Neurophysiologic markers in laryngeal muscles indicate functional anatomy of laryngeal primary motor cortex and premotor cortex in the caudal opercular part of inferior frontal gyrus

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\textbf{Abstract}

Objective: The aim of this study was to identify neurophysiologic markers generated by primary motor and premotor cortex for laryngeal muscles, recorded from laryngeal muscle.

Methods: Ten right-handed healthy subjects underwent navigated transcranial magnetic stimulation (nTMS) and 18 patients underwent direct cortical stimulation (DCS) over the left hemisphere, while recording neurophysiologic markers, short latency response (SLR) and long latency response (LLR) from cricothyroid muscle. Both healthy subjects and patients were engaged in the visual object-naming task. In healthy subjects, the stimulation was time-locked at 10–300 ms after picture presentation while in the patients it was at zero time.

Results: The latency of SLR in healthy subjects was 12.66 ± 1.09 ms and in patients 12.67 ± 1.23 ms. The latency of LLR in healthy subjects was 58.5 ± 5.9 ms, while in patients 54.25 ± 3.69 ms. SLR elicited by stimulation of M1 for laryngeal muscles corresponded to induced dysarthria, while LLR elicited by stimulation of the premotor cortex in the caudal opercular part of inferior frontal gyrus, recorded from laryngeal muscle, corresponded to speech arrest in patients and speech arrest and/or language disturbances in healthy subjects.

Conclusion: In both groups, SLR indicated location of M1 for laryngeal muscles, and LLR location of premotor cortex in the caudal opercular part of inferior frontal gyrus, recorded from laryngeal muscle, while stimulation of these areas in the dominant hemisphere induced transient speech disruptions.

Significance: Described methodology can be used in preoperative mapping, and it is expected to facilitate surgical planning and intraoperative mapping, preserving these areas from injuries.

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1. Introduction

Mapping of primary motor cortex and Broca’s area during awake craniotomy is not always the best option, especially in children and uncooperative patients; it would be important to find a neurophysiologic methodology for intraoperative mapping and monitoring anatomic and functional integrity of these cortical areas. The development of a methodology for preoperative identification using navigated transcranial magnetic stimulation (nTMS) would facilitate surgical planning and intraoperative mapping.

The primary motor cortex (M1) for laryngeal muscles has a role in execution and motor speech control, while the posterior inferior frontal gyrus, namely Broca’s area, is regarded as an important motor speech cortical area having a role in all stages of word encoding and their unification (Sahin et al., 2009), as well as sending coded “commands” to M1.

After electrical stimulation of Broca’s area, postsynaptic potentials of high amplitudes are recorded in the lateral part of the M1 (Greenlee et al., 2004). These results indirectly indicate a functional connection of Broca’s area and M1 for face, mouth, pharynx, and larynx. It has been proposed that the critical parts of M1 needed to control vocalization are closely and uniquely associated with the laryngeal muscles (Corballis, 2003). The direct functional connectivity of M1 for laryngeal muscles was demonstrated in our studies (Deletis et al., 2008, 2009, 2011; Espadaler et al. 2012). In these studies, we have developed methodologies for stimulating M1 for laryngeal muscles and recording corticobulbar motor-evoked potentials (MEPs) from vocal and cricothyroid muscles. Corticobulbar MEPs can be regarded as a synonym for short latency response (SLR) representing a neurophysiologic marker of M1 for laryngeal muscles. It has been also reported that long latency response (LLR) can be recorded from laryngeal muscles after magnetic stimulation of the frontal cortex (Ahammad et al., 1988; Ertelen et al., 2001). We also recorded LLRs intraoperatively but we neither studied this systematically nor investigated or speculated about its exact origin (Ahammad et al., 1988; Ertelen et al., 2001; Deletis et al., 2008, 2011).

The standard intraoperative neurophysiologic method for mapping of Broca’s area and M1 for orofacial, pharyngeal, and laryngeal muscles consists of electrical stimulation of these areas and inducing transient speech disruptions: (a) speech arrest and/or language disturbances while stimulating Broca’s area and (b) dysarthria while stimulating M1 for orofacial, pharyngeal, and laryngeal muscles. Electrical stimulation with 50–60 Hz is used during surgery or through the subdural grid electrodes. In addition to 50–Hz stimulation for 1–3 s, speech arrest was also elicited with a short train of stimuli technique (5 pulses, 3-ms interstimulus interval) (Axelson et al., 2009). Furthermore, high-frequency repetitive transcranial magnetic stimulation with a repetition rate of 2–25 Hz was also used, but so far, only three groups of authors reported induced speech arrest (Epstein et al., 1996, 1999; Pascual-Leone et al., 1991). Recently, Pich et al. (2013) showed a good overall correlation between repetitive navigated (nTMS) and direct cortical stimulation (DCS) during awake surgery for the identification of language-related areas in patients with left-hemisphere lesions.

We hypothesized that time-locked electrical activity is recorded in laryngeal muscles after the stimulation of M1 for laryngeal muscles and of premotor cortex in the caudal opercular part of inferior frontal gyrus.

Therefore, the goal of this study was to investigate the relationship between cortical spots generating neurophysiologic markers recorded in laryngeal muscle and the functional role of these cortical areas by clinically producing transient speech disruptions.

2. Methodology

2.1. Healthy subjects/patients

Ten right-handed healthy subjects, three male and seven female, average age 31 ± 13.32 (range 22–66 years), and 18 right-handed patients, 10 male and eight female, average age 46.2 ± 13.69, range 27–68 years, were included in the study. The Edinburgh Inventory Questionnaire test (Oldfield, 1971) was used for assessment of handedness. All healthy subjects and patients signed informed consent forms to participate in the study. Healthy subjects received a small honorarium. The study was approved by the Ethical Committees of School of Medicine, University of Split, Croatia, and University Hospital Bellvitge, Barcelona, Spain.

2.2. Healthy subjects group

2.2.1. Magnetic resonance imaging (MRI) and navigated transcranial magnetic stimulation (nTMS)

Magnetic resonance imaging (MRI) of the head for each subject was performed with Siemens Magneton Avanto, Tim (76 × 18) strength 1.5 T. MRI images were recorded by specific MRI requirements for nTMS-NBS (Navigated Brain Stimulation), Nexstim System 4 (Helsinki, Finland), including the head, visible ears, and nose. MRI images are integrated in the nTMS machine with a three-dimensional navigation system display of the subjects’ brain. Sophisticated real-time data processing allows the precise display of the induced electric field (E-field) within the brain tissue. Targeting tools available on-screen are the following: grid for systematic brain mapping, targeting tool for optimal coil placement, aiming tool for precise repetition of given stimuli, and automated stimulation (location controlled). The subject wears an optical head tracker and by using a pointer, 12 points are registered on the subject’s scalp. An air-cooled eight-shaped figure coil was used, generating a biphasic pulse of 289 μs pulse length. The maximum E-field is 172 V/m below the Nexstim Focal coil in the spherical conductor model representing the human head.

The nTMS mapping procedure is as follows:

1. Eliciting MEP resting threshold for hand muscle, abductor pollicis brevis (APB);
2. Mapping of the very lateral part of M1 and recording SLR in cricothyroid muscle during the visual object-naming task;
3. Mapping of the inferior frontal gyrus and recording LLR in cricothyroid muscle during the visual object-naming task;
4. Stimulation of cortical spot which elicited SLR to produce transient speech disruption during the visual object-naming task;
5. Stimulation of cortical spot which elicited LLR to produce transient speech disruptions during the visual object-naming task.

Mapping of the M1 for hand muscle is the standard method in nTMS studies dealing with mapping of M1 (Epstein et al., 1999; Schmidt et al., 2009; Jøkunen et al., 2011) and its vicinity (Jennum et al., 1994; Michelucci et al., 1994; Epstein et al., 1999; Stewart et al., 2001), and as a standard intraoperative procedure preceding mapping of primary motor cortex and Broca’s area (Duffau, 2008; Kim et al., 2009; Lubrano et al., 2010). Mapping over the left M1 for APB was determined by the “omega knob” on axial MRI images, or a “hook structure” at the sagittal MRI (Yousry et al., 1997). The central sulcus was also used as a landmark while moving the coil in the anterior–posterior direction in order to map the hot spot for M1 for APB. The MEP resting threshold was defined as the lowest stimulus intensity for eliciting at least five MEPs in the APB muscle.
with an amplitude of at least 50 µV in a series of 10 consecutive trails. Mapping over the left M1 for laryngeal muscle was performed after mapping of M1 for APB muscle. The threshold and location of M1 for laryngeal muscle was determined by recording corticobulbar MEPs from the cricothyroid muscle. Mapping over the left M1 for cricothyroid muscle was performed using into account our recently reported data on Euclidian distance of 25.19 ± 6.51 mm between the cortical representation of M1 for APB and M1 for cricothyroid muscle, with corticobulbar muscle lateral to APB (Espadaler et al., 2012). The resting threshold of corticobulbar MEP was defined as the lowest stimulus intensity for eliciting at least five corticobulbar MEPs in cricothyroid muscle with an amplitude of 50 µV in a series of 10 consecutive trails of TMS.

For mapping of the inferior part of frontal gyrus, the guideline topography of Broca’s area was used according to Ebeling et al. (1989). We also used as a guideline the distance between M1 for cricothyroid muscle and of inferior frontal gyrus generating transient speech disruptions area of 17.23 ± 4.73 mm (Rogić et al., 2014). For mapping of the inferior frontal gyrus, the stimulation intensity was adjusted using the resting threshold for corticobulbar MEPs in cricothyroid muscle. If this intensity was unpleasant to the subject, the stimulus intensity was reduced in steps of 1–2% of maximum stimulator output.

2.2.2. Stimulation parameters and speech task procedure
Patterned bursts of magnetic stimuli were used, described in our previous study (Rogić et al., 2014). Briefly, two paradigms of patterned repetitive stimulation were used:

The “first paradigm” consisted of four bursts of five stimuli each, 6 ms apart, burst repetition rate of 4 Hz.

The “second paradigm” consisted of 16 bursts of four stimuli each, 6 ms apart, burst repetition rate of 12 Hz.

All healthy subjects underwent two testing sessions separated by 4 months.

First testing session. The first paradigm of stimulation was used for eliciting: (a) a neurophysiologic marker of M1 for laryngeal muscles obtained by mapping of the lateral part of M1 and recording of SLR from cricothyroid muscle and (b) a neurophysiologic marker of premotor cortex of inferior frontal gyrus, recorded from laryngeal muscle, obtained by mapping of the caudal opercular part of inferior frontal gyrus and recording of LLR from cricothyroid muscle. Due to the slight jittering in LLR responses, the mean values of five consecutive latencies of LLRs have been calculated.

Second testing session. For testing the functional role of cortical spots generating neurophysiologic markers in cricothyroid muscle, the second paradigm of stimulation was used in order to elicit transient speech disruptions while stimulating the cortical spot which elicited neurophysiologic markers.

Stimulation was applied while subjects were engaged in the visual object-naming task. The methodology for visually presented objects (VPOs)/pictures were taken from a normative study by Brodeur et al. (2010). Fifty pictures were presented during measurement in a randomized manner in one session. The pictures were presented on a computer monitor (LG 22” “LCD” with 1920 × 1080 resolution) using the Presentation program (Neurobehavioral Systems, Albany, CA, USA). The picture was presented on a white background while the edges of the screen were black for the photo sensor to indicate the start and end of the presented picture. VPO was presented for 3000 ms, followed by 2390 ms of blank screen. The time from VPO until the presentation of the next VPO was 5390 ms. The Presentation program triggered nTMS 0–350 ms after VPO. In the first testing session in eight subjects, the beginning of stimulation was 300 ms after VPO, in one subject 350 ms, and in another 150 ms after VPO. In the second testing session, the Presentation program triggered nTMS at zero time with the VPO.

In the first testing session, neurophysiologic markers were recorded from cricothyroid muscle, while in the second testing session, stimulation of identical cortical spots generating markers were repeated to elicit transient speech disruptions, without placing electrodes in cricothyroid muscle. One subject (Table 1, No. 1) was an exception in whom in both testing sessions the electrodes were placed in cricothyroid muscle to record neurophysiologic markers and speech-related activity. In this subject, the second paradigm of stimulation was used to elicit neurophysiologic markers and transient speech disruptions.

According to Abel et al. (2009), errors can happen incidentally in healthy subjects during the visual object-naming task; therefore, before the nTMS stimulation session started, each subject participated in a visual object-naming task in two control sessions. In the second control session, we discarded “problematic” pictures, and did not use them during the nTMS session.

Control measurement with nTMS was performed in four subjects with the coil set away from the subject’s head during the visual object-naming task in the first testing session. This measurement was performed to exclude possible influence of micro-reflexes, especially of sonomotor origin (Bickford et al. 1964; Bickford, 1966).

Table 1
SLR and LLR parameters in a group of healthy subjects and the type of transient speech disruption by stimulating cortical spot which elicited SLR in cricothyroid muscle.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Gender</th>
<th>Age</th>
<th>First testing session</th>
<th>Second testing session</th>
<th>First testing session</th>
<th>Second testing session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SLR Latency (ms)</td>
<td>Intensity (%)</td>
<td>LLR Latency (ms)</td>
<td>Intensity (%)</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>29</td>
<td>11.57</td>
<td>38</td>
<td>Dysarthria</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>66</td>
<td>13.57</td>
<td>45</td>
<td>Dysarthria</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>27</td>
<td>12.53</td>
<td>34</td>
<td>Dysarthria</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>39</td>
<td>11.65</td>
<td>42</td>
<td>Dysarthria</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>27</td>
<td>12.54</td>
<td>34</td>
<td>Dysarthria</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>26</td>
<td>14.96</td>
<td>40</td>
<td>Dysarthria</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>30</td>
<td>11.9</td>
<td>40</td>
<td>Dysarthria</td>
<td>43</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>21</td>
<td>11.98</td>
<td>34</td>
<td>Dysarthria</td>
<td>35</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>23</td>
<td>13.7</td>
<td>37</td>
<td>Dysarthria</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>22</td>
<td>12.2</td>
<td>39</td>
<td>Dysarthria</td>
<td>29</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>31 ± 13.32</td>
<td>12.66 ± 1.09</td>
<td>38.3 ± 3.7</td>
<td>33.8 ± 7.08</td>
</tr>
</tbody>
</table>

No = transient language disruption elicited number of times.

* Subject did not participate in the second testing session of the study.
2.2.3. Recordings

The recordings of neurophysiologic markers from cricothyroid muscles were done using a five-channel EMG amplifier integrated with E-field navigation and TMS stimulation of Nexstim NBS system (Nexstim NBS System 4, Helsinki, Finland). The channels are: (a) EMG from APB muscle, (b) EMG from cricothyroid muscle, (c) beginning and end of VPO, (d) stimulator trigger, and e) microphone recordings.

Surface electrodes Neurione 720 (Ambu Inc, Glen Burnie, MD, USA) were placed over the right APB muscle. The method for the percutaneous placement of electrodes into cricothyroid muscle has been described in our previous studies (Deletis et al., 2011; Espadaler et al., 2012). Briefly described, for recording SLR and LLR from right cricothyroid muscle, two hook-wire electrodes were used, each consisting of a Teflon-coated stainless steel wire, 76 µm in diameter passing through 27-gauge needles (0.4 mm) and of 13 mm length (hook-wire electrodes, specially modified, Viasys Healthcare WI, MA, USA). The recording wires have a stripped Teflon isolation of 2 mm at their tip and curved to form the hook for anchoring them.

In the second testing session, modified methods of video and audio recordings (Panasonic HDC-SDT750) (Lioumis et al., 2012) were taken for off-line analysis.

2.3. Patient group

2.3.1. Intraoperative DCS

The patients presented with pathology in the left hemisphere (Table 2), located in or nearby the eloquent cortical areas. A monopolar handheld probe was used to stimulate the exposed cortex with a stimulation paradigm of bursts (monopolar stimulator instrument dissector 1.2 mm curved 30°, INOMED, Germany), while a bipolar electrode for 50 Hz stimulation. The electrodes were placed in the cricothyroid muscle and recordings were made during all mapping procedures for M1 for laryngeal muscles and premotor cortex of inferior frontal gyrus.

2.3.2. DCS procedure

The procedure includes the following:

1. Mapping of somatosensory cortex by using phase reversal median somatosensory-evoked potentials
2. Mapping of M1 for hand, leg, and facial muscles to produce muscle contractions
3. Mapping of the inferior frontal gyrus in order to produce speech arrest
4. Positive spots from mapping step 2 were repeated for eliciting MEPs in hand, leg, and facial muscles – very lateral part of M1 was also mapped to elicit SLR in cricothyroid muscle
5. Positive spots from mapping step 3 were repeated for eliciting LLR of premotor cortex in the caudal opercular part of inferior frontal gyrus, recorded from laryngeal muscle.

2.3.3. Stimulation parameters and speech task procedure

All patients underwent electrical stimulation of the exposed cortex during awake craniotomy using the following stimulating parameters:

a) Stimulation paradigm of 50 Hz, biphasic pulses of 4 ms duration, 3 s of train, intensity up to 15 mA for mapping of M1 for upper (hand muscles) and lower extremities (leg muscles) and facial muscles in order to produce muscle contractions were applied. The same paradigm of stimulation was applied in order to elicit speech arrest while mapping the inferior frontal gyrus. During surgery after identification of the Rolandic cortex, a series of line-drawing images were presented on the computer screen to the patients as a visual object-naming task. Images were time-locked at zero time with the electrical stimulation. Before the surgery, patients were acquainted with the visual object-naming task and problematic pictures were excluded from the intraoperative testing. The patient was instructed to name the object in combination with the beginning sentence: “This is a...” during cortical stimulation.

All cortical sites were stimulated three times as a standard procedure during intraoperative mapping of these cortical areas. The cortical spot was considered “positive” if DCS elicited speech arrest in at least two of three times after testing of identical point.

b) Stimulation paradigm of bursts consists of four monophasic pulses of 0.5 ms duration each, 4 ms apart, burst repetition rate of 4 Hz, intensity up to 15 mA for eliciting SLR, and LLR recorded from cricothyroid muscle. Stimulation was applied while subjects were in rest (not engaged in the visual object-naming task). Due to the slight jittering in LLR responses, the mean values of five consecutive latencies of LLRs have been calculated.

Table 2

Latencies of SLR and LLR recorded in cricothyroid muscle in a group of patients.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender</th>
<th>Age</th>
<th>Pathology</th>
<th>SLR latency (ms)</th>
<th>LLR latency (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>59</td>
<td>Left frontal GBM</td>
<td>10.49</td>
<td>55.2</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>67</td>
<td>Left frontal M1</td>
<td>12.2</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>68</td>
<td>Left temporal GBM</td>
<td>12.6</td>
<td>56.76</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>32</td>
<td>Left frontal glioma</td>
<td>12.1</td>
<td>60.35</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>43</td>
<td>Left temporal GBM</td>
<td>11</td>
<td>51.45</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>35</td>
<td>Left frontal cavernoma</td>
<td>12.35</td>
<td>58.7</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>60</td>
<td>Left frontal M1</td>
<td>12.95</td>
<td>59.05</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>38</td>
<td>Left frontal cavernoma</td>
<td>14.23</td>
<td>58.7</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>26</td>
<td>Left parietal glioma</td>
<td>12.15</td>
<td>50.15</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>39</td>
<td>Left fronto-temporal astrocioma</td>
<td>15.7</td>
<td>50.05</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>55</td>
<td>Left frontal oligodendroglia</td>
<td>14.35</td>
<td>52.45</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>27</td>
<td>Left frontal MAV</td>
<td>13.45</td>
<td>55.1</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>59</td>
<td>Left frontal GBM</td>
<td>12.9</td>
<td>50.05</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>43</td>
<td>Left fronto-temporal astrocioma</td>
<td>13.45</td>
<td>50.15</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>34</td>
<td>Left temporal cavernoma</td>
<td>12.9</td>
<td>53.95</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>61</td>
<td>Left fronto-opercular glioma</td>
<td>11.6</td>
<td>50.15</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>38</td>
<td>Left temporal-insular astrocioma</td>
<td>11.65</td>
<td>58.95</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>48</td>
<td>Left parietal cavernoma</td>
<td>11.6</td>
<td>52.45</td>
</tr>
</tbody>
</table>

Mean ± SD 46.22 ± 13.69 12.67 ± 1.23 54.25 ± 3.69

Range 27–68 10.49–15.7 50.05–60.35
In the patient group, the electrodes were placed in the cricothyroid muscle and recordings were made during all mapping procedures.

2.3.4. Recordings

A pair of twisted subdermal needles (Neuroline-Ambu, 0.5” x 27 G) were placed in: orbicularis oris, extensor digitorum communis and APB, tibialis anterior, and abductor hallucis. The method for the percutaneous placement of electrodes into cricothyroid muscle was identical to that described in the group of healthy subjects. In the patient group, we used a modified Synergy (Viasys Healthcare) 10-channel EMG-evoked potential unit.

The team consisted of a neurophysiologist and neuropsychologist observing and documenting elicited transient speech disruptions.

2.4. Anesthesia

All patients underwent a standard technique of asleep–awake–asleep anesthesia, with conscious sedation using propofol and remifentanil, and assisted ventilation with laryngeal mask (LMA). After craniotomy was performed, the patients were gradually awakened by weaning their sedation and LMA was removed, and reinserted after completion of the mapping procedure.

3. Results

3.1. Short latency response

In all healthy subjects and patients, SLR was successfully recorded from cricothyroid muscle while stimulating the very lateral
part of M1. The latency of SLR elicited by magnetic stimulation in healthy subjects was 12.66 ± 1.09 ms (Table 1 and Fig. 1), and by electrical stimulation in patients 12.67 ± 1.23 ms (Table 2, Fig. 2). The functional role of this area was determined by stimulation of cortical spot which elicited SLR and clinically producing dysarthria (Table 1 and Table 2), accompanied by contractions of facial and masticatory muscles.

**Long Latency Response.** In all healthy subjects and patients, LLR was successfully recorded from cricothyroid muscle while mapping the caudal opercular part of inferior frontal gyrus. The latency of LLR elicited by magnetic stimulation in healthy subjects was 58.5 ± 5.9 ms (Table 1, Fig. 1), while by electrical stimulation in patients it was 54.25 ± 3.69 ms (Table 2, Fig. 2). The functional role of this area was tested by stimulation of cortical spot that elicited LLR and clinically produced transient speech disruptions (Table 1 and Table 2). Intraoperatively, speech arrest was elicited in all patients, while in healthy subjects speech arrest and/or language disturbances were elicited (Table 1).

Speech arrest was elicited in seven healthy subjects (77.8%). In those seven healthy subjects in whom speech arrest was induced once, twice, or more times, language disturbances (semantic errors) were also induced stimulating the same cortical spot. In two subjects (22.2%) (No. 2 and No. 10), language disturbances (semantic errors) (Table 1) were elicited (kruska (pear)/instead/grožde (grapes)/, etc.). In healthy subjects, speech arrest was characterized as a complete cessation of speech, confirmed by absent microphone recordings together with an off-line analysis of video recordings showing no facial and masticatory muscle contractions. Semantic errors (subjects gave semantically similar word for the object) were also confirmed by an off-line analysis of video recordings.

In healthy subjects, when M1 for cricothyroid muscle and inferior frontal gyrus were mapped, the electrical activity was recorded in cricothyroid muscle in the pre-speech (pre-EMG period) and speech execution period (contractions related to the speech). The SLRs and LLRs are elicited in cricothyroid muscle and can be detected in the period before the pre-speech phase. By stimulating the same LLR cortical spot which elicited speech arrest, semantic paraphasias could also be elicited with LLR recordings before pre-speech, and with electrical activity in pre-speech and speech execution period. When semantic paraphasias were elicited from other parts of inferior frontal gyrus (not LLR cortical spot), LLR was not recorded in cricothyroid muscle; only electrical activity in the pre-speech and speech execution phase was recorded in cricothyroid muscle.

In one healthy subject (Table 1, No. 1), in whom speech arrest was elicited while simultaneously recording activity from cricothyroid muscle, LLRs were present in EMG, but voice was absent in microphone recordings (Fig. 3). The subject did not give any response for object presenting/vješalica (coat hanger)/which was confirmed with absent activity recorded with the microphone. The off-line video analysis also showed absent activity in facial and masticatory muscles. The latency of LLRs, indicated with the red stars in Fig. 3 was 57.6 ± 0.71 ms.

LLR was elicited in all healthy subjects 423.50 ± 115.31 ms from the beginning of the visual object presentation. The speech onset reaction time was 684.07 ± 91.48 ms. The repeatability of eliciting LLR when mapping the caudal opercular part of inferior frontal gyrus is shown for one healthy subject No. 3 in Fig. 4.

In the patient group, M1 was mapped first applying electrical stimulation with 50 Hz in order to elicit tonic contractions in the
orofacial muscles during rest and dysarthric speech. With the same stimulation paradigm, inferior frontal cortex was mapped, and only a specific area in the caudal opercular part of inferior frontal gyrus, which elicited speech arrest, also elicited LLR from cricothyroid muscle (Fig. 2). Stimulation of larger and more rostral parts of the opercular inferior frontal gyrus elicited speech arrest or paraphasia without eliciting LLR in cricothyroid muscle (Figs. 2 and 5).

4. Discussion

For the first time, we have shown that it is possible to elicit neurophysiologic markers of M1 and premotor cortex in the caudal opercular part of inferior frontal gyrus for laryngeal muscles, applying a specific paradigm of stimulation. To elicit SLR and LLR, a patterned burst of magnetic stimuli were used in healthy subjects (Rogić et al., 2014), while a short train of electrical stimuli was used in patients. The functional role of these areas was tested by stimulation of cortical spot that elicited SLR and LLR and clinically producing transient speech disruptions, respectively.

SLR can be regarded as a neurophysiologic marker of M1 for laryngeal muscles, according to the following evidence:

1) SLRs are elicited from the vocal and cricothyroid muscles during electrical and magnetic stimulation of the M1 for laryngeal muscles in a group of patients and healthy subjects.
SLR corresponds/equal to corticobulbar MEP.

2) The results of this study have shown that in all healthy subjects and patients, nTMS and DCS of cortical spot, which elicited SLR, clinically produced dysarthria.

3) M1 for laryngeal muscles is anatomically represented at the most lateral part of M1. Recently, we have reported that the distance between the cortical representation of M1 for APB and M1 for cricothyroid muscle was 25.19 ± 6.51 mm, with cricothyroid muscle lateral to APB (Espadaler et al., 2012).

During speech disruptions induced by stimulation of SLR cortical spot, dysarthric speech was observed accompanied with contractions of the orofacial and masticatory muscles visible on the video recordings. Contractions of orofacial and masticatory muscles were presumably produced due to the spread of the current from primary motor cortex for laryngeal muscles to the nearby primary motor cortical representations for other cranial muscles.

While the results demonstrate the SLR spot’s role in speech execution, it probably also has functional roles in other voluntary laryngeal movements that we did not test, such as coughing, throat clearing, swallowing, crying, moaning, or humming.

The following are evidence for LLR being the most likely neurophysiologic marker of premotor cortex for laryngeal muscles:

1) The results of this study have shown that DCS and nTMS of the stimulated cortical spot, which elicited LLR, clinically produced speech arrest in all patients and speech arrest and/or language disturbances in healthy subjects. Anatomically, this cortical spot corresponds to a small region of the caudal opercular inferior frontal gyrus detectable on an MRI of healthy subjects and in the patients by intraoperative inspection.
After electrical stimulation of Broca’s area, post-synaptic potentials of high amplitude were recorded in the lateral part of the M1 (Greenlee et al., 2004). These data can be regarded as indirect evidence of an anatomical connection between the premotor cortex in the caudal opercular part of inferior frontal gyrus and M1 for laryngeal muscle. There are also possible contributions of other pathways to LLR origin, other than through M1.

LLR was recorded in cricothyroid muscle when speech arrest was elicited (Fig. 3) in one healthy subject since electrodes were inserted in the second session only in that subject. By stimulating the same LLR cortical spot which elicited speech arrest, semantic paraphasias could also be elicited with LLR recordings before pre-speech, and with electrical activity in pre-speech and speech execution period. Stimulus spread to nearby Broca’s area language cortex likely explains these observations. When semantic paraphasias were elicited by stimulating other parts of the dominant opercular inferior frontal gyrus (not LLR cortical spot), LLR was not recorded in cricothyroid muscle; only electrical activity in pre-speech and speech execution phase was recorded in cricothyroid muscle. Dysphasia due to Broca’s area language cortex disruption likely explains this kind of speech arrest without elicited LLR. Furthermore, even though we did not test the nondominant hemisphere, it is possible that cortical spot eliciting LLR by stimulation of nondominant hemisphere is likely part of the relays for emotive expression (e.g., prosody and singing). What functional role the LLR spot of either hemisphere may play in other voluntary laryngeal movements, such as humming, etc., is unknown because we tested only speech.

In the patient group, electrical stimulation with 50 Hz was first applied in order to elicit contractions in the orofacial muscles during rest and dysarthric speech when mapping M1, and to elicit speech arrest and/or language disturbances when mapping the dominant inferior frontal gyrus. Dysarthric speech was presumably accompanied with dysphonic/aphonic speech as well.

Speech arrest and/or phonological or semantic paraphasias were elicited by mapping specific parts of premotor cortex in the caudal opercular part of inferior frontal gyrus, and tonic contractions could be elicited from cricothyroid muscle. After that, bursts consisting of four stimuli each, 4 ms apart, burst repetition rate of 4 Hz, were applied to the cortical spots which have previously elicited speech arrest. Cortical spots which elicit semantic or phonological paraphasias and/or speech arrest without LLR recording in cricothyroid muscle when mapping the dominant inferior frontal gyrus can be regarded as Broca’s area language cortex (Figs. 2 and 5). We suggest that dysphasia/aphasia might be likely contributing mechanism to semantic or phonological paraphasias and/or speech arrest without LLR recording in cricothyroid muscle.

The spot in the caudal opercular part of dominant inferior frontal gyrus generating LLR and speech arrest after stimulation can be considered only as premotor cortex for laryngeal muscle, but not as motor language cortex. This spot cannot be a safe resection margin, but an orientation spot for close proximity to the language producer Broca’s area.

We believe that we are dealing with small neuronal pools where language as a mental process gets transformed into a coded message to the M1 in order to activate muscles involved in speech. It is a matter of fact that no methodology, so far, to look at the electrical activity of laryngeal muscles during speech arrest has been published.

During mapping procedures for eliciting neurophysiologic markers, only a very limited area, approximately 5 × 5 mm of stimulated cerebral cortices, by either nTMS or DCS (Deletis et al., 2008), generates specific neurophysiologic markers recordable in the laryngeal muscles. Therefore, we consider these markers as anatomical and functional indicators of primary motor and premotor cortex for laryngeal muscles. In our previous studies (Deletis et al., 2008, 2011), we have also showed the possibility to elicit SLR and LLR in laryngeal muscles by transcranial electrical stimulation (TES) of the right hemisphere with the TES montage C3 versus Cz and C4 versus Cz. TES stimulating parameters were a short train of electric stimuli consisting of three to five stimuli having a duration of 0.5 ms each. These stimuli were spaced 2 ms apart with a train repetition rate of 2 Hz and intensity up to 120 mA.

Thus, neither spot can be specific to language or speech and in order to use the LLR as a guide to nearby Broca’s area language cortex, one must know through other means that the dominant hemisphere is being stimulated.

As we have mentioned earlier in the methodological part, we made an effort to exclude micro-reflexes of Bickford (Bickford et al., 1964; Bickford, 1966) as a potential source of SLR or LLR in the nTMS measurement of healthy subjects. The sonomotor micro-reflex can be activated via corticoreticular neurons as has been already shown (Fisher et al., 2012). We excluded micro-reflexes by moving the coil away from the head during the stimulation. By this approach, SLR and LLR were not recorded. Furthermore, if SLR and LLR are micro-reflexes due to TMS, or activation of the auditory system by click produced by magnetic coil, micro-reflex would be activated by the coil away from the head. We have shown that is not the case. We have presented further evidence that SLR and LLR are cortical origins and specific for the particular region in our data. SLR can only be elicited if M1 for laryngeal muscles is stimulated and LLR can only be elicited if premotor cortex in the caudal opercular part of inferior frontal gyrus is stimulated.

Our explanation of the mechanism of SLR and LLR generation during speech execution is as follows: when the coded signal from the premotor cortex in the caudal opercular part of inferior frontal gyrus reaches the M1 motoneurons of the muscles involved in speech, it gets transmitted further via the corticobulbar pathway to the motor neurons of the cranial nerves in the brain stem. From the motoneurons of the cranial nerves and via cranial motor nerves, targeted muscles get activated. If M1 for laryngeal muscles and premotor cortex in the caudal opercular part of inferior frontal gyrus.
gyrus are stimulated during their “working state,” when their excitability is increased, we can easily induce synchronized activity of their neurons, and record this activity in the laryngeal muscles as SLR or LLR, depending on the neural structure being stimulated. Increased excitability of Broca’s area during motor speech processing has been shown in a temporal domain of 300 ms during the execution of specific speech tasks. This was shown by an invasive study of recording EPSPs from Broca’s area in epileptic patients (Sahin et al., 2009), and noninvasively (Schuhmann et al., 2009; 2012; Rogić et al., 2014). In this study, for most of the healthy subjects the delay of 300 ms for triggering of nTMS after picture presentation has been shown to be optimal for eliciting SLR and LLR.

Presumably, more synapses are involved when stimulating premotor cortex in the caudal opercular part of inferior frontal gyrus, compared to M1 for laryngeal muscles; therefore, the latency of LLR is longer than for SLR. Not only latency difference but also jittering is more pronounced in LLR than SLR. After analysis of response latencies of LLR elicited by magnetic stimulation in healthy subjects and by electric stimulation in patients, we found out that LLR have longer latencies when elicited by magnetic versus electric stimulation. The latency of LLR elicited by magnetic versus electric stimulation were 58.5 ± 5.9 and 54.25 ± 3.69 ms. Furthermore, it was easier to elicit both SLR and LLR electrically than magnetically. This was the reason why we needed to introduce a time-locked visual object-naming task when applying nTMS in healthy subjects to facilitate eliciting of SLR and LLR. Intraoperatively, patients were in the rest state when eliciting SLR and LLR.

4. Conclusion

In all healthy subjects and patients, neurophysiologic markers of specific cortical areas are identified: (a) a neurophysiologic marker of M1 for laryngeal muscles obtained by mapping of the lateral part of M1 and recording of SLR from cricothyroid muscle and (b) a neurophysiologic marker of premotor cortex for laryngeal muscles obtained by mapping of the caudal opercular part of inferior frontal gyrus and recording of LLR from cricothyroid muscle. Stimulation of the SLR spot clinically produced dysarthria (and probably also dysphonia). Stimulation of the dominant hemisphere’s LLR spot produced speech arrest likely by disrupting phonation and, in some healthy controls, produced language disturbances likely by unavoidably also exciting nearby Broca’s area language cortex. LLR cortical spot in the dominant hemisphere may be a useful guide to the nearby proximity of Broca’s area language cortex. It might also help approximate the likely nearby location of Broca’s area language cortex for patients unable to tolerate language mapping under local anesthesia. Further studies are needed on the same group of patients undergoing nTMS and DCS in order to replicate the data obtained in this study. The nondominant hemisphere should also be tested on the group of healthy subjects and patients, since it is possible that nondominant SLR or LLR might disrupt speech execution, which is not specific to the dominant hemisphere.

In addition, the possible functional role of the SLR and LLR spots in executing voluntary phonations and laryngeal movements other than speech could be evaluated by specific testing.

5. Significance

Novel methodology for testing the anatomic and functional integrity of primary motor and premotor cortex for laryngeal muscles can be used in preoperative and intraoperative mapping. Given the close proximity of laryngeal premotor cortex to Broca’s language cortex in the dominant hemisphere, the methodology could facilitate or compliment surgical planning and intraoperative mapping intended to help avoid motor speech deficits.

References


