Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation

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Todd, Gabrielle, Janet L. Taylor, and S. C. Gandevia. Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. J Appl Physiol 97: 236–242, 2004. First published March 19, 2004; 10.1152/japplphysiol.01336.2003.—Voluntary activation of muscle is commonly quantified by comparison of the extra force added by motor nerve stimulation during a contraction [superimposed twitch (SIT)] with that produced at rest by the same stimulus (resting twitch). An inability to achieve 100% voluntary activation implies that failure to produce maximal force output from the muscle must have occurred at a site at or above the level of the motoneurons. We have used cortical stimulation to quantify voluntary activation. Here, incomplete activation implies a failure at or above the level of motor cortical output. With cortical stimulation, it is inappropriate to compare extra force evoked during a contraction with the twitch evoked in resting muscle because motor cortical and spinal cord excitability both increase with activity. However, an appropriate “resting twitch” can be estimated. We previously estimated its amplitude by extrapolation of the linear relation between SIT amplitude and voluntary torque calculated from 35 contractions of >50% maximum (Todd G, Taylor JL, and Gandevia SC. J Physiol 551: 661–671, 2003). In this study, we improved the utility of this method to enable evaluation of voluntary activation when it may be changing over time, such as during the development of fatigue, or in patients who may be unable to perform large numbers of contractions. We have reduced the number of contractions required to only three. Estimation of the resting twitch from three contractions was reliable over time with low variability. Furthermore, its reliability and variability were similar to the resting twitch estimated from 30 contractions and to that evoked by conventional motor nerve stimulation.

VOLUNTARY ACTIVATION DESCRIBES the level of neural drive to muscle during exercise and is commonly estimated with the twitch interpolation method (11). This method, first proposed by Merton in 1954 (18), involves interpolation of a single supramaximal electrical stimulus to the motor nerve during an isometric voluntary contraction. The resultant superimposed twitch decreases in size with increasing voluntary force (1, 4, 7, 18). A realistic model of twitch interpolation, developed by Herbert and Gandevia (14) for adductor pollicis, showed this relationship to be linear, even when axonal factors such as refractoriness and antidromic collision were incorporated.

During maximal voluntary contractions (MVCs), the presence of a superimposed twitch produced by motor nerve stimulation demonstrates that force produced by the muscle is less than maximal and suggests that either the stimulated axons are not all recruited voluntarily or they are discharging at subtetanic rates. Hence, voluntary activation must be less than maximal (e.g., Refs. 4, 9, 14, 18). Voluntary activation is quantified by comparing the amplitude of the superimposed twitch with the force evoked by the same stimulus at rest, immediately after a contraction (termed “resting twitch”: e.g., Refs. 3, 5, 7, 23). The prior contraction potentiates the muscle. If voluntary activation is incomplete, then failure to drive the muscle must have occurred at or above the site of stimulation of the motor axons.

More recently, stimulation of the motor cortex has been used to estimate voluntary activation to further localize the site of failure of voluntary drive. Here, the presence of a superimposed twitch implies that output from the motor cortex was not optimal to drive the muscle maximally at the time of stimulation. Thus one site of failure of voluntary drive must be at or above the level of motor cortical output. This method has revealed submaximal voluntary activation of muscles during maximal voluntary efforts of respiratory muscles (12) and subsequently of thenar (13), elbow extensor (24), and elbow flexor muscles (26). It has also revealed central fatigue, a progressive decline in voluntary activation, during maximal efforts (10, 16, 22, 25). Voluntary activation tested by motor cortical stimulation is not necessarily equivalent to that tested by motor nerve stimulation. For example, with pathology of the corticospinal neurons or motoneurons, a subject may be unable to drive the muscle fully. In such a case, motor nerve stimulation distal to the pathology would reveal impaired voluntary activation, whereas motor cortical stimulation would not. Thus comparison of the two measures may help localize the site of impairment of voluntary drive.

Quantification of voluntary activation with motor cortical stimulation is difficult. The motoneuronal output evoked by the cortical stimulus with the muscle at rest is less than that during a contraction because motor cortical and motoneuronal “excitability” increase with activity (Refs. 8, 15, 27; for review, see Ref. 20). Thus the same magnetic stimulus evokes less cortical output, which recruits fewer motor units during rest than during voluntary activity. Therefore, it is inappropriate to normalize the superimposed twitch to that evoked at rest. Other studies have normalized the superimposed twitch to the background voluntary force before the stimulus (10, 16, 22, 25). This method is also problematic because background voluntary force production is affected by changes that occur in the central nervous system and the muscle (e.g., during fatigue). To avoid these difficulties, we recently proposed a method to estimate the resting twitch that would be evoked by motor cortical stimulation if cortical and motoneuronal excitability were maintained during rest. We then used this to measure voluntary...
activation in a new way (26). This estimation is based on extrapolation of the relationship between the size of the superimposed twitch evoked by motor cortical stimulation and voluntary force (24, 26). This relationship is negative and linear for elbow flexion forces above 50% of maximum whether the muscle is fresh or fatigued by isometric exercise (26). The extrapolation was calculated from data derived from cortical stimulation during 35 contractions. However, the performance of 35 contractions is time consuming and not practical for experimental conditions where voluntary activation may be changing over time, such as during the development of, or recovery, from fatigue or in patient groups who are unable to perform such large numbers of contractions. In the present study, we have refined this method to reduce the number of contractions required to estimate the resting twitch and to improve its practical application. We have shown that the resting twitch can be reliably estimated with as few as two or three contractions and that its variability is similar to the resting twitch evoked by motor nerve stimulation.

MATERIALS AND METHODS

Voluntary activation of the elbow flexor muscles was assessed on 2 days with the use of motor cortical stimulation. Subjects \(n = 8, 37\) yr (SD 12) sat with the right arm held firmly at the wrist in an isometric myograph, which measured elbow flexion torque. Subjects were positioned with the shoulder and elbow flexed at 90° with the forearm vertical and fully supinated. All experimental procedures were approved by the institutional ethics committee and conducted according to the Declaration of Helsinki. Written, informed consent was obtained.

Force and EMG Recordings

Isometric elbow flexion torque was measured by using a linear strain gauge (Xtran, Melbourne, Australia: linear to 2 kN). Electromyographic (EMG) activity was recorded with surface electrodes (Ag-AgCl, 10-mm diameter) positioned over the tendon and middle of the muscle belly of biceps brachii and the long head of triceps brachii. Surface EMG signals were amplified (100–300×), filtered (16–1,000 Hz), and sampled (2,000 Hz) for later analysis using a data acquisition system (CED 1401 interface with Signal software, Cambridge Electronic Design, Cambridge, UK).

Stimulation

Three forms of stimulation were used. These included stimulation of the brachial plexus, stimulation of the biceps and brachialis motor nerve, and transcortical magnetic stimulation of the motor cortex (TMS).

Stimulation of the brachial plexus. In the experiment setup before voluntary contractions, the size of the resting maximal compound muscle action potential (M_max) was determined with surface EMG. It was used as a reference for the size of the EMG responses evoked by TMS. Single electrical stimuli of 100-μs duration were delivered to the brachial plexus while the subjects were at rest via a cathode in the suprascapular fossa (Erb’s point) and an anode on the acromion.

Fig. 1. A: experimental protocol. Arrows indicate the timing of the motor cortical (TMS) or motor nerve (MN) stimulus. B: single-subject data showing the average elbow flexion torque trace after motor cortical stimulation during 50, 75, and 100% maximal voluntary contraction (MVCs). The increment in torque after the stimulus is known as a superimposed twitch. Background torques have been offset to allow comparison of the twitches. C: raw electromyograph trace showing a motor-evoked potential (MEP). The MEP area was measured between set cursors (dashed lines).
Stimulation of the biceps brachii and brachialis motor nerve. Single electrical stimuli of 100-μs duration were delivered (constant current, DS7, Digitimer) to biceps and brachialis via a surface cathode located midway between the anterior edge of the deltoid and the elbow crease with the elbow flexed at 90° and via a surface anode positioned over the bicipital tendon. The stimulus intensity (120–480 mA) was set 20% above the level required to produce a resting twitch of maximal amplitude in unpotentiated muscle (i.e., muscle not potentiated by a prior contraction).

TMS. A circular coil (13.5-cm outside diameter) positioned over the vertex evoked motor-evoked potentials (MEPs) recorded from biceps and triceps (Magstim 200, Magstim, Dyfed, UK). The direction of current flow in the coil preferentially activated the left motor cortex. The stimulator output (50–90% of maximum) was set to produce a large MEP in biceps (minimum amplitude 50% of M_{max}) during brief MVCs of the elbow flexor muscles and only a small MEP in triceps (usually <10–15% of M_{max}). Stimulus intensity remained constant throughout the protocol.

Protocol

Subjects participated in two identical experiments that were performed at least 24 h apart. Each experiment started with up to three brief (1–2 s) control MVCs, from which the peak torque was measured. Two submaximal target torques of 50 and 75% of the largest maximal control effort were displayed on a visual-feedback device. Subjects performed 10 sets of contractions with 1–2 min of rest between sets to avoid fatigue. Each set involved a brief MVC (1–2 s) followed in a pseudorandom order by either a brief 50% MVC or a 75% MVC and then another brief contraction to the other target torque (Fig. 1A). Contractions in a set were separated by rests of 6 s. A single motor cortical stimulus was delivered during each contraction, and a motor nerve stimulus was delivered at rest between the maximal effort and the first submaximal contraction (2 s after completion of the MVC).

Data Analysis

Voluntary activation was quantified by measurement of the torque responses to motor cortical stimulation. Any twitch increment in elbow flexion torque evoked during a contraction (superimposed twitch; e.g., Fig. 1B) was expressed as a fraction of the estimated amplitude of the response that could be evoked by the same stimulus in relaxed muscle (“estimated resting twitch”). We then quantified the level of drive as a percentage using the following equation: voluntary activation (%) = (1 − superimposed twitch/estimated resting twitch) × 100.

Four methods were used to estimate the amplitude of the resting twitch evoked by motor cortical stimulation for each subject. Each involved linear regression between the amplitude of the superimposed twitch and voluntary torque. The y-intercept was taken as the estimated amplitude of the resting twitch (see MATERIALS AND METHODS).

Fig. 2. Single-subject data showing the amplitude of the superimposed twitch evoked by motor cortical stimulation during 50, 75, and 100% MVCs. Four methods were used to estimate the size of the resting twitch evoked by motor cortical stimulation for each subject. The four methods involved linear regressions between the amplitude of the superimposed twitch and voluntary torque. The y-intercept was taken as the estimated amplitude of the resting twitch (see MATERIALS AND METHODS). A: method 1. A single linear regression was performed on data from all contractions (1 regression per subject). B: method 2. A linear regression was performed for each set of three contractions (10 regressions per subject). Methods 3 and 4 involved a linear regression for each pair of contractions, a MVC followed by a 50% MVC (C) or a MVC followed by a 75% MVC (D), respectively (5 regressions per subject per method).
regression for each pair of contractions, MVC followed by a 50% MVC or MVC followed by a 75% MVC, respectively (5 regressions per subject per method; Fig. 2, C and D).

The areas of MEPs (Fig. 1C) and \( M_{\text{max}} \) were measured between set cursors for the biceps and triceps muscles. For between-subject comparisons, MEPs were normalized to \( M_{\text{max}} \) elicited at rest. For each subject, torque was normalized to the largest MVC torque recorded during the 10 sets of contractions.

Statistics

In the text and table, group data are presented as means ± SD, whereas in the figures, means ± SE are shown. Statistical analysis involved two-way repeated-measures ANOVA for comparison of the MEP size during the 50, 75, and 100% MVCs performed in the two testing sessions. Two-way repeated-measures ANOVA was also used for comparison between the resting twitch evoked by motor nerve stimulation and the four types of estimated resting twitch evoked by motor cortical stimulation in the two testing sessions. To assess variability within a session, coefficients of variation (CV; SD/mean × 100; in %) were calculated for each subject. Comparison of the variability in size of the resting twitch evoked by motor nerve stimulation and the estimated resting twitches evoked by TMS was made with one-way repeated-measures ANOVA. Post hoc discrimination between means was made with the Student-Newman-Keuls procedure. A Friedman repeated-measures ANOVA on ranks was used for comparison of the variability in voluntary activation derived from the four estimated resting twitches because these data were not normally distributed. Equivalence tests were performed to determine whether the estimated resting twitches calculated with methods 2, 3, and 4 were similar to that calculated with method 1. Each equivalence test used 10% margins and involved two one-sided \( t \)-tests on the difference in the estimated resting twitch between method 1 and the other method. The null hypothesis for these tests was that the mean difference was greater than ±10% of the average resting twitch calculated with method 1 (i.e., the original method, Ref. 26). The reproducibility of the resting twitch between sessions was quantified with the intraclass correlation coefficient (ICC\(_{2,1}\)) based on a repeated-measures ANOVA with session as the independent variable (21). Statistical significance was set at the 5% level.

RESULTS

Torque and EMG responses to stimulation of the motor cortex were measured during brief contractions of the elbow flexor muscles. To calculate voluntary activation, the size of the resting twitch evoked by motor cortical stimulation was estimated (26). Four methods were used to estimate the resting twitch, each based on linear regression between the superimposed twitch and voluntary torque. The aim was to determine whether a small number of contractions (as few as 2) could be

Table 1. Average size, reproducibility, and variability of the estimated resting twitch evoked by TMS and the resting twitch evoked by motor nerve stimulation

<table>
<thead>
<tr>
<th>Method</th>
<th>Average Session 1, %MVC</th>
<th>Average Session 2, %MVC</th>
<th>Average Difference, %MVC</th>
<th>Within-Session CV, %</th>
<th>ICC(_{2,1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS method 1: 30 contractions</td>
<td>21.6±4.3</td>
<td>22.4±4.7</td>
<td>1.0±1.2</td>
<td>10.7±5.2</td>
<td>0.99</td>
</tr>
<tr>
<td>TMS method 2: 3 contractions</td>
<td>21.6±4.3</td>
<td>22.5±4.7</td>
<td>1.0±1.2</td>
<td>6.2±3.5</td>
<td>0.99</td>
</tr>
<tr>
<td>TMS method 3: 2 contractions 50% MVC</td>
<td>21.4±4.3</td>
<td>22.8±5.0</td>
<td>1.5±1.0</td>
<td>11.3±6.2</td>
<td>0.98</td>
</tr>
<tr>
<td>TMS method 4: 2 contractions 75% MVC</td>
<td>20.8±5.0</td>
<td>20.2±5.2</td>
<td>4.1±3.0</td>
<td>25.7±24.9</td>
<td>0.86</td>
</tr>
<tr>
<td>Motor nerve</td>
<td>7.7±2.1</td>
<td>8.0±2.5</td>
<td>0.3±0.7</td>
<td>5.7±2.2</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Values are means ± SD. Twitches were estimated or measured directly in 2 testing sessions performed on different days. The average difference in the size of the twitches between sessions is shown. To estimate the resting twitch evoked by motor cortical stimulation (see MATERIALS AND METHODS), extrapolation of the linear regression between superimposed twitch amplitude and voluntary torque was performed on data collected during 2, 3, or 30 contractions. TMS, transcranial magnetic stimulation of the motor cortex; CV, coefficient of variation, ICC\(_{2,1}\), intraclass correlation coefficient.
used to accurately estimate the resting twitch. The reproducibility and variability of each method were determined, and the values were compared with those for the resting twitch evoked by motor nerve stimulation.

Superimposed Twitch and Voluntary Torque

For each subject, the size of the superimposed twitch evoked by motor cortical stimulation decreased linearly with increasing contraction strength for contractions of 50–100% of maximum (day 1: average $r^2 = 0.92 \pm 0.07$; day 2: average $r^2 = 0.95 \pm 0.03$; $P < 0.001$). Figure 3A shows this relationship for one subject. In the first session, the average size of the superimposed twitch was $7.4 \pm 2.6$ N·m (11.3 ± 2.1% MVC) during the 50% MVC and $0.8 \pm 0.7$ N·m (1.2 ± 1.3% MVC) during maximal efforts (Fig. 3B). Its size was similar in the two testing sessions ($P = 0.75$; ICC$_{2,1} = 0.98$).

Resting Muscle Twitch

Four methods were used to estimate the size of the resting twitch evoked by motor cortical stimulation for each subject (see Materials and Methods; Fig. 2). Table 1 shows the average estimated resting twitch calculated with each method. Methods 2 and 3 yielded an estimated resting twitch of similar size to method 1 (test of equivalence, $P < 0.01$ for each t-test), with comparable results between days ($P = 0.44$; ICC$_{2,1} > 0.98$). However, the estimated resting twitch calculated with method 4 (MVC followed by a 75% MVC) was not similar to method 1 (test of equivalence, $P > 0.05$ for each test). It also had a greater within- and between-session variability ($P < 0.05$).

For motor nerve stimulation, the average amplitude of the unpotentiated resting muscle twitch was $3.3 \pm 1.0$ N·m. As expected, the amplitude increased to $4.9 \pm 1.5$ N·m (7.7 ± 2.1% MVC) when the muscle was potentiated by a prior maximal contraction. The potentiated resting twitch evoked by motor nerve stimulation was significantly smaller than the estimated twitch evoked by motor cortical stimulation ($P < 0.001$). The twitch evoked by motor nerve stimulation was reproducible between sessions (ICC$_{2,1} = 0.91$). The within- and between-session variability was not different to the estimated resting twitch evoked by TMS when calculated with methods 1, 2, or 3 ($P > 0.05$).

Voluntary Activation Measured With Motor Cortical Stimulation

As expected, for each subject, voluntary activation measured with the use of motor cortical stimulation increased linearly with increasing voluntary contraction strength regardless of the method used to estimate the size of the resting twitch (method

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**Fig. 4.** Group data (means ± SE) showing voluntary activation measured with the use of motor cortical stimulation during 50, 75, and 100% MVCs performed on 2 days (●, session 1; ○, session 2). Voluntary activation was calculated with the following formula: $(1 - \text{superimposed twitch/estimated resting twitch}) \times 100$. The resting twitch was estimated by 4 methods (see Materials and Methods and Fig. 2). A: method 1. B: method 2. C: method 3. D: method 4.
Figure 4 shows the average increase in voluntary activation with torque. Across sessions, voluntary activation was high during maximal efforts (method 1: 94.1 ± 6.1%; method 2: 93.9 ± 6.5%; method 3: 94.1 ± 6.0%; method 4: 94.4 ± 4.8%) and was reproducible between sessions for each method of estimation of the resting twitch (ICC2,1 > 0.86). During MVCs, the average variability of voluntary activation within a session was low for methods 1 (CV = 2.9 ± 1.9%), 2 (CV = 3.7 ± 3.9%), and 3 (CV = 2.8 ± 2.0%), but it was significantly higher for method 4 (CV = 8.9 ± 19.6%; P < 0.05).

**DISCUSSION**

We recently proposed a new method for measurement of voluntary activation with motor cortical stimulation that involved estimation of the resting twitch rather than direct measurement (26). The resting twitch was estimated by extrapolation of the linear relation between superimposed twitch amplitude and voluntary torque. In the present study, data from different combinations of voluntary contractions were used in the regression in an attempt to determine whether a small number of contractions can be used to accurately quantify voluntary activation. We contrasted the reproducibility and variability of this estimated resting twitch with that produced by motor nerve stimulation. Three aspects of the results will be discussed: first, the torque and EMG responses to TMS, which underlie estimation of the resting twitch; second, the reproducibility and variability of the estimated resting twitch when calculated from 2, 3, or 30 contractions; and third, the reproducibility and variability of voluntary activation calculated with the use of the estimated twitch.

The MEP size for biceps and its antagonist triceps muscle was reproducible between sessions for contractions of 50–100% of maximum. This suggests that the stimulus was similar on both occasions. During 50% MVCs, when the superimposed twitch is largest, the size of the MEP in biceps approached that of M\text{max}. This suggests that under these conditions most motoneurons were activated by the motor cortical stimulus. During maximal efforts, the MEP size is smaller and the superimposed twitch is very small or in some cases completely absent (10, 22, 26). The smaller MEP may reflect the inability of some motoneurons to fire in response to the stimulus. With higher firing rates in the stronger contractions, more motoneurons may be effectively “refractory” because of their intrinsic properties and the trajectory of the afterhyperpolarization (see Refs. 17, 26). The relatively small size of the triceps MEP at all contraction strengths is important for measurement of voluntary activation because activation of triceps produces extension torque at the elbow and would reduce the size of the flexion twitch. This reduction of the superimposed twitch during elbow flexion contractions would result in an overestimation of voluntary activation of elbow flexor muscles. However, because the response in triceps is small, the effect on the measurement of voluntary activation is also small. The propensity of TMS to activate elbow flexors rather than extensors has been noted previously (19).

The superimposed twitch evoked by motor cortical stimulation decreases linearly with voluntary force over the contraction range studied (26). In the present study, we have confirmed this finding and shown that the relationship is reproducible between sessions. We used this robust relationship to estimate the size of the resting twitch by extrapolation of data collected from 2, 3, or 30 contractions. The average estimated resting twitch calculated from 30 contractions in one testing session was similar to the average calculated from 3 contractions or even 2 contractions if a MVC and 50% MVC were used. For methods 1, 2, and 3, the estimated resting twitch was reproducible between sessions with a low between-session variability (<1.5% MVC). However, the estimated resting twitch calculated with method 4 (MVC and a 75% MVC) was not similar to that calculated with method 1 and had considerably greater within- and between-session variabilities than the other methods. The reproducibility and variability between sessions for methods 1, 2, and 3 based on cortical stimulation were similar to the measures for the resting twitch produced by motor nerve stimulation. This occurred although the twitch produced by motor nerve stimulation was smaller in size because it only reflects torque produced by biceps and brachialis, which contribute about one-half of total elbow flexion torque (2). In contrast, the twitch evoked by motor cortical stimulation reflects the net output from all muscles that generate elbow flexion torque. Furthermore, it is the result of
multiple descending volleys that can sometimes activate individual motoneurons more than once (6).

To derive voluntary activation using motor cortical stimulation, we compared the size of the superimposed twitch with the estimated resting twitch (26). As expected, voluntary activation increased linearly with increasing contraction strength for contractions between 50 and 100% of maximum. This occurred for all four methods of estimation of the resting twitch (see Fig. 4).

For each method, calculated voluntary activation was high during maximal efforts (>93%) and was reproducible between sessions with lower within-session variability for methods 1, 2, and 3 (<4%) than for method 4 (9%). Incomplete voluntary activation during a maximal effort implies that, at the moment of stimulation, voluntary motor cortical output was not sufficient to recruit all motoneurons or to drive them at a sufficiently fast rate to produce maximal force. Furthermore, extra output from the motor cortex was available, as the motor cortical stimulus was able to evoke extra torque that the subject could not access voluntarily (13, 26). In addition to further localizing the site of failure of voluntary drive, measurement of the EMG responses to motor cortical stimulation enables simultaneous measurement of cortical excitability and inhibition.

The present results have practical implications for the assessment of motor performance with motor cortical stimulation. Of the methods tested, voluntary activation is best derived with estimation of the resting twitch based on a large number of contractions (method 1) for assessment of healthy subjects before and after an intervention or to compare two groups of subjects. For patient groups or when voluntary activation is changing rapidly over time, estimation of the resting twitch based on three contractions (a MVC, 50% MVC, and 75% MVC) will provide an accurate and reliable measure of voluntary activation. If there is not enough time (~15 s) to perform three contractions without disruption of the experimental paradigm, then a good estimate of the resting twitch can be derived from only two contractions if a MVC and 50% MVC are used. Our previous studies showed that the submaximal contraction must be at least 50% MVC (26).

In summary, provided at least two contractions that differ widely in strength are used, estimation of the resting twitch evoked by motor cortical stimulation is reproducible between days with low variability. As a result, voluntary activation measured with cortical stimulation is reliable over time. Because the size of the resting twitch can be estimated accurately with small numbers of contractions, motor cortical stimulation can now be used for measurement of voluntary activation in a range of experimental circumstances when neural drive to the muscles may be changing rapidly.

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GRANTS

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