Reproducible Measurement of Human Motoneuron Excitability With Magnetic Stimulation of the Corticospinal Tract

Peter G. Martin, Anna L. Hudson, Simon C. Gandevia, and Janet L. Taylor

Prince of Wales Medical Research Institute and the University of New South Wales, Sydney, Australia

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Martin PG, Hudson AL, Gandevia SC, Taylor JL. Reproducible measurement of human motoneuron excitability with magnetic stimulation of the corticospinal tract. J Neurophysiol 102: 606–613, 2009. First published April 29, 2009; doi:10.1152/jn.91348.2008. It is difficult to test responses of human motoneurons in a controlled way or to make longitudinal assessments of adaptive changes at the motoneuron level. These studies assessed the reliability of responses produced by magnetic stimulation of the corticospinal tract. Cervicomedullary motor evoked potentials (CMEPs) were recorded in the first dorsal interosseus (FDI) on 2 separate days. On each day, four sets of stimuli were delivered at the maximal output of the stimulator, with the final two sets of stimuli delivered at different stimulus intensities to obtain stimulus-response curves. In addition, on the second day, responses at different stimulus intensities were evoked during weak voluntary contractions. Responses were normalized to the maximal muscle compound action potential (Mmax). CMEPs evoked in the relaxed FDI were small, even when stimulus intensity was maximal (3.6 ± 2.5% Mmax) but much larger during a weak contraction (e.g., 26.2 ± 10.2% Mmax). CMEPs evoked in the relaxed muscle at the maximal output of the stimulator were highly reproducible both within (ICC = 0.83, session 1; ICC = 0.87, session 2) and between sessions (ICC = 0.87). ICCs for parameters of the input-output curves, which included measures of motor threshold, slope, and maximal response size, ranged between 0.87 and 0.62. These results suggest that responses to magnetic stimulation of the corticospinal tract can be assessed in relaxation and contraction and can be reliably obtained for longitudinal studies of motoneuronal excitability.

INTRODUCTION

All motor behaviors activate alpha motoneurons, the final common pathway of the CNS. In humans, it is difficult to test responses of motoneurons in a controlled way because the techniques commonly used to describe behavior in the human motor pathway, such as the motor evoked potential [the muscle response to transcranial magnetic stimulation (TMS)], the H-reflex (the muscle response to activation of Ia afferents), and the F-wave (the muscle response to antidromic activation of motoneurons) each have characteristics that limit their effectiveness as a test of motoneuron excitability (for reviews, see Gandevia 2001; Pierrot-Deseilligny and Burke 2005). The best available method is to record EMG responses to stimulation of descending corticospinal axons at a subcortical level (for reviews, see Taylor 2006; Taylor and Gandevia 2004). An electrical pulse passed between the mastoids elicits a single descending volley, which in turn evokes a motor response in some muscles. Responses are difficult to evoke in distal muscles of the upper limb (Ugawa et al. 1991) but are readily evoked in proximal muscles (Gandevia et al. 1999; Martin et al. 2006a,b, 2008). A significant proportion of the motoneuronal response is monosynaptic for biceps brachii and first dorsal interosseous (FDI) (Petersen et al. 2002; Ugawa et al. 1991), and there is evidence that descending tracts are not subject to presynaptic inhibition (Jackson et al. 2006; Nielsen and Petersen 1994). These characteristics make it the most direct assessment of a motoneuron’s response to synaptic input in awake humans.

However, electrical stimulation of descending pathways cannot be used to assess changes at the motoneurons across multiple testing sessions performed on different days because it is impossible to relocate the stimulating electrodes accurately to ensure that the stimulus activates the same corticospinal axons on each occasion. Therefore the synaptic input received by the motoneurons is unlikely to remain constant across sessions. Other techniques that use electrical stimulation, such as the H-reflex and the F-wave, have a similar problem. The lack of an available tool for assessing motoneuron behavior over time means that knowledge of the adaptive processes that occur in the human corticospinal pathway with interventions such as training is limited and has proven very difficult to advance (Enoka and Gandevia 2006).

“Corticospinal” excitability can be assessed in a reproducible way using TMS (Carroll et al. 2001; Christie et al. 2007; Devanne et al. 1997; Malcolm et al. 2006). The principle is that the optimal location (or “hot spot”) for stimulating the motor cortex projecting to a given muscle can be found on each occasion. Location of this spot maximizes the likelihood that a given stimulus intensity will activate a similar set of cortical neurons on each occasion. Using optimal stimulation location, we developed a new method for testing motoneuron excitability across multiple test sessions performed on different days. Magnetic stimulation over the back of the head using a double-cone coil can evoke muscle responses with the same latencies as those evoked by electrical stimulation of the cervicomedullary junction (Butler et al. 2003; Levenez et al. 2008; Matsunoto et al. 2008; Roy and Gorassini 2008; Ugawa et al. 1994). We hypothesized that magnetic stimulation of the corticospinal tract at the cervicomedullary level would evoke reproducible responses within a test session and across sessions if the stimulating coil was always located in a position that produced the largest responses attainable in the target muscle.

METHODS

Subjects

Sixteen subjects (7 women) were screened to determine whether magnetic stimulation over the back of the skull could produce EMG...
responses in the relaxed FDI. It was not possible to evoke responses in the right or left FDI in eight subjects, regardless of the location of the stimulating coil. Although responses were evoked in these subjects during a weak voluntary contraction of the target muscle, the primary purpose of the study was to assess the efficacy of the technique for monitoring responses in hand muscles at rest, and hence these subjects were excluded from the main study. The remaining eight subjects [3 women; 31 ± 12 (SD) yr] took part in two sessions that were ≥3 days apart. All but one subject was right handed. The procedures were approved by the local ethics committee, and the study was conducted according to the Declaration of Helsinki. All subjects gave their informed written consent.

**Experimental setup**

Subjects were seated with their arms relaxed and placed on a pillow across their lap. For assessment of responses during voluntary activity (session 2 only) the index finger was placed firmly in a ring which was connected to an immobile bar and strain gauge to measure the force of index finger abduction (see Fig. 2B in Martin et al. 2006a). Feedback of force was provided on a LED device. EMG was recorded from the FDI using self-adhesive electrodes (Ag–AgCl, 10 mm diam). One electrode was placed over the muscle belly and the other over the second metacarpophalangeal joint. EMG signals were amplified, filtered (16–1,000 Hz), and collected at 2 KHz for off-line analysis using customized software (CED 1401 with Signal software, Cambridge Electronic Design, Cambridge, UK). Force signals were collected at 2 KHz during voluntary activity.

**Stimulation**

**CORTICOSPINAL TRACT STIMULATION.** Stimuli were delivered to the corticospinal tract close to the cervicomedullary junction. All subjects wore earplugs throughout the stimulation. Responses evoked by cervicomedullary stimulation are termed cervicomedullary motor evoked potentials (CMEPs). CMEPS were evoked using two Magstim 200 magnetic stimulators coupled together through a Y-piece to a double-cone coil (11 cm OD, Magstim, Dyfed, UK). The same 200 magnetic stimulators coupled together through a Y-piece to evoke responses in muscles of the right hand and arm. A position to the left of the inion produces responses in muscles of the right hand and arm. The white arrow indicates the direction of current in the coil. Modified with permission (Taylor and Gandevia 2004). B: examples of the sigmoid fit to the size of cervicomedullary motor evoked potentials (CMEPs) and stimulus intensity for a single subject and the parameters of interest. Each circle represents the mean area of CMEPs (% of the maximal response to ulnar nerve stimulation, Mmax) relaxed 1st dorsal interosseous (FDI) produced at a given stimulus intensity.

- **FDI of the left and right hand by electrical stimulation** (duration, 100 μs; constant current, Digitimer D57AH, Welwyn Garden City, Hertfordshire, UK) of the ulnar nerves innervating each hand via surface electrodes (Ag–AgCl, 10 mm diam) fixed just proximal to the wrist. The intensity of stimulation was increased until there was no increase in the peak-to-peak amplitude of the M wave with increasing intensity (30–170 mA). Mmax amplitude was 22.1 ± 4.2 (SD) mV (peak-to-peak amplitude).

**ADDITIONAL STIMULI.** Before each experiment, the latencies of responses to magnetic stimulation of the motor cortex [motor evoked potentials (MEPs)] and of the cervical roots were established and used as a guide to the expected latencies of CMEPs. For stimulation of the motor cortex, a circular coil (13.5 cm OD, Magstim 200) was positioned over the vertex and oriented so that...
the direction of current flow in the coil preferentially activated the left motor cortex to evoke responses in right FDI and the right motor cortex to evoke responses in left FDI. The MEP latency was established during a weak voluntary contraction of target muscle. For stimulation of the cervical roots, the circular coil was positioned over the cervical spinal enlargement which produced responses in both left and right FDI at rest.

Protocol

Stimuli to the corticospinal tract were delivered in sets of 5 (at 0.1 Hz). Sets were ≥1 min apart, and the coil was removed from the head and replaced between sets.

SESSION 1. The first session had two parts. For part 1, stimuli were delivered to produce responses from the optimal side (see above). Two sets were delivered at the maximal output of the stimulator (100% on both stimulators). The stimulus intensity was reduced in 5% increments (reduced on both stimulators), and single sets of stimuli were delivered at each level. This procedure continued until the stimulus intensity was insufficient to evoke a CMEP (i.e., below resting motor threshold). After a break of ≥5 min, two additional sets were delivered at the maximal stimulator output. For part 2, stimuli were delivered to produce responses from the nonoptimal side. A single set of responses was collected at the maximal stimulator output. The intensity was reduced in 5% increments with a set at each level until stimulator output was insufficient to produce CMEPs.

SESSION 2. Part 1 of the second session was identical to session 1. For part 2, all responses were evoked during a contraction of the FDI on the optimal side. Subjects maintained a contraction of 10% of their maximal index finger abduction force. A single set of responses was collected at the maximal output of the stimulator. The intensity was reduced in 10% increments with a set at each level until stimulator output was insufficient to produce a CMEP (i.e., below active motor threshold).

Additional observations

RESPONSES EVOKED FROM PROXIMAL MUSCLES. Preliminary experiments indicated that stimulation over the optimal sites for producing responses in FDI could also produce EMG responses in biceps brachii indicating that it may be possible to assess multiple muscles simultaneously. Hence, although FDI was the primary muscle investigated, EMG responses in right and left biceps were also collected with EMG electrodes placed over the belly and tendon of each muscle (Ag–AgCl, 10 mm diam). To provide an index of the size of CMEPs with EMG electrodes placed over the belly and tendon of each muscle, responses in right and left biceps were also collected to provide an index of the size of CMEPs with EMG electrodes placed over the belly and tendon of each muscle.

For stimulation of the cervical roots, the circular coil was positioned over the cervical spinal enlargement which produced responses in both left and right FDI at rest.

RESULTS

The optimal position for evoking responses in the FDI was 4.2 ± 0.9 cm (range, 3.0–5.0 cm) lateral and 2.8 ± 1.2 cm (range, 0.5–3.0 cm) caudal to the inion (relative to the center junction of the coil). Responses were evoked in the muscle ipsilateral to the stimulus site. In relaxation, the latency of responses was 18.1 ± 1.5 ms, which was midway between the latency of responses to TMS (21.2 ± 1.6 ms) and stimulation of the cervical roots (14.6 ± 1.0 ms).

CMEPs at the maximal output of the stimulator

Figure 2A shows typical superimposed EMG responses in FDI elicited by magnetic stimulation of the corticospinal tract at the maximal output of the stimulator. For this subject, the
area of potentials (normalized to $M_{\text{max}}$) was consistent across the eight sets delivered across the two sessions (Fig. 2B). For the group (Fig. 3), CMEPs were consistent across the four sets delivered at the maximal output of the stimulator in the first session [ICC (A,1) = 0.83] and also in the second session [ICC (A,1) = 0.87]. Furthermore CMEPs were consistent across the two sessions [ICC (A,2) = 0.87].

CMEPs at different intensities of stimulator output

Although the size of the CMEP did not plateau with the available stimulus intensities, the relationship between CMEP size and stimulus intensity was sigmoidal (Fig. 4). The median proportion of the variance that was accounted for by the sigmoid fit was 97% (as opposed to, for example, 95% for an exponential function or 86% for a linear fit). The ICC (A,2) for the two sessions ranged from 0.62 to 0.87 for parameters derived directly from the experimental data and from the sigmoid function (Table 1).
The optimal location for the stimulating coil was several centimeters lateral to the inion. Stimulation to the right of the inion produced responses in muscles on the right side of the body, whereas muscles on the left side were activated by a position to the left of the inion. Stimulation on one side of the skull rarely evoked bilateral responses. The predominance of ipsilateral responses suggests activation at a site distal to the pyramidal decussation and is compatible with the idea that activation occurs at the foramen magnum (Ugawa 2002; Ugawa et al. 1994).

For most subjects, there was a clear bias in the ability to evoke responses on a particular side of the body. Presumably, in each subject, the individual anatomy, including the orientation and distance of the cervicomedullary junction from the magnetic coil, as well as the routes of different axons within the pyramidal decussation, determines the likelihood of responses in different muscles (Taylor 2006). In fact, of the 16 subjects screened for the main experiments, only 8 subjects had responses in the relaxed hand muscle. Therefore as a secondary aim, we studied whether a different waveform shape for the output of the stimulator would improve the efficacy of the technique. For TMS of the motor cortex, the shape of the waveform makes a substantial difference to the ability to activate corticospinal neurons and hence evoke responses in muscles (Brazil-Neto et al. 1992; Kammer et al. 2001; Niehaus et al. 2000).

A biphasic waveform has been reported to reduce the threshold for excitation and increase the response amplitude for a given stimulus intensity (Maccabee et al. 1998; Sommer et al. 2006). We found that Magstim Super Rapid stimulator (biphasic waveform) was far less effective at activating the corticospinal tract than the linked Magstim 200 stimulators (monophasic waveform). The most likely reason is that the maximal energy of the Magstim Super Rapid stimulator (252 J stored in the capacitor) is much less than the Magstim 200 (720 J for a single stimulator) (Kammer et al. 2001). Indeed, the improved efficacy of activating cortical cells with biphasic compared with monophasic TMS is only evident when the two machines have the same output energy. Another way of improving the technique could be to use pairs of stimuli. In a recent study, Matsumoto et al. (2008) used pairs (1.5–10 ms apart) of

### TABLE 1. ICC for the relationship between CMEP size and stimulus intensity

<table>
<thead>
<tr>
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<th>Mean Session 1</th>
<th>Mean Session 2</th>
<th>ICC (A.2)</th>
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<tbody>
<tr>
<td>CMEP100%stim, % M max</td>
<td>3.7 ± 2.4</td>
<td>3.4 ± 2.4</td>
<td>0.67</td>
</tr>
<tr>
<td>S max, % stimulator output</td>
<td>89 ± 3</td>
<td>89 ± 3</td>
<td>0.62</td>
</tr>
<tr>
<td>Peak slope, mV/% stimulator output</td>
<td>0.32 ± 0.15</td>
<td>0.30 ± 0.15</td>
<td>0.74</td>
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<tr>
<td>Function threshold, % stimulator output</td>
<td>70 ± 7</td>
<td>79 ± 12</td>
<td>0.82</td>
</tr>
<tr>
<td>Measured threshold, % stimulator output</td>
<td>77 ± 8</td>
<td>75 ± 11</td>
<td>0.74</td>
</tr>
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</table>

Values are means ± SE. CMEP, cervicomedullary motor evoked potential; ICC (A.2), reliability of the parameters over the two sessions; CMEP100%stim, the mean response across sets delivered at the maximal output of the stimulators; S max, the stimulus intensity at which the CMEP size is 50% of the maximal CMEP that could be evoked, peak slope, maximal rate of increase in CMEP magnitude with stimulus intensity; measured threshold, the lowest stimulus intensity to elicit CMEPs ≥ 50 μV determined during the session; function threshold, stimulus intensity defined by the sigmoid function when CMEP = 50 μV.
magnetic stimuli to activate the descending pathways at the cervicomedullary level. Although responses were 15 times larger when using a pair of stimuli delivered 2 ms apart compared with a single stimulus, responses in relaxation were still much smaller (~0.2 mV, peak-to-peak amplitude) than those reported in this study (0.8 mV). Moreover, pairs of stimuli were ineffective at increasing the size of responses in contraction (Matsumoto et al. 2008).

Disadvantages and precautions

Magnetic stimulation is less painful than electrical stimulation but the local muscle contraction and loud noise means that it remains unpleasant. Stimulation of motor roots can be a problem with magnetic stimulation because the wings of the double-cone coil lie over the upper neck and can induce sufficient current to activate the motor roots. This is less of a problem when testing distal muscles because they are innervated by lower cervical roots. Furthermore, the problem can be easily identified. First, a small voluntary contraction should cause a dramatic increase in the size of the response if it is evoked transsynaptically. Second, a jump in the latency of the stimulus of ~2 ms occurs if the stimulus has spread to the cervical roots. Another problem can arise if the wings of the coil lie too close to the motor cortex, which can evoke responses with latencies consistent with motor cortically evoked responses. Such spread is easily identified, not only because of the longer latency of responses (~22 vs. ~18 ms for FDI) but also because cortically evoked potentials always appear initially in the muscles of the contralateral side, whereas CMEPs are best seen in muscles of the ipsilateral side.

Advantages: a method for probing spinal cord plasticity

Spinal cord circuitry is modified in response to a variety of different motor experiences, including skill, strength, balance, and endurance training, as well as detraining (for reviews, see Aagaard 2003; Adkins et al. 2006; Nielsen and Cohen 2008; Wolpaw and Carp 2006; Wolpaw and Tennisen 2001; Zehr 2006). Evidence from animals suggests that this spinal cord plasticity may include changes in motoneuron properties (for review, see Gardiner et al. 2006) and, although there is some indirect evidence that changes may also occur in humans (Adam et al. 1998; Van Cutsem et al. 1998), current methods do not allow direct assessment in awake behaving humans. Changes at the spinal cord have largely been described using the H-reflex (Aagaard et al. 2002; Carroll et al. 2002; Voigt et al. 1998). Although the H-reflex provides a means of describing spinal adaptations, it is influenced by many factors, including presynaptic inhibition, disynaptic inhibition, axonal excitability of both motor and sensory axons, as well as the procedures used to normalize the size of the reflex (for reviews, see Gandevia 2001; Pierrot-Deseilligny and Burke 2005). The demonstration of a change in the H-reflex following an intervention is usually described as a change in net spinal excitability. Therefore knowledge of processes involved in spinal cord plasticity remains rudimentary (Enoka and Gandevia 2006). In this study, we showed an approach that provides a means of evaluating a specific aspect of adaptation at the spinal cord, namely alterations at the level of the motoneuron pool. The characteristics of the CMEP mean that after appropriate normalization to $M_{\text{max}}$, alterations in its size can be interpreted as relating to changes in the excitability of the motoneuron pool. The efficacy of the corticospinal-motoneuronal synapse may change, but only acute modifications related to repetitive activity have thus far been shown in humans (Gandevia et al. 1999; Petersen et al. 2003).

There is evidence that MEPs evoked by TMS are also altered by interventions such as skill and strength training. Interpretation of these changes is often difficult because they can reflect changes at the motor cortex and/or the motoneurons (Jensen et al. 2005; Pascual-Leone et al. 1995; Perez et al. 2004). Hence, it is impossible to interpret MEP changes in terms of what is occurring at the motor cortex without an accurate measure of changes in excitability at the subcortical level. Ugawa (2002) compares the advantages of a number of methods of testing spinal cord excitability. Cervicomedullary stimulation provides the most appropriate control for TMS because the two types of stimulation activate many of the same axons (Gandevia et al. 1999; Taylor et al. 2002; Ugawa et al. 1991, 1994).

A major advantage of this technique is that CMEPs can be used to test motoneuron behavior in a wide variety of tasks (Carson et al. 2007; Levenez et al. 2008; Martin et al. 2006b). There is evidence that measures of spinal cord excitability at rest may not adequately reflect the state of the spinal circuitry during activity, indicating that measurements should be performed in functional contraction tasks and not solely in the resting muscle (Aagaard et al. 2002; Voigt et al. 1998). CMEPs increased significantly during contraction. That is, the response of the motoneuron pool to the same descending input became greater. Other tests are comparatively limited in their usefulness in testing the behavior of motoneurons when they are activated voluntarily. F-waves and H-reflexes also tend to increase with increased voluntary activity (e.g., Espiritu et al. 2003). However, F-waves will fail to test some of the active motoneurons because of collision between orthodromic voluntary potentials and the antidromic volley used to elicit the response. Activation of Ia afferents also accompanies voluntary contraction and the magnitude of H-reflex increase can be affected by consequent homosynaptic postactivation depression (Crone and Nielsen 1989) and with changes in the size of the afferent volley caused by activity-dependent changes in axonal excitability (Burke and Gandevia 1999; Vagg et al. 1998). Presynaptic inhibition is also altered by voluntary effort (Hultborn et al. 1987). The demonstration of an increase in response size with voluntary contraction at all stimulus intensities suggests that magnetic stimulation of the corticospinal pathway allows the possibility of testing motoneuron behavior at rest and during voluntary contraction, including during the performance of complex tasks.

In summary, magnetic stimulation of descending pathways produces responses which are reproducible both within a session and across multiple sessions. This approach provides a means for evaluating adaptive changes in spinal cord circuitry, specifically at the level of the motoneuron pool, to interventions such as exercise training.
Innovative Methodology


GRANTS

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REFERENCES


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