2007; Restuccia et al., 2009), showed a clear fronto-central negative shift in response to an infrequent change of vibration stimulus, peaking at 150-250ms, mimicking the MMN caused by auditory stimulation, and was predominant on the side contralateral to stimulation location. The standard stimuli also showed a clear N60 component which is similar to N1 in auditory stimulation (Shinozaki et al., 1998). Intracranial recordings and fMRI data point to the principal generators of somatosensory MMN being within the parietal lobe, with a possible additional frontal component (Chen et al., 2010; Spackman et al., 2006). The role of the frontal cortex in sMMN is further supported by MMN findings from patients with frontal lesions and intracranial recordings (Rosburg et al., 2005). Recently, this evidence has been added to by Kim et al, who showed decreased MMN strength in frontal and temporal cortices in patients with schizophrenia and in people at high
risk of psychosis (Kim et al. 2017). These disorders are characterized by frontal dysfunctions, especially functional disconnection between the temporal and frontal cortices (Gaebler et al., 2015). Garrido et al have proposed, using dynamic causal modelling of fMRI data of MMN tasks, a predictive coding model of MMN where mismatch responses produced by the comparison of standard stimuli to unexpected deviants are explained by prediction error minimization schemes (Garrido et al., 2007, 2008), and are likely to be mediated by asymmetrical changes in intrinsic and extrinsic effective connectivity within a hierarchical network (Dietz et al., 2014; Garrido et al., 2008, 2009). This system relies on plastic changes happening within synapses at different levels of an hierarchical network, in other words adaptive changes caused by the predictability of the standard stimulus.

Our results suggest that the integrity of this network is altered in dystonia, but it is not possible to determine how with the data from this study. Certainly abnormalities of neuronal plasticity have been proposed to be important in the pathophysiology of dystonia, though responses to experimental probes of synaptic plasticity in patients with dystonia are very variable and can be normal. More recently, cerebellar dysfunction has been suggested to be important in the pathophysiology of dystonia. We have previously demonstrated that non-invasive stimulation of the cerebellum can alter sMMN (Chen et al, 2014), in keeping with a small study of patients with cerebellar hemisphere lesions where sMMN was abnormal. Further studies of sMMN in dystonia using fMRI or MEG might be able to determine more precisely the nature of the network dysfunction causing sMMN.

There is a further interesting question raised by these data: could the abnormality in sMMN underlie some of the previously reported abnormalities in somatosensory function reported in dystonia. We have recently suggested that previously reported abnormalities in somatosensory
temporal discrimination in dystonia might be due to a more general deficit in criterion setting rather than a deficit in millisecond timing processes. One could hypothesize a deficit in pre-conscious orientation towards potentially salient signals might lead to a more conservative threshold for decision-making on the nature of somatosensory signals. Further investigation of the relationship between deficits in sMMN and other somatosensory abnormalities in dystonia would certainly be of interest. In conclusion, our results bring an additional evidence regarding the role of sensory error prediction in the pathophysiology of dystonia. However, the implicated neuronal populations and the interaction between sensory abnormalities and the specific motor dysfunction seen in dystonia are still far from being defined. It is hoped that the application of novel methods and analysis will provide better tools to identify disease-specific abnormalities in the sensory domain with ensuing insight into the pathophysiology of dystonia and other movement disorders.

References


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Figures legend

**Figure 1:** (A) Grand average of standard, deviant, and MMN ERPs at C3 in the vibratory testing (upper row) and at F4 in the auditory testing (lower row) across 18 subjects/patients. 

(B) Scalp topographies of standard, deviant, and MMN ERPs at C3 in the vibratory testing (upper row) and at F4 in the auditory testing (lower row) across 18 subjects/patients. Maps are based on mean amplitudes of a 50 ms interval around individually defined MMN peaks in a time window of 150-250ms after stimulus onset. Consistent left central-frontal maximal effects of the MMNs were noted in vibratory testing and fronto-central maximal effects in auditory testing. 

(C) MMN peak amplitudes between normal control and dystonia patients for the vibratory stimulus condition at C3 electrode and the auditory stimulus condition at F4 electrode. 

(D) MMN peak latencies between normal control and dystonia patients for the vibratory stimulus condition at C3 electrode and the auditory stimulus condition at F4 electrode. 

Error bars indicate 1 SEM. Somatosensory MMN peak amplitudes of dystonia patients were significant smaller as compared to age-matched control subjects.