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Effect of muscular activation on surrounding motor networks in developmental stuttering: A TMS study

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ABSTRACT

Previous studies regarding developmental stuttering (DS) suggest that motor neural networks are strongly affected. Transcranial magnetic stimulation (TMS) was used to investigate neural activation of the primary motor cortex in DS during movement execution, and the influence of muscle representations involved in movements on "*surrounding*" ones. TMS was applied over the contralateral *abductor digiti minimi* (ADM) motor representation, at rest and during the movement of homologue first dorsal interosseous muscles (tonic contraction, phasic movements cued by acoustic signalling, and "*self-paced*" movements). Results highlighted a lower cortico-spinal excitability of ADM in the left hemisphere of stutterers, and an enhanced intracortical inhibition in their right motor cortex (in comparison to fluent speakers). Abnormal intracortical functioning was especially evident during phasic contractions cued by "*external*" acoustic signals. An exaggerated inhibition of muscles not directly involved in intended movements, in stuttering, may be useful to obtain more efficient motor control. This was stronger during contractions cued by "*external*" signals, highlighting mechanisms likely used by stutterers during fluency-evoking conditions.

1. Introduction

Developmental stuttering (DS) is a disturbance in which the rhythm of speech and its normal flow is impaired. It is characterized by symptoms such as blocks and/or repetitions, especially at the start of words and sentences, but also by secondary symptoms such as associated jerks and/or grimaces of the oro-facial muscular districts.

DS usually appears during childhood; it may spontaneously recover, but may also persist in adulthood. The causal factors are not fully clarified, but it is now evident that DS is a multi-factorial disorder involving genetic factors as well as neurological impairments (Chang, Garnett, Etchell, & Chow, 2019; Etchell, Civier, Ballard, & Sowman, 2018). More specifically, previous studies have demonstrated that DS is characterized by broad impairments of neural networks (especially in the left hemisphere) involving brain regions such as the left inferior frontal cortex, the associative and the primary motor cortices, and temporo-parietal regions (Chang et al., 2018, 2019; Etchell et al., 2018). Impairments are evident at a structural (e.g. abnormal grey and white matter connections) and functional level (e.g. impaired/lower excitability of the sensorimotor networks of the left hemisphere *vs.* augmented activity of homologue regions of the right one; Chang et al., 2019; Etchell et al., 2018).

In this context, DS should be more appropriately considered as a "*dynamic*" disorder of motor control, where an impaired/delayed exchange of information is evident in neural networks, thus easily resulting in motor disruptions and stuttering symptoms (e.g. Busan et al., 2019; Ludlow & Loucks, 2003; Salmelin, Schnitzler, Schmitz, & Freund, 2000). It has been demonstrated that DS is also characterized by

Abbreviations: ADM, Abductor Digiti Minimi; AAF/DAF, Altered/Delayed Auditory Feedback; DS, Developmental Stuttering; EMG, Electromyography; FDI, First Dorsal Interosseous; ISI, Interstimulus Interval; MEP, Motor Evoked Potential; RMT, Resting Motor Threshold; SD, Standard Deviation; SSI-4, Stuttering Severity Instrument-4; SMA, Supplementary Motor Area; TMS, Transcranial Magnetic Stimulation

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abnormal/impaired functioning of basal ganglia and the related cortico-basal-thalamo-cortical system (e.g. Alm, 2004; Craig-McQuaide, Akram, Zrinzo, & Tripoliti, 2014; Giraud et al., 2008; Lu et al., 2010; Toyomura, Fujii, & Kuriki, 2011, 2015). Dopamine alterations are evident in basal ganglia of people who stutter (e.g. Wu, Maguire, Riley, & Lee, 1997; see for example Maguire, Riley, Franklin, & Gottschalk, 2000; Maguire et al., 2004; Tavano, Busan, Borelli, & Pelamatti, 2011, for a pharmacological perspective). Compatibly, DS may easily result in impairments when elaborating volitional motor information, i.e. managed by an "*internal timing network*", mainly relying on the correct functioning of structures such as the basal ganglia and supplementary motor cortex. In opposition, motor information elaborated on the basis of "*external*" cues is related to the correct functioning of different systems involving structures such as the cerebellum and/or premotor regions (compare with Alm, 2004; Etchell, Johnson, & Sowman, 2014, for a perspective in stuttering).

Therefore, neural networks of cortico-basal-thalamo-cortical systems are strongly involved in the preparation, execution, and control of voluntary motor acts or in complex motor sequences, such as speech (e.g. Hertrich, Dietrich, & Ackermann, 2016, for a perspective about cortico-basal-thalamo-cortical systems and their principal cortical "*hub*", i.e. supplementary motor area). These networks modulate the correct release of the "*intended*" (i.e. voluntary) movement, as well as the correct inhibition of all competing (i.e. "*unintended*") motor actions, thus influencing also the neural activity of muscular representations that are different but (often) close to the activated ones (e.g. Calabresi, Picconi, Tozzi, Ghiglieri, & Di Filippo, 2014). As a consequence, these systems are fundamental in the management of neural information (mainly) related to the "*timing*" of motor sequence release or inhibition, and this may be evident in all muscular districts participating in the implementation/execution of the intended (volitional) movements.

In this context, the brain dynamics related to "*muscular interplay*" (i.e. the neural influence that effectors may have on the closer ones, especially during motor implementation and execution) have been not clarified in stuttering: their comprehension may help to obtain a better understanding of the physiopathology underlying this disturbance. As a matter of fact, stuttering can be an overt symptom of a more general motor syndrome (i.e. easily resulting in clinically evident motor/speech disruptions; see Busan, Battaglini, & Sommer, 2017, for a recent revision of motor excitability in different muscular districts -i.e. hand and speech muscles-, in DS). As already reported above, previous studies have suggested that stuttering is a motor/speech problem characterized by aberrant or defective (i.e. "*delayed*") exchange of neural information in sensorimotor networks (e.g. Busan et al., 2019; Ludlow & Loucks, 2003; Salmelin et al., 2000): the abnormal functioning of discrete brain regions (e.g. the left inferior frontal cortex; see Desai et al., 2017), as well as abnormalities in white matter structure (e.g. Connally, Ward, Howell, & Watkins, 2014; Sommer et al., 2002; Watkins, Smith, Davis, & Howell, 2008), and the abnormal activation of discrete neural circuits (e.g. the cortico-striato-thalamo-cortical system; see Alm, 2004; Craig-McQuaide et al., 2014), may continuously interact, in DS (compare with Alm, 2004; Etchell et al., 2014; Ludlow & Loucks, 2003). As a consequence, abnormal neural patterns may easily result in impaired neural "*synchronization*", with respect to fluent speakers: this suggestion is supported by findings showing that motor activity may be found as "*anticipated*" or "*delayed*", in DS (mainly depending from tasks and experimental settings; e.g. Busan et al., 2019; Ning, Peng, Liu, & Yang, 2017; Salmelin et al., 2000), finally resulting in defective activations of muscular effectors (see Busan et al., 2017).

Compatibly, Transcranial Magnetic Stimulation (TMS) can be successfully used to evaluate the presence of alterations in cortical excitability and in cortical inhibition of motor networks (see Busan et al., 2017, for a perspective in stuttering). TMS has been employed in different basal ganglia-related disorders (e.g. Parkinson's disease, Tourette syndrome and focal dystonia), showing the presence of altered motor mechanisms (e.g. Cantello, Tarletti, & Civardi, 2002; Sohn & Hallett, 2004a; Shin, Kang, & Sohn, 2007; compare also with Jahanshahi & Rothwell, 2017). Thus, TMS may help to highlight the presence of alterations in cortical mechanisms of movement preparation/execution and in motor control, especially when considering primary motor cortex and its intracortical networks (see Busan et al., 2017, for a perspective in stuttering). As a matter of fact, TMS has already highlighted the presence of abnormal/impaired modulations of motor circuits in stuttering, at rest and during very simple motor tasks (e.g. Busan et al., 2009, 2016; Neef, Paulus, Neef, von Gudenberg, & Sommer, 2011): a defective regulation of the excitability of primary motor cortex of speech/hand muscles is evident, usually represented by a lower cortico-spinal/cortico-bulbar excitability of the left motor cortex (with respect to the right one, or with respect to fluent speakers; see Busan et al., 2017). In this context, a defective balance in intracortical motor networks may lead to the incorrect implementation and realization of "*complex*" motor acts (such as speech). Compatibly, an altered equilibrium in excitatory/inhibitory intracortical motor networks is evident in DS (e.g. Alm, Karlsson, Sundberg, & Axelson, 2013; Busan et al., 2009, 2013Busan et al., 2016; Neef, Jung, et al., 2011; Neef, Paulus, et al., 2011; Sommer et al., 2002). However, TMS studies of stuttering did not investigate the mutual influence among muscular districts, during the preparation/execution of a movement. This matter has been largely investigated in previous TMS studies of other basal ganglia-related motor disorders, showing that defective inhibition is evident in muscles not currently involved in the requested motor act (e.g. Sohn & Hallett, 2004a; Shin et al., 2007). Thus, we hypothesize that this may be true also in stuttering.

As a consequence, in the present work, TMS was used to evaluate "*muscular interplay*" in stuttering, by investigating motor representations of hand muscles during the execution of finger movements. We were interested in better understanding the neural influence of the activated muscular districts on muscles that may be "*potentially*" involved in successive movements (as usually happens in speech). We hypothesize that DS may result in abnormal/impaired mechanisms of intracortical modulation of muscles that are not directly involved in actual movements, thus resulting in abnormal/disrupted neural signalling and/or motor excitability, impairing the "*correct*" implementation/execution of movements. In this context, we evaluated participants at rest and during tonic muscular contractions, as well as during "*self-paced*" and "*cue-related*" phasic movements. These different tasks have been selected in order to have the possibility to compare i) "*sustained*" *vs*. "*dynamic*" (i.e. tonic *vs*. phasic) motor acts, as well as ii) "*volitional*"/internally generated movements (i.e. more relying on "*internal timing network*") *vs.* movements driven by sensorial cues (i.e. more relying on "*external timing network*"). This selection was useful to define if different neural dynamics may be evident in distinct movement categories, in DS.

2. Materials and methods

2.1. Recruitment of participants and behavioural evaluation

Thirty-one right-handed male participants were recruited for this study. Fifteen (age range 24–47 years, mean 32.3, standard deviation -SD- ± 8.0) were stutterers, while 16 (age range 21–48 years, mean 29.8, SD ± 7.4) were fluent speakers. Fourteen DS participants (age range 24–47 years, mean 32.3 years, SD ± 8.4) and 14 fluent speakers (age range 21–48 years, mean 29.8 years, SD ± 7.5) were able to give meaningful data from experimental tasks: one DS participant and two fluent speakers dropped out after the initial TMS evaluation at 150% of resting motor threshold (RMT; see below) due to excessive discomfort. One DS participant was not able to realize "*self-paced*" evaluation (see below) for technical problems.

The two groups were comparable considering variables such as age, handedness (Edinburgh Handedness Inventory; Oldfield, 1971; see Table 1), educational level, musical instruments expertise, physical activity, smoking habits, depressive symptoms (Beck Depression

Table 1
Comparison of characteristics of groups. Data about age (years), education (years), and handedness (Oldfield, 1971) are reported by using means (± SD). Categorical data such as smoke habits, migraine, training with musical instruments, and sport habits are indicated by reporting ratios of participants (yes/no; single/paired pulse TMS sub-groups in brackets).

Characteristics/Groups	Stuttering	Fluent Speakers	p	p (single/paired pulse TMS sub-groups)
Age	32.3 ± 8.0	29.8 ± 7.4	p = 0.38	p = 0.52
Education	17.2 ± 3.6	15.8 ± 2.1	p = 0.33	p = 0.11
Handedness	85.4 ± 12.0	85.2 ± 12.2	p = 0.82	p = 0.69
Smoke Habits	4/11 (4/10)	4/12 (4/10)	p = 0.76	p = 1
Migraine	2/13 (2/12)	1/15 (1/13)	p = 0.95	p = 1
Training with Musical Instruments	5/10 (5/9)	4/12 (4/10)	p = 0.91	p = 1
Physical Training	7/8 (7/7)	13/3 (11/3)	p = 0.10	p = 0.24
BDI-II	4.2 ± 4.7	2.9 ± 3.9	p = 0.53	p = 0.53
BigCAT	**23 ± 10.0**	**4.4 ± 3.3**	**p < 0.001**	**p < 0.001**

Inventory-II; Beck, Steer, & Brown, 1996), and migraine. DS participants were stutterers since childhood, with no associated neurological/psychiatric conditions. Fluent speakers reported no history of stuttering or other fluency disorders (even in childhood and/or adolescence), and had no associated neurological/psychiatric conditions. Participants were not taking drugs that act on the central nervous system, and had no contraindications to TMS (see Rossi, Hallett, Rossini, & Pascual-Leone, 2009; Rossi, Hallett, Rossini, & Pascual-Leone, 2011). Stuttering participants were evaluated using the Stuttering Severity Instrument-4 (SSI-4; Riley, 2009). An audio–video sample of spontaneous speech (about three-four minutes) and a reading passage was obtained to evaluate the percentage of stuttered syllables, the longest block durations, and movements associated with dysfluencies. Samples of about 250 words (after disregarding the initial 25 words) were always evaluated and rated by the same researcher (P.B.). The scoring was then confirmed by a therapist specialized in fluency disorders (S.B.). All participants completed a questionnaire about speech-related attitudes (BigCAT; Vanryckeghem & Brutten, 2012).

Experimental procedures were approved by the regional Ethics Committee of the Friuli-Venezia Giulia region. All procedures were in accordance with the Declaration of Helsinki, and participants signed a written informed consent form.

2.2. Transcranial magnetic stimulation settings and experimental tasks

TMS (Medtronic MagPro R30) was administered using a figure-of-eight coil (Medtronic C-B60; antero-posterior direction of the first phase of the current in the coil; biphasic wave). Participants wore a tissue cap where a grid of 1 cm-spaced points was drawn. Self-adhesive disposable electrodes (Ag/AgCl) were placed on a tendon belly montage over *abductor digit minimi* (ADM) and first dorsal interosseous (FDI) muscles, of every hand. Electromyography (EMG) was recorded using a digital band pass filtering of 20–2000 Hz (sampling rate 8000 HZ). TMS coil was maintained at 45° with respect to the inter-hemispheric fissure, with the handle pointing backwards.

First, the position on the scalp (hot-spot) that allowed to obtain the highest and more reproducible motor evoked potentials (MEPs) from the contralateral ADM muscle was individuated, in every hemisphere (participants at rest, open eyes). Thus, resting motor threshold (RMT) was bilaterally individuated as the stimulation intensity resulting in a MEP of at least 50 μV in half of 8–10 consecutive trials, when stimulating the hot-spot. Resting state was always verified by EMG on-line visual inspection. Successively, five MEPs (see Cavaleri, Schabrun, & Chipchase, 2017) were recorded by stimulating the contralateral ADM motor representation at 150% of RMT (at rest, open eyes), in every hemisphere, to replicate observations obtained in Busan et al. (2013). Thus, single and paired pulses protocols were administered.

Single pulse TMS allows a reliable evaluation of the cortico-spinal excitability of the stimulated motor pathways, especially when stimulation intensities represent a discrete interval well above RMT (e.g. Christie, Fling, Crews, Mulwitz, & Kamen, 2007; Hashemirad, Zoghi, Fitzgerald, & Jaberzadeh, 2017). On the other hand, paired pulse TMS protocols allow to evaluate the influence of intracortical networks on motor outputs, as well as their functioning (see Kujirai et al., 1993). These protocols have been administered stimulating the contralateral ADM primary motor cortex representations, at rest and during contraction of the FDI muscle of the same hand, in different conditions (always with open eyes). More specifically, MEPs at 130% of RMT were bilaterally recorded at rest. Then, in the same condition (i.e. rest), a paired pulse protocol was used: the (first) conditioning stimulus was delivered at 70% of RMT and the (second) test stimulus at 130% of RMT. Interstimulus intervals (ISIs) were at 3 and 5 ms. These different time intervals were mainly used to evaluate short intracortical inhibition (compare with the original publication of Kujirai et al., 1993): the shorter interval should result in more reliable levels of intracortical suppression, while the longer one is used as a control, e.g. to evaluate possible unspecific effects of stimulation. Thus, measures obtained at rest represent a baseline level of the recorded indexes, useful for comparisons with motor tasks. In fact, these protocols were also applied during the tonic contraction of FDI (i.e. pushing the index finger against a surface, maintaining its contraction; left and right hand, separately; maximal voluntary contraction) and during the phasic contraction of the same muscle (about 30–40% of maximal voluntary contraction). In the second case, two different conditions were investigated: the phasic contraction was "*cued*" by an acoustic signal (duration 0.23 sec, frequency 650 Hz), allowing participants to move the index finger and pushing it against a surface; otherwise, participants were asked to accomplish the same movement in a "*self-paced*" condition (i.e. they were allowed to decide when execute the movement, without "*external*" signal).

TMS delivery during phasic contractions was managed by a customized electronic circuit that sent the magnetic pulse about 10–60 ms after the passage of the index finger beyond an optic sensor, thus resulting in (random) stimulations in a time range (normally) comprised between 100 and 200 ms after the start of EMG activity in FDI (i.e. always stimulating during movement execution). The adopted timing is compatible with the start of a successive movement in a fast finger tapping sequence (compare with Repp, 2005), thus allowing to evaluate the neural influence of the activated muscular districts on muscles that may be involved in successive movements. Participants were always asked to activate the FDI muscle minimizing activity in ADM, and favouring accuracy of movement over speed. Conditions were presented in a randomized order; the contralateral and ipsilateral ADM and FDI muscles were always recorded (to verify contraction and relaxation levels). Five MEPs for each condition were recorded (see Cavaleri et al., 2017) and stored for successive analysis. The entire procedure lasted 90–120 min. Experimental setting is summarized in Fig. 1.

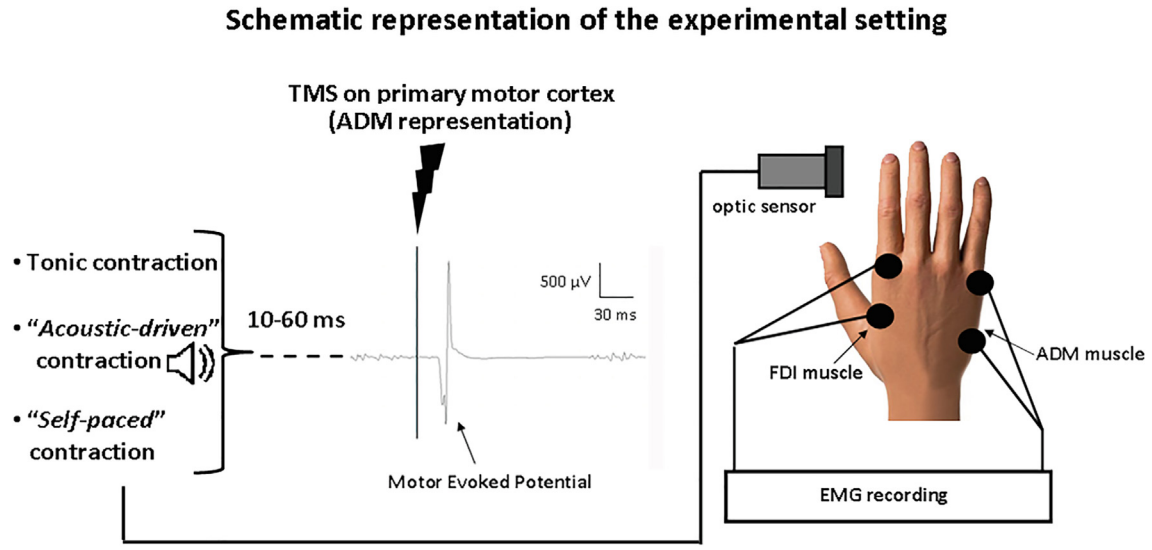

Fig. 1. Schematic representation of the experimental setting. A schematic representation of experimental tasks, recorded muscles, TMS timing and delivery is reported.

2.3. Data elaboration and statistics

RMT was expressed as the percentage of the maximum stimulation intensity of TMS. MEPs were calculated from ADM and FDI channels. MEPs allowed to evaluate the excitability of the cortico-spinal pathways activated by the magnetic pulse. Thus, peak-to-peak MEPs amplitude (μV) and MEPs area (V/s) of each recorded trace and condition were computed during off-line analysis. MEPs latency (ms) was also calculated when considering single pulse TMS. Peak-to-peak amplitudes may be useful to characterize the "*magnitude*" of neural firing (i.e. higher excitability results in higher amplitudes); MEPs latency is used to characterize the "*speed*" of this firing (i.e. higher excitability results in "*faster*" or lower latencies), while MEPs area may be useful to combine information about the "*magnitude*" of neural firing and the "*duration*" of the response, allowing to obtain further and more complete information about cortico-spinal excitability. Intracortical modulation values were computed by calculating the ratio of the value obtained by the test stimulus *vs.* the value previously obtained when using the unconditioned stimulus (i.e. single pulse TMS) in the same condition. Pre-stimulus EMG activities (i.e. 60 ms before TMS delivery) of the ADM and FDI muscles were also recorded in each condition and channel, computed, and expressed in V/s.

Different levels of statistical analyses were conducted: behavioural data (as well as variables such as age, handedness, education, etc.) were compared using Student's *t*-test (normally distributed and homogenous data), Welch's *t*-test (normally distributed but not homogeneous data), Mann-Whitney non-parametric test (not normally distributed data), or Chi-square statistic (Yates correction, categorical data).

RMT, MEPs, and pre-TMS EMG data were always analyzed using a linear mixed model approach (see Faraway, 2006; West, Welch, & Galecki, 2006). Overall, mixed model analysis is preferable considering that it may better account for random effects (i.e. sources of variability in data), and it may be quite robust also when assumptions are not optimally suited (e.g. when considering distribution of random effects or residual errors). Some major advantages of mixed model analysis are that it doesn't need to assume the presence of independence in data (i.e. correlations can be evident within a unit or a cluster, as it could be the case for present MEPs data), and it can be able to successfully manage datasets with missing observations.

When considering RMT, the effects of main factors (i.e. groups and stimulated hemisphere) and their interaction were evaluated. MEPs obtained at 150% of RMT (ADM; amplitudes, areas and latencies) were evaluated by considering the effects of main factors (i.e. groups and stimulated hemisphere), as well as their interaction. MEPs amplitudes, areas, and latencies obtained by using single pulse TMS delivered at 130% of RMT, and paired pulse TMS -ISIs 3 and 5 ms-, were evaluated on ADM muscles by calculating the effect of main factors (groups, stimulated hemisphere, and recording condition -rest, tonic contraction, phasic "*acoustic-driven*" contraction, phasic "*self-paced*" contraction-), as well as their interactions. Pre-TMS EMG data were also analyzed (ADM and FDI muscles) by considering the effect of main factors (groups, side of contraction -left *vs.* right hand-, and recording condition -rest, tonic contraction, phasic "*acoustic-driven*" contraction, phasic "*self-paced*" contraction-), as well as their interactions. When considering post-hoc analysis (i.e. Student's *t*-test, Mann-Whitney test, or Wilcoxon test, depending on data characteristics), we were also able to report an estimate of the corresponding effect size when significant "*two-means*" comparisons were encountered. This was done depending on conditions such as data normality, "*between*" or "*within*" statistical design. Effects (refer to Lenhard & Lenhard, 2016) were reported in absolute values using *Hedges' g/Cohen's* $d_{unbiased}$, d ($0.2 < d_{unbiased}, d < 0.5$ = small effect; $0.5 < d_{unbiased}, d < 0.8$ = medium effect; $d_{unbiased}, d > 0.8$ = large effect), or r values ($0.1 < r < 0.3$ corresponds to small effect size; $0.3 < r < 0.5$ corresponds to medium effect size; $r > 0.5$ corresponds to large effect size). In non-parametric comparisons both r and $d_{unbiased}$ -or d- are reported to allow a more complete evaluation of the effect. Control analyses were also performed (when appropriate) by considering similar data obtained from FDI (statistics performed by using Student's *t*-test, Mann-Whitney test, or Wilcoxon test, depending on data characteristics). A two-tailed $p < 0.05$ was always considered as significant ($0.05 < p < 0.10$: trend toward significance).

Finally, a correlation analysis was also performed to evaluate if relations may exist among indexes of stuttering severity, speech attitude, TMS, and pre-TMS EMG data. It was computed using Pearson's correlation (normally distributed data), Spearman's correlation (not normally distributed data), or Gamma (Γ) correlation (not normally distributed data and "*tied*" observations). A $p < 0.05$ was considered significant.

Pre-TMS EMG data analysis, control analysis on TMS data of FDI muscles, and correlation analysis have been computed in an explorative way for a better understanding and interpretation of the main findings (i.e. the effect of the neural influence of activated muscular districts on muscles "*potentially*" involved in successive movements). As a

Table 2
Results obtained from Stuttering Severity Instrument-4 (SSI-4) and BigCAT.

Participants	SSI-4 value	Percentile	Classification	BigCAT
A	12	1–4	Very mild	7
B	13	5–11	Very mild	7
C	18	12–23	Mild	15
D	21	24–40	Mild	9
E	23	24–40	Mild	25
F	25	41–60	Moderate	32
G	25	41–60	Moderate	21
H	26	41–60	Moderate	27
I	28	61–77	Moderate	14
J	30	61–77	Moderate	32
K	31	61–77	Moderate	32
L	31	61–77	Severe	33
M	32	78–88	Severe	32
N	32	78–88	Severe	32
O	36	89–95	Severe	27

consequence, for expositive clarity, these analyses have been included in the Supplementary Material.

3. Results

3.1. Behavioural evaluation

SSI-4 (Riley, 2009) was used to measure stuttering severity in DS participants: three resulted as severe, seven as moderate, three as mild, and two as very mild. Experimental and control groups significantly differed when considering speech and communication attitudes (BigCAT; Vanryckeghem & Brutten, 2012), indicating higher difficulties in the DS group (Mann-Whitney test, $p < 0.001$; $r = 0.812$, large effect size; *Hedges' g/Cohen's* $d_{unbiased} = 2.537$, large effect size). Data are summarized in Table 1 and in Table 2.

3.2. RMTs in primary motor cortex ADM representations

RMTs of ADM muscles representations in primary motor cortex did not result in significant differences between groups (DS *vs.* fluent speakers) or stimulated hemisphere (left *vs.* right), as well as in their interaction.

3.3. Single pulse stimulation of primary motor cortex ADM representations: MEPs at 150% of RMT

Peak-to-peak MEP amplitudes, obtained at 150% of RMT when stimulating the contralateral representations of ADM in primary motor cortex, resulted in a significant effect with the statistical model ($p < 0.001$) indicating the presence of a difference between stimulated hemispheres ($t_{26} = -3.23$, $p = 0.003$; difference not supported by post-hoc analysis), but, more importantly, significance was highlighted in the interaction between groups and stimulated hemisphere ($t_{24} = 2.43$, $p = 0.023$). This indicates that DS was characterized by lower MEPs when stimulating the left hemisphere, compared to the right one (Wilcoxon test, $p = 0.022$, $r = 0.612$, large effect size; $d = 1.551$, large effect size), as well as with respect to the left hemisphere of fluent speakers (Mann-Whitney test, $p = 0.007$; $r = 0.475$, medium effect size; *Hedges' g/Cohen's* $d_{unbiased} = 0.916$, large effect size). Similar findings were evident when considering MEP areas (significant effect of the statistical model, $p = 0.021$; main effect of the stimulated hemisphere, $t_{26} = -3.20$, $p = 0.004$ -lower MEPs in the left hemisphere than in the right one, Wilcoxon test $p = 0.079$, $r = 0.321$, medium effect size; $d = 0.453$, small effect size-; interaction between groups and stimulated hemisphere, $t_{24} = 2.44$, $p = 0.022$ -DS was characterized by lower MEPs when stimulating the left hemisphere, compared to the right one [Wilcoxon test $p = 0.009$, $r = 0.696$, large effect size; $d = 2.009$, large effect size], and with respect to the left hemisphere of fluent speakers [Mann-Whitney test $p = 0.011$, $r = 0.454$, medium effect size; *Hedges' g/Cohen's* $d_{unbiased} = 0.925$, large effect size]-). No differences were evident when considering MEP latencies. Data are summarized in Table 3.

3.4. Single pulse stimulation of primary motor cortex ADM representations: MEPs at 130% of RMT

3.4.1. Peak-to-peak MEP amplitudes

When considering peak-to-peak MEP amplitudes, the statistical model resulted in significant differences ($p < 0.001$), indicating an effect of the main factor related to the requested task ($t_{24} = 2.55$, $p = 0.018$; $t_{24} = 3.08$, $p = 0.005$; $t_{24} = 2.96$, $p = 0.007$; in general, lower MEPs were recorded at rest *vs*. tonic and phasic movements in both groups). A significant interaction among groups, tasks, and the stimulated hemisphere was also found ($t_{20} = 2.62$, $p = 0.016$), suggesting that lower cortico-spinal excitability was evident in DS (compared to fluent speakers; see Fig. 2 and Table 4), when stimulating the left hemisphere: more specifically, this was true when considering MEPs obtained at rest (Mann-Whitney test, $p < 0.001$, $r = 0.608$, large effect size; *Hedges' g/Cohen's* $d_{unbiased} = 1.161$, large effect size), MEPs recorded during "*acoustic-driven*" phasic movements (Mann-Whitney test, $p = 0.009$, $r = 0.486$, large effect size; *Hedges' g/Cohen's* $d_{unbiased} = 1.215$, large effect size), and during "*self-paced*" phasic condition ($t_{25} = 2.62$, $p = 0.015$, *Hedges' g/Cohen's* $d_{unbiased} = 1.021$, large effect size). Phasic movements were also characterized by higher MEPs recorded in the left hemisphere of fluent speakers compared to the right one ("*acoustic-driven*": $t_{13} = 4.18$, $p = 0.001$, $d = 0.945$, large effect size; "*self-paced*": $t_{13} = 2.80$, $p = 0.015$, $d = 0.665$, medium effect size). Tonic contractions did not result in significant differences.

3.4.2. MEP areas

Similar findings were evident when considering MEP areas. Here, the statistical model yielded a significant difference ($p < 0.001$), indicating an effect of the main factor related to the requested tasks ($t_{24} = 2.14$, $p = 0.043$; $t_{24} = 3.07$, $p = 0.005$; $t_{24} = 2.57$, $p = 0.017$;

Table 3
TMS findings obtained when recording from the contralateral ADM muscles in DS and fluent speakers: resting motor thresholds (RMTs) and single pulse MEPs obtained at 150% of RMT. Significant comparisons are reported in bold. Data are represented reporting mean ± standard deviation, for every condition.

TMS index/Groups	Stuttering		Fluent Speakers	
	Left Hemisphere	Right Hemisphere	Left Hemisphere	Right Hemisphere
RMT (%)	46.5 ± 10.4	49.1 ± 11.4	49.9 ± 8.5	48.7 ± 7.2
150% RMT MEPs Ampl. (μV)	**791.4 ± 743.9***,**	**2075.0 ± 1864.5***	**1734.3 ± 1237.5****	1686.5 ± 1112.3
150% RMT MEPs Area (V/s)	**3476.3 ± 3311.3***,**	**11289.4 ± 11260.8***	**8520.0 ± 6871.1****	8140.8 ± 6161.8
150% RMT MEPs Latency (ms)	21.5 ± 1.0	21.6 ± 1.6	20.8 ± 1.3	21.4 ± 1.4

Abbreviations: Motor Evoked Potentials (MEPs), Resting Motor Threshold (RMT), Transcranial Magnetic Stimulation (TMS).
* Groups per stimulated hemisphere interaction, left hemisphere *vs.* right hemisphere in DS, $p = 0.022$ (ampl.), $p = 0.009$ (area).
** Groups per stimulated hemisphere interaction, left hemisphere, DS *vs.* fluent speakers, $p = 0.007$ (ampl.), $p = 0.011$ (area).

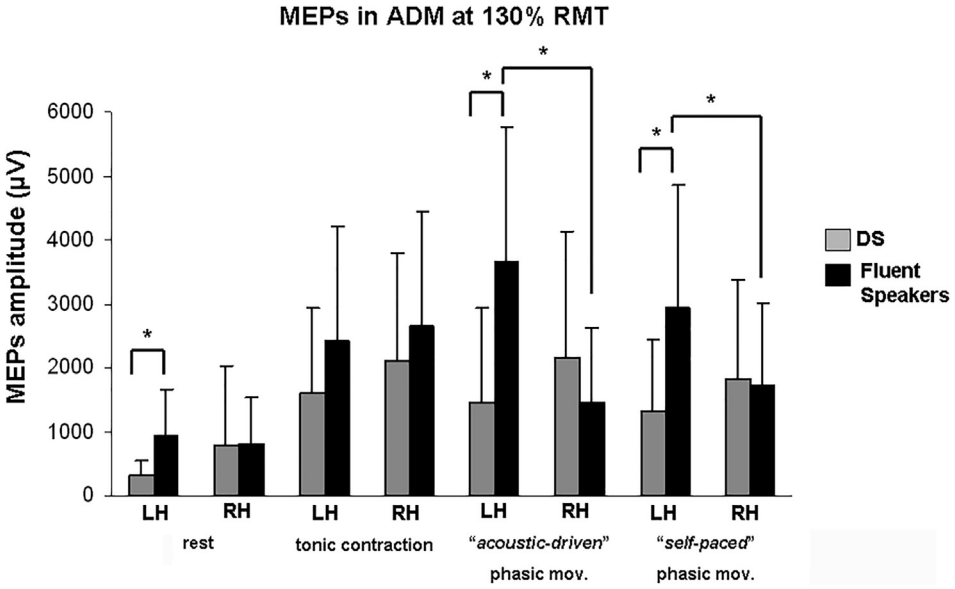

Fig. 2. Differences between DS and fluent speakers when single pulse TMS was applied on primary motor cortex ADM representation at rest and during tonic/phasic movements of the FDI muscle of the same hand. Data referring to peak-to-peak MEP amplitudes (μV) are reported (error bars representing standard deviations); fundamental (i.e. significant) differences are indicated by an asterisk (*).

in general, lower MEPs were evident at rest with respect to tonic/phasic movements). An effect of the interaction among groups, requested tasks, and stimulated hemispheres was also evident (t_{20} = 2.94, p = 0.008), again showing lower cortico-spinal excitability in DS, when stimulating the left hemisphere (compared to fluent speakers). This was evident during rest (Mann-Whitney test, p < 0.001; r = 0.616, large effect size; *Hedges' g/Cohen's* $d_{unbiased}$ = 1.168, large effect size), during "*acoustic-driven*" phasic movements (Mann-Whitney test, p = 0.006; r = 0.512, large effect size; *Hedges' g/Cohen's* $d_{unbiased}$ = 1.184, large effect size), as well as during "*self-paced*" phasic movements (t_{25} = 2.84, p = 0.009; *Hedges' g/Cohen's* $d_{unbiased}$ = 1.111, large effect size). Phasic movements were also characterized by higher MEP areas in the left hemisphere of fluent speakers, compared to the right one ("*acoustic-driven*": t_{13} = 4.26, p < 0.001; d = 0.966, large effect size; "*self-paced*": Wilcoxon test, p = 0.013; r = 0.663, large effect size; d = 0.686, medium effect size). Finally, at rest, MEP areas of the left hemisphere of DS participants were lower than the right one (Wilcoxon test, p = 0.074; r = 0.479, intermediate effect size; d = 0.450, small effect size). Again, tonic contractions did not result in significant differences.

Table 4
TMS findings obtained when recording from the contralateral ADM muscles in DS and fluent speakers: single pulse MEPs obtained at 130% of RMT. Significant comparisons are reported in bold, trends toward significance are reported in *italic*. Data are represented reporting mean ± standard deviation, for every condition.

TMS index/Groups	Stuttering		Fluent Speakers	
Resting condition				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
130% RMT MEPs Ampl. (μV)	**317.7 ± 222.0***	778.3 ± 1257.1	**942.0 ± 727.2***	805.0 ± 733.8
130% RMT MEPs Area (V/s)	***1152.3 ± 706.6**,°**	*3813.9 ± 6964.3°*	**4175.0 ± 3590.8***	3577.5 ± 3607.0
130% RMT MEPs Latency (ms)	22.8 ± 1.4	22.8 ± 1.5	21.7 ± 1.30	22.2 ± 1.4
Tonic muscular contraction				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
130% RMT MEPs Ampl.	1618.3 ± 1306.9	2101.3 ± 1688.6	2408.6 ± 1787.2	2653.6 ± 1784.2
130% RMT MEPs Area	7206.7 ± 5995.2	10313.1 ± 9339.8	11389.4 ± 9167.9	12588.1 ± 9204.0
130% RMT MEPs Latency	21.2 ± 1.8	21.6 ± 1.5	20.7 ± 1.5	20.8 ± 1.7
"Acoustic-driven" phasic contraction				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
130% RMT MEPs Ampl.	**1447.2 ± 1494.0***	2166.6 ± 1975.2	**3661.4 ± 2100.6*,****	**1449.9 ± 1168.1****
130% RMT MEPs Area	**6835.6 ± 8503.1***	11672.1 ± 12203.0	**19834.3 ± 12994.4*,****	**6556.3 ± 5435.2****
130% RMT MEPs Latency	21.0 ± 1.3	20.9 ± 1.2	20.6 ± 1.4	20.9 ± 1.6
"Self-paced" phasic contraction				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
130% RMT MEPs Ampl.	**1321.8 ± 1117.5***	1825.4 ± 1559.6	**2932.0 ± 1929.8*,****	**1715.3 ± 1300.0****
130% RMT MEPs Area	**6001.7 ± 5604.7***	8647.2 ± 8284.6	**15896.0 ± 11275.5*,****	**8481.2 ± 7158.2****
130% RMT MEPs Latency	21.2 ± 1.2	20.9 ± 2.0	20.8 ± 1.3	21.0 ± 1.1

Abbreviations: Motor Evoked Potentials (MEPs), Resting Motor Threshold (RMT), Transcranial Magnetic Stimulation (TMS).

* Groups per stimulated hemisphere per task interaction, left hemisphere, DS *vs.* fluent speakers; rest: p < 0.001 (ampl. and area); "acoustic-driven" phasic contraction: p = 0.009 (ampl.), p = 0.006 (area); "self-paced" phasic contraction: p = 0.015 (ampl.), p = 0.009 (area).

** Groups per stimulated hemisphere per task interaction, left hemisphere *vs.* right hemisphere in fluent speakers; "acoustic-driven" phasic contraction: p = 0.001 (ampl. and area); "self-paced" phasic contraction: p = 0.015 (ampl.), p = 0.013 (area).

° Groups per stimulated hemisphere per task interaction, left hemisphere *vs.* right hemisphere in DS, p = 0.074.

3.4.3. MEP latencies

Finally, when considering MEP latencies, the significance of the statistical model ($p < 0.001$) showed an effect related to the requested task ($t_{24} = -4.06$, $p < 0.001$; $t_{24} = -4.57$, $p < 0.001$; t_{24} = -2.85, $p = 0.009$): in general, MEPs obtained at rest were characterized by higher latencies (i.e. lower excitability) *vs*. tonic/phasic muscular contractions.

Findings are summarized in Table 4 and in Fig. 2.

3.5. Paired pulse stimulation at 3 ms ISI of primary motor cortex ADM representations

3.5.1. Peak-to-peak MEP amplitudes

Paired pulse stimulations delivered on the primary motor cortex ADM representations at an ISI of 3 ms resulted in significance of the statistical model ($p = 0.011$), especially when considering the interaction between groups and stimulated hemisphere ($t_{24} = -3.19$, $p = 0.004$), indicating that DS was characterized by higher intracortical inhibition when the right hemisphere was stimulated compared to fluent speakers (Mann-Whitney test, $p = 0.007$; $r = 0.504$, large effect size; *Hedges' g/Cohen's* $d_{unbiased} = 1.014$, large effect size). Additionally, fluent speakers had greater inhibition in intracortical motor circuits of the left hemisphere compared to the right one (Wilcoxon test, $p = 0.064$; $r = 0.495$, medium effect size; $d = 0.446$, small effect size).

3.5.2. MEP areas

Similar findings were evident when considering MEP areas (significance of the statistical model, $p = 0.048$): an effect of the interaction between groups and stimulated hemisphere was evident ($t_{24} = -2.86$, $p = 0.009$), but also the interaction among groups, stimulated hemisphere, and requested tasks resulted in a trend toward significant difference ($t_{20} = 1.89$, $p = 0.07$). More specifically, higher intracortical inhibition was evident in DS (*vs*. fluent speakers; Fig. 2 and Table 3) when the right hemisphere was stimulated (Mann-Whitney test, $p = 0.002$; $r = 0.565$, large effect size; *Hedges' g/Cohen's* $d_{unbiased} = 1.112$, large effect size): this was found when considering data obtained from tonic contractions ($t_{26} = 2.56$, $p = 0.008$; *Hedges' g/Cohen's* $d_{unbiased} = 1.082$, large effect size) and during "*self-paced*" phasic contractions ($t_{25} = 2.69$, $p = 0.012$; *Hedges' g/Cohen's* $d_{unbiased} = 1.058$, large effect size), but, more evidently, during "*acoustic-driven*" phasic contractions ($t_{26} = 4.01$, $p < 0.001$; *Hedges' g/Cohen's* $d_{unbiased} = 1.630$, large effect size). In addition, fluent speakers were characterized by higher inhibition when stimulating the left hemisphere compared to the right one (Wilcoxon test, $p = 0.056$; $r = 0.512$, large effect size; $d = 0.465$, small effect size), especially during "*acoustic-driven*" phasic movements (Wilcoxon test, $p = 0.004$; $r = 0.512$, large effect size; $d = 1.471$, large effect size). Finally, higher inhibition was generally evident at rest in fluent speakers, compared to tonic and/or phasic movements, in both hemispheres (statistics not reported).

3.6. Paired pulse stimulation at 5 ms ISI of primary motor cortex ADM representations

Paired pulse stimulations delivered in primary motor cortex ADM representations with an ISI of 5 ms did not result in significant differences of main factors or interactions.

All TMS findings are summarized in Fig. 2, Fig. 3, Table 3, Table 4 and in Table 5. As previously indicated, analysis referring to pre-TMS EMG activity (ADM and FDI), control analysis performed on FDI TMS data, and correlation findings are reported in the Supplementary Material.

4. Discussion

4.1. Summary of results

The main finding of the present work is that a simple movement of the index finger (activating the FDI muscle), results, in DS, in an exaggerated intracortical inhibition of the ADM muscle (i.e. a closer hand muscle not involved in the requested task). More specifically, this is evident in the contralateral districts, when the ADM representation of the right primary motor cortex is stimulated (paired pulse TMS, ISI 3 ms). This finding is also modulated by the requested task: it was highlighted during tonic contractions, during "*self-paced*" phasic movements but, more evidently, during "*acoustic-driven*" phasic movements. DS and fluent speakers showed reversed patterns of intracortical inhibition: fluent speakers resulted in higher intracortical inhibition of the left primary motor cortex, while DS resulted in higher intracortical inhibition of the right one. In general, higher inhibition was evident at rest in fluent speakers, when compared to motor tasks. Finally, DS resulted in a (generally) lower cortico-spinal excitability of the left primary motor cortex, as evaluated by single pulse stimulations. Complementary and control analyses reported in the Supplementary Material support these observations. In the following, present findings will be discussed considering the currently available evidence about neural mechanisms in stuttering, also focusing on the influence of cortico-striato-thalamo-cortical pathways on motor outputs.

4.2. Previous evidence of neural abnormalities in DS: relevance of present findings

DS is characterized by neural hallmarks that have been consistently reproduced using different techniques (e.g. Brown, Ingham, Ingham, Laird, & Fox, 2005; Neef, Anwander, & Friederici, 2015). In general, abnormal/impaired activity of speech/motor regions of the left hemisphere is evident, accompanied by over-activation of the homologue regions of the right one, involving complex fronto-temporo-parietal networks (see Etchell et al., 2018, for a review). This may be evident during speech, during not speech-related motor tasks, as well as during rest. These functional hallmarks are associated with impaired and/or abnormal white matter fascicles that connect speech/motor regions as well as anterior and posterior cortical sites (e.g. Chang, Erickson, Ambrose, Hasegawa-Johnson, & Ludlow, 2008; Chang, Horwitz, Ostuni, Reynolds, & Ludlow, 2011; Chang, Zhu, Choo, & Angstadt, 2015; Chow & Chang, 2017; Cieslak, Ingham, Ingham, & Grafton, 2015; Connally et al., 2014; Cykowski, Fox, Ingham, Ingham, & Robin, 2010; Sommer et al., 2002; Watkins et al., 2008; compare also with Chang et al., 2018). Thus, the most accepted vision is that DS relies on dysfunction of the left hemisphere, while neural networks of the right one may work to compensate these deficits (e.g. Busan et al., 2019; Chang et al., 2008; Garnett et al., 2018; Kell et al., 2009; compare with Chang et al., 2019; Neumann et al., 2003; Preibisch et al., 2003). Functional and anatomical abnormalities of the right hemisphere may be the result of compensatory strategies, or, more properly, may represent "*maladaptive*" processes. In this context, there is some evidence suggesting a more causative role of the right hemisphere in the physiopathology of DS (see Neef et al., 2016, 2018). At the end, motor output discharged by primary motor cortex may be continuously modulated by these abnormal neural interactions. Impaired or abnormal activity of related intracortical motor networks is also present (see Section 4.4). Compatibly, the finding seen herein of general lower excitability of cortico-spinal pathways of the left primary motor cortex (during rest and movement execution) sustain the vision that these pathways (and related networks) are defective in DS (this is confirmed also when considering pre-TMS EMG activity of the moving muscle -i.e. FDI-, in Supplementary Material).

On the other hand, the present findings regarding the modulation of intracortical motor networks during movement execution (especially

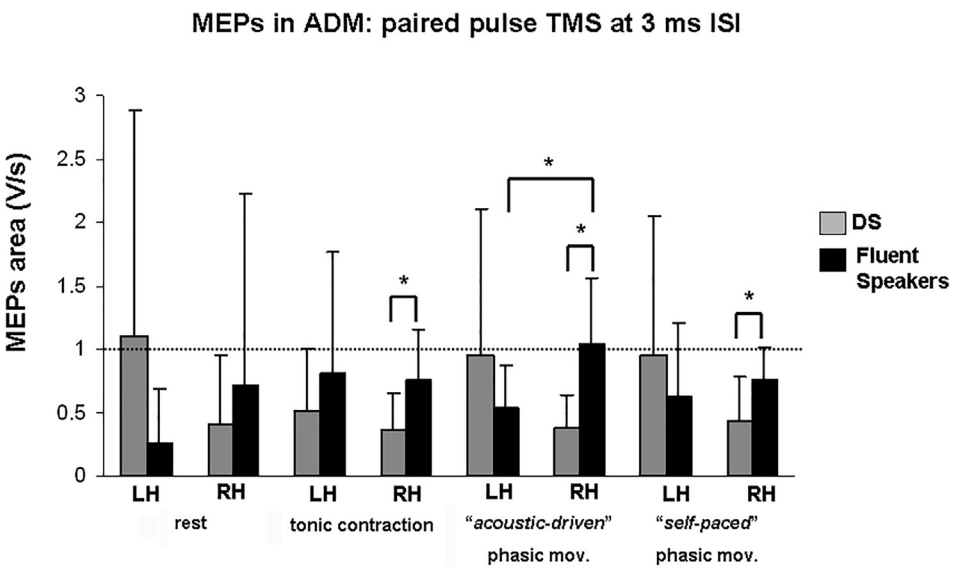

Fig. 3. Differences between DS and fluent speakers when paired pulse TMS (ISI: 3 ms) was applied on primary motor cortex ADM representations at rest and during tonic/phasic movements of the FDI muscle of the same hand. Data referring to MEP areas (V/s) are reported (error bars representing standard deviations); fundamental (i.e. significant and/or trends toward significance) differences are indicated by an asterisk (*).

when basing on "*acoustic-driven*", external, sensorial cues) suggest more complex interactions among the components of the motor system, especially when considering cortical and sub-cortical networks. They suggest that intracortical networks of the right primary motor cortex may have a role in exaggeratedly inhibiting muscles that are not directly involved in movement execution (ADM). This evidence may be an attempt to lower "*neural noise*" during movement execution, especially when based on sensorial/external cues, trying to facilitate actual motor acts in the form of ("*maladaptive*") compensation. These concepts will be developed in the next section.

Table 5
TMS findings obtained when recording from the contralateral ADM muscles in DS and fluent speakers: paired pulse TMS. Significant comparisons (main findings) are reported in bold, trends toward significance (main findings) are reported in *italic*. Data are represented reporting mean ± standard deviation, for every condition.

TMS index/Groups	Stuttering		Fluent Speakers	
Resting condition				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
Paired Pulse ISI 3 ms MEPs Ampl.(ratio)	0.87 ± 1.3	0.50 ± 0.8*	0.30 ± 0.4°	0.66 ± 1.1*,°
Paired Pulse ISI 3 ms MEPs Area (ratio)	1.10 ± 1.8	0.41 ± 0.5*	0.27 ± 0.4°	0.71 ± 1.5*,°
Paired Pulse ISI 5 ms MEPs Ampl. (ratio)	1.40 ± 1.8	1.71 ± 2.0	1.14 ± 1.0	1.34 ± 1.9
Paired Pulse ISI 5 ms MEPs Area (ratio)	1.71 ± 2.8	1.66 ± 2.0	1.26 ± 1.5	1.54 ± 2.5
Tonic muscular contraction				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
Paired Pulse ISI 3 ms MEPs Ampl. (ratio)	0.48 ± 0.43	0.39 ± 0.3*	0.75 ± 0.8°	0.74 ± 0.4*,°
Paired Pulse ISI 3 ms MEPs Area (ratio)	0.50 ± 0.50	***0.37*** ± ***0.3****,**	0.81 ± 1.0°	***0.75*** ± ***0.4****,**,°
Paired Pulse ISI 5 ms MEPs Ampl. (ratio)	0.90 ± 0.6	0.99 ± 0.6	1.17 ± 1.0	1.21 ± 0.9
Paired Pulse ISI 5 ms MEPs Area (ratio)	0.95 ± 0.7	0.94 ± 0.6	1.27 ± 1.3	1.31 ± 1.1
"Acoustic-driven" phasic contraction				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
Paired Pulse ISI 3 ms MEPs Ampl. (ratio)	0.86 ± 1.1	0.35 ± 0.20*	0.60 ± 0.3°	1.01 ± 0.5*,°
Paired Pulse ISI 3 ms MEPs Area (ratio)	0.94 ± 1.2	***0.37*** ± ***0.3****,**	***0.53*** ± ***0.3*** ***,°	***1.04*** ± ***0.5****,**,***,°
Paired Pulse ISI 5 ms MEPs Ampl. (ratio)	1.61 ± 2.2	1.24 ± 1.0	1.02 ± 0.4	1.60 ± 1.4
Paired Pulse ISI 5 ms MEPs Area (ratio)	1.74 ± 2.2	1.26 ± 1.2	1.04 ± 0.6	1.86 ± 2.2
"Self-paced" phasic contraction				
	Left Hemisphere	*Right Hemisphere*	*Left Hemisphere*	*Right Hemisphere*
Paired Pulse ISI 3 ms MEPs Ampl. (ratio)	0.80 ± 0.7	0.43 ± 0.4*	0.73 ± 0.5°	0.77 ± 0.3*,°
Paired Pulse ISI 3 ms MEPs Area (ratio)	0.95 ± 1.1	***0.42*** ± ***0.4****,**	0.63 ± 0.6°	***0.75*** ± ***0.3****,**,°
Paired Pulse ISI 5 ms MEPs Ampl. (ratio)	1.77 ± 2.1	0.96 ± 0.8	1.42 ± 1.1	1.57 ± 1.2
Paired Pulse ISI 5 ms MEPs Area (ratio)	1.84 ± 1.8	0.99 ± 0.7	1.54 ± 1.4	1.84 ± 1.7

Abbreviations: Interstimulus Interval (ISI), Motor Evoked Potential (MEP), Resting Motor Threshold (RMT), Transcranial Magnetic Stimulation (TMS).

* Groups per stimulated hemisphere interaction, right hemisphere, DS *vs.* fluent speakers, p = 0.007 (ampl.), p = 0.002 (area).

** Groups per stimulated hemisphere per task interaction, right hemisphere, DS *vs.* fluent speakers; tonic contraction, p = 0.008; "acoustic-driven" phasic contraction, p < 0.001; "self-paced" phasic contraction, p = 0.012.

*** Groups per stimulated hemisphere per task interaction, left hemisphere *vs.* right hemisphere in fluent speakers, p = 0.004.

° Groups per stimulated hemisphere interaction, left hemisphere *vs.* right hemisphere in fluent speakers, p = 0.064 (ampl.), p = 0.056 (area).

4.3. Cortico-basal-thalamo-cortical loops in motor control: intracortical inhibition in movement execution of DS and clinical relevance of present findings

The main finding of the present work is that DS results in increased intracortical inhibition of the right primary motor cortex representation of the ADM muscle when participants were asked to execute a movement with their left index finger (thus activating FDI). This was evident when participants carried "*self-paced*" phasic movements and tonic contractions, but effect size was maximal when the phasic movement was cued by an external "*acoustic*" stimulation. The primary motor cortex is the final gate of a complex system that manages movement preparation/execution. This system comprises structures such as associative motor cortices (supplementary motor area -SMA- and premotor cortex), as well as sub-cortical structures such as basal ganglia and cerebellum. Thus, an "*internal timing network*", mainly relying on the SMA "*complex*" and basal ganglia, is fundamental for the correct implementation of motor acts performed on a voluntary basis, especially when considering correct/timely activation or inhibition (see Etchell et al., 2014, for a perspective in DS). An "*external timing network*" also exists, mainly relying on structures such as the cerebellum and the premotor cortex, which is involved in motor tasks based on external ("*sensorial*") cues (see Etchell et al., 2014). Thus, these systems are fundamental to regulate neural commands to promote the desired movements and, contemporaneously, inhibiting the competing ones (e.g. Friend & Kravitz, 2014; Mink, 2018). For example, cortico-basal-thalamo-cortical circuits, projecting to motor areas, are important in modulating planning/execution of complex motor sequences, such as speech (e.g. Jin & Costa, 2015; Jin, Tecuapetla, & Costa, 2014). Altered functioning of these networks is evident in DS (see Alm, 2004; Craig-McQuaide et al., 2014; Etchell et al., 2014, 2018; Smits-Bandstra & De Nil, 2007; see Wu et al., 1997, in relation to abnormal dopaminergic activity).

The present findings suggest that, in DS, intracortical networks of the right primary motor cortex are able to strongly inhibit surrounding muscles that are not directly involved in actual movement execution. As already suggested, this augmented inhibition may be an attempt of the system to dampen activation of muscles not directly involved in task execution, thus lowering "*neural noise*" and favouring motor acts during compensation attempts. Thus, augmented inhibition of "*competing*" muscular districts may help to better counteract motor impairment in DS, especially when the system is actively trying to overcome difficulties (e.g. a stronger beta suppression may be needed to start speech movements; Mersov, Jobst, Cheyne, & De Nil, 2016), resulting in a generally increased neural/motor effort (and thus, in a likely less "*specific*" motor recruitment; compare, for example, with De Nil et al., 2008; Mersov et al., 2016; Toyomura et al., 2011; Sommer, Omer, Wolff von Gudenberg, & Paulus, 2019). This could be managed by "*internal/external timing networks*", and their cortico-striato-thalamo-cortical (e.g. SMA and basal ganglia) or cortico-cortical loops (e.g. cerebellum and premotor cortex). Effect magnitudes suggest that augmented inhibition of "*competing*" muscular districts is maximal when the movement is phasic and cued by "*acoustic*" (external) stimulation, followed by "*self-paced*" (voluntary/internal) phasic movements, and, finally, by tonic contraction. As a consequence, external (sensorial) cues and the realization of movements on a voluntary (internal) basis may modulate the effectiveness of the motor system, in DS. Compatibly, previous investigations have suggested that DS is a speech timing and motor sequencing disorder (e.g. Alm, 2004; Civier, Bullock, Max, & Guenther, 2013; Civier, Tasko, & Guenther, 2010; Howell, 2004; Kent, 1984; Ludlow & Loucks, 2003; Max, 2004; Max, Guenther, Gracco, Ghosh, & Wallace, 2004; Packman, Code, & Onslow, 2007; Perkins, Kent, & Curlee, 1991; Postma & Kolk, 1993; Smits-Bandstra & De Nil, 2007). Fluency enhancements are possible when external, sensorial cues are provided such as choral speech, metronome, altered/delayed auditory feedback (AAF/DAF), etc. For example, Toyomura et al. (2015) demonstrated that training with a metronome helps to regain normal neural activity in DS (i.e. left lateralized neural patterns). Kalinowski and Saltuklaroglu (2003) suggested that choral speech may be effective in DS because it is able to activate wider neural systems, also involving "*mirror neurons*" mechanisms. Fluency is further favoured when more general, external, sensorial cues are provided (e.g. not overlapping or temporally altered choral speech, conversational babbling, visual cues, etc.; compare with Park & Logan, 2015), easily resulting in a slower speaking rate and higher temporal entrainment with the cue. As a consequence, in these cases, neural patterns of motor activation should be more effective, in DS. Compatibly, pre-TMS EMG in ADM (please refer to the Supplementary Material) indicated that (especially when using the right primary motor cortex) DS was generally related to higher EMG activations during "*self-paced*" phasic movements, lower EMG activations during tonic contractions, and normal EMG activations during "*acoustic-driven*" phasic movements. This suggests that augmented intracortical inhibition of the ADM representation in the right primary motor cortex may be useful when trying to regulate FDI movements of the same hand, thus resulting in better ratios of EMG activations when faced with externally-driven sensorial cues (in this case, "*acoustic*"). Interestingly, while tonic contractions may continue to result in "*insufficient*" levels of EMG activation (as evident also in the moving FDI muscle), the "*self-paced*" (voluntary) phasic condition may result in a higher level of muscular (neural) "*noise*" in effectors that are not involved in the requested movement (ADM). Increased intracortical inhibition in the right primary motor cortex may help to adjust these abnormal ratios of neural "*noisy*" activity, trying to reduce motor difficulties in stuttering. The data suggest that this procedure will be effective when sustained by externally-driven sensorial cues (i.e. recruiting "*external timing network*" *-vs.* "*internal timing network*"-). Thus, intracortical mechanisms of the right primary motor cortex may have a role in helping to recover effective motor execution, in DS.

In this context, Busan et al. (2019) showed that, after single-pulse TMS of SMA (part of an "*internal timing network*"), DS participants were not able to effectively recruit it and related speech/motor regions of the left hemisphere such as ventral premotor cortex, inferior frontal regions, and parietal cortex. After a certain delay, they were able to activate fronto-temporal regions of the right one, finally allowing "*re-activation*" of right premotor regions and the SMA "*complex*". This delay in neural timing may result in increased "*neural noise*" or less "*focused/synchronized*" motor plans. Thus, it may explain symptoms reported in DS such as blocks or repetitions (i.e. ineffective start or execution of timely motor sequences), offering a possible neural basis for better understanding of the effects of techniques based on external sensorial cues (e.g choral speech, metronome, AAF/DAF) in improving DS, favouring the re-gain of neural "*synchrony*". Compatibly, choral speech is more effective when temporally expanded and less effective when temporally compressed (Guntupalli, Kalinowski, Saltuklaroglu, & Nanjundeswaran, 2005). The present findings on the intracortical mechanisms of DS may be related to this evidence, speculatively showing the final influence of these systems on motor outputs. Interestingly, AAF/DAF augments motor/speech variability in fluent speakers: Kittilstved et al. (2018) demonstrated that AAF/DAF-induced sensorial asynchrony or variability may favour fluent speakers to recruit right hemispheric regions (with respect to the "*classical*" left motor/speech networks), thus resembling what happens in DS.

Thus, recruiting of the right hemisphere may have a compensatory role ("*adaptive*" or "*maladaptive*"), in DS, also considering its involvement in the correct management of action timing (e.g. Pflug, Gompf, Muthuraman, Groppa, & Kell, 2019). Impairments in motor/speech structures of the left hemisphere are evident in children with DS (e.g. Garnett et al., 2018), while typical right hemispheric increments are not present or less evident (e.g. Chang et al., 2008). Thus, the augmented inhibition observed in intracortical networks of the right primary motor cortex in muscles not directly involved in movement execution may represent a mechanism that the DS brain develops over a life-long

period, trying to counteract defective motor functioning (especially of the left one).

In conclusion, excitatory and inhibitory signals are needed to implement motor commands. Release (i.e. excitation) of planned actions is normally associated with stopping (i.e. inhibition) of inappropriate/competing movements. Intracortical inhibition seems to rely on GABAergic transmission. Compatibly, short-interval intracortical inhibition in the primary motor cortex has been suggested to mediate inhibitory signals directed toward muscles that are not strictly involved in actual movement execution (e.g. Beck et al., 2008; Sohn & Hallett, 2004b; Stinear & Byblow, 2003; Zoghi, Pearce, & Nordstrom, 2003), but associative/contralateral (motor) regions may also play a role (compare with Greenhouse, Sias, Labruna, & Ivry, 2015). Separate basal ganglia pathways may be involved in these inhibitory mechanisms, mediating motor selection/initiation (compare with Duque, Greenhouse, Labruna, & Ivry, 2017; Greenhouse et al., 2015). In the present findings, the evidence of augmented intracortical inhibition (in the right hemisphere) of the closer/surrounding muscular districts (ADM), not actually and directly involved in action execution, may represent a strategy that is useful to obtain better signal-to-noise ratios or better "*gain*" during movement execution and compensatory attempts (see Duque et al., 2017; Greenhouse et al., 2015). Increased intracortical inhibition of less involved muscles may facilitate independent movements as well as spatial/temporal coordination of different or sequential movements, trying to promote a more rapid and effective preparation/implementation of (complex) actions (compare with Duque et al., 2017). External sensorial cues may favour these mechanisms and, as a consequence, they may favour fluency in DS by improving (and "*synchronizing*") multimodal sensorimotor integration (compare with Kent, 1984; Neumann et al., 2003; Park & Logan, 2015; Saltuklaroglu, Kalinowski, & Guntupalli, 2004).

4.4. Previous TMS works in DS: relevance of present findings

As already reported, previous TMS studies in DS have rarely investigated cortico-muscular excitability during the preparation/execution of a movement. DS may rely on a defective regulation of the excitability of motor cortices representing speech/hand muscles (e.g. lower excitability of the left motor cortex in contraposition to heightened excitability of the right motor one; see Busan et al., 2017, for a review). This may be true for cortico-spinal/cortico-bulbar pathways as well as for intracortical motor networks (especially when considering speech muscles), where an altered balance between excitatory and inhibitory networks may be bilaterally evident in relation with stuttering severity (e.g. Alm et al., 2013; Busan et al., 2009, 2013, 2016; Neef, Jung, et al., 2011; Neef, Paulus, et al., 2011; Sommer, Wischer, Tergau, & Paulus, 2003; Sommer et al., 2019). For example, Neef, Paulus, et al. (2011) showed that intracortical modulation is abnormal in tongue muscle representations of DS (i.e. lower facilitation, bilaterally; lower/delayed inhibition, especially in the right hemisphere), during tonic contractions. Neef, Hoang, Neef, Paulus, and Sommer (2015) also demonstrated that excitability of the left primary motor cortex of the tongue was dampened during speech execution in DS; excitability was inversely related to stuttering severity. In this regard, Whillier et al. (2018) demonstrated (by measuring TMS-induced MEPs before speech onset) that DS may result in a lower speech facilitation during immediate speech, delayed speech without pacing, and delayed speech with predefined pacing. Thus, an equilibrium among excitatory and inhibitory neural signals is needed in order to realize "*effective*" motor tasks. This equilibrium may be altered in stuttering, especially when facing complex and/or "*demanding*" acts (see Busan et al., 2016, 2017), such as speech. Sommer et al. (2019) showed that cortico-spinal excitability of hand muscles increases during spontaneous speech in DS, but it is lower during non-verbal oro-facial movements. This suggests a stronger involvement of muscles not strictly related to speech during language implementation and execution, in DS. This may be the result of "*uncontrolled*" motor activations but it may also be related to compensatory processes.

As a matter of fact, speech involves the punctual activation and the fast coordination of a series of different muscles of the oral district, that needs to be timely recruited in order to fulfil a fluent language. In this context, the present work deepened the investigation of the existing relations between different muscular representations of the primary motor cortex during motor execution, in DS, trying to shed light on their neural dynamics and exchange of information. More specifically, we showed that DS may rely on processes of heightened intracortical inhibition of muscular districts not involved in the requested movement: this may be useful to obtain a higher signal-to-noise ratio at a neural level, trying to favour muscular districts effectively involved in the requested motor task. This is more evident when relying on "*acoustic-driven*" sensorial cues, thus resembling commonly effective fluency-inducing techniques (e.g. metronome), suggesting the compensatory meaning of this process. This is also indirectly confirmed by the previous observations of Neef, Jung, et al. (2011): they demonstrated that DS participants were not able to synchronize the motor performance of their hands with isochronous sequences of acoustic cues, after using inhibitory repetitive TMS on the right premotor cortex (fluent speakers were more affected by stimulation of the left hemisphere). These mechanisms may be used to counteract the lower neural excitability generally observed in effectors at rest and during motor/speech tasks, especially in the primary motor cortex of the left hemisphere (e.g. Alm et al., 2013; Busan et al., 2013, 2016; Neef, Paulus, et al., 2011; Neef, Anwander, et al., 2015; Sommer et al., 2003, 2019; Whillier et al., 2018).

4.5. Muscular interplay in other basal-ganglia related motor impairments

An altered influence of intracortical networks on the final motor output is common in various basal ganglia-related motor impairments. This may result from impaired modulation of intracortical inhibition (see Cantello et al., 2002; Stinear & Byblow, 2004), but is especially evident when movements modulate muscles that are not directly involved in actual execution (e.g. Shin et al., 2007; Sohn & Hallett, 2004a). This is evident in disturbances such as Parkinson's disease (e.g. Cantello et al., 2002; Shin et al., 2007) and dystonia (e.g. Sohn & Hallett, 2004a; Stinear & Byblow, 2004), thus confirming the fundamental role of cortico-striato-thalamo-cortical systems in motor planning and execution, especially when "*interplay*" among different muscles (or effectors) is requested. Motor interactions among muscles may be modulated by different allocation of attention (e.g. Kuhn, Keller, Lauber, & Taube, 2018; Kuhn, Keller, Ruffieux & Taube, 2017a, 2017b): this may be used in DS when trying to manage dysfluencies (e.g. switching from an "*internal timing network*" system to an "*external timing network*" one; compare for example with Etchell et al., 2014). An atypical influence of a moving muscle on closer or surrounding motor representations is often reported in Parkinson's disease: lower inhibitory influence may be evident, resulting in less effective or less precise movements (e.g. Shin et al., 2007). Similar findings may be highlighted in dystonia (e.g. Beck et al., 2008; Beck, Schubert, Richardson, & Hallett, 2009; Bütefisch, Boroojerdi, Chen, Battaglia, & Hallett, 2005; Hallett, 2011; Molloy, Sohn, & Hallett, 2002; Sohn & Hallett, 2004a; Stinear & Byblow, 2004). Effective neural communication is crucial to warrant the correct functioning of these systems: for example, when successful dopamine transmission is requested (e.g. Mink, 2003). In fact, similar to other basal ganglia-related motor impairments, DS may be characterized by alterations in dopamine (e.g. Wu et al., 1997). Compatibly, dopamine-related abnormalities of cortico-striato-thalamo-cortical systems may result in impaired modulation of both intracortical motor networks and motor cortical outputs (e.g. Cantello et al., 2002; Ziemann, Tergau, Bruns, Baudewig, & Paulus, 1997).

4.6. Limitations of the study

The present work investigated neural dynamics of muscular "*interplay*" in persistent DS using TMS. However, it has some limitations that should be considered. Due to the characteristics of the experimental settings, we were unable to directly evaluate effects on speech muscles. In each case, the evidence for differences in hand muscles supports the hypotheses that DS may be a general motor disorder, and that symptoms may be evident during complex/demanding motor tasks (such as speech), where very fast/timed/coordinated activation of muscles is requested to realize successful communication. Our protocol did not allow to directly evaluate the inhibitory signals that the neural representation of the moving muscle is able to send to the closer ones at the exact moment of the start of the action (i.e. "*classical*" surround inhibition; e.g. Sohn & Hallett, 2004b). In every case, especially when considering basal ganglia-related motor diseases (e.g. Parkinson's Disease and dystonia), the influence of the moving muscles on surrounding ones may also be evident hundreds of milliseconds after movement initiation (e.g. Nakamura, Sakamoto, Ueno, Hirano, & Toge, 2017; Shin et al., 2007). Thus, this allowed to evaluate long lasting influences of the moving muscle (in this case FDI), on mechanically/synergically related muscular districts (Häger-Ross & Schieber, 2000; Sohn & Hallett, 2004b), as well as effects on muscular districts that may be successively asked to intervene in a motor sequence (in this case ADM), similarly to speech execution. In fact, the proposed timing of stimulation used herein overlaps with the time needed to pass through two different steps of a fast motor sequence, such as finger tapping (Repp, 2005). Thus, basic dynamics toward complex motor interactions may be unravelled: from a neural point of view, this is a fundamental aspect in DS (Busan et al., 2019). However, in the present work, we evaluated a simple motor condition (i.e. not resulting in complex motor sequences, with discrete allocations of cognitive/attentional resources). The modulatory effect of more complex tasks on neural networks that are useful for motor control (e.g. Bütefisch et al., 2005) could be investigated in DS starting from present findings (e.g. the effect of "*time pressure*" on motor execution). The influence of the moving muscles on the closer/surrounding ones (not directly involved in the requested motor act) is known to be modulated by a series of variables such as attention/volition (e.g. Kuhn et al., 2017a, 2017b, 2018; Sohn, Wiltz, & Hallett, 2002), task difficulty (e.g. Liepert, Classen, Cohen, & Hallett, 1998), exercise (e.g. Kang, Hallett, & Sohn, 2013), evaluated muscle (e.g. Sohn & Hallett, 2004b), force (e.g. Beck et al., 2009), current direction in the TMS coil (Thirugnanasambandam, Khera, Wang, Kukke, & Hallett, 2015; Zoghi et al., 2003), or hemispheric dominance (Shin, Sohn, & Hallett, 2009).

When considering paired-pulse stimulation, we stimulated at 70% of RMT (conditioning stimulus): this intensity is normally below active motor threshold, and thus it should be sufficient to prevent overt muscular activations, even during movements (compare, for a perspective in DS, with Busan et al., 2013, 2016). Compatibly, MEP latencies obtained during paired pulse protocols (ISIs 3 and 5 ms) were normally longer than single pulse stimulations. Similarly, paired pulse MEP amplitudes/areas normally resulted in activations that were well above motor thresholds. Reaction times of movements were not recorded: it is not rare that DS may result in higher reaction times, even when considering hand movements, thus confirming that DS may be a subtle and general motor impairment (compare for example with Etchell et al., 2014, and with Smits-Bandstra & De Nil, 2007, for brief reviews of the available literature). Finally, it could be useful to re-evaluate the present findings by using TMS indexes that allow obtaining different data, such as recruitment curves; similarly, using different ISIs, different stimulation intensities, or measures related to intracortical facilitation (paired pulse TMS), may also give further insights to muscular "*interplay*", in DS.

5. Conclusions and future directions

This work investigated cortical excitability and intracortical networks involved in control of muscular districts that are not directly recruited in actual movement execution in DS. We report a reduction of contralateral MEPs after stimulation of the left (dominant) hand primary motor cortex at rest and during the planning/execution of phasic movements, suggesting that a general decrease of cortico-spinal excitability is present. We also tested the hypothesis that modulation of intracortical networks, useful to manage voluntary movements, may be altered in DS. Higher intracortical inhibition of the ADM motor representation of the right hemisphere of DS was evident in motor tasks requesting FDI activation (i.e. tonic contractions, "*acoustic-driven*" and "*self-paced*" phasic movements). The physiological mechanisms underlying this altered muscular *"interplay*" needs to be unravelled: effective modulation of intracortical circuits is fundamental for the correct execution of skilled motor acts. This augmented inhibition of the right hemisphere may represent a compensatory ("*adaptive*" or "*maladaptive*") mechanism, as the consequence of the diffuse structural/functional impairments of the left one (e.g. cortico-basal-thalamo-cortical or white matter deficiencies, which are unable to drive the proper motor planning/execution and the proper neural "*balance*" between motor excitation and inhibition). Moreover, previous reports (see Alm, 2004; Etchell et al., 2014) suggest that deficiency of "*internal timing networks*" has a key role in DS. Compatibly, fluency enhancements (i.e. "*effective*" motor execution, possibly characterized by desired levels of excitatory/inhibitory neural ratios) are observed when stutterers mainly rely on "*external timing networks*", in conditions such as choral speech or by using a metronome (see Kalinowski & Saltuklaroglu, 2003; Toyomura et al., 2011, 2015). The evidence of higher levels of intracortical inhibition (accompanied by normal levels of pre-TMS EMG activation), in ADM, when phasic contraction of the left index finger was cued by "*acoustic*" (external) stimulation, support this hypothesis.

Relation of present findings with neural processes involved in inhibition (or stopping) of "*on-going*" actions should be also further investigated. A network composed by the right inferior frontal cortex and the pre-SMA (targeting the primary motor cortex via GABA-ergic inhibition) may be useful to "*stop*" planned movements (e.g. Xue, Aron, & Poldrack, 2008). Motor structures of the right hemisphere may exert inhibitory control also by using properties of beta band oscillations (Swann et al., 2009). Interestingly, frontal structures of the right hemisphere may be over-activated or over-represented in DS, representing "*compensatory*", "*maladaptive*", or pathological mechanisms (e.g. Neef et al., 2016, 2018). Fronto-temporo-parietal regions of the right hemisphere may also help in managing rhythmic/temporal "*cues*" (e.g. Bueti, van Dongen, & Walsh, 2008; Koch, Oliveri, & Caltagirone, 2009; Pflug et al., 2019), perhaps favouring the fluency-inducing effect of "*external*" sensory cues in DS (i.e. resulting in more effective movement execution or inhibition of muscular districts not involved in requested motor acts). External sensory cueing also seems to modulate beta frequencies in the brain (e.g. Pflug et al., 2019). As a consequence, future investigations could be useful to examine if acting on beta frequencies of the brain by means of neuro-modulation (especially at a motor level), can be useful to obtain a further facilitatory effect on fluency, in DS (see Etchell, Johnson, & Sowman, 2015).

In conclusion, this study further deepens the understanding of the neurophysiology of the motor system of persistent DS, suggesting that exaggerated right hemisphere intracortical modulation is evident in motor representations of muscles not directly involved in the actual motor act, likely trying to favour it. As a consequence, the present findings may be useful to better understand the neural dynamics of fluency-inducing conditions in stuttering in order to define more focused rehabilitative solutions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bandl.2020.104774.

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