Increased probability of repetitive spinal motoneuron activation by transcranial magnetic stimulation after muscle fatigue in healthy subjects

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Andersen B, Felding UA, Krarup C. Increased probability of repetitive spinal motoneuron activation by transcranial magnetic stimulation after muscle fatigue in healthy subjects. J Appl Physiol 112: 832–840, 2012. First published December 15, 2011; doi:10.1152/japplphysiol.00917.2009.—Triple stimulation technique (TST) has previously shown that transcranial magnetic stimulation (TMS) fails to activate a proportion of spinal motoneurons (MNs) during motor fatigue. The depression in size of the TST response, but no attenuation of the conventional motor-evoked potential, suggested increased probability of repetitive spinal MN activation during exercise, even if some MNs failed to discharge by the brain stimulus. Here we used a modified TST [quadruple stimulation (QuadS) and quintuple stimulation (QuintS)] to examine the influence of fatigue on second and third MN discharges after a single TMS in healthy subjects. This method allows an estimation of the percentage of double and triple discharging MNs. Following a sustained contraction of the abductor digiti minimi muscle at 50% maximal force maintained to exhaustion, the size of QuadS and QuintS responses increased markedly, reflecting that a greater proportion of spinal MNs was activated two or three times by the transcranial stimulus. The size of QuadS responses did not return to precontraction levels during 10-min observation time, indicating long-lasting increase in excitatory input to spinal MNs. In addition, the postexercise behavior of QuadS responses was related to the duration of the contraction, pointing to a correlation between repeated activation of MNs and the subject’s ability to maintain force. In conclusion, the study confirmed that an increased fraction of spinal MNs fire more than once in response to TMS when the muscle is fatigued. Repetitive MN firing may provide an adaptive mechanism to maintain motor unit excitability; triple stimulation; collision technique

FAILURE TO MAINTAIN MUSCLE force develops during the course of continued muscle contraction. It partly depends on changes within muscle fibers, but central factors at spinal and supraspinal levels also play a role in the decline of force. Central fatigue implies that activation of motoneurons (MNs) is inadequate and is defined as a progressive exercise-induced reduction in voluntary activation or neural drive to the muscle (for review, see Refs. 18, 40).

Electromyographic (EMG) responses to transcranial magnetic stimulation (TMS) are altered during muscle fatigue produced by either maximal or submaximal voluntary contractions. During a fatiguing contraction, the motor-evoked potential (MEP) increased in size (37, 38), and a progressive reduction in short-interval intracortical inhibition was observed (5). At the same time, the subsequent period of EMG silence (silent period) was prolonged (27, 37, 38). Also, when MEPs were evoked in relaxed, fatigued muscle, a short-duration facilitation of the MEP was followed by a long-lasting depression of the MEP (9, 19, 24, 28). All of these changes in the MEP were believed to reflect cortical phenomena and suggest paradoxically both increased excitation and increased inhibition in the motor cortex during fatigue.

In a previous study, we used the triple stimulation technique (TST) to study changes in corticospinal excitability after a submaximal contraction sustained to exhaustion (3). This method employs a collision technique that eliminates the effects of MN discharge desynchronization on the size of the MEP, and the TST response reflects the proportion of spinal MNs activated by a transcranial stimulus (10, 25, 26). We found that a maximal cortical stimulus failed to activate a large proportion of spinal MNs immediately after a sustained fatiguing contraction, while, at the same time, the depression of the TST response was not associated with attenuation of the conventionally evoked MEP. This aspect of our results was puzzling and pointed to factors other than the number of activated spinal MNs coming into play. After a single cortical stimulus, spinal MNs sometimes fire, not just once but repetitively (6, 13, 22, 29). Repetitive MN discharges are caused by excitatory inputs impinging on spinal MNs and are influenced by stimulation intensity and the degree of facilitatory muscle contraction (13). We hypothesize that the probability of repetitive MN activation may have increased during exercise, even if a proportion of MNs in the pool failed to discharge on arrival of the first volley set up by the brain stimulus. Hence, complex interactions between failure of activation of some MNs and compensatory repetitive discharges of other MNs may contribute to the maintenance of muscle force during sustained voluntary activity. In the TST recordings, multiple MN discharges are seen, but they do not contribute to the size of the TST response. Thus the TST response reflects only the first MN discharges succeeding a cortical stimulus. The recently introduced quadruple stimulation (QuadS) or quintuple stimulation (QuintS) techniques combine the TST with one or two additional nerve stimuli in the periphery and permits a quantitative assessment of the number of spinal MNs discharging two or three times (44). Here, we used this modified TST to examine the influence of fatiguing exercise on responses to the second and third MN discharges after TMS in healthy volunteers. It was the aim to test the hypothesis that a greater fraction of spinal MNs are activated, not just once, but repetitively during fatiguing exercise, hence providing further quantitative information on activity-dependent changes in excitability of the corticomotoneuronal pathway. Preliminary results of this study have been presented elsewhere (2).
METHODS

Subjects

Thirty-one healthy subjects (18 women, 13 men; age range 19–31 yr) took part in the study and were examined with QuadS and QuintS protocols. Five of the subjects were studied on two occasions. All gave written, informed consent for the procedures, which were approved by the local Ethics Committee of Frederiksberg and Copenhagen.

Force and EMG Recordings

The experimental procedures and recording techniques were similar to those described previously (3). The subjects were lying comfortably on a bed with the right hand and forearm held in supinated position. The forearm was carefully fixed in a splint and strapped to a horizontal arm table to avoid movements during the experiments and to minimize the contribution of stabilizing forearm muscles during digit V abduction. Digits II–IV were taped together and strapped to the table in extended position and further fixed between two metal blocks, adjusted according to the width of the hand. A strain gauge was placed against the proximal interphalangeal joint of digit V to record the force output of abductor digiti minimi muscle (ADM) during the contractions. The output of the strain gauge was fed to a personal computer via a data acquisition system. Visual feedback of the force output was displayed on a personal computer monitor placed in front of the subject, and verbal encouragements were given throughout the contractions. The maximal voluntary force (MVC) of the ADM was positioned along the arm and hand during all experiments.

The experiments were carried out using a Nicolet Viking IV EMG apparatus (Nicolet, Madison, WI) to record the compound muscle action potential (CMAP) from the right ADM using surface electrodes in a belly tendon montage (band-pass filter range 5 Hz to 5 kHz). Constant-current stimulators were used, 0.2-ms stimulus duration at wrist, and 0.5-ms stimulus duration at Erb’s point. Dedicated protocols were developed in collaboration with Judex AS and Cephalon AS (Denmark) for automated delivery of stimuli sequences and measurements of responses. A ground electrode was taped to the dorsum of the hand. The skin temperature at wrist was checked throughout the experiments and kept above 34°C. An infrared heating lamp was placed against the proximal interphalangeal joint of digit V to standardize skin temperature at wrist, and one to the brachial plexus at Erb’s point to measure the internal region of the suprascapular fossa. The motor threshold at Erb’s point (CMAPErb) and at the wrist (CMAPwrist) was defined as the stimulus intensity that produced reproducible responses. To ensure supramaximal stimulation of the brachial plexus at Erb’s point, stimuli were applied over the scalp using a circular 90-mm coil fixed to a stand. The center of the coil was positioned at the vertex or slightly lateral toward the stimulated hemisphere. The coil was oriented with the coil current flowing anticlockwise (when viewed from above) to excite the left hemisphere preferentially. The motor threshold was defined as the stimulus intensity that produced reproducible responses with amplitude exceeding 100 μV (baseline to peak) at a facilitation level of 5% MVC in 6 of 10 stimuli. The stimulus strength for the QuadS and QuintS experiments was set to double threshold intensity. The high stimulus intensity was chosen since repetitive MN discharges have a high threshold and notable amounts of repetitive MN discharges occur only when some 75% of MNs in the pool are activated (44). Also, using twice the motor threshold intensity, we previously showed that almost 100% of spinal MNs are activated by TMS in a healthy subject (see also Ref. 3). Particular care was taken to maintain the optimal position of the coil throughout the experiments.

TMS. TMS was performed using a Magstim 200 magnetic stimulator (Magstim, Dyfed, UK). The intensity of the magnetic pulse was expressed as a percentage of the maximal output (2.0 T). Stimuli were applied over the scalp using a circular 90-mm coil fixed to a stand. The center of the coil was positioned at the vertex or slightly lateral toward the stimulated hemisphere. The coil was oriented with the coil current flowing anticlockwise (when viewed from above) to excite the left hemisphere preferentially. The motor threshold was defined as the stimulus intensity that produced reproducible responses with amplitude exceeding 100 μV (baseline to peak) at a facilitation level of 5% MVC in 6 of 10 stimuli. The stimulus strength for the QuadS and QuintS experiments was set to double threshold intensity. The high stimulus intensity was chosen since repetitive MN discharges have a high threshold and notable amounts of repetitive MN discharges occur only when some 75% of MNs in the pool are activated (44). Also, using twice the motor threshold intensity, we previously showed that almost 100% of spinal MNs are activated by TMS in a healthy subject (see also Ref. 3). Particular care was taken to maintain the optimal position of the coil throughout the experiments.

QuadS and QuintS.

To further secure that all electrical stimuli were supramaximal before the contraction we measured a QuadS “control” response in all subjects. In this response, the brain stimulus was replaced by a proximal peripheral nerve stimulus at Erb’s point. The succession of the four stimuli was Erb-wrist 1-wrist 2-Erb. The delay between
Fig. 1. Principle of the triple (TST) and quadruple stimulation (QuadS) techniques. A–C: on the left, the motor tract is simplified to 3 spinal motoneurons (MNs). Horizontal lines depict muscle fibers of the 3 motor units. Arrows represent action potentials; solid arrows represent action potentials giving rise to a recorded trace deflection from the abductor digiti minimi (ADM), whereas open arrows do not. TST and QuadS recordings are shown on the right. The sweep of the traces is delayed and starts at the time of the second stimulus (wrist). A: 1: TMS causes 3/3 MNs to discharge and 3 desynchronized action potentials descend. MNs (a) and (b) discharge twice so that a second action potential descends (*). 2: After a delay of ~17–19 ms an electrical stimulus is applied to the wrist (W). Descending orthodromic potentials evoke the first deflection of the TST response (compound muscle action potential, CMAPwrist) while the antidromic potentials collide with descending activity from the brain stimulus. The second action potentials (*) descending on MN (a) and (b) are not cancelled and continue to descend (multiple discharges, see traces A, right). 3: After a further delay of ~10 ms, a third electrical stimulus is applied at Erb’s point (E), and action potentials descend along 3/3 axons. 4 and 5: as a result of E stimulation a synchronized response is recorded as the second main deflection of the recording trace (TSTtest). B: the TST control response TSTcontrol is recorded by replacing the cortical stimulus by a stimulus at E with appropriate adjustments of the delays. The ratio (amplitude and area) TSTtest/TSTcontrol estimates the proportion of spinal MNs that are excited by TMS, which is 100% in this example. C: 1: TMS causes 3/3 MNs to discharge and 3 desynchronized action potentials descend. MN (a) and (b) discharge twice (*). 2: After a delay, an electrical stimulus is applied to the wrist (W1). It evokes the first deflection of the QuadS response (CMAPwrist), while antidromic potentials collide and cancel with descending activity from the brain stimulus. The second action potentials (*) continue to descend. 3: After 3 ms, a second electrical stimulus is applied to the wrist (W2). It gives rise to a negative deflection of the recording trace, melting with the first one. The antidromic potentials collide with descending activity from the second MN discharges and surviving activity on axon (c) ascends toward E. 4: After a delay, an electrical stimulus is applied to E, and action potentials descend along axon (a) and (b), while a collision occurs in axon (c). 5: As a result of E stimulation, a synchronized response from the 2 MNs that conducted repetitive MN discharges (but were occluded by the second wrist stimulation) is recorded as the second main deflection of the QuadS recording trace (QuadStest). The ratio (amplitude and area) QuadStest/TSTcontrol estimates the proportion of spinal MNs that are activated twice by TMS, in this example 2/3 MNs.
Erb-wrist 1 was similar to wrist 2-Erb. As a result, no response (no second deflection) was recorded in the control trace. In some of the subjects, the TST was combined with two additional stimuli applied to the wrist as to quantify the number of spinal MNs discharging three times (QuintS). The sequence of stimuli was cortex-wrist 1-wrist 2-wrist 3-Erb’s point. The delays of cortex-wrist 1-wrist 2-Erb’s point were the same as those used in the QuadS mode. The interstimulus interval between wrist 2 and wrist 3 was 3 ms. The second negative deflection of the QuintS response (QuintSmax) then represents a resynchronized response that reflects the amount of third MN discharges. By comparing it to the TSTcontrol, an estimation of the percentage of spinal MNs that are activated three times after the transcranial stimulus was calculated.

**Experimental Protocol**

In each subject, baseline measurements consisted of two to three TSTtest responses, one to two TSTcontrol responses, and three QuadS and/or three QuintS responses using a magnetic stimulator output of double-threshold intensity. The transcranial stimuli were applied while the subject contracted the target ADM at 5% MVC. The stimulus intensity and level of precontraction were kept constant throughout an experiment. Visual feedback of the precontraction level was provided.

Different exercise protocols were used. 1) The subjects were instructed to perform a sustained effort at 50% MVC as long as possible. At the endurance point where the target force could no longer be maintained and the contraction became “maximal”, the subjects were encouraged to continue the contraction until the force was further reduced to 30% MVC, defined as exhaustion and end of exercise. 2) The subjects performed a sustained contraction at 50% MVC for 15 s and/or 30 s.

**Main QuadS study**. The recovery period after exercise at 50% MVC sustained to exhaustion was studied in 20 subjects using QuadS. At the point of exhaustion (30% MVC), the subject relaxed on instruction and immediately thereafter contracted the ADM at a 5% MVC facilitation level. “Sets” of QuadS stimulations were delivered within 3–4 s (time 0), at 30 and 60 s after the contraction, at 1-min intervals up to 4 min, and thereafter at 2-min intervals up to 10 min total recovery time. A set of QuadS recordings consisted of 1 × TSTtest and 2 × TSTcontrol responses. We were particularly aware of changes in the peripheral TSTcontrol, which calibrates the QuadSmax and, for that reason, QuadStest responses were always superimposed upon control responses recorded successively at short intervals at a maximum of 10–20 s. At time 0, the interval between the recordings was no more than 5 s, since our previous study showed marked changes in the size of both TSTtest and TSTcontrol responses at the point of exhaustion (3). In subsequent sets of QuadS recordings, the interval between the recordings was 10 s. The slightly longer interval was used to limit the subjects’ discomfort. Since rapid changes in size of the TST response occur after exercise, only two QuadStest recordings were done at each time point. The mean value of the amplitude and area ratio calculated from the two QuadStest responses in a recording set was used for analysis at this time point. Following every one or two sets of QuadS recordings, a wrist stimulus was applied to serve as a baseline for measurements of the QuadS amplitude and area ratio. A computer triggered the QuadS trials and wrist stimulations automatically throughout the recovery period.

**Effects of short submaximal exercise duration on QuadS responses**. On separate days, further QuadS studies were undertaken to examine if a situation in which the subjects were capable to maintain the target force would influence the size of theQuadS response. In 14 subjects, QuadS responses were studied after exercise, while the subjects maintained a submaximal target force at 50% MVC for 15-s duration (n = 12) and 30-s duration (n = 10). QuadS responses were recorded using a similar protocol to that of the main QuadS study, apart from a shorter recovery period (4 min). In eight subjects who participated in both exercise tests, the two studies were separated by at least 15 min of rest.

**QuintS study**. In seven subjects who had a large QuadS response, QuintS responses were recorded using a similar protocol to that of the main QuadS study (see above). The subjects chosen for the QuintS study had QuadS amplitude ratio around the mean value or higher, because we would not expect a QuintS response in subjects with very low QuadS amplitude ratio (45).

**Additional control experiments**. We have used a precontraction facilitation level at 5% MVC in all experiments. However, maintaining a 5% MVC force may require stronger effort when the muscle is fatigued than in the nonfatigued muscle. Since the number of multiple MN discharges is influenced by the degree of target muscle contraction, this may have been a source of error in our results. Therefore, in five subjects, we measured QuadS responses at a precontraction level of 17% MVC before the contraction at 50% MVC (as described in the main QuadS study) and at a precontraction level of 5% MVC after the contraction at time 0 when the subject could no longer maintain 30% MVC. A 5% MVC force in the fatigued muscle, when 30% MVC can no longer be produced, corresponds to a 17% MVC in the nonfatigued muscle. Furthermore, we measured the force production and QuadS responses at a precontraction level of 17% MVC after 10 min of recovery.

In the same five subjects, QuadS responses were measured in the relaxed muscle before and after a 50% MVC.

**Statistical Analysis**

Nonparametric repeated-measures analysis of variance (Friedman’s test) was performed to test changes in both the main QuadS data set. The Wilcoxon signed-ranks test for paired comparisons examined the size of QuadS responses and QuintS responses before and after fatigue at time 0 and in the control experiments also after 10-min recovery. To test differences between group means at different time points after exercise, the Mann-Whitney test compared differences between independent groups of the different contraction protocols.

In addition to nonparametric statistics, we added parametric statistics with special reference to identify differences between means of the recovery period of the QuadS data sets. For that purpose, repeated-measures analysis of variance (one-way ANOVA) with post hoc testing (Tukey’s B) was performed.

Significance was set at the 5% level. In the text, group data are stated as means ± SD, unless indicated otherwise, whereas in figures, means ± SE are shown.

**RESULTS**

All 31 subjects tolerated the measurements well, and no adverse effects occurred.

**TST Responses Before Exercise**

Before exercise, the average ratios of the best possible superimposition of TSTtest and TSTcontrol recordings using a 5% precontraction level and double-threshold stimulation intensity were 98 ± 3% for both the amplitude and area ratio. These findings confirm previous results (3, 26) and imply that, during a fixed facilitation level of 5% MVC and high-stimulus intensity, TMS achieves depolarization of nearly all spinal MNs to the target ADM muscle in healthy subjects.

**Changes in QuadS Responses After Exercise at 50% MVC Sustained to Exhaustion**

Similar to the TST, the QuadS evokes a response that is composed of two main negative deflections. The first negative deflection is caused by the two successive wrist stimuli (second
and third stimulus of the QuadS procedure, Fig. 1C), while the second main deflection of the response stems from the Erb’s point stimulus (fourth stimulus of the QuadS procedure, Fig. 1C) and represents the number of MNs discharging twice. Since this response is compared with the second negative deflection of the peripheral TSTcontrol Response, influences of desynchronization on its size are eliminated.

Twenty subjects, who exercised until exhaustion, maintained the target force at 50% MVC for a mean duration of 81 s (range 32–133 s) with a mean duration of the total exercise time (to 30% MVC) of 93 s (range 41–154 s). Before exercise, the average ratios of the QuadS test and TSTcontrol response were 35 ± 21% for the amplitude and 35 ± 20% for the area. The recruitment of QuadS test responses was highly variable between the subjects, and the QuadS test-to-TSTcontrol ratio (i.e., the actual percentage of recruited MNs discharging two times) ranged from 0 to 75%. In one subject, double-discharging MNs could be recorded only after exercise. The observed percentage of double discharging MNs was slightly higher in our subjects than that found by Z’graggen et al. (45) during their condition, corresponding of increasing effort. Following 15- and 30-s contraction, there was a trend toward a minor increase in size of the QuadS amplitude ratio, although these increases in response size did not reach statistical significance (P = 0.07 and 0.06, respectively). Particularly after 30 s of contraction, a marked increase in the QuadS amplitude ratio was observed in some of the subjects. At time 0 (within 3–4 s after the contraction), the mean QuadS amplitude and area ratio increased from the precontraction value by a factor of 1.8 ± 0.2 (SE) (P < 0.0005 for the amplitude and P < 0.002 for the area), reflecting that a greater proportion of spinal MNs were activated twice by the transcranial stimulus. This increase in size of the QuadS responses did not return to precontraction values during the 10-min observation time (repeated-measurements ANOVA, P < 0.02 and post hoc Tukey’s B; Fig. 3).

Changes in QuadS Responses After Exercise at 50% MVC Sustained for 15 and 30 s

To determine whether the increase in size of the QuadS responses observed after exercise sustained to exhaustion was related to inability of the subject to maintain the target force, we measured QuadS responses after shorter exercise durations of 15 and 30 s. All subjects were capable of maintaining the contractions at 50% MVC for these exercise durations. Yet, at the end of the 30-s exercise period, some of the subjects reported difficulty in maintaining the target force level, suggestive of increasing effort. Following 15- and 30-s contraction, there was a trend toward a minor increase in size of the QuadS amplitude ratio, although these increases in response size did not reach statistical significance (P = 0.07 and 0.06, respectively). Particularly after 30 s of contraction, a marked increase in the QuadS amplitude ratio was observed in some of the subjects. At time 0 (within 3–4 s after the contraction), the mean QuadS amplitude and area ratio increased from the precontraction value by a factor of 1.8 ± 0.2 (SE) (P < 0.0005 for the amplitude and P < 0.002 for the area), reflecting that a greater proportion of spinal MNs were activated twice by the transcranial stimulus. This increase in size of the QuadS responses did not return to precontraction values during the 10-min observation time (repeated-measurements ANOVA, P < 0.02 and post hoc Tukey’s B; Fig. 3).

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the subjects. The relationship between changes in size of QuadS responses after 15- and 30-s contraction and after 50% MVC sustained to exhaustion is depicted in Fig. 3. While differences in size of the QuadS responses following the different contraction protocols did not reach statistical significance at time 0 immediately after the contractions due to great variation between the subjects, the behavior of the QuadS responses differed markedly during the rest of the recovery period ($P \leq 0.01$; Fig. 3). The size of QuadS responses after 15 and 30 s of contraction equaled preexercise values, while QuadS responses after strong contraction (50% MVC sustained to exhaustion) remained increased in size. Thus the number of double-firing MNs remained elevated only in a situation in which the muscle force could no longer be maintained during a sustained contraction. This suggests an association between loss of force and repetitive firing of MNs.

**Changes in QuintS Responses After 50% MVC Sustained to Exhaustion**

To see if some MNs discharged more than twice in response to TMS, the QuintS was applied in seven subjects. These subjects were chosen because they all had large QuadS responses before exercise. During this procedure, three stimuli were applied to the wrist so that both the first and second action potentials on each axon were eliminated. Hence, the QuintS test response represented a measure of the number of spinal MNs discharging three times.

Before exercise, a discernible QuintS response could be recorded in five of the seven subjects. The precontraction values of the QuintS responses were much smaller than the QuadS responses, and in two subjects a QuintS response could be measured only after exercise. The average ratios of the QuintS and TSTcontrol response before exercise were 12 ± 15% (range 0–44%) for the amplitude and 14 ± 18% (range 0–53%) for the area. The size of the QuintS responses varied even more between the subjects than the QuadS responses. Following the contraction, an increase in size of the QuintS response was observed in all subjects. At time 0, the mean QuintS amplitude ratio had increased to 29 ± 23% ($P < 0.02$), and the QuintS area ratio was 30 ± 23% ($P < 0.02$). The increase in size of the QuintS response was only significant immediately after the contraction at time 0 (one-way ANOVA, $P < 0.002$ and post hoc Tukey’s B). The normalized data pooled for the group of subjects with discernible QuintS responses before the contraction ($n = 5$) are depicted in Fig. 4.

In one of the subjects who had a decrease in size of the QuadS response after exercise, a large increase in size of the QuintS response was observed. In this subject, the QuintS ratio increased from 8% before the contraction to 30% after the contraction. Hence, even if the number of single- and double-firing MNs is depressed during fatigue, an increased number of MNs in the pool may fire three times. We did not have the opportunity to perform a QuintS study on the second subject who had a decrease in size of the QuadS response following fatigue.

**Control Experiments**

In five subjects, QuadS responses were measured at a pre-contraction level of 17% MVC before the fatiguing contraction at 50% MVC maintained to exhaustion. After the contraction, when 30% MVC could no longer be maintained, the mean QuadS amplitude ratio measured at a precontraction level of 5% MVC increased from the precontraction value by a factor 1.6 ± 0.2 (SE) ($P < 0.05$). This is only slightly lower than the mean increase in size of QuadS responses observed using similar precontraction levels of 5% MVC before and after the contraction. Hence the slightly stronger force that may be needed producing a 5% MVC in the fatigued muscle does not explain the increased probability of MNs to fire more than once in the fatigued muscle. Following a 10-min recovery period, the maximal force output reached 100% in four of the five subjects, while one subject reached 80% MVC. When measuring QuadS responses 10 min after the contraction at a precontraction level of 17% MVC in the four fully recovered subjects and at 14% MVC in the one subject who recovered to 80% MVC, the mean QuadS amplitude ratio remained increased from the precontraction value by a factor of 2.2 ± 0.6 (SE) ($P < 0.05$). The further increase in QuadS amplitude ratio observed after 10 min of recovery compared with the increase observed at time 0 did not reach statistical significance.

In the relaxed muscle, QuadS responses could not be recorded, either before or after a fatiguing contraction. Thus repetitive MN discharges have a relatively high threshold and require facilitation, as also observed in previous studies (44, 45).

**DISCUSSION**

Our study showed that, following a sustained fatiguing contraction, there was a marked increase in the proportion of spinal MNs that fired two or three times after a transcranial stimulus. This result was achieved using a modified TST (QuadS and QuintS), which estimates the percentage of the second and third discharges of the MN. By using high-stimulus intensities and a precontraction level of 5% MVC, the number of double-discharging MNs was found to be about one-third of MNs in the pool before exercise, while only some 10% of the MNs fired three times. After the fatiguing exercise, both the QuadS and QuintS responses increased markedly in size, suggesting increased excitatory input converging on the spinal MNs, resulting in repetitive firing. It was previously shown that TMS failed to activate a large proportion of spinal MNs when the muscle had become fatigued following sustained or repetitive contractions (3, 34). Those studies suggested an important...
supraspinal contribution to the drop of the TST response or central conduction index (a slightly modified TST response; see Ref. 34) during fatigue. Importantly, the central conduction index reduction was particularly large when voluntary corticospinal activity is involved during an exercise, while the contribution of spinal mechanisms appeared rather small (34). Thus, in a situation with depression of corticospinal effects on spinal MNs leading to failure of activation of some MNs in the pool, a greater proportion of double- and triple-discharging MNs may reflect an adaptive mechanism acting to increase output to the exercising muscle. Before consideration of possible mechanisms for these phenomena, some technical and measurement issues regarding the TST and QuadS need to be addressed.

**Technical Issues**

Anterior horn cell discharges after a cortical magnetic stimulus are not synchronous, and, consequently, phase cancellation (negative phases of individual motor unit potentials canceled by positive phases of others) occurs and reduces the size of the compound response. The main effect of the TST is to resynchronize the temporal dispersion of MN discharges; hence the TST response reflects the percentage of target MNs driven to discharge by the brain stimulus. A second effect of the TST is that the amplitudes of TST responses are not influenced by repetitive MN discharges because only the first descending action potential on each MN is involved in the collisions (for detailed discussion, see Refs. 10, 26). As the first MN discharges are desynchronized, it is a reasonable assumption that repeated MN discharges are desynchronized as well. The QuadS and QuintS use the TST principle to resynchronize the second and third MN discharges descending on the MNs. Therefore, quantification of the percentage of these discharges has become possible (44, 45). The QuadS response originates solely from the second MN discharges, while QuintS responses originate from triple-discharging MNs. Peripheral factors influencing the muscle response are controlled by the TST control, which calibrates the test responses of the TST, QuadS, and QuintS. Furthermore, the short interval between test and control responses in the present study eliminated the effects of changes in the periphery that might have occurred during exercise. The interstimulus intervals between the wrist stimuli in QuadS and QuintS were set at 3 ms, because it is shorter than the estimated arrival of the earliest repetitive MN discharges and longer than the refractory period of peripheral nerves (see also Ref. 44). However, underestimation of the increase in the number of repetitively firing MNs because of some very short or extremely long intervals cannot be totally excluded. Extremes of firing intervals have not been further evaluated in our work.

In the present study, we attempted to minimize methodological factors that may have an effect on response variability. It is known that the number of multiple MN discharges is influenced by the degree of target muscle contraction (13, 44). Therefore, the degree of voluntary background contraction was carefully controlled and kept at a constant level of 5% MVC during TMS in all experiments. However, maintaining a 5% MVC in the fatigued muscle may require more voluntary drive than in the nonfatigued muscle, and this could have increased the facilitatory effect and been a source of error in our results.

The outcome of our additional control experiment concluded that this methodological error was only minimal and could not explain the present results. Also, particular care was taken to keep the position of the stimulating coil constant by marking the coil position on the head and, if necessary, readjusting the coil position following stimulation. This was needed because the Erb’s point stimulation involved in the TST and QuadS may lead to head movements due to stimulation of neck muscles.

**Possible Mechanisms to Account for Increased Amount of Repeated MN Activation**

Repetitive MN discharges are the consequence of the succession of excitatory D and I waves of the descending corticospinal volley after TMS. These converge on the spinal MNs (32) and may cause them to discharge repetitively (6, 12, 13). Repeated activation of MNs was shown to occur after brain stimulation, but not after stimulation at the level of the brain stem, pointing to the importance of D and I waves driving the MNs to discharge more than once in response to TMS (6, 42). From a physiological aspect, repetitive MN discharges may be considered a measure of excitability of the cortico-motoneuronal pathway. Thus the increased probability of double- and triple-discharging MNs observed in the present study could reflect an increased excitability of the motor cortex, or the MN pool, or both. Several kinds of actions at the MN pool might lead to repetitive firing of MNs during fatigue: the brain stimulus evokes greater net excitatory output from the cortex, synaptic inputs to MNs from muscle afferents or recurrent inhibition may change, or the responsiveness of MNs through a change in their intrinsic properties may increase. Although it is likely that all of those actions may play a role, the latter explanation seems less important, since a number of observations suggest that MNs become less excitable during a fatiguing contraction: 1) during a sustained maximal effort, MN firing rates declined (7, 8, 43), and, while different mechanisms may contribute to this drop, a decreased responsiveness of MNs to synaptic input was thought to be most significant (for review, see Ref. 39); 2) the duration of the silent period after peripheral nerve stimulation increased (38); and, importantly, 3) EMG responses to stimulation of the corticospinal tract at a subcortical level (cerebromedullary stimulation) decreased in size (11, 19). As the cervicomedullary responses have a large monosynaptic component (33) and are unaffected by classical presynaptic inhibition (31), it was concluded that these responses reflect MN excitability (see also Ref. 39).

If MNs become less excitable during a fatiguing contraction, then extra drive to the MNs might be required to maintain activation. It is very likely that the increased probability of some MNs in the pool to fire repetitively after a fatiguing contraction is caused by a net increase of the excitatory outflow from the motor cortex. The cortical events underlying such a mechanism will most likely be an increased amount of generated I waves after exercise, a mechanism that was also proposed by Gandevia (18) to account for the growth in MEP size observed during a sustained MVC (18, 38). The association of repetitive MN discharges with handedness and the skill for precision movements supports the hypothesis of a supraspinal and probably cortical origin of these discharges (45).
In the present study, we observed marked differences in the behavior of QuadS responses after short durations of contraction (15 and 30 s) and strong contraction sustained to exhaustion, pointing to a correlation between the proportion of double-firing MNs and the subjects’ ability to drive the muscle and produce force. The maintained increase in the number of double-firing MNs during the 10-min recovery period occurred only when the contraction was held beyond the endurance point and the subjects could no longer sustain the target force (see Fig. 3). This suggests a long-lasting increase in excitability of the cortical neurons that may reflect a central adaptive mechanism acting to increase the descending drive to the MNs during recovery from fatigue. In a study by Benwell et al. (4), it was shown that, by raising cortical excitability using a paired-pulse TMS protocol designed to induce I-wave facilitation (41), MEP amplitude recorded with slight background contraction increased significantly during a 15-min intervention period and remained increased for more than 10 min postintervention. Also, by producing a further increase in corticomotor excitability, functional performance improved with a reduction in the rate of force loss during a maximal effort. This effect was still present when tested 8 min after the intervention period when the cortical excitability was still raised. The similarities between MEP behavior observed in their study and the remained increase in size of QuadS responses observed here after fatigue may indirectly support the view that increased excitatory input on spinal MNs from the motor cortex generating more repetitive discharges may reflect a compensatory mechanism acting to maintain cortical output and task performance.

Failure of MN Activation Contrasts With Increased Cortical Excitability

In parallel with the increased amount of observed double firing of MNs, previous studies showed that the TST disclosed failure to activate a proportion of spinal MNs during and after exercise when the force-generating capacity of the target muscle was depressed (3, 34). A clear relationship between reduction in size of the TST response and strength and duration of the contraction was observed and was thought to reflect alterations in cortical function and loss of force-producing capacity (3). Also, in the study by Rösler et al. (34), it was concluded that failure of activation was critical to the drop of the TST response during fatigue, suggesting that most of the reduction was caused by supraspinal mechanisms. Hence, in the present study, the dissociation between sizes of the QuadS and TST responses suggests that both excitatory and inhibitory events within the motor cortex are coming into play during muscle fatigue.

There is convincing evidence that I waves originate in the motor cortex mainly through activation of corticocortical circuits presynaptic to the corticospinal cells (1, 32). Also, using TMS, I waves were found to originate in different cortical circuits (12, 15–17, 20, 21, 30, 36, for review, see also Ref. 14), which may suggest that these circuits might be differentially modulated during muscle fatigue. Consequently, some corticospinal cells could be inhibited, while others were more excitable during fatigue, resulting in differential cortical input on MNs, leading to failure of activation of some MNs in the pool or repetitive firing of others. Thus our present findings of increased probability of some MNs in the pool to fire repetitively after a sustained contraction while, at the same time, other MNs did not discharge by TMS (3) may reflect complex interactions within cortical circuits generating I waves during fatigue. It cannot, however, be excluded that excitability changes at the spinal segmental level contribute to the TST response depression (discussed in detail in Ref 3, see also Refs. 11, 34).

In conclusion, the results of the present study confirmed that an increased fraction of spinal MNs fire not just once, but repetitively in response to TMS after fatiguing exercise. This increase in repetitive MN discharges was related to duration of the contraction, suggestive of a compensatory response to muscle fatigue. It is likely that repetitive spinal MN discharges have a supraspinal origin; hence, a net increase in corticospinal actions on spinal MNs occurs after fatiguing exercise. The results add further evidence to the view that repetitive MN firing may provide a central adaptive mechanism acting to maintain motor unit activation and task performance during sustained voluntary activity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

B.A., U.A.F., and C.K. conception and design of research; B.A. and U.A.F. performed experiments; B.A., U.A.F., and C.K. analyzed data; B.A., U.A.F., and C.K. interpreted results of experiments; B.A. and U.A.F. prepared figures; B.A. drafted manuscript; B.A. and C.K. edited and revised manuscript; B.A., U.A.F., and C.K. approved final version of manuscript.

REFERENCES

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