Motor excitability evaluation in developmental stuttering: A transcranial magnetic stimulation study

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Article info
Article history:
Received 29 December 2010
Reviewed 25 March 2011
Revised 15 July 2011
Accepted 7 December 2011
Action editor Pia Rotshtein
Published online 16 December 2011

Keywords:
Left hemisphere
Motor deficit
Motor evoked potentials
Pharmacological treatment of stuttering
Stuttering

Abstract
Introduction: Developmental stuttering (DS) is viewed as a motor speech-specific disorder, although several lines of research suggest that DS is a symptom of a broader motor disorder. We investigated corticospinal excitability in adult DS and normal speakers.

Methods: Transcranial magnetic stimulation (TMS) was administered over left/right hand representation of the motor cortex while recording motor evoked potentials (MEPs) from the contralateral first dorsal interosseous (FDI) muscle. Resting, active motor thresholds, silent period threshold and duration were measured. A stimulus–response curve at resting was also obtained to evaluate MEP amplitudes.

Results: Lower corticospinal responses in the left hemisphere of DS were found, as indicated by a reduction of peak-to-peak MEP amplitudes compared to normal speakers.

Conclusions: This provides further evidence that DS may be a general motor deficit that also involves motor non-speech-related structures. Moreover, our results confirm that DS may be related to left hemisphere hypoactivation and/or lower left hemisphere dominance. The present data and protocol may be useful for diagnosis of subtypes of DS that may benefit from pharmacological treatment by targeting the general level of cortical excitability.

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1. Introduction

Stuttering is defined as a disruption of the rhythm of speech and language articulation, where the subject knows what he/she wants to say, but is unable to utter the intended word or phrase fluently (World Health Organization, 1977). Developmental stuttering (DS) is the most common form of stuttering, and appears during childhood. A percentage of children recover from DS, while others remain persistent stutters in adulthood even if DS may spontaneously disappear years after its onset (Kell et al., 2009). The principal symptoms of DS are blocks and/or repetitions at the beginning of phrases and/or words (Bloodstein, 1995). It is usually accompanied by evident movements and spasms, especially of the oro-facial muscular...
districts, in order to overcome disfluencies (Mulligan et al., 2003; Riva-Posse et al., 2008). DS is considered as a complex and multi-factorial disorder (Ambrose et al., 1993; Ambrose et al., 1997; Ludlow and Loucks, 2003; Maguire et al., 2002; Yairi et al., 1996). Orton (1928) and Travis (1978) theorized that DS was the result of an incomplete left language lateralization in the brain, followed by over-activation of the right one, resulting in a conflict for the execution of speech motor tasks. Recent functional magnetic resonance imaging (fMRI) studies have confirmed this hypothesis (Blomgren et al., 2003; Braun et al., 1997; Chang et al., 2009; De Nil et al., 2000; Fox et al., 2000, 1996; Ingham et al., 2004; Kell et al., 2009; Neumann et al., 2003; Preibisch et al., 2003; Sommer et al., 2002).

Anatomically, the stuttering brain also shows differences compared with that of fluent subjects. For example, stutterers fail to show the normal hemispheric asymmetries that are present in prefrontal and occipital lobes (Foundas et al., 2003) or in the frontal operculum and planum temporale (Foundas et al., 2001). Thus, it has been suggested that stutterers may have a different pattern of neural connections compared to fluent speakers (Cykowski et al., 2010; Ludlow and Loucks, 2003; Lu et al., 2010b, 2009; Salmelin et al., 2000; Sommer et al., 2002; Watkins et al., 2008).

It is not clear if these abnormalities are a prerequisite for the appearance of stuttering, or if they are the result of long-term stuttering in adults. However, it can be suggested that overt disfluencies are not needed to differentiate the stuttering brain from that of fluent individuals. In fact, it appears that the brain in stutterers is characterized by dopamine-related abnormalities (Wu et al., 1997, 1995). In this regard, it is clear that stuttering deficits remit after administration of anti-dopaminergic and/or serotonergic drugs (Boldrini et al., 2003; Busan et al., 2009; Kumar and Balan, 2007; Maguire et al., 2004, 2000; Murray et al., 1977). However, although the chemical equilibrium in the brain may be an important factor in stuttering (Schreiber and Pick, 1997), contrasting reports (Guthrie and Grunhaus, 1990; Lee et al., 2001; Linazasoro and Van Blercom, 2007) suggest that different subgroups of stutterers may exist (Alm, 2004). This is especially true if it is considered that both dopamine and serotonin are important for the modulation of motor output (Cantello et al., 2002; Loubinoux et al., 2002, 1999; Pariente et al., 2001).

Taken together, these studies suggest that DS is an incompletely understood neurological problem, wherein disfluency is only one symptom of a more complex and subtle motor syndrome (Büchel and Sommer, 2004; Saltuklaroglu et al., 2009). This has been confirmed by recent investigations (Chang et al., 2009) demonstrating that stutterers show less BOLD signal change than control subjects during motor planning for both speech- and non-speech-related tasks.

In agreement with such a non-speech specific origin of DS, investigations have been conducted on general motor skills in stutterers (Webster, 1990a, 1990b, 1989) which have shown that stutterers may have difficulties in motor skills that are unrelated to speech (Brown et al., 1990; Forster and Webster, 2001; Jones et al., 2002; Smits-Bandstra and De Nil, 2007; Smits-Bandstra et al., 2006; Starkweather et al., 1984; Vaughn and Webster, 1989; Webster, 1990a, 1990b, 1989, 1986; Zelaznik et al., 1997).

As a consequence, we postulated that DS might also show some secondary indexes of motor abnormalities, as measured by transcranial magnetic stimulation (TMS; Cantello et al., 2002). Specifically, we investigated if DS might affect non-speech specific motor representations (e.g., hand muscle representation). Herein, we studied hand muscle representations allowing a direct comparison with previous publications (Busan et al., 2009; Sommer et al., 2009, 2003). Earlier studies showed that resting and active motor thresholds (AMTs) are increased in stuttering, suggesting the presence of a more widespread general motor cortical inhibition in DS with respect to normal speakers (Sommer et al., 2003). However, inter-hemispheric inhibition, intra-cortical inhibition (ICI) and facilitation (ICF) appear to be normal in DS when considering hand representation (Sommer et al., 2009, 2003). Weaker inhibition (in the right hemisphere) and a reduced facilitation (bilaterally) may be evident in the tongue motor representation of stutterers, accompanied by a steeper stimulus–response curve during muscular activation of the same districts (Neef et al., 2011b). Furthermore, a previous study (Busan et al., 2009) showed that the cortical silent period (believed to be an index of intra-cortical inhibition) was significantly reduced in a group of adult DS after the administration of paroxetine.

In the present study, we measured several indices of corticospinal excitability, some of which were not previously evaluated in DS subjects. Specifically, we measured bilateral resting and AMTs, cortical silent period threshold and duration and a resting motor evoked potential (MEP) stimulus–response curve. These measures were obtained from bilateral motor hand representations to directly investigate whether: (i) stuttering is a wider motor disorder and not exclusively a motor speech-related abnormality; (ii) stuttering is characterized by abnormal left hemisphere corticospinal activation.

### Materials and methods

#### 2.1. Subjects

A total of 40 subjects were recruited: 17 were developmental stutterers from childhood (6 females, age range: 19–46 years, mean: 26.5, standard deviation (SD): 6.9, 16 with right hand preference, 6 smokers), while 23 were sex-, age- and handedness-matched normal speakers (6 females, age range: 20–43 years, mean: 26.3, SD: 6.2, 21 with right hand preference, 7 smokers). Recruitment was conducted at two different centres (Ferrara and Trieste; Table 1) to obtain a sufficient number of stuttering subjects. Experimental groups were matched for similar characteristics, with particular attention to maintain a good balance between groups in their totality (stutterers vs normal speakers), considering mean age, handedness scores and gender, as in previous studies on stuttering (Braun et al., 1997; Cykowski et al., 2008). This led to

<table>
<thead>
<tr>
<th>Subjects/centres</th>
<th>Ferrara</th>
<th>Trieste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stutterers</td>
<td>5 males/4 females</td>
<td>6 males/2 females</td>
</tr>
<tr>
<td>Normal speakers</td>
<td>7 males/5 females</td>
<td>10 males/1 female</td>
</tr>
</tbody>
</table>
the presence of different numbers of subjects in the two groups. Moreover, when selecting experimental subjects, we considered smoking habits since chronic smokers have different cortical excitability (Lang et al., 2008). Two stutterers suffered from migraine attacks, and two subjects with similar characteristics were also present in the normal speaker group (Khedr et al., 2006; Van der Kamp et al., 1996). Moreover, subjects in both groups had comparable amounts of continuous training with musical instruments (Rosenkranz et al., 2007). Finally, menstrual cycle and hormones are known to affect cortical excitability (Inghilleri et al., 2004), and this was also taken into consideration (see Supplementary Material). Handedness was evaluated before the experiment with adapted tests (Edinburgh Inventory; Oldfield, 1971). Stuttering was assessed by using the stuttering severity instrument (SSI; Riley, 1980). According to the SSI, stutterers were classified as very mild (n = 3), mild (n = 7), moderate (n = 6) and severe (n = 1; Table 2). Each control subject was interviewed by a trained researcher to exclude undetected stuttering. No major neurological deficits were detectable; moreover, none of the subjects were under pharmacological treatment with antidepressants or antipsychotic drugs. Normal speakers reported no familial history of DS. Procedures received the favourable judgement of the local ethics committee and were in accordance with the Declaration of Helsinki. They were fully explained to study participants, who provided informed consent.

2.2. Electromyographic recording and TMS

The present study was conducted at two centres, where two different magnetic stimulators were available. In Ferrara, we used TMS monophasic single pulses (Magstim, Whitland, UK) through a figure-of-eight coil (wing diameter, about 70 mm; anterior to posterior flowing of the current in the coil). In Trieste, TMS biphasic single pulses were delivered (Medtronic MagPro R30) through a figure-of-eight coil (wing diameter, about 70 mm; posterior to anterior flowing of the first phase of the current in the coil).

During the stimulations, the coil was manually maintained at a 45° angle with respect to the inter-hemispheric fissure and pointing backward. In Ferrara, MEPs were acquired with a wireless electromyography (EMG) system (Aurion, ZeroWave EMG) using a tendon-belly montage with Ag/AgCl electrodes. Signal was band-pass filtered at 10–1000 Hz and digitized at 2000 Hz. EMG traces were acquired and stored using the commercial software Signal 3.09 (Signal Software Services, Corp.). In Trieste, a Galileo Sirius system (EBNeuro, Florence, Italy; signal band-pass filtered at 20–2000 Hz and digitized at 4000 Hz) was used to acquire and store data, using the same tendon-belly montage. It is evident that different results could be obtained with different instrumental set-ups (Sommer et al., 2006); however, this potential confounding factor was controlled by using a comparable ratio between groups (stutterers vs normal speakers) in the two laboratories. Moreover, all measures were transformed in z-scores before statistical analyses compared to the averaged raw measures obtained in the different experimental settings.

2.3. Study protocol

Subjects were comfortably seated during the experiment. A tight tissue cap with a grid of points spaced 1 cm, centred on the vertex, was used to individuate the hot spot (the point on the scalp evoking the maximal muscular response) of the first dorsal interosseous (FDI) muscle of the contralateral hand on both hemispheres. Subjects were instructed not to speak, and to remain relaxed (except when a muscular contraction was requested).

Resting motor threshold (RMT) and AMT were defined as the intensity of the stimulator output eliciting a MEP of at least 50 μV in half of 10 consecutive stimulations for RMT and a MEP of at least 200 μV in half of 10 consecutive stimulations for AMT (Rossini et al., 1994). Subsequently, we also evaluated the silent period threshold (spMT; Fritz et al., 1997).

Silent period duration was acquired at stimulation intensities of 150%, 160% and 175% of the spMT, recording 8 repetitions for each intensity on both hemispheres. The silent period duration was defined as the time elapsing from the start of MEP until the clear restoration of muscular activity (Saisinen et al., 2008).

MEP stimulus–response curves were obtained at resting to evaluate possible baseline differences between groups, since they should have a prevalent cortical component, whereas curves obtained during muscular pre-activation are largely expected to reflect the spinal contribution (Aminoff, 1992; Oishi et al., 2008). TMS was administered at 90%, 110%, 130% and 150% of the RMT. For each intensity, 5 repetitions were recorded for both hemispheres. Peak-to-peak mean amplitudes and maximal MEP amplitude (MEPmax) were calculated.

After thresholds determination for both hemispheres, all experimental procedures were randomly presented (88 TMS pulses were delivered in total) during the experiment. Thirty-nine subjects completed all experimental sessions, while in 1 stuttering subject only thresholds and the resting MEP stimulus–response curve were assessed because of reported

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Centre of recruitment</th>
<th>Sex</th>
<th>Stuttering severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>Ferrara</td>
<td>M</td>
<td>16 (very mild)</td>
</tr>
<tr>
<td>N2</td>
<td>Ferrara</td>
<td>M</td>
<td>18 (mild)</td>
</tr>
<tr>
<td>N3</td>
<td>Ferrara</td>
<td>F</td>
<td>21 (mild)</td>
</tr>
<tr>
<td>N4</td>
<td>Ferrara</td>
<td>M</td>
<td>27 (moderate)</td>
</tr>
<tr>
<td>N5</td>
<td>Ferrara</td>
<td>F</td>
<td>26 (moderate)</td>
</tr>
<tr>
<td>N6</td>
<td>Ferrara</td>
<td>F</td>
<td>8 (very mild)</td>
</tr>
<tr>
<td>N7</td>
<td>Ferrara</td>
<td>M</td>
<td>20 (mild)</td>
</tr>
<tr>
<td>N8</td>
<td>Ferrara</td>
<td>M</td>
<td>21 (mild)</td>
</tr>
<tr>
<td>N9</td>
<td>Ferrara</td>
<td>F</td>
<td>19 (mild)</td>
</tr>
<tr>
<td>N10</td>
<td>Trieste</td>
<td>M</td>
<td>29 (moderate)</td>
</tr>
<tr>
<td>N11</td>
<td>Trieste</td>
<td>F</td>
<td>29 (moderate)</td>
</tr>
<tr>
<td>N12</td>
<td>Trieste</td>
<td>M</td>
<td>18 (mild)</td>
</tr>
<tr>
<td>N13</td>
<td>Trieste</td>
<td>M</td>
<td>32 (severe)</td>
</tr>
<tr>
<td>N14</td>
<td>Trieste</td>
<td>M</td>
<td>13 (very mild)</td>
</tr>
<tr>
<td>N15</td>
<td>Trieste</td>
<td>F</td>
<td>24 (moderate)</td>
</tr>
<tr>
<td>N16</td>
<td>Trieste</td>
<td>M</td>
<td>25 (moderate)</td>
</tr>
<tr>
<td>N17</td>
<td>Trieste</td>
<td>M</td>
<td>19 (mild)</td>
</tr>
</tbody>
</table>
discomfort during TMS. A summary of the main excitability measures obtained is provided in Table 3.

2.4. Statistical analysis

RMT, AMT and spMT were expressed as percentages with respect to the maximal stimulation intensity of the magnetic stimulators. Silent period duration was expressed in msec, while MEP stimulus–response curves and MEPmax were expressed in microvolts. All measures were successively transformed in z-scores before statistical analyses. To provide a statistical model of the phenomena, due to the non-independent repeated measures longitudinal scheme of the experiment, the mixed model analysis technique (West et al., 2006) was chosen. Normality was assessed using the Shapiro–Wilk test. To assess differences in non-normally distributed data, we performed a non-parametrical Wilcoxon test, while in normally distributed data differences in means were assessed by Student’s t-test.

MEP amplitudes analysis was initially conducted considering groups (stutterers vs normal speakers) as a between-subjects factor. Hemispheres (left vs right) and stimulation intensities (90%, 110%, 130% and 150% of RMT) were considered as within-subject factors, while the handedness score (also transformed in z-scores) was considered as a covariate. All interactions among factors were also considered. The same statistical procedure was implemented for the silent period duration analysis. In this case, stimulation intensities were 150%, 160% and 175% of spMT. RMT, AMT, spMT and MEPmax analysis were implemented considering only groups, hemispheres and handedness (covariate factor) as factors for singular and interaction effects. When significance was obtained in factor or interaction analysis, a backward simplification of the model was conducted.

Suitable degrees of freedom for the mixed model analysis were approximated considering the sample size and subtracting free parameters. An additional analysis was performed to determine if a relation exists between the neurophysiological indexes registered and the severity of stuttering (SSI), or between the handedness score of each stuttering subject and her/his degree of stuttering. Correlation analysis was performed with Pearson’s correlation when normally distributed data were available, and using Spearman’s correlation (or Gamma statistic when more appropriate) for non-normally distributed results, with cortical excitability measures expressed as z-scores.

See the Supplementary Material for the supplementary analyses performed (with the same methods used for main analyses, if not otherwise specified). A p < .05 was considered significant (analyses were performed with a two-tailed assumption). Analyses were carried out using the statistical software R (R Development Core Team, 2008) and the statistical package Statistica 6.0 (StatSoft Italia srl).

3. Results

No significant relations were seen when considering measures of excitability registered and the severity of stuttering compared to centre of recruitment (analyses conducted for males and females together and for males only; all p > .05). The only exception was a positive correlation [r(7) = .675, p = .046] obtained when considering left hemisphere silent period threshold for subjects recruited in Ferrara (both males and females), and a positive correlation between stuttering degree and MEP amplitudes averaged for each intensity of stimulation when considering only males recruited in Ferrara [rs = .90, p = .04]. Correlations and probabilities that were significant when grouping together subjects recruited at the two centres (see the Results section and the Supplementary Material) showed comparable and high values (even if not significant, except for the positive correlation between the degree of stuttering and AMTs in the left hemisphere for stuttering subjects recruited in Ferrara, both males and females, r(7) = .692, p = .04, and the inverse correlations with silent period durations for stuttering males subjects recruited in Trieste at 160% spMT and 175% spMT: r(9) = −.912, p = .03; r(9) = −.933, p = .02, respectively) when analyses were subdivided in the two recruitment groups.

<table>
<thead>
<tr>
<th>Table 3 – Summary of the main excitability indexes measured.</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td><strong>RMT</strong></td>
<td>The intensity of stimulation eliciting a muscular response of at least 50 µV in half of 10 stimulations at rest; it is useful to define the threshold for corticospinal activation at resting</td>
</tr>
<tr>
<td><strong>AMT</strong></td>
<td>The intensity of stimulation eliciting a muscular response of at least 200 µV in half of 10 stimulations during muscular pre-activation; it is useful to define the threshold for corticospinal activation during muscular pre-activation</td>
</tr>
<tr>
<td><strong>Silent period threshold (spMT)</strong></td>
<td>The minimal intensity of stimulation inducing a “silence” in muscular activation: it is useful to define the threshold for evoking a silent period (an index of intra-cortical inhibition)</td>
</tr>
<tr>
<td><strong>Silent period stimulus–response curve</strong></td>
<td>Duration of the silent period evoked during muscular pre-activation stimulating at 150%, 160% and 175% of spMT; it is useful to characterize the trend of intra-cortical inhibition as measured by silent period</td>
</tr>
<tr>
<td><strong>MEP stimulus–response curve</strong></td>
<td>Amplitude (measured in microvolts) of the muscular response obtained when stimulating at 90%, 110%, 130% and 150% of RMT at resting; it is useful to characterize the trend of corticospinal activation</td>
</tr>
<tr>
<td><strong>Maximal amplitude of MEPs (MEPmax)</strong></td>
<td>Maximal muscular response (amplitude measured in microvolts) obtained during MEP stimulus–response curve; it is useful to determine the maximal response that could be obtained from corticospinal activation during present stimulus-response curve measurements</td>
</tr>
</tbody>
</table>
The dominance measured by the handedness score in the stuttering group was +72 (n = 17, SD: 43), while it was +76 (n = 23, SD: 47) in the normal speaker group. There was no significant difference between groups considering handedness scores (p > .1).

These results are summarized in Table 4.

### 3.1. Resting and AMTs

No significant differences were found in RMT in the stuttering group compared to normal speakers. In fact, no significant differences were seen between groups, hemispheres, handedness score or interaction analyses (all p > .1).

AMT showed a significant interaction in a hemispheres versus handedness score [t(36) = −2.425, p = .02], indicating that this excitability measure is related with handedness depending on hemispheres, but independently from groups. Considering that this finding was beyond the main objective of the present study, this effect was not further investigated in simplification of the model. No significant differences were seen between groups, hemispheres, handedness score effects or in the remaining interaction analyses (all p > .1).

### 3.2. Silent period

When considering the silent period duration, a significant result was obtained in the stimulation intensities effect [t(35) = 5.882, p < .001] indicating that higher stimulation was associated with a longer silent period. No differences were seen for the silent period duration in groups, hemispheres or handedness score effects or in interaction analyses (always p > .1).

No differences were present between groups, hemispheres and handedness score effects or in interaction analyses in spMT (all p > .1).

### 3.3. MEP amplitudes

Mixed models analysis showed that the stimulation intensities effect was significant [t(36) = 6.427, p < .001], suggesting that higher stimulation intensities were associated with higher responses in MEP amplitudes. This effect was modulated by an interaction with hemispheres and handedness score [three-way interaction: t(36) = 2.183, p = .04], indicating that the handedness score may be related with MEP amplitudes independently of groups, but with different effects on the two hemispheres and depending on stimulation intensity. This was not further investigated since it was beyond the principal aim of the study. When considering two-way interactions, significant findings were observed for the hemispheres versus handedness score interaction [t(36) = 2.827, p = .008], further indicating that there may be a relationship between MEP amplitudes and handedness score that depends on the hemispheres (and independently of groups). However, this finding was not further investigated as it was beyond the main scope of the study, but the influence of handedness was investigated (see below).

Interestingly, a groups versus hemispheres interaction was also observed [t(36) = 2.207, p = .03]. No other significant effects were observed (all p > .1). Simplification of the model on this last effect showed a significant result (Wilcoxon test, p = .04), indicating that when stimulating the left hemisphere of the stuttering group, lower MEP amplitudes (averaged for every intensity of stimulation) were observed compared to the left hemisphere of normal speakers.

We investigated the possibility that this result was influenced by the handedness score also considering a trend suggesting that handedness score could have a different influence compared to hemispheres and groups [three-way interaction: t(36) = −1.908, p = .06]. In this regard, probability analyses showed that an inverse relation between MEP amplitudes averaged for each intensity of stimulation and the

<table>
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<tr>
<th>Table 4 – Corticospinal measures obtained using TMS.</th>
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<tbody>
<tr>
<td>TMS Measurements</td>
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<tr>
<td></td>
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<tr>
<td>RMT</td>
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<tr>
<td>AMT</td>
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<tr>
<td>spMT</td>
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<tr>
<td>Silent period at 150% spMT</td>
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<tr>
<td>Silent period at 160% spMT</td>
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<tr>
<td>Silent period at 175% spMT</td>
</tr>
<tr>
<td>MEP amplitudes at resting 90% RMT</td>
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<tr>
<td>MEP amplitudes at resting 110% RMT</td>
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<tr>
<td>MEP amplitudes at resting 130% RMT</td>
</tr>
<tr>
<td>MEP amplitudes at resting 150% RMT</td>
</tr>
<tr>
<td>MEP amplitudes at every intensity of stimulation</td>
</tr>
</tbody>
</table>

Results obtained for TMS measurements in stuttering and normal speaker groups. Data are reported according to the left or right hemisphere for each group, and are expressed as raw mean values (in parenthesis, SD; stutterers n = 17 – except for silent period durations, n = 16 – normal speakers n = 23). Thresholds are expressed as percentages with respect to the maximum stimulator output, and silent period durations are expressed in msec. MEP amplitudes are reported in microvolts. Significant differences are shown in bold. Asterisks indicate a significant positive relation with stuttering degree.
correspondent handedness score of stutterers could be present in the right hemisphere ($I^2 = -0.355, p = 0.065$), providing control for the trend in the above indicated three-way interaction. However, the lack of significant differences between groups in the MEP amplitudes of the right hemisphere did not allow further considerations of this possibility. No further relationships were evident when considering handedness score of both groups compared to MEP amplitudes averaged for each intensity of stimulation of the left or the right hemisphere for each subject, or when considering the handedness score of the stuttering group or normal speakers compared to the correspondent MEP amplitudes averaged for each intensity of stimulation of the left or right hemisphere for each subject (all $p > 0.1$). This allowed for control of the handedness score versus hemispheres interaction. The significant results are summarized in Fig. 1 and Table 4.

3.4. MEPmax amplitudes

The mixed models analysis revealed a significant finding in the hemispheres versus handedness score interaction [$t_{150} = 2.245, p = 0.03$], indicating that this measure of excitability is related with handedness score, depending on hemispheres but independent of groups. Considering that this finding was beyond the main objectives of the study, it was not further investigated. No significant findings for main effects were observed. Additionally, the remaining interaction analyses failed to show any significant findings (all $p > 0.1$).

3.5. Correlation and probability analyses

Analyses performed to investigate the relationships between the neurophysiologic indexes in stutterers and the degree of stuttering showed that a significant positive correlation was present between AMT in both hemispheres of stutterers and SSI [left hemisphere: $r_{15} = 0.566, p = 0.02$; right hemisphere: $r_{15} = 0.513, p = 0.035$]. This indicates that higher degrees of stuttering were related with higher AMTs (Fig. 2). Significance was never reached (all $p > 0.05$) in the remaining analyses when considering the entire sample of stuttering subjects. In addition, no relation was evident between handedness score and the degree of stuttering ($p > 0.1$).

4. Discussion

4.1. Summary of main results, interpretation and relation to previous findings

The present study demonstrates that left hemisphere hand corticospinal excitability in stuttering participants is lower than that of normal speakers. Therefore, this provides direct evidence that DS may have lower left corticospinal excitability (possibly compensated by the right hemisphere; Morgan et al., 2008; Neef et al., 2011a), which is in agreement with several aspects of previous findings (see Introduction). More importantly, this abnormal excitability is highlighted by the results of hand motor representation, suggesting that stuttering might be only one aspect of a broader motor disorder.

Our main result is based on an altered MEP stimulus–response curve. This measure is thought to provide information about the strength of the corticospinal pathway (Devanne et al., 1997; Ridding and Rothwell, 1997) in terms of density of cortical projecting neurons, the number of spinal alpha motor neurons influenced by the descending volley and the synchronicity of activation of both (Wassermann et al., 2008). As a consequence, the significant differences highlighted herein could be related to lower strength and recruitment of the corticospinal pathway, or with a smaller number and density of cortical projecting neurons in stutterers. This effect could be hypothetically related with defects in modulations of Na$^+$ and Ca$^{2+}$ channels, but also, for example, of mono-aminergic systems (Boroojerdi et al., 2001), as already
suggested by Sommer et al. (2003). Moreover, the present results are in good agreement with the proposal that differences in MEP input–output curves could be due either to a decreased number of spinal motoneurons being activated synchronously, or to the activation of the same number of motoneurons in a less synchronous manner (Pitcher et al., 2003).

It is commonly believed that stuttering relies principally on several types of cerebral deficits (Khedr et al., 2000; Maguire et al., 2002; Watkins et al., 2008), although spinal influences have also been proposed (Lastovka, 1970). MEP stimulus–response curves at resting are assumed to have a prevalent cortical component, whereas curves obtained during muscular pre-activation should more largely reflect the spinal contribution (Aminoff, 1992; Oishi et al., 2008). In this regard, they are considered as the most sensitive index of cortical excitability (Boroojerdi et al., 2001). Nevertheless, it is very difficult to completely isolate a central effect from a spinal component (Devanne et al., 1997), also considering the direct influence of supra-segmental structures on spinal circuitry and vice versa (Di Lazzaro et al., 2010; Huang, 2010; Mori et al., 2009).

With specific reference to the stuttering syndrome, DS showed hypoactivation and/or deactivation of motor language-related areas in the dominant left hemisphere in various fMRI studies, both during stuttered speech (Braun et al., 1997; Fox et al., 2000, 1996), fluent speech (Braun et al., 1997) and covert speech (Blomgren et al., 2003), with an anomalous activation and/or an over-activation of the homologue right hemisphere areas (Braun et al., 1997; Kell et al., 2009; Preibisch et al., 2003), which could be considered as either dysfunctional (Chang et al., 2009; Fox et al., 2000, 1996; Ingham et al., 2004) or as the result of a compensatory strategy (Braun et al., 1997; Kell et al., 2009; Preibisch et al., 2003; Sommer et al., 2002).

Our results confirm the observation of left hemisphere hypoactivation using a direct neurophysiological measure, and suggest that the same mechanism of left hemisphere depression may also be present for centres controlling the hand. These findings may be considered as an indirect confirmation of the hypothesis that an atypical balance of cerebral dominance may be present in DS (Travis, 1978).

4.2. Secondary results and limitations of the study

The lower MEP amplitudes in the left hemisphere of stutterers compared to normal speakers were decidedly more evident with higher intensities of stimulation in the stuttering males subgroup — who are more frequently affected by DS (Yairi et al., 1996) — and also evident when excluding left-handed subjects (see Supplementary Material). An inverse relation between the right hemisphere silent period duration and the degree of stuttering was also found at all intensities of stimulation (when considering only males, see Supplementary Material), even if this result could be biased from the high number of analyses. In general, this could be seen as a limitation of the study, even if there is on-going debate about this topic (Aickin, 1999; Bender and Lange, 1999; Perneger, 1999, 1998). However, this finding suggests that more severe DS symptoms are associated with lower intra-cortical inhibition of the right hemisphere. Similar findings have also been reported by Neef et al. (2011b). The AMT of both hemispheres and severity of stuttering was also related, in agreement with Sommer et al. (2003) who demonstrated that RMT and AMT may be increased in stuttering, suggesting a reduction in general motor excitability in severe stutterers.

In general, the negative results of the present study speculatively suggest that, for example, intra-cortical inhibitory circuits could be affected (see also Busan et al., 2009; Neef et al., 2011b) differently in stutterers compared to indexes associated with modulations of Na+ and Ca2+ channels and/or mono-aminergic systems (MEP stimulus–response curves; Boroojerdi et al., 2001). The lack of significant differences in MEPmax amplitudes between groups (despite a clear reduction in MEPmax in the stuttering group) could indicate that this disturbance is subtle and varies with time. Finally, the general lack of significant relations between DS severity and indexes of cortical excitability could indicate that stuttering severity is not always fundamental to observe specific variations in cortical excitability.

A possible confounding factor might be hemispheric dominance. In the present study, several relationships between handedness and other independent variables were found. However, no associations were present between handedness scores and significant MEPs comparisons, although hand preference was shown to be related with measures of cortical excitability (De Gennaro et al., 2004) and with hand area size in a magnetoencephalography study (Volkmann et al., 1998). However, no significant differences were evident between groups in resting and AMTs, silent period threshold and duration or MEPmax amplitudes. The fact that there was no change in thresholds reduces the
likelihood that hemispheric dominance significantly contributes to the pattern of the present results.

Another possible confounding factor is related with the possibility that different subgroups of stutterers exist (Alm, 2004). As a consequence, a greater variability among them could be observed, as well as differences in experimental results. Moreover, different settings of brain stimulation show different profiles of brain activation (Sommer et al., 2006): this should be always kept in mind. Finally, an effect of facilitation or depression of post-exercise MEPs (Samii et al., 1996) cannot be ruled out. Indeed, this element, as in other basal ganglia-related disturbances, merits consideration (Khedr et al., 2007).

4.3. DS as a general motor dysfunction

Several authors (Chang et al., 2009; Preibisch et al., 2003) have proposed that DS may be not only a specific motor speech-related disorder, but a general motor disorder involving the entire motor system. This claim is supported by behavioural studies indicating that DS subjects show: (i) difficulties in bimanual coordination (Vaughn and Webster, 1989; Webster, 1990a, 1990b), (ii) increase in reaction times (Jones et al., 2002; Starkweather et al., 1984; Vaughn and Webster, 1989; Webster, 1990b, 1989) and (iii) difficulties in performing unpredictable and complex motor sequences (Brown et al., 1990; Smits-Bandstra and De Nil, 2007; Webster, 1986). Difficulties in finger-tapping (Smits-Bandstra et al., 2006) and finger movement sequencing (Forster and Webster, 2001) have also been reported. These types of tasks can be considered analogous to motor speech implementation, and probably involve a cortico-basal ganglia-thalamo-cortical circuit and/or a cerebello-cortical circuit that may be defective in stutterers (Lu et al., 2010a, 2010b, 2009; Smits-Bandstra and De Nil, 2007). However, it should also be considered that these problems could be seen as a consequence in a failure of the recruitment of left frontal regions (Kell et al., 2009). Nonetheless, published results are often in contrast, with some studies showing positive and others negative results (Postma and Kolk, 1991; Webster, 1985; Zelaznik et al., 1994). It is also possible that general motor skills are defective in stuttering, independently of the presence of verbal disfluencies (Chang et al., 2009; Smits-Bandstra and De Nil, 2007). In support of this interpretation is the fact that our main results were obtained while stutterers were at rest, suggesting that the DS deficit is rather general. Finally, it can be suggested that stuttering is only a symptom of a subtle and complex motor disorder that becomes evident during speech control due to its dynamic complexity. As a consequence, DS deficits should be more evident when a complex motor act is about to begin, for example when a stutterer is requested to speak (Bloodstein, 1995). In this regard, it has been reported that abnormalities in stuttering may reflect a more widespread difficulty in movement initiation, principally involving the supplementary motor cortex, basal ganglia and the anterior parts of the cerebral cortex (Caruso, 1991; Webster, 1988).

However, it should also be pointed out that speech production can modulate hand muscle activity (Sparing et al., 2007). Several proposals suggest that speech might have evolved from hand gesture control (Corballis, 2002; Rizzolatti and Arbib, 1998), predominantly involving the left hemisphere (Meguerditchian and Vauclair, 2006; Meguerditchian et al., 2010). If this is true, a link between hand areas and articulatory muscles is likely to have remained, explaining the subtle differences between stutterers and normal speakers in manual non-communicative tasks. In this view, hand-related motor deficits may be a secondary effect of DS, which occurs as a function of the central interconnectivity between speech and manual functions (Saltuklaroglu et al., 2009). Deficits in stutterers have been reported in the left hemisphere, which is usually dominant for language and hand preference in right-handed people (Khedr et al., 2002). In this regard, the present findings are interesting if compared to those of Neef et al. (2011b) that show steeper stimulus–response curves in the tongue motor representation of stutterers during muscular activation. In fact, it could be suggested that different muscles may have different relations with DS symptoms. However, Neef et al. (2011b), recorded tongue motor indexes during muscular activation, while in the present investigation the MEP stimulus–response curve was obtained at resting. Therefore, this aspect could hypothetically play a role (Benwell et al., 2007; Samii et al., 1996). These apparently conflicting results may also be found in different neurological illnesses, as for example in multiple sclerosis (Kale et al., 2009; Thickbroom et al., 2006) and Parkinson’s disease (Tremblay and Tremblay, 2002), even if the results regarding motor activity may be muscle–dependent (Vacherot et al., 2010).

Interestingly, Wu et al. (1997) suggested that stutterers have an overactive dopaminergic system in basal ganglia. In fact, the present results are also similar to those found in Tourette’s Syndrome (Orth et al., 2008), another basal ganglia-related disorder analogous to DS (Mulligan et al., 2003). Giraud et al. (2008) have shown that a negative correlation between severity of disfluencies and basal ganglia activity in DS is present, even if also a positive correlation between stuttering severity and basal ganglia activity was reported (Kell et al., 2009). This latter experiment suggests that abnormal functioning of the basal ganglia in stuttering could be the response to a defect in the left inferior frontal region and not vice versa.

Connectivity studies in DS support this hypothesis, showing abnormal/weaker connections (Lu et al., 2010b, 2009; Neef et al., 2011a; Salmelin et al., 2000), lower density of white matter fibres (Chang et al., 2011, 2008; Cykowski et al., 2010; Sommer et al., 2002; Watkins et al., 2008), and/or grey matter (Kell et al., 2009) in regions including the left inferior frontal gyrus (Chang et al., 2011; Kell et al., 2009), left motor and premotor regions (Chang et al., 2011; Cykowski et al., 2010) and the bilateral corticospinal tract (Chang et al., 2008). However, patterns of abnormally increased white matter fibres in neighbouring regions have also been reported (Kell et al., 2009; Watkins et al., 2008), including the right hemisphere (Chang et al., 2011, 2008). Finally, the possibility of a different anatomical constitution of the stuttering brain should be kept in mind (Foundas et al., 2001; Lu et al., 2010b; Watkins et al., 2008). This could be hypothetically related also to a different response to magnetic stimulation (Wagner et al., 2008, 2006), compared to a non-stuttering individual. This would help to explain the present results from a physical point of view, but confirms the evidence that the brain in stutterers may be different from normal speakers.
4.4. Clinical considerations on DS and conclusions

The present study provides further evidence that DS is a condition characterized by general motor signs (Chang et al., 2009; Smits- van Gunsteren et al., 2009). If any case, future research is needed on other corticospinal districts, such as the orofacial area (despite the challenging methodological aspects related with its registration, see Neef et al., 2011b), or those related to control of legs. In fact, coupling between leg muscle activity and speech has been reported (Liuzzi et al., 2008), similar to the functional connection with hand/arm muscular districts (Bernardis et al., 2008; Gentilucci et al., 2004; Gentilucci and Dalla Volta, 2008). Moreover, while hand/arm activations have clearly been found to have an effect on tongue activations (both speech- and non speech-related; Gentilucci et al., 2009), it cannot be excluded that other districts have a similar but indirect effect (Gentilucci et al., 2009).

However, our results may be relevant in terms of re-evaluating the management of stuttering syndrome when a rehabilitation program or a pharmacological treatment is proposed. In these cases, TMS could be used to evaluate possible changes in corticospinal excitability induced by rehabilitation and/or pharmacological treatment. Finally, it could be used to investigate the possibility that subgroups of stutterers exist, characterized by a lower excitability when stimulating the left motor areas with respect to normal speakers. Before these results can be applied in a clinical setting, the present observations will require further investigation on the role played by other social and personality aspects often associated with stuttering (e.g., social phobia, anxiety, arousal levels, attention, developmental and/or personality disorders or depressive symptoms; Ardila et al., 1994; Blumgart et al., 2010; Iverach et al., 2010, 2009). Moreover, confirmation is needed to relate the present observations with behavioural outcomes and to deal with the different subtypes of stutterers (Alm, 2004), and the possibility of different cortical excitability profiles. Finally, it is very important to specify that the present results should be applied only to adult stutterers. It would however be interesting to extend the investigation to children to determine whether these findings could be related to a pre-existing neural deficit or with long-term alterations in motor control and/or a loss of stuttering.

In conclusion, the present study suggests that DS might have lower left corticospinal excitability (speculatively compensated by the right hemisphere), in agreement with several lines of evidence from previous research (see above). Importantly, this abnormal excitability is evident in hand motor representation, suggesting that stuttering might be a broader motor disorder.

Acknowledgements

The authors are very grateful to Dr. Pia Rothstein and to the anonymous reviewers that helped to improve the effective presentation of results, comprehension and readability of the paper.

Supplementary material


References


Perneger TV. Adjusting for multiple testing in studies is less important than other concerns. British Medical Journal, 318(7193): 1288, 1999.


