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RESEARCH REPORT

Neurophysiologic markers of primary motor cortex for laryngeal muscles and premotor cortex in caudal opercular part of inferior frontal gyrus investigated in motor speech disorder: a navigated transcranial magnetic stimulation (TMS) study

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Abstract Transcranial magnetic stimulation studies have so far reported the results of mapping the primary motor cortex (M1) for hand and tongue muscles in stuttering disorder. This study was designed to evaluate the feasibility of repetitive navigated transcranial magnetic stimulation (rTMS) for locating the M1 for laryngeal muscle and premotor cortical area in the caudal opercular part of inferior frontal gyrus, corresponding to Broca's area in stuttering subjects by applying new methodology for mapping these motor speech areas. Sixteen stuttering and eleven control subjects underwent rTMS motor speech mapping using modified patterned rTMS. The subjects performed visual object naming task during rTMS applied to the (a) left M1 for laryngeal muscles for recording corticobulbar motor-evoked potentials (CoMEP) from cricothyroid muscle and (b) left premotor cortical area in the caudal opercular part of inferior frontal gyrus while recording long latency responses (LLR) from cricothyroid muscle. The latency of CoMEP in control subjects was 11.75 ± 2.07 ms and CoMEP amplitude was 294.47 ± 208.87 μV, and in stuttering subjects CoMEP latency was 12.13 ± 0.75 ms and 504.64 ± 487.93 μV CoMEP amplitude. The latency of LLR in control subjects was 52.8 ± 8.6 ms and 54.95 ± 4.86 in stuttering subjects. No significant differences were found in CoMEP latency, CoMEP amplitude, and LLR latency between stuttering and control-fluent speakers. These results indicate there are probably no differences in stuttering compared to controls in functional anatomy of the pathway used for transmission of information from premotor cortex to the M1 cortices for

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laryngeal muscle representation and from there via corticobulbar tract to laryngeal muscles.

Keywords Motor speech disorder · Stuttering · Primary motor cortex · Premotor cortex · Laryngeal muscles · Transcranial magnetic stimulation

Introduction

Stuttering is a motor speech disorder in which the flow of speech is disrupted by involuntary repetitions and prolongations of sounds, syllables, and words, as well as involuntary silent pauses or blocks in which the person who stutters is unable to produce sounds. Often it is accompanied by movements, tremors and spasms of oro-facial and laryngeal muscles (Kelly et al. 1995; Smith et al. 1993) as well as abnormal involuntary movements (ticks) (Mulligan et al. 2003; Riva-Posse et al. 2008). Stuttering is commonly a developmental disorder of speech production, beginning in early childhood, typically at the age of 2–4. The incidence of stuttering is approximately 5 %, with majority of affected children showing spontaneous recovery (Yairi and Ambrose 2005). About 1 % of the general population continues to suffer from severe stuttering in adulthood with male-to-female ratio of 3:1 (Fox et al. 1996). The linkage and association studies have begun to reveal contributing genes to the stuttering disorder (Kraft and Yairi 2012). However, the etiology of stuttering is not fully understood, and the neurophysiological characteristics of the central nervous system functioning are yet to be investigated.

Transcranial magnetic stimulation (TMS) studies on the neurophysiologic mechanisms of stuttering have pointed out altered cortical excitability of the M1 for the hand (Sommer et al. 2003, 2009; Busan et al. 2009, 2013; Alm et al. 2013) and tongue muscle representations (Neef et al. 2011, 2015) in subjects with stuttering. According to studies of Sommer et al. (2003, 2009), interhemispheric inhibition, intra-cortical inhibition, and intra-cortical facilitation appear to be normal for bilateral hand muscle representation in subjects with stuttering. The motor threshold tends to be increased for the left hemisphere in stuttering (Alm et al. 2013; Sommer et al. 2003), and peak-to-peak amplitudes of motor-evoked potentials (MEPs) recorded from the hand muscles were shown to be reduced in the left hemisphere in subjects with stuttering (Busan et al. 2013). Weaker inhibition of the M1 for tongue muscles was shown in the right hemisphere, with a reduced facilitation for this cortical area bilaterally (Neef et al. 2011). Neef et al. (2015) reported a speech-induced facilitation in the left hemisphere in fluent speakers and the lack of this facilitation in adults with stuttering. An index of intra-cortical inhibition, the cortical silent period was shown to be reduced in hand muscles in stuttering subjects after administration of antidepressant drug paroxetine (Busan et al. 2009). Thus, with the exception of one research group (Neef et al. 2011, 2015) that investigated corticobulbar excitability for tongue muscles, no TMS study investigated corticobulbar excitability for laryngeal muscles. A number of investigators using EMG provided convincing evidence that disfluent speech of stuttering subjects is often associated with tremor characterized by abnormal oscillations of EMG activity in oro-facial and laryngeal muscles (Fibiger 1971; McClean et al. 1984; Smith 1989; Smith et al. 1993; Kelly et al. 1995). Smith et al. (1993) recorded the activity of two intrinsic laryngeal muscles, thyroarytenoid and cricothyroid, in ten adults with stuttering, and reported oscillations of EMG typically occurring in a frequency band of 5–15 Hz.

In several previous studies, TMS was used for testing the excitability of corticobulbar projections to laryngeal muscles by mapping the M1 for laryngeal muscles representation and recording corticobulbar motor-evoked potentials (CoMEPs) from laryngeal muscles in patients with neurological diagnosis and control subjects (Amassian et al. 1988; Ertekin et al. 2001; Khedr and Aref 2002; Rödel et al. 2004). Recently, a methodology was established for mapping the M1 for laryngeal muscles using three-dimensional (3D) magnetic resonance imaging (MRI) navigated transcranial magnetic stimulation (nTMS) and by recording CoMEPs from cricothyroid muscles in healthy subjects (Espadaler et al. 2012). Stimulation over the M1 for the cricothyroid muscle elicited CoMEPs in contralateral cricothyroid muscle with a mean latency of 11.89 ± 1.26 ms. Furthermore, a neurophysiologic marker of motor speech cortical area in the premotor cortex in the caudal opercular part of inferior frontal gyrus corresponding to Broca's area was detected. The neurophysiologic marker of this area was detected with nTMS in control subjects, and intraoperatively in patients by applying stimulation with transcranial electrical and direct cortical stimulation and recording long latency responses from cricothyroid muscle (Deletis et al. 2014). The LLR latency in the control group was 58.5 ± 5.9 ms, while in patients 54.25 ± 3.69 ms. Magnetic stimulation of these motor speech-related cortical areas (M1 for laryngeal muscles and premotor cortex in the caudal opercular part of inferior frontal gyrus), generating evoked responses/neurophysiologic markers in cricothyroid muscle, elicited also transient speech impairments (Rogić et al. 2014; Deletis et al. 2014). Therefore, the differences in the latencies indicate functional anatomy of the M1 for laryngeal muscles (CoMEP) and the premotor cortex in the caudal opercular part of inferior frontal gyrus (LLR) (Deletis et al. 2014). Furthermore, the amplitudes of contralateral and ipsilateral CoMEP responses were analyzed and contralateral

corticobulbar projections to cricothyroid muscle showed to be dominant in regard to weak ipsilateral projections (Rogić Vidaković et al. 2015).

The aim of this study was to evaluate the feasibility of our developed rTMS methodology (Espadaler et al. 2012; Deletis et al. 2014; Rogić et al. 2014) for locating the left M1 for laryngeal muscle and premotor cortical area in the caudal opercular part of inferior frontal gyrus, corresponding to Broca's area in stuttering and control-fluent speakers. So far, it was not possible to map these motor speech cortical areas in stuttering due to methodological reasons. In our previous studies, we have shown that CoMEPs and LLR could be elicited in cricothyroid muscle by stimulating the M1 for laryngeal muscles and premotor cortices during engagement of the participants in a specific speech task (i.e., visual object naming task) (Espadaler et al. 2012; Deletis et al. 2014). The visual object naming task is frequently used for mapping the Broca's area and the M1 for oro-facial muscles to interfere with speech and language processing, intraoperatively during awake craniotomy with direct cortical stimulation (Picht et al. 2013), and preoperatively with nTMS (Lioumis et al. 2012; Krieg et al. 2015).

Therefore, in this study the same task was used in both study groups to facilitate generation of CoMEPs and LLRs during nTMS mapping of the M1 for laryngeal muscles and premotor cortex in the left hemisphere in stuttering and control group. By application of modified patterned rTMS protocol (bursts of stimuli) (Rogić et al. 2014) at an exact time when visual object is presented, the CoMEPs and LLRs were recorded from cricothyroid muscle. The CoMEP latency, CoMEP amplitude, and LLR latency were analyzed in both groups.

Materials and methods

Study subjects

Sixteen adults with stuttering (11 males, 5 females, mean age 27.56 ± 8.56 years) and eleven fluent speakers (eight males, three females, mean age 27.55 ± 8.72 years) participated in the study. Adults who stutter were recruited by advertisement and from Department for diagnostics and rehabilitation of hearing and speech, Clinic for rehabilitation of persons with disabilities, Split, Croatia. Fluent speakers were recruited by advertisement. All participants were right handed, except one female stuttering subject. Informed consent was obtained from all participants. The Edinburgh Handedness Inventory (Oldfield 1971) was used to assess the hand dominance. None of the participants was taking any medication that could affect cortical excitability. All participants met the exclusion criteria for TMS such as the presence of metal objects (i.e., denture) or cardiac pacemaker, epileptic seizures, or a history of epileptic seizures.

The stuttering severity was assessed by the Stuttering Severity Instrument (SSI-3) (Riley 1994). Speech samples were video recorded by Panasonic HDC-SDT750 for off-line analysis performed by qualified speech and language pathologist (one of the authors of this study). Each fluent speaker was interviewed to exclude undetected stuttering and none reported familial history of persistent developmental stuttering.

Overall study design

The MRI of the head was obtained for all subjects on 1 day with previous evaluation by the SSI-3 in stuttering subjects and an interview conducted with control subjects. Mapping with nTMS was performed approximately 1 week following the MRI scanning. In each subject, the mapping of the M1 for hand muscle representation was performed by single TMS, and was followed by rTMS mapping of two motor speech cortical areas while participating in visual object naming task. The mapping session (not including the time needed for insertion of the hook wire and surface electrodes, co-registration process, and behavioral testing) lasted approximately 1 h. All mappings were conducted by the first author, who obtained manufacturers certification 6 years ago.

Ethics

This study was performed in accordance with the ethical standards of the World Medical Association (2013) and approved by the ethics committee of the School of Medicine University of Split, Croatia.

MRI acquisition

The MRIs of the head for each subject were performed with Philips Magnetic Resonance Achieva 1.5 T A-series, Head Coil 8 channel (Polyclinic Sunce, Split, Croatia). MRI images were obtained to suit nTMS requirements and were integrated in the nTMS system and used for the 3D reconstruction of individual's brain (Ruohonen and Karhu 2010).

rTMS mapping

Experimental setup

Each participant underwent rTMS mapping with the Nexstim eXimia NBS system 4.1. (Nexstim Ltd., Helsinki,

Finland), including a magnetic stimulator and focal biphasic figure-of-eight cool coil with a mean winding diameter of 50 mm, and outer winding diameter of 70 mm. The shape of the biphasic pulse is ca 280 μs pulse length. The trigger and synchronization output line: gate out signal (for synchronizing EMG device): 100 μs before the stimulus pulse, width 500 μs, amplitude 5 V TTL positive polarity, and output impedance 1500 Ω.

At first, mapping over the left M1 for hand muscle (abductor pollicis brevis, APB) was performed to determine the resting motor threshold (RMT) for eliciting the MEP responses in APB muscle (Yousry et al. 1997; Schmidt et al. 2009; Julkunen et al. 2011). The RMT was defined as the lowest stimulus intensity for eliciting at least five MEPs in APB muscle with the amplitude of at least 50 μV in a series of 10 consecutive trails (Rossini et al. 1994). For mapping the M1 for APB muscle, single TMS pulses were used with an interstimulus interval of 5 s. The rTMS motor speech mapping was performed afterward, using modified patterned rTMS protocol consisting of 4 bursts of 4–5 stimuli each, with an interstimulus interval of 6 ms, and a burst repetition rate of 4 Hz (Rogić et al. 2014). Two motor cortical areas were stimulated, the left M1 for laryngeal muscle and the left premotor cortical area in the caudal opercular part of inferior frontal gyrus, corresponding to Broca's area, while recording evoked responses from cricothyroid muscle (Espadaler et al. 2012; Deletis et al. 2014; Rogić et al. 2014). The CoMEPs and LLR responses were recorded from the cricothyroid muscle, CoMEPs when stimulating the M1 for laryngeal muscle, and LLR while stimulating the premotor cortical area.

Recordings

For recording the responses from the cricothyroid muscle, two hook wire electrodes (type 003-400160-6) (SGM d.o.o., Croatia) were inserted into the right cricothyroid muscle according to published methodology (Deletis et al. 2011; Espadaler et al. 2012). Surface electromyography electrodes (Ambu® Blue Sensor BR, BR-50-K/12) were attached in a belly tendon fashion over the right APB muscle with the ground electrode over the dorsal surface of the right APB muscle. A brief explanation of the methodology can be found below:

Anatomical guidelines for the insertion of the hook wire electrodes into the cricothyroid muscle were described according to the methodology of Hirano and Ohala (1969). Before insertion of the electrodes individually, the subject needs to slightly extend the neck and produce a high-pitch-sound (i.e./iiii…/). During this slight facilitation, it is helpful to palpate the contracted cricothyroid muscle belly between the thyroid and cricoid cartilages with marking this spot with the marker. Then, a hook wire electrode is inserted in the cricothyroid muscle under the angle of 30°–40°. Each hook wire electrode consist of teflon-coated stainless steel wire 76 μm in diameter passing through 27-gauge needles (0.4 mm) and 13 mm of length. The recording wires have a stripped teflon isolation of 2 mm at their tip and curved to form the hook for anchoring them. After the wire insertion in the cricothyroid muscles, the needles are withdrawn and wires braded. The correct position of the electrode insertion is verified by asking the subject to slightly produce a high-pitch vocalization and by visually inspecting the EMG activity from the cricothyroid muscle. Figure 1 (on the right) illustrates the placement of the electrodes into the right cricothyroid muscle. The evoked responses from APB and cricothyroid muscles were recorded by using the EMG amplifier integrated with the n TMS system. An additional channel was used to record the audio signals of speech simultaneously with the EMG recordings. The Nexstim EMG amplifier has six channels, one common ground EMG amplifier (external module) with TMS-artifact rejection circuitry. The sampling rate was 3 kHz per channel, resolution 0.3 μV, scale −7.5–7.5 mV, CMRR > 90 dB, noise <5 μV peak-to-peak, frequency band 10–500 Hz.

Stimulus presentation and response recording was controlled via presentation software Presentation® (©Neurobehavioural Systems, Inc., version 14.7 11.10.10), running a custom-made script.

Stimulation mapping procedure

Mapping of the M1 for laryngeal muscle was performed according to published methodology (Espadaler et al. 2012). After finding the RMT for APB muscle, the coil was moved laterally using the central sulcus as a landmark in steps of a couple of millimeters. The coil was moved in an anterior–posterior direction in order to map the hot spots for the M1 for cricothyroid muscle. For initial mapping, the RMT intensity was used and in subjects who felt discomfort the intensity was lowered 5–10 %. When not eliciting CoMEP responses from the cricothyroid muscle, in subjects without discomfort, the TMS intensity was raised 1–20 % (Espadaler et al. 2012). Minimum five to ten CoMEP responses were obtained, and in the offline analysis CoMEP responses were visually checked and those with existing pre- and post-stimulus EMG activity were excluded. The CoMEPs were elicited after single bursts, before the speech onset (Deletis et al. 2014) (Fig. 2 upper). After mapping the M1 for laryngeal muscle, the premotor cortex in the caudal opercular part of inferior frontal gyrus was mapped by recording LLRs from the cricothyroid muscle (Deletis et al. 2014). The same modified patterned rTMS protocol was used for stimulating this area as for mapping the M1 for laryngeal muscle. First, the same

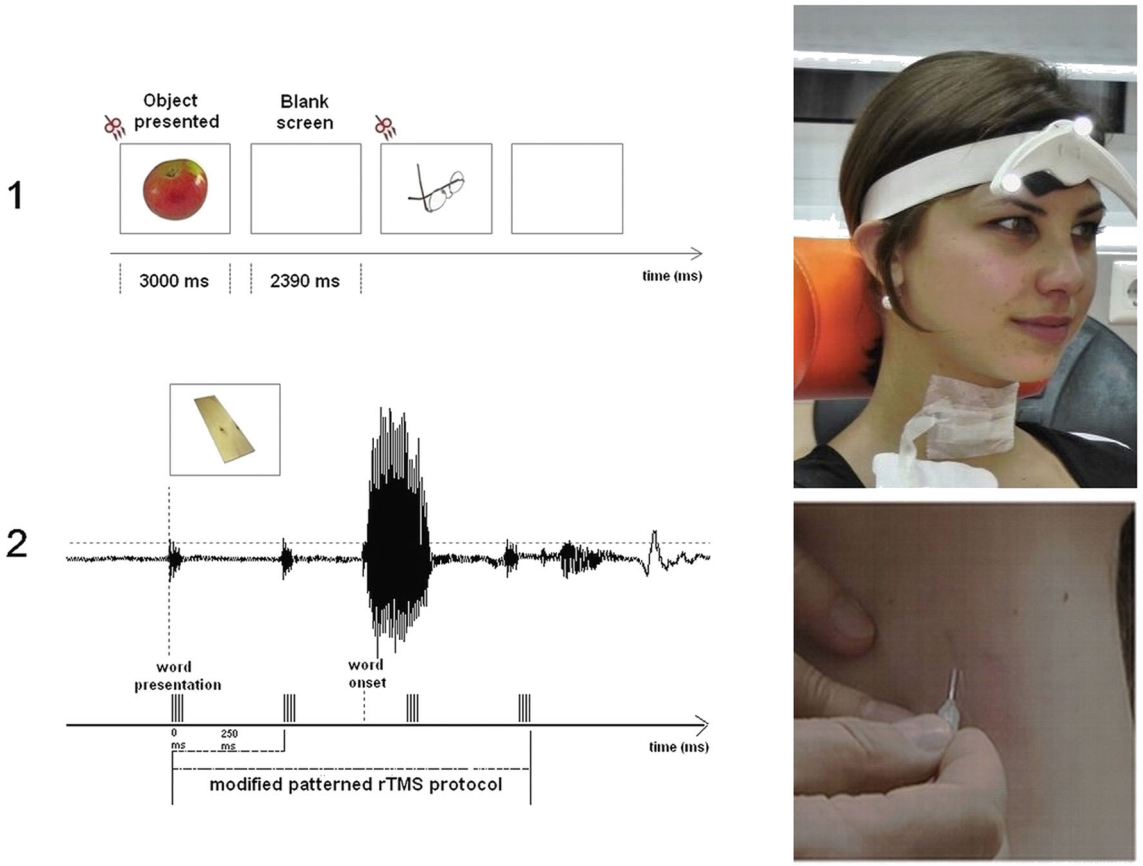

Fig. 1 Experimental setup and procedure. (*1*) A trial consisting of the presentation of object/picture for 3000 ms, followed by a blank screen of 2390 ms. (*2*) Modified patterned rTMS pulses applied at zero time with the picture presentation. Stimulation protocol consist of 4 bursts of 4 stimuli each, 6 ms apart, burst repetition rate of 4 Hz. Recorded participant's response is shown for one word with depicted stimulation artifacts before speech and after speech onset. *On the right* illustration of electrode attachment into cricothyroid muscle. (*Upper*) Inserted electrode into the right cricothyroid muscle and covered with sterile gauze and adhesive tape in one control participant. (*Lower*) Insertion of two hook wire electrode into cricothyroid muscle. The single hook wire electrode (type 003-400160-6) (SGM d.o.o., Croatia) consists of 40 cm PTFE insulated stainless steel wire AISI 302 (0.08 mm d, 40 G) threaded through a hypodermic needle

intensity used for mapping the M1 for laryngeal muscles representation was used for mapping the premotor cortex. If it caused discomfort to the subject (mainly due to activation of temporal muscle), the intensity was lowered 5–20 %, and in only one subject the intensity was slightly increased. Minimum five to ten responses were taken. In the offline analysis, LLR responses were visually checked and those with existing pre-EMG and post-EMG speech-related muscle activity were excluded. The LLRs were elicited after single bursts, before the speech onset (Deletis et al. 2014) (Fig. 2 lower).

Speech task procedure

When mapping the M1 for cricothyroid muscle representation and caudal opercular part of the inferior frontal gyrus, magnetic stimulation was applied during the subject's engagement in visual object naming task. The reason for introducing visual object naming task during the application of patterned rTMS protocol was to elicit CoMEPs and LLRs from cricothyroid muscle in the phase before speech onset (Deletis et al. 2014; Rogić Vidaković et al. 2015) (Figs. 1*2*, 2). For each participant, the total of 20 pictures in one session were randomly presented on a computer monitor DELL Inc. (Dell Inc., 2007FPB, 1600 × 1200) using the Presentation® software (©Neurobehavioural Systems, Inc., version 14.7 11.10.10) from the pool of 150 pictures (Brodeur et al. 2010). Presentation program triggered the onset of magnetic stimulation at zero time with the picture presentation on the computer screen. The picture was presented on a white background for 3000 ms, followed by 2390 ms of blank screen (Fig. 1*1*). The time from presentation of one object until the presentation of the next one was 5390 ms. Therefore, an interstimulus interval of the rTMS bursts was 5390 ms when mapping the M1 for laryngeal muscles and premotor cortex. These 20 pictures were presented for approximately three times per stimulated cortical area.

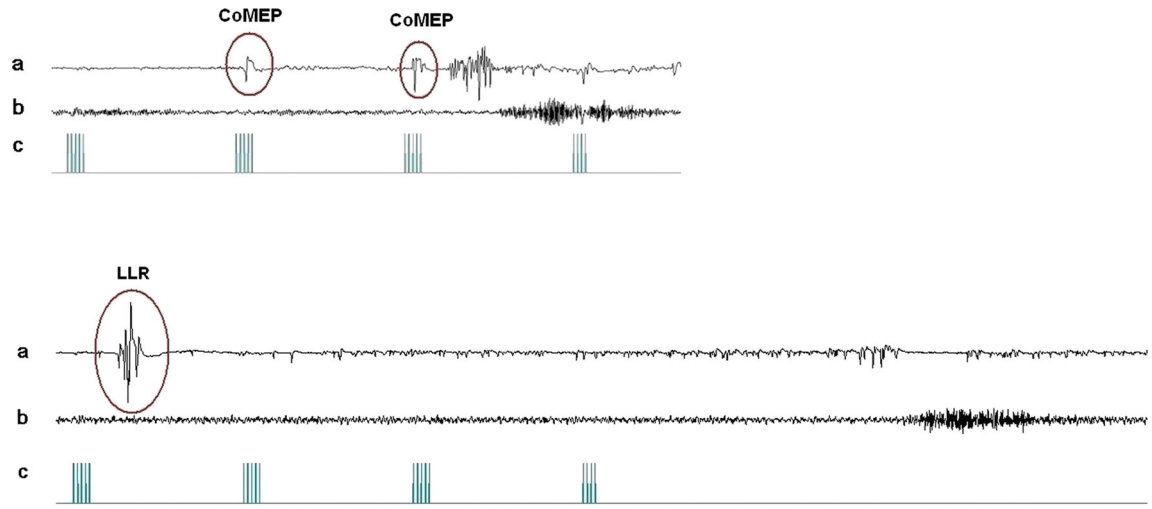

Fig. 2 Trial by trial analysis of CoMEP and LLR responses recorded from laryngeal muscle. (*Upper*) Stimulation of M1 for laryngeal muscles elicits CoMEP responses from cricothyroid muscle depicted on channel (*a*). Stimulation artifacts could not be seen on channel (*b*) due to increased distance from participants's mouth to the microphone. (*Lower*) Stimulation of the caudal opercular part of inferior frontal gyrus elicits LLR response depicted on channel (*a*). *Legend* (*a*) Electromiographic recording from cricothyroid muscle; (*b*) microphone recording; (*c*) stimulation artifacts

The nTMS measurement was combined with video recording (Panasonic HDC-SDT750) (Lioumis et al. 2012). Figure 1 illustrates the experimental paradigm of visual object presentation and the time course of a single trial of the visual object naming task. Control measurement with rTMS was also performed in all subjects with the coil set away from the subject's head during visual object naming task. This measurement was performed to exclude possible influence of micro reflexes, especially of sonomotor origin (Bickford et al. 1964; Bickford 1966).

Data analysis

Visual inspection of recordings and time intervals detection was done using free, open-source platform EDFbrowser. Peak-to-peak CoMEP amplitudes, CoMEP latencies, and LLR latencies were extracted separately in each trail. Figure 2 illustrates CoMEP (upper) and LLR (lower) responses elicited by applying patterned rTMS protocol. Signal presented in the Figs. 2 and 3 is obtained by Matlab R2012 from the recorded corresponding EDF files. Time is set to zero at the start of the stimulation signal for the presented trails.

Descriptive statistical analysis was performed on the relevant variables. When appropriate, the descriptive parameters are reported as mean value ± standard deviation (SD). The *t* test for independent samples was used in order to determine the differences in RMT, CoMEP latency, CoMEP amplitude, LLR latency, and intensity of stimulation between groups. Nonparametric (Chi-square and Spearman's rank-order correlation coefficients) were used when appropriate. Chi-square coefficient was used to test the differences in frequency of induced CoMEP and LLR responses between stuttering and control group when mapping the M1 for laryngeal muscles and premotor cortex in the caudal opercular part of inferior frontal gyrus, respectively. To examine whether there is any relationship in results between stuttering severity and CoMEP amplitude, CoMEP latency and LLR measures, Spearman's rank-order correlation coefficient was used. Considering that stuttering severity is expressed on ordinal scale, all measures were appropriately transformed to *z* scores. A threshold of $p < .05$ was used for determining the level of effect significance. All statistical analyses were performed using STATISTICA 12 (StatSoft, Inc., Tulsa, USA). The ACDSee v4.0 digital imaging software was for the preparation of graphical images.

Results

According to stuttering severity instrument, stuttering was classified as very mild, mild, moderate, severe, and very severe. Nine adults with stuttering were classified as having mild stuttering, four having moderate stuttering, one with severe stuttering, and two with very severe stuttering. All demographic data together with TMS data (RMT, CoMEP latency, CoMEP amplitude, LLR latency and intensities used for stimulation of M1 for laryngeal muscle and premotor cortical area) are presented in Table 1 for control group and in Table 2 for stuttering group. Figure 3 illustrates the repeatability of CoMEPs recorded from

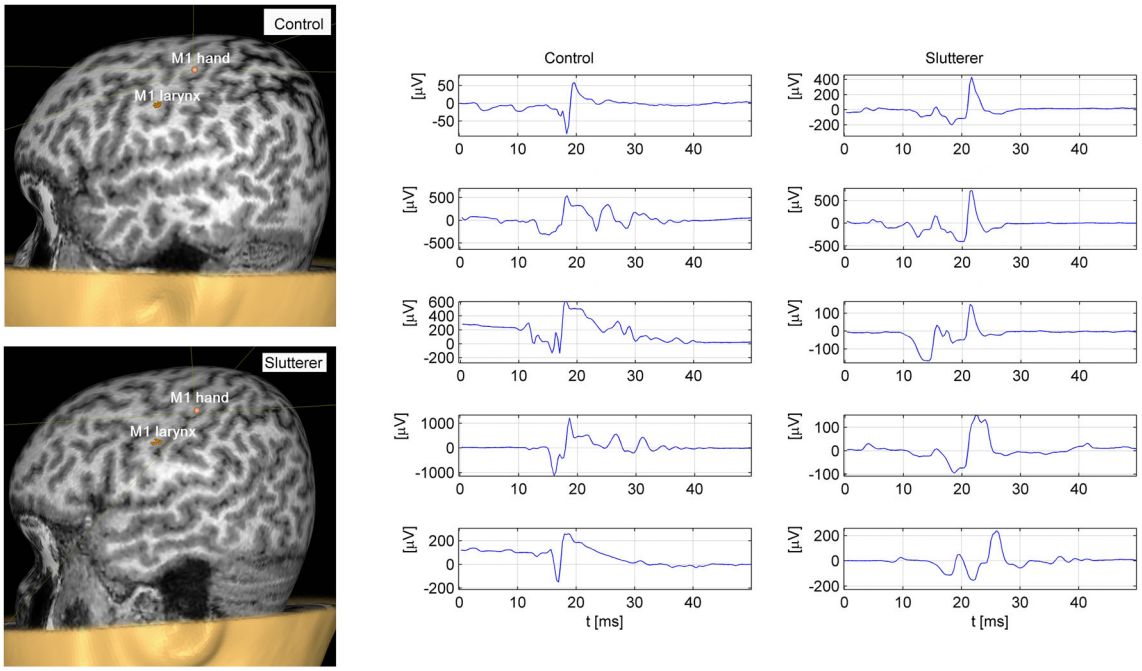

Fig. 3 Repeatability of CoMEP responses recorded in cricothyroid muscle during rTMS of the *left* M1 for laryngeal muscles. *Left* Stimulated cortical spots in the *left* M1 for laryngeal muscles in control (No. 9) and stuttering subject (No. 16) (*yellow spots*) with the M1 APB reference spot. *Right* repeatability of CoMEPs recorded from cricothyroid muscle in same control and stuttering subject. The latency values (*x*-axis) are presented in milliseconds (ms) and amplitude values in microvolts (μV) (color figure online)

cricothyroid muscle with stimulated M1 cortical spots for cricothyroid muscle in the left hemisphere for both groups. The repeatability of LLRs recorded from cricothyroid muscle is shown in Fig. 4 with stimulated cortical spots in the left premotor cortex in the caudal opercular part of inferior frontal gyrus for both groups.

In regards to RMT, no significant differences [$t(25) = .21$, $p = .83$] were found between stuttering and control group. When mapping the left M1 for laryngeal muscle no significant differences were found between groups with respect to CoMEP latency [$t(11) = .42$, $p = .68$] and CoMEP amplitude [$t(11) = 1.04$, $p = .32$]. In regards to stuttering group, frequency of induced CoMEP responses was not significantly different from the control group [χ^2 $(1) = .13$, $p = .7$]. With respect to the stimulation of the left caudal opercular part of inferior frontal gyrus, no statistically significant differences were found in LLR latencies between groups [$t(20) = .71$, $p = .48$]. The latency of CoMEP in control subjects was 11.75 ± 2.07 ms and CoMEP amplitude was 294.47 ± 208.87 μV, and in stuttering subjects CoMEP latency was 12.13 ± 0.75 ms and CoMEP amplitude 504.64 ± 487.93 μV. The latency of LLR in control subjects was 52.8 ± 8.6 ms and 54.95 ± 4.86 in stuttering subjects. Statistically significant difference was found in the frequency of induced LLR responses [χ^2 $(1) = 4.22$, $p = .04$] between tested groups, with significantly higher number of stuttering subjects in whom LLR response could not be elicited. Furthermore, no differences [laryngeal muscles: $t(1) = -.91$, $p = .53$; premotor cortices: $t(1) = -2.3$, $p = .26$] were found regard to the stimulation intensities used for mapping the M1 for laryngeal muscles and premotor cortices between tested groups.

We were also able to elicit stuttering moments during visual object naming task in one male subject with stuttering (No. 9) (Table 2) while mapping the M1 for laryngeal muscles representation in the left hemisphere. The CoMEP responses could not be elicited in this subject.

Stimulation of the cortical spots which induced CoMEP responses generated dysarthric-like speech characterized by visible contractions in oro-facial muscles in both group of subjects, while cortical spots inducing LLR responses generated semantic paraphasias and/or speech arrest in both groups (Tables 1, 2).

Results show that while mapping the M1 for laryngeal muscles, there were no significant correlations between stuttering severity and CoMEP latency ($r = -0.001$, $p > .05$) and CoMEP amplitude ($r = -0.005$, $p > .05$). Furthermore, no significant correlations were found between stuttering severity and LLR latency ($r = -0.29$, $p > .05$) while mapping premotor cortex in the caudal opercular part of inferior frontal gyrus.

Table 1 Demographic and rTMS data for the control group of subjects

	Gender (M/F)	Handedness (R/L)	rTMS							
			Primary motor cortex for laryngeal muscle					Premotor cortical area		
			RMT (%)	CoMEP latency (ms)	CoMEP amplitude (μV)	Intensity (%)	Qualitative error	LLR latency (ms)	Intensity (%)	Qualitative error
1	M	R	33	10.69	412.6	33	Dysarthria	62.62	31	–
2	M	R	36	13.76	238.07	36	Dysarthria	50.85	34	–
3	F	R	30	–	–	26	Dysarthria	51.54	24	–
4	M	R	41	10.23	112.94	36	Dysarthria	47.93	34	–
5	M	R	40	–	–	41	Dysarthria	41.2	38	–
6	M	R	28	10.3	677.18	32	Dysarthria	52.35	27	Semantic paraphasia
7	F	L	32	14.28	39.21	32	Dysarthria	72.82	30	Semantic paraphasia; Speech arrest
8	F	R	37	–	–	35	Dysarthria	44.22	35	Semantic paraphasia; Speech arrest
9	M	R	37	13.68	288.39	35	Dysarthria	50.65	28	–
10	M	R	33	–	–	30	Dysarthria	53.96	29	–
11	M	R	30	9.3	292.95	26	Dysarthria	52.7	24	–
Mean ± SD (range)			34.27 ± 4 24 (28–41)	11.75 ± 2.07 (9.3–14.28)	294.47 ± 208.87 (39.21–677.18)	32.91 ± 4.46 (26–41)		52.8 ± 8.6 (41.2–62.62)	30.36 ± 4.54 (24–38)	

The data are presented as mean ± standard deviation (SD). CoMEP amplitudes are expressed in microvolts (μV) and CoMEP and LLR latency in milliseconds (ms). Intensity of stimulation is expressed as percent (%) of maximal stimulator

M male, *F* female, *R* right, *L* left, *RMT* resting motor threshold, *CoMEP* corticobulbar motor- evoked potentials, *LLR* long latency response

Table 2 Demographic and rTMS data for the stuttering group of subjects

	Gender (M/F)	Handedness (R/L)	Age of stuttering	Stuttering severity	rTMS							
					Primary motor cortex for laryngeal muscle					Premotor cortical area		
					RMT (%)	CoMEP latency (μV)	CoMEP amplitude (μV)	Intensity (%)	Qualitative error	LLR latency (ms)	Intensity (%)	Qualitative error
1	M	R	6 or 7	Moderate	36	–	–	37	Dysarthria	53.96	31	–
2	M	R	12	Mild	46	–	–	46	Dysarthria	53.62	41	Semantic para.
3	F	R	9	Mild	34	12.66	668.6	34	Dysarthria	51.13	32	–
4	M	R	6 or 7	Moderate	44	11.62	1420	39	Dysarthria	57.13	37	–
5	M	R	15	Mild	36	11.3	324.2	36	Dysarthria	62.86	34	–
6	M	R	4	Severe	28	12.63	81.19	34	Dysarthria	–	27	Semantic para.
7	F	L	4	Mild	28	–	–	28	Dysarthria	63.36	25	–
8	F	R	3	Mild	23	–	–	23	Dysarthria	–	25	–
9	M	R	10 or 11	Very severe	38	–	–	38	Dysarthria/ Stuttering	–	29	Speech arrest
10	M	R	**	Moderate	46	–	–	46	Dysarthria	55.35	36	–
11	M	R	**	Very severe	43	–	–	43	Dysarthria	–	39	Semantic para.
12	M	R	**	Mild	28	–	–	28	Dysarthria	46.68	27	–
13	F	R	7	Mild	36	–	–	36	Dysarthria	–	34	–
14	M	R	5 or 6	Mild	38	13.1	247.65	38	Dysarthria	53.86	36	–
15	M	R	4	Mild	25	–	–	25	Dysarthria	54.92	23	–
16	F	R	3	Moderate	28	11.46	286.2	28	Dysarthria	51.57	27	Semantic para.
Mean ± SD (range)					34.8 ± 7.52 (23–46)	12.13 ± 0.76 (11.3–13.1)	504.64 ± 487.93 (81.19–1420)	34.94 ± 7.02 (25–46)		54.95 ± 4.86 (51.13–63.36)	31.44 ± 5.5 (23–41)	

The data are presented as mean ± standard deviation (SD). CoMEP amplitudes are expressed in microvolts (μV) and CoMEP and LLR latency in milliseconds (ms). Intensity of stimulation is expressed as percent (%) of maximal stimulator

M male, *F* female, *R* right, *L* left, *RMT* resting motor threshold, *CoMEP* corticobulbar motor-evoked potentials, *LLR* long latency response, *Semantic parap.* Semantic paraphasia

**The subject could not provide information regarding age of stuttering onset

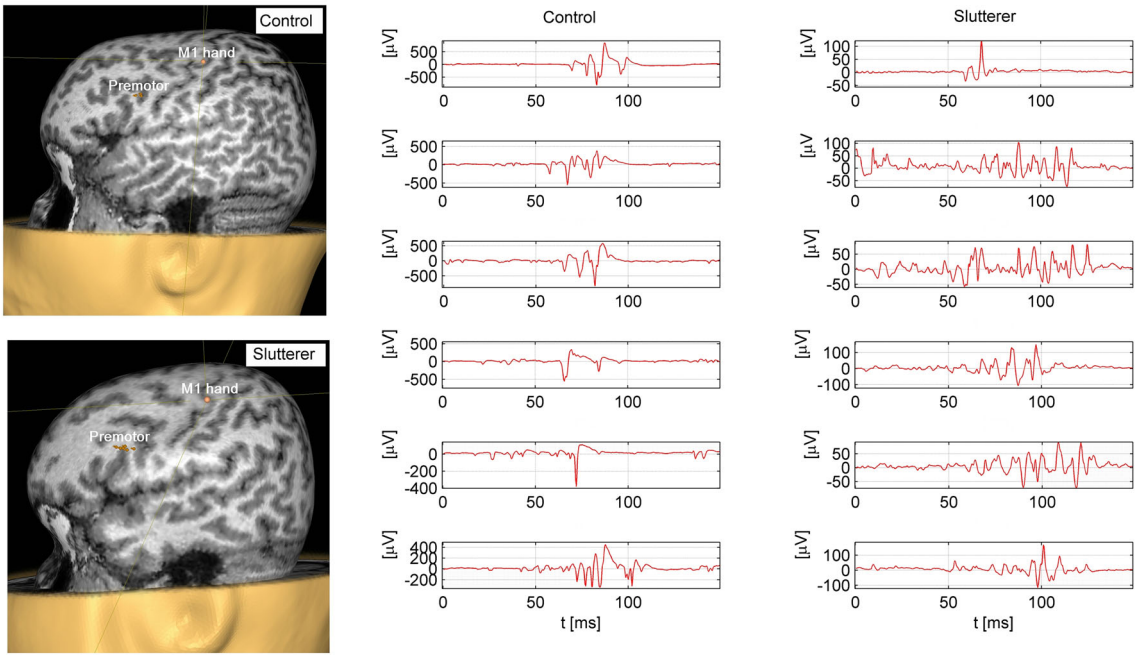

Fig. 4 Repeatability of LLR responses recorded in cricothyroid muscle during rTMS of the *left* premotor cortex in caudal opercular part of inferior frontal gyrus. *Left* stimulated cortical spots in the *left* the *left* premotor cortex in caudal opercular part of inferior frontal gyrus in control (No. 3) and stuttering (No. 16) subject (*yellow spots*) with the M1 APB reference spot. *Right* repeatability of LLRs recorded from cricothyroid muscle in the same control and stuttering subject. The latency values (*x*-axis) are presented in milliseconds (ms) and amplitude values in microvolts (μV) (color figure online)

Discussion

Summary of main results and interpretation

In our study, we tested the excitability of M1 for laryngeal muscles and premotor cortical area in the caudal opercular part of inferior frontal gyrus, corresponding to Broca's area, in stuttering and control-fluent subjects by applying recently developed methodology for mapping these motor speech cortical areas. The M1 for laryngeal muscles has an important role in execution of motor speech movements, and it receives information through association fibers from the premotor cortex (Greenlee et al. 2004; Brodal 2010; Friederici 2015). The posterior part of inferior frontal gyrus, corresponding to Broca's area, is regarded as an important motor speech/language-related cortical area involved in motor speech planning (Penfield and Roberts 1959; Ojemann 1992; Sahin et al. 2009).

We did not find significant differences between stuttering and control group in latency and amplitude of CoMEPs recorded from cricothyroid muscle while mapping the M1 for cricothyroid muscle representation, as well as no differences in latency of LLRs recorded from cricothyroid muscle while mapping the premotor cortex in caudal opercular part of inferior frontal gyrus. The data suggest there are no differences in stuttering compared to controls in functional anatomy of the pathway used for transmission of information from the caudal opercular part of inferior frontal gyrus to the M1 cortices for cricothyroid muscle representation and from there via corticobulbar tract to cricothyroid muscle. The explanation of the mechanisms of CoMEP and LLR generation was previously reported in detail (Deletis et al. 2014), and a brief explanation is given further in the text. The coded signal is transmitted from the premotor cortical area in the caudal opercular part of inferior frontal gyrus to the M1 motoneurons involved in motor speech execution, and from there the signal gets transmitted via corticobulbar pathways to the motoneurons in the brainstem, and from there via cranial nerves to speech target muscles. The excitability of the M1 for laryngeal muscles and premotor cortical area can be increased while the subject is participating in speech task, and we can induce synchronized activity of their neurons and record this activity in the laryngeal muscles as CoMEP and LLR, depending on the neural structure being stimulated. Most probably more synapses are implicated when stimulating the premotor cortical area compared to the M1 for laryngeal muscles; therefore, the latency and the jittering of the LLR is more pronounced compared to CoMEP. Similar to previous findings (Deletis et al. 2014), stimulation of the M1 for laryngeal muscle induced dysarthric-like speech together with CoMEPs recorded from

laryngeal muscle, while stimulation of the premotor cortical spot induced speech arrest and/or semantic paraphasia together with LLRs recorded from cricothyroid muscle. Even though we did not test the non-dominant hemisphere in this study, it is possible to elicit LLR by stimulation of the non-dominant hemisphere according to intraoperative data of Deletis et al. (2011).

Limitation of the study and future guidelines

The reason why CoMEP and LLR responses could not be elicited in all subjects might be related to the stimulation intensity which possibly is not optimal for inducing the responses in some individuals. It could also be related to the discomfort elicited by activation of temporal muscles when applying rTMS. For initial mapping of the M1 for laryngeal muscles, the RMT intensity was used, and in subjects who felt discomfort the intensity was lowered 5–10 %. Only in two subjects the intensity was raised. In our previous study, the stimulation intensities related to RMT were raised 5–10 % and CoMEP responses were obtained in all subjects (Espadaler et al. 2012). Individual differences in scalp to cortex distances (Cvetković and Poljak 2015; Cvetković et al. 2015) of the stimulated cortical areas might also be an additional factor related to the intensity of stimulation that was used. Therefore, we suggest future studies to use an intensity of stimulation higher than RMT (similar to Espadaler et al. 2012) when mapping the M1 for laryngeal muscles, and exploring the ways for tilting the coil for optimal position to reduce the discomfort often elicited by the activation of temporal muscle.

One of the lacks of our study might be the different severities of stuttering of adults enrolled in the study. Therefore, we believe future studies should take a homogeneous group (i.e., only severe stuttering) and apply developed setup for locating the M1 for laryngeal muscles and the premotor cortical area in the caudal opercular part of inferior frontal gyrus in children and adults with stuttering. Furthermore, according to previous findings of studies indicating structural abnormalities in white matter tracts in the premotor frontal cortical areas (Sommer et al. 2002; Chang et al. 2008; Cykowski et al. 2010; Cai et al. 2014) in stuttering, studies could combine the methodology for eliciting neurophysiologic markers of M1 for laryngeal muscles and premotor cortex together with diffusion tensor imaging technique. Likewise, according to previous findings on disturbed timing of cortical activation sequences of brain motor speech areas in the left hemisphere in stuttering shown by MEG (Salmelin et al. 2000), studies should consider using the chronometric TMS and rTMS protocol to chart the time points at which neural activity in these cortical areas functionally contributes to speech process in stuttering. Also, the indexes of cortical excitability of the CoMEP and LLR can also be investigated by rTMS during engagement of stuttering subjects in specific motor speech tasks.

Regarding the silent period considered as a probe of motor cortical inhibition and evaluated in TMS studies investigating the excitability of corticospinal tract in stuttering (Busan et al. 2009, 2013), we currently do not have evidences about the actual existence of silent period when mapping the M1 for laryngeal muscles.

Potential mechanisms underlying stuttering

Generation of speech depends on the functional integrity of corticobulbar tract input to the brainstem nuclei, which innervates the musculature (supralaryngeal and laryngeal muscles) for speech production (Dick et al. 2014). Further, the functional integrity of the corticospinal tract input to the spinal nuclei, which innervates the respiratory musculature, has supportive role in speech execution (Darley et al. 1975; Ludlow 2005). Precise regulation and coordination of the excitability of the M1 cortices in both hemispheres is crucial for the successful execution of speech movements (Watkins et al. 2003; Devlin and Watkins 2008). The neurons in the M1 cortices receive input from a large distributed network and finally integrate received information to provide coordinated speech movements (Sahin et al. 2009; Flinker et al. 2015). Apart from this voluntary motor descending system activated in speech, speech is also generated by activating the emotional motor system including the prefrontal—periaqueductal gray—nucleus retroambiguus—motoneuronal pathway (Holstege and Subramanian 2015). Frontal-thalamic circuits, as well as cerebellar connections with premotor and association cortices, are associated with speech generation (Barbas et al. 2013).

The question that arises is what the key neuronal sources are underlying stuttering. The data across different studies (EMG, neuroimaging and electrophysiologic-diffusion tensor imaging, functional MRI, magnetoencephalography, TMS, electroencephalography, positron emission tomography) provide currently a basis for assumption on the pathophysiologic mechanisms of complex movement disorder affecting speech motor systems. The hypothesis range from structural and functional connectivity deficits found in the Broca's area (BA 44/6) and the premotor regions of the left hemisphere (Fox et al. 1996; Braun et al. 1997; Foundas et al. 2001; Sommer et al. 2002; Chang et al. 2008; Cykowski et al. 2010; Chang et al. 2011; Cai et al. 2014; Beal et al. 2015), alterations in auditory processing system (Liotti et al. 2010; Kikuchi et al. 2011; Jansson-Verkasalo et al. 2014), disturbed timing of cortical activation sequences of brain motor speech areas in the left

hemisphere (Salmelin et al. 2000), increased excitability in the right hemisphere suggested to reflect a compensatory mechanism in stuttering (Braun et al. 1997; Preibisch et al. 2003; Chang et al. 2008; Lu et al. 2010; Kikuchi et al. 2011; Lu et al. 2012), impairments in basal ganglia-thalamo-cortical circuit (Wu et al. 1995, 1997; Giraud et al. 2008; Chang and Zhu 2013; Civier et al. 2013; Foundas et al. 2013; Kemerdere et al. 2016), abnormal oscillations of EMG activity in oro-facial and laryngeal muscles (Fibiger 1971; McClean et al. 1984; Smith 1989; Smith et al. 1993; Kelly et al. 1995), and alterations in cortical excitability of M1 cortices (Sommer et al. 2003, 2009; Busan et al. 2009, 2013; Alm et al. 2013; Neef et al. 2011, 2015). The findings of our study investigating premotor and corticobulbar excitability for laryngeal muscles complement the results of previously published TMS studies on stuttering.

Conclusion

The present study demonstrates the feasibility of locating the M1 for laryngeal muscles and the premotor cortical area in the caudal opercular part of inferior frontal gyrus, corresponding to Broca's area, via rTMS in stuttering subjects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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