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Short communication

Test–retest reliability of motor evoked potentials (MEPs) at the submental muscle group during volitional swallowing

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ABSTRACT

Motor evoked potentials (MEPs) recorded from pharyngeal and anterior hyo-mandibular (submental) muscles at rest have been used to evaluate treatment effects on neural pathways underlying swallowing. This study documents a novel methodological approach of recording reliable intra- and inter-session MEPs at the submental muscle group during task-related volitional swallowing. MEPs were elicited by single-pulse transcranial magnetic stimulation (TMS), triggered by a custom-made system when a pre-set level of surface electromyographic activity in the target muscles was breached. Fifteen MEPs were recorded during each of four sessions. Intraclass correlation coefficients (ICCs) were used to assess test–retest reliability within and across sessions for blocks of 3, 5, 10 and 15 trials. Highly reliable intra-session reliability was achieved, maximal for blocks of five trials (0.915). Inter-session reliability varied between 0.474 (three trials per block) and 0.909 (10 trials per block). Surface electromyography-triggered TMS allows reliable measurement of MEP amplitude at the submental muscle group within and across sessions when muscles are pre-activated during volitional swallowing. This methodology will be useful for future investigations on the effects of pathology and modulation of swallowing neural pathways.

1. Introduction

The evaluation of transcranial magnetic stimulation (TMS) triggered motor evoked potentials (MEPs) has recently emerged as a measure of the excitability of the corticobulbar pathways involved in swallowing. Recent studies have employed this approach to investigate the effects of electrical stimulation of the pharynx (Fraser et al., 2002) and faucial pillars (Power et al., 2004) on corticobulbar excitability. A reliability analysis of TMS for mapping the swallowing musculature has documented moderate to good test–retest reliability for motor map volume and maximal MEP size location, as well as motor map area, maximal MEP site location, maximal MEP site size and motor threshold, respectively (Plowman-Prine et al., 2008). In these studies, MEPs were recorded when the peripheral muscles of interest were at rest. MEPs have not previously been recorded from the oropharyngeal muscles during performance of a specific task, e.g. swallowing. Investigation of MEPs triggered in these muscles during a task-specific context is important, however, as they may provide insight into the excitability of the corticobulbar pathways during swallowing and increase our understanding of corticobulbar contribution to volitional swallowing behavior.

Triggering MEPs of the submental muscle group during swallowing also yields additional advantages. MEP amplitudes reported previously for the pharynx ($99 \pm 24\,\mu V$) and the mylohyoid muscle ($152 \pm 37\,\mu V$) were small and required relatively high TMS intensities [1.7 ± 0.1 T] (Hamdy et al., 1997). As background muscle contraction has a facilitatory effect on MEP amplitude in a variety of upper limb muscles (Rothwell et al., 1991; Maertens de Noordhout et al., 1992; Lim and Yiannikas, 1992), we posited that the same could be found in the context of oropharyngeal muscles. For example, McMillan et al. (2001) documented a positive relationship between isometric muscle pre-activation and MEP amplitudes

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evoked in the masseter (facial) muscle. These researchers also supported earlier observations (Macaluso et al., 1990) that MEPs are not consistently elicited in resting masseter muscle. Our group observed a similar phenomenon in some research participants during preliminary MEP recordings at the anterior hyomandibular (submental) muscles using surface electromyography (sEMG). Recent research into the effects of pre-activation in a different context, that of the abductor digiti minimi muscle, also suggests that good reliability of recording the mean MEP peak-to-peak amplitude is found when a sufficiently large number of trials are averaged for analysis (Christie et al., 2007).

We report a new method of using surface electromyography recorded during volitional swallowing to trigger TMS evoked motor potentials, thus allowing for muscle pre-activation in a functional context. This study further established the reliability of MEP peak-to-peak amplitude measures, recorded with sEMG at the submental muscle group, during volitional swallowing. We applied similar statistical analyses to those reported previously for the establishment of reliability measures of MEPs recorded from muscles innervated by corticospinal projections (Christie et al., 2007) to document the reliability of MEPs recorded from muscles innervated by the corticobulbar tract.

2. Methods

Ten young healthy adults participated in this project [mean age: 27.5 years; (S.D. 2.9 years), 7 females, 7 right-handed (Oldfield, 1971)]. The participants attended four sessions at a free-standing brain research institute. This project was approved by the appropriate Regional Health and Disability Ethics Committee. Participants provided written informed consent and expressed full comprehension of the research procedures. Participants reported no medical history, current symptoms of dysphagia or neurological impairment and no drug use that would potentially affect their swallowing or neurological function. All participants were naïve in regards to procedure, MEP recordings and analysis.

2.1. Data recording

Two surface electrodes (BRS-50K, Blue Sensor^{TM}, Ambu, Denmark) were positioned at the midline over the submental muscle group (anterior belly of digastric, mylohyoid, and geniohyoid). Intersession variation in electrode placement was minimized by always placing the anterior electrode first, with its anterior edge located directly behind the interior spine of the mandible. The second electrode was mounted thereafter, with a gap of 5 mm in-between electrodes. One reference electrode was positioned over the bony aspect of the mandible. The surface electrodes (approximately 2 cm × 1.5 cm in size) were placed with the longer edge perpendicular to midline, thus allowing an overlap of 1 cm on either side of midline. The electrodes thus recorded collective sEMG activity from left and right digastric muscles (anterior belly), portions of the left and right mylohyoid muscles and left and right geniohyoid muscles. The electrodes were connected to an amplifier (Dual Bio Amp, ML 135^{TM}, ADInstruments, Castle Hill, Australia) and recording system (Powerlab 8/30^{TM}, ML 870, ADInstruments). Data were acquired at a rate of 10 kHz, with high pass filtering of 10 Hz. Discharge of the magnetic stimulator (Magstim 200^{TM}, Magstim Company Limited, Whitland, Wales) triggered the Scope^{TM} software package to record a sweep of 200 ms duration. Each sweep recorded data 50 ms pre- and 150 ms post-trigger.

2.2. Trigger system

Transcranial magnetic stimulation was triggered from submental sEMG activity. In order to establish the trigger threshold, peak sEMG activity of the submental muscle group was recorded across 10 swallows for each individual. The trigger threshold for TMS was subsequently set to 75% of each individual's mean peak sEMG amplitude. Triggering TMS at this level of submental background activation assured discharge of the stimulator and subsequent elicitation of a MEP at the onset of the pharyngeal phase of the volitional swallow.

This threshold was determined at the beginning of each session to correct for slight differences in electrode placement between sessions. A custom-built trigger device monitored the continuous sEMG signal (Fig. 1). A single transistor–transistor logic (TTL) impulse was produced when the recorded sEMG signal breached the pre-set trigger threshold. The trigger device was automatically disabled for 10 s after TMS discharge to avoid unintentional production of a secondary trigger stimulus. Research participants thus swallowed no more frequently than approximately every 12 s. Participants were asked to swallow their saliva after the rest period

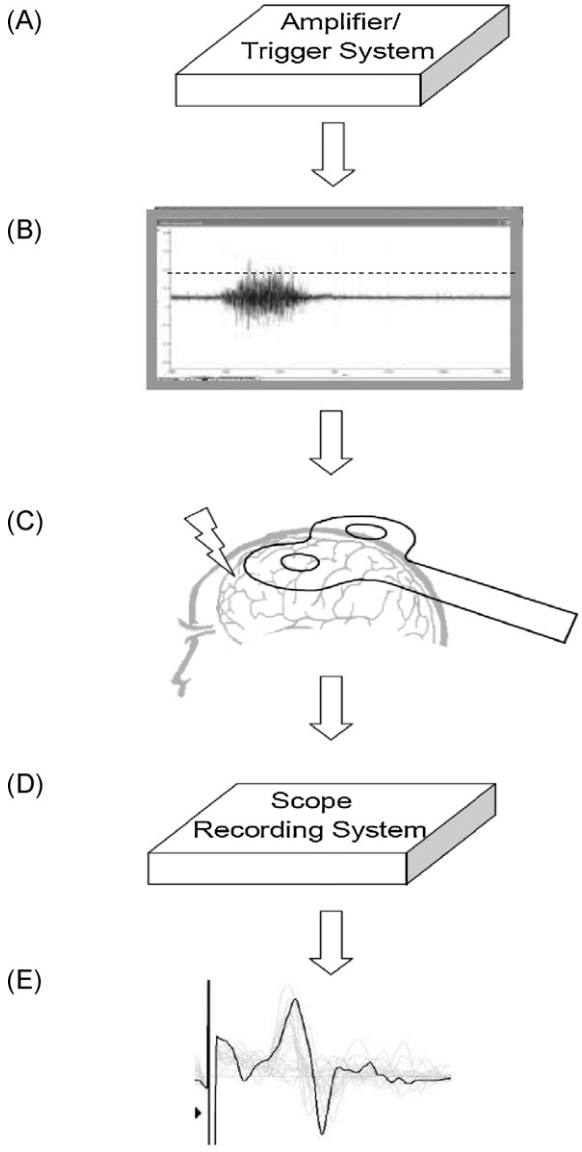

Fig. 1. Illustration of triggering system. An amplifier and custom-made triggering system (A) monitor surface electromyographic activity during muscle activation (B). Upon breach of a pre-set threshold, a TTL pulse is generated to trigger the magnetic stimulator (C) which subsequently triggers the recording of a 200-ms sweep in the Scope recording software (D). (E) Displays the averaged MEP signal across 15 trials of one research participant.

had ended and to keep their tongue as still and relaxed as possible. This minimized contamination of the sEMG signal by voluntary tongue movements immediately prior to a swallow. Participants were allowed to practice this task at the beginning of each data collection session.

2.3. Transcranial magnetic stimulation

A figure-8-coil with an outer diameter of 70 mm and a maximal output of 2.2 T was used for focal cortical stimulation. The optimal site for consistently eliciting MEPs was identified at a TMS intensity of 60% maximal stimulator output. An area of 4 cm anterior and 8 cm lateral to the cranial vertex (Cz) was searched in 1 cm increments for the site producing largest MEP peak-to-peak amplitude. During this procedure, TMS was triggered from sEMG activity when the preset trigger threshold, as identified at the beginning of each session, was breached during volitional muscle contraction. The stimulating coil was positioned with the handle pointing posteriorly and with the coil positioned at a 45° angle to the nasion–inion plane. If no MEPs could be detected at this stimulation intensity, stimulator output level was increased until MEPs were detectable or maximal stimulator output was reached.

Subsequently, maximal MEP amplitude was identified by increasing MEP amplitude in 10% increments until no increase in MEP amplitude was observed or maximal stimulator output was reached. The same procedure was undertaken over both hemispheres to determine the hemisphere over which the largest MEPs could be evoked, which was used for all subsequent sessions. TMS intensity for further testing was set to the value at which half maximal MEP amplitudes were elicited and remained constant across all data acquisition sessions. This value was identified by reducing stimulator output to a level at which at least three consecutive half-maximal MEPs could be recorded. During each of four data collection sessions, 15 MEPs were recorded and averaged for offline statistical analysis. Sessions were performed on separate days, at least 48 h apart and at approximately the same time of day.

2.4. Data analysis

To determine the within-session reliability of MEP peak-to-peak amplitudes, the 15 trials recorded in the first session were divided into three blocks of five trials. Intraclass correlation coefficients (ICCs) were calculated on these data. Additionally, ICCs were calculated for three blocks containing fewer trials per block (only the first three or only the first four trials) to determine which number of trials produced the greatest intra-session reliability.

To examine inter-session variability, a two-way repeated measures Analysis of Variance (ANOVA) was performed first to identify influences of block-within-session (15 trials divided into 3 blocks of 5 trials for each session), four sessions and the interaction between these factors. Subsequently, inter-session reliability of MEP amplitudes was determined between session 1 and each of the three subsequent sessions. ICCs were calculated for blocks of the first 5, 10 and all 15 trials in order to identify the number of trials which produced optimal inter-session reliability. Further, ICCs for inter-session reliability between all four sessions were calculated, again using blocks of the first 5, 10 and all 15 trials.

3. Results

Two of the 10 participants did not display identifiable MEPs during the swallowing task and were excluded. In five of the remaining participants, MEPs were recorded from the left hemisphere. For the other three participants MEPs were recorded from the right hemisphere. Fig. 2 shows representative MEP waveforms from one subject.

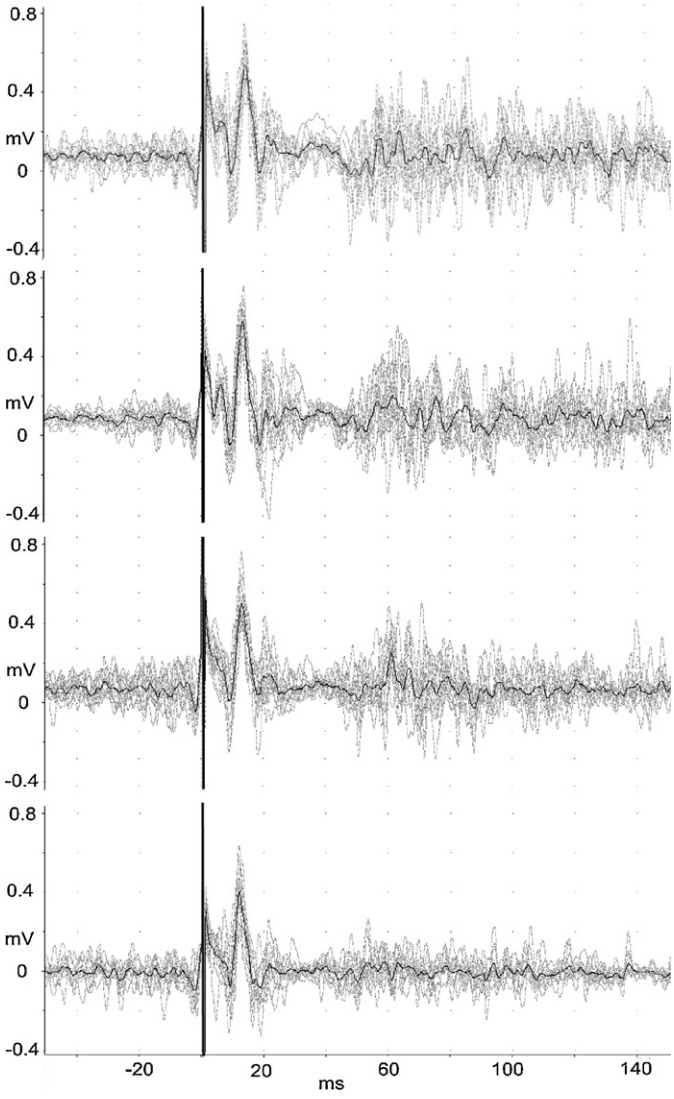

Fig. 2. Illustration of MEP waveforms of one representative participant. MEPs were triggered from swallowing-related sEMG activity at the submental muscle group. Displayed are 15 samples recorded across a 200 ms time-window at four separate sessions with the means highlighted in bold.

3.1. Intra-session reliability

The ICCs for the three blocks of five trials recorded in the first session revealed high within-session reliability, ICC = 0.915. Decreasing the number of trials per block led to a progressively mild reduction in ICC measures (blocks of four: ICC = 0.888; blocks of three: ICC = 0.797).

Table 1
Intraclass correlation coefficients (ICCs) for inter-session reliability between session 1 and each of three subsequent sessions and across all four sessions. ICCs are presented for blocks of 5, 10 and 15 trials. Largest ICC values of each comparison are in bold.

ICC values for	Inter-session reliability			
	Sessions 1 and 2	Sessions 1 and 3	Sessions 1 and 4	All four sessions
5 trials	**0.641**	0.887	0.486	0.657
10 trials	0.609	0.**909**	**0.649**	**0.716**
15 trials	0.553	0.811	0.642	0.690

Table 2
Mean and standard deviation (S.D.) of MEP amplitude (in μV) for first, second and third blocks of five trials for each session.

	Mean MEP Amplitude (μV)			
	Session 1	Session 2	Session 3	Session 4
1st block	511.41 (226.12)	541.36 (278.38)	603.24 (228.82)	605.82 (339.69)
2nd block	572.15 (290.67)	520.98 (148.37)	512.28 (198.18)	555.41 (229.44)
3rd block	585.1 (313.86)	494.2 (178.16)	496.89 (170.95)	585.24 (299.4)

3.2. Inter-session reliability

Repeated measures ANOVA revealed no significant influence of block ($F_{[2,14]} = 1.0$, $p > .05$ $p = 0.4$)) or sessions ($F_{[3,21]} =< 1.0$, $p = 0.8$), and no significant interaction between these factors ($F_{[6,42]} = 1.8$, $p = 0.12$). MEP amplitudes for all blocks and sessions are presented in Table 1. As no changes in mean levels of performance were evident across blocks or sessions, the ICCs provide a robust estimate of inter-session reliability in the context of stable MEPs. Correlation coefficients for all comparisons are presented in Table 2. Inter-session reliability was calculated between session 1 and each of the subsequent three sessions, for blocks of the first 5, 10 and all 15 trials. ICC measures ranged from 0.641 (five trials per block) to 0.909 (10 trials per block). Interestingly, marginally higher ICCs were achieved for blocks of 10 trials in two out of three comparisons and for all four sessions combined.

4. Discussion

4.1. Intra-session reliability

Atkinson and Nevill (1998) have characterized the quality of reliability measures, defining that ICC measures above 0.9 indicate 'high' reliability, while those between 0.7 and 0.8 indicate 'good' reliability. In this study, reliability of MEP amplitudes recorded within a single session is high, when blocks of five trials are used to establish ICCs. Correlation coefficients decreased as the number of trials per block decreased, but even the lowest ICC value can still be considered 'good'. These data are in agreement with prior research on MEPs derived from the abductor digiti minimi muscle (Christie et al., 2007), and indicate that five trials should optimally be included in peak-to-peak amplitude analyses.

4.2. Inter-session reliability

Inter-session reliability coefficients established between session 1 and each of the subsequent sessions ranged from moderate for five trials per block to high for 10 trials per block. Interestingly, reliability measures reached optimal values when 10 trials were included in the analysis, with a slight drop when all 15 trials were considered. As highest reliability was achieved for blocks of 10 trials, it appears necessary to include 10 trials into data analysis when the research paradigm includes multiple independent sessions for data collection.

Reliability measures were slightly lower for inter-session comparisons compared to intra-session comparisons. It is possible that a small degree of variability in coil placement has been introduced because of the necessity to identify the cortical hotspot during several data collection sessions. Further, establishing TMS output levels at the beginning of each session may have increased inter-session reliability to a certain degree. Studies including large numbers of sessions for data collection on the same research participant which are not conducted on the same day, i.e. which require multiple identifications of the optimal TMS stimulation site, thus need to take particular care in identifying this site.

5. Conclusions

The reliability of swallowing-related MEPs recorded from the submental muscle group using the novel methods described here is similar to that reported previously for hand muscles (Christie et al., 2007). We conclude from our data that for within-session comparisons, analysis of five trials produces high reliability. When data are collected in two or more sessions, it is advisable to perform data analysis on an average of 10 trials.

This study explored a sEMG-based TMS-trigger system which, to our knowledge, is a novel approach to MEP acquisition in swallowing research. Based on the data presented here, MEPs triggered by volitional swallowing can be recorded reliably at the submental muscle group across multiple sessions, when the level of background activation is controlled for by means of threshold triggering from sEMG activity. Investigation of task-related MEPs provides insight into corticobulbar excitability during muscle contraction and will be a valuable method for investigating the effects of rehabilitative treatment approaches.

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