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RESEARCH NOTE

**Eric M. Wassermann · Ali Samii · Bruno Mercuri
Katsunori Ikoma · Daniele Oddo · Stephen E. Grill
Mark Hallett**

Responses to paired transcranial magnetic stimuli in resting, active, and recently activated muscles

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Abstract Transcranial magnetic stimulation (TMS) causes the corticospinal system to become refractory to subsequent stimuli for up to 200 ms. We examined the phenomenon of paired pulse inhibition with TMS under conditions of rest, ongoing voluntary activation (isometric force generation), and at variable delays following activation (postactivation) of the wrist extensors of seven normal subjects. Paired stimuli were delivered to the motor cortex with a circular coil at 1.1 times motor evoked potential (MEP) threshold, with various interstimulus intervals. Voluntary activation caused a marked decrease in the variability of the ratio of the amplitude of the MEP evoked by the test pulse to that of the MEP evoked by the conditioning pulse. Marked inhibition of the MEP evoked by the test pulse was still present. Postactivation, however, caused a dramatic reversal of the inhibitory effect of the conditioning pulse in all subjects at interstimulus intervals ranging from 40 to 120 ms. This effect lasted for up to 10 s following the cessation of activation. MEPs to transcranial electrical stimulation were also inhibited by conditioning TMS, but postactivation did not reverse this inhibition, indicating that the reversal of paired pulse inhibition is intracortical. We conjecture that paired pulse inhibition reflects activity of inhibitory interneurons or inhibitory connections between cortical output cells that are inactivated in the postactivation state.

Key words Motor cortex · Exercise · Transcranial magnetic stimulation · Inhibition · Paired stimuli · Human

E. M. Wassermann (✉)[1] · A. Samii · B. Mercuri · K. Ikoma
D. Oddo · S. E. Grill · M. Hallett
Human Motor Control Section, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md., USA

Present address:
[1] Building 10, Room 5N226, 10 Center DR MSC 1428, Bethesda MD 20892–1428, USA

Introduction

Inhibition of the corticospinal motor system following transcranial magnetic stimulation (TMS) is by now a familiar phenomenon which has attracted numerous investigators (Calancie et al. 1987; Cowan et al. 1986; Fuhr et al. 1991; Mills 1988; Roick et al. 1993; Valls-Solé et al. 1992; Wassermann et al. 1993), who have found evidence of both spinal and cortical mechanisms. In a previous report (Valls-Solé et al. 1992), some of us described the time course of the excitatory and inhibitory effects of a conditioning TMS pulse on the response to a subsequent pulse. When the conditioning pulse was strong enough to produce a motor evoked potential (MEP) in the target muscle, there was strong inhibition of responses to the test stimulus at interstimulus intervals (ISIs) longer than about 30 ms. The inhibitory effect began later and lasted longer when the intensity of stimulation was increased.

Voluntary activation of the target muscle causes a large increase in the size of the MEP evoked by a cortical stimulus. Recently, we found that the amplitude of MEPs to TMS is also increased in relaxed muscles shortly after the cessation of voluntary muscle activation (postexercise facilitation; Brasil-Neto et al. 1994; Samii et al., in press). The mechanism of this effect is unknown but may be related to posttetanic potentiation (Samii et al., in press). In the present study we used the paradigm of Valls-Solé et al. (1992) to see whether the inhibitory effect of a conditioning TMS pulse could be modulated by either ongoing or recent muscle activation.

Materials and methods

Subjects were eight normal volunteers (two women, six men) aged 28 to 60 years, including four of the authors. All gave written informed consent, and the research protocol was approved by the Institutional Review Board. Subjects were seated with the right arm resting in the apparatus used for measuring isometric force. This device consisted of a rigid frame, which held a strain gauge over the subject's arm and a padded platform to support the arm. Thus, the subject could raise the dorsum of the hand against the plunger

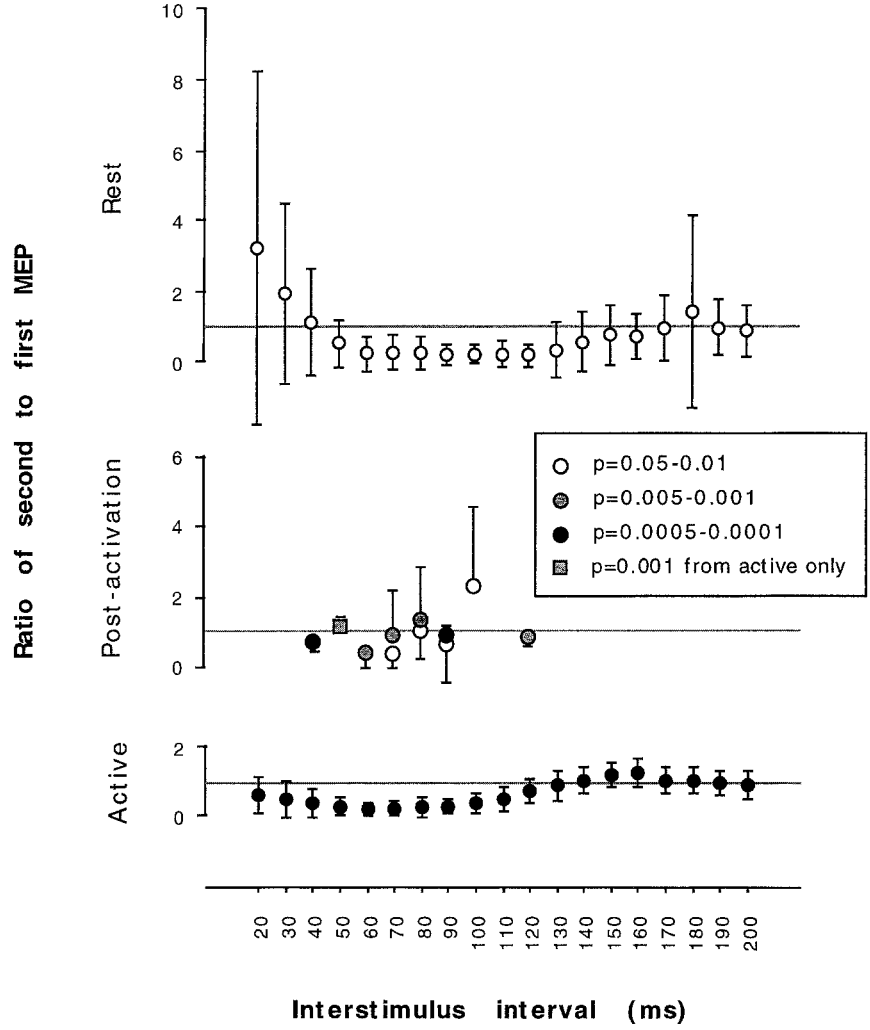

Fig. 1 *Top and bottom* The ratio of the motor evoked potential (*MEP*) evoked by the test transcranial magnetic stimulus to that evoked by the conditioning pulse at different interstimulus intervals (*ISIs*) under conditions of rest (*open circles*) and voluntary activation (*filled circles*). *Points* represent the grand mean of ten trials at each ISI for all five subjects. *Bars* show SD. *Middle* Data from individual subjects showing the ratio of the MEP evoked by the test stimulus to that evoked by the conditioning stimulus under the postactivation condition. Each *point* shows the mean and SD from ten trials in a single subject. All points except one differed significantly from both the resting and active ratios in the same subject

of the strain gauge by the isolated action of the wrist extensors. The output of the strain gauge was led to an oscilloscope for feedback and to the computer that controlled the experiments and stored the data.

Electromyographic activity (EMG) was recorded from the wrist extensors of the right arm and amplified by an electromyograph with filter settings of 100 and 2000 Hz. The analog signal was digitized at 5 kHz.

TMS pulses were administered through a 9-cm circular coil with a Cadwell high-speed magnetic stimulator. The coil was placed at the optimal position on the scalp for producing a MEP in the target muscle (near or slightly lateral to the vertex).

Experiment 1

In five subjects, pairs of stimuli were delivered with ISIs of 20–200 ms in increments of 10 ms. ISIs were randomized and were presented in two blocks of 95 stimuli containing five trials at each ISI. The intertrial interval was 7 s. This range of ISIs was chosen based on the results of Valls-Solé et al. (1992), who found inhibition at these intervals. One block of trials was performed with the subject at rest and was followed by a block during which the subject was instructed to extend the wrist with 10% of maximum effort as measured by the strain gauge. Subjects were instructed to avoid fatigue by resting between trials. The sequence of blocks was repeated during rest and activation for a total of 380 trials. TMS intensity was set at approximately 1.1 times the MEP threshold, which was defined as the intensity required to evoke a MEP greater than or equal to 50 μV on five out of ten trials at rest. The peak-to-peak amplitude of MEPs was measured off-line. We then computed the mean ratio of the amplitude of the MEP evoked by the second (test) stimulus to that evoked by the first (conditioning) stimulus at each ISI. This enabled us to find the ISIs where maximum inhibition of the test MEP was produced in each subject.

Experiment 2

The effect of recent muscle activation (postactivation) on paired pulse inhibition was studied in the subjects from experiment 1 as follows: the subject was instructed to extend the wrist with 20% of maximal effort for 10 s and then to relax rapidly but passively. The computer was programmed to wait 500 ms after the output of the strain gauge returned to zero and then to deliver a pair of stimuli. The 500-ms wait time was found in preliminary experiments to be the shortest period of time by which all of the subjects could relax after a mild contraction. ISIs producing maximal inhibition of the test MEP were chosen for each subject based on the results of experiment 1. These ranged from 40 to 120 ms. Three subjects were tested with two ISIs, and two subjects were tested with three ISIs. Ten trials were given at each ISI, with a rest period of at least 120 s between trials. No more than two ISIs were tested in a single subject on the same day. Mean ratios of the test to the conditioning MEP were computed as in experiment 1 and compared with the resting and active ratios in the same individuals by two-tailed *t*-tests.

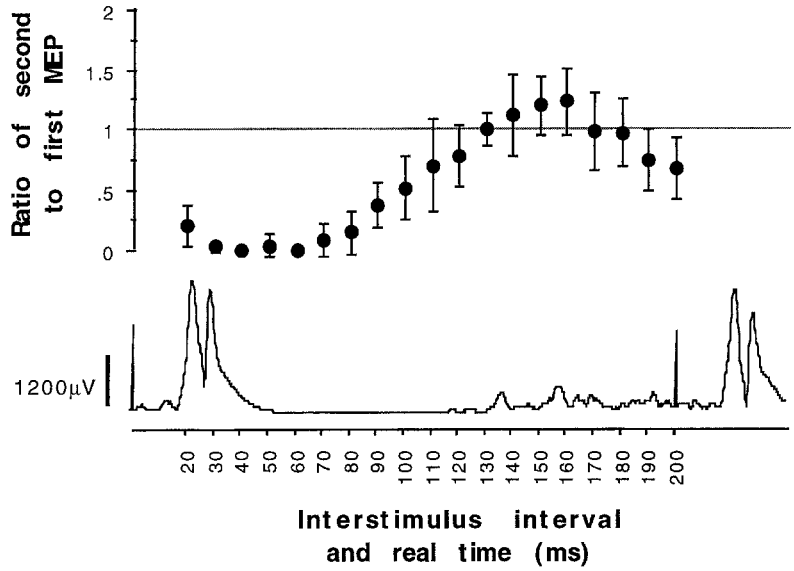

Fig. 2 *Top* The ratio of the MEP evoked by the test transcranial magnetic stimulus to that evoked by the conditioning pulse at different ISIs with activation from one subject. *Points* represent the mean and SD of ten trials at each ISI. *Bottom* Rectified averaged EMG from ten trials of paired pulse stimulation in the same subject with an ISI of 200 ms

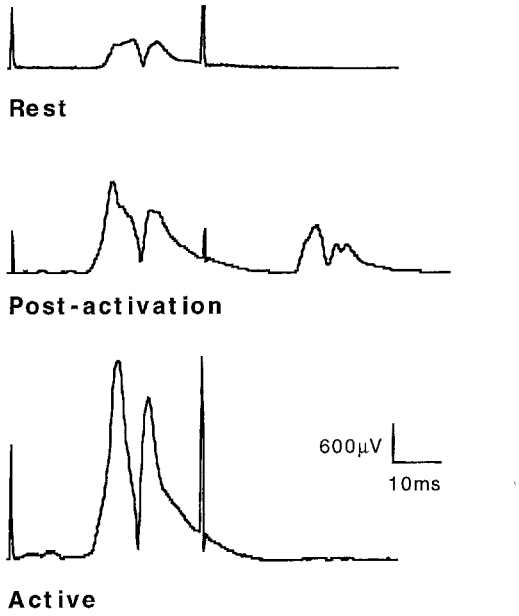

Fig. 3 Rectified averaged traces from ten trials of paired pulse transcranial magnetic stimulation (ISI 40 ms) from a single subject (whose data are shown in Fig. 2) under conditions of rest, activation, and postactivation

Experiment 3

In seven subjects, the time course of the postactivation effect was studied by giving a series of pulse pairs with an ISI of 80 ms during rest and postactivation. Four pairs were given at each of several delays (500 ms, 1 s, 3 s, 5 s, 10 s, and 20 s) following the return of voluntary force to zero. EMG was played through a loudspeaker in order to monitor relaxation during the delay period. Trials where muscle activation occurred during the delay period were discarded.

Experiment 4

Transcranial electrical stimulation (TES) experiments were performed in four subjects at rest and postactivation. The design was similar to that of the TMS studies except that TES was substituted for the test stimulus. Stimuli were administered with a Digitimer D180 stimulator through gold cup electrodes affixed to the scalp at the vertex (cathode) and 6 cm lateral. The intensities of both TMS and TES were set at approximately 1.1 times the MEP threshold. An ISI of 40 ms was tested in the three subjects who had inhibition of the TMS test pulse at this ISI, because Inghilleri et al. (1990) found inhibition of a test TES MEP at this interval. These subjects were also tested at longer ISIs, where profound inhibition of the TMS test MEP was found: one subject was tested at 60 ms, one at 70 ms, and the other at 60 and 90 ms. The fourth subject was tested only with an ISI of 70 ms.

Results

The time course of inhibition of the test pulse under conditions of rest and activation is shown in Fig. 1. In all subjects when the wrist extensors were at rest, inhibition of the test MEP began at ISIs of about 30–50 ms. At shorter ISIs, the inhibition was sometimes overridden by facilitation of the test MEP, which was quite variable across trials within subjects and across subjects. The inhibition of the test MEP at ISIs from about 50–130 ms was often virtually complete. At longer ISIs (130–150 ms) the inhibition began to wane and there was occasional facilitation of the test MEP (Fig. 1). During activation, early facilitation did not occur, but instead inhibition was apparent at the 20-ms ISI in all subjects (Fig. 1). During activation, the period of inhibition also ended earlier than during rest in all subjects and was followed by some evidence of facilitation. All subjects had at least one ISI in the range 130–200 ms at which there was facilitation of the test MEP (Fig. 2), but this tendency, while apparent at ISIs of 150 and 160 ms, was not statistically significant in the grand averaged data (Fig. 1). The period of inhibition coincided in each subject with the depression of voluntary activation, or "silent period," following the conditioning MEP (Fig. 2). Voluntary activation markedly reduced the variability of the ratio of the second to the first MEP within and across subjects (Fig. 1).

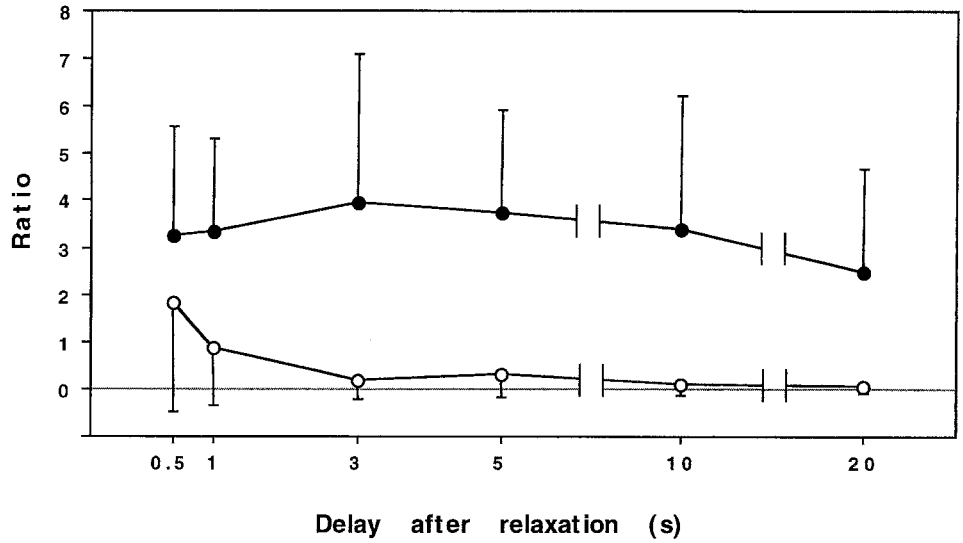

Fig. 4 Pooled, averaged data from seven subjects showing the time course of the decay of the effect of postactivation on paired pulse inhibition (*open circles*) and the facilitatory effect on the conditioning MEP (*filled circles*). *Open circles* represent the mean of the ratio of the second to the first MEP from pulse pairs (ISI 80 ms) delivered at different delays after muscle relaxation. *Filled circles* represent the mean ratio of the amplitude of the conditioning MEP at each delay after relaxation to the the subject's mean resting MEP. *Bars* show SD

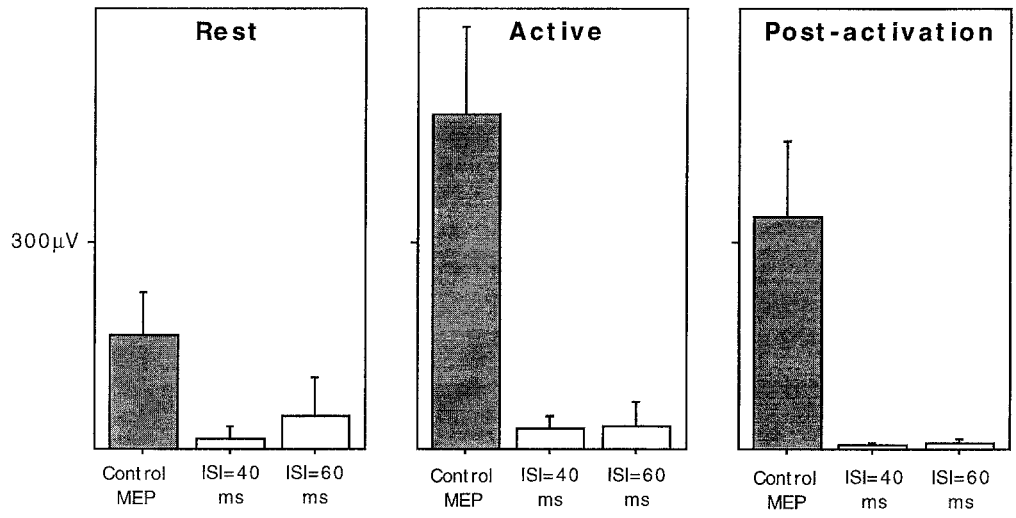

Fig. 5 The amplitude of the unconditioned MEP to transcranial electrical stimulation (TES) (*shaded bars*) and the amplitudes of MEPs to TES after a conditioning transcranial magnetic pulse at ISIs of 40 and 60 ms, under conditions of rest, activation and postactivation. Data are from the subject whose data also appear in Figs. 2 and 3. *Bars* represent the mean of ten trials. *Error bars* show SD

Postactivation had dramatic effects on the inhibition of the test pulse (Figs. 1, 3). At all ISIs tested in all subjects the ratio of the test to the conditioning MEP was greater in the postactivation state than at rest or during activation. All subjects had at least one ISI where the postactivation ratio was higher than both the rest and the active ratios at the $P<0.01$ level (t-test), and four out of five subjects had at least one ISI that differed from rest and activation at the $P<0.005$ level. Only one subject had a postactivation ratio at any ISI that did not differ from both the activation and rest ratios at the $P<0.05$ level (square point in Fig. 1, middle). Some subjects showed frank facilitation of the test MEP at some ISIs where it was inhibited during rest and activation (Fig. 1). In one subject, the test MEP with postactivation was more than 4 times larger than the conditioning MEP at an ISI where the test MEP was completely suppressed at rest.

Whereas activation produced a massive increase in the amplitude of the conditioning MEP, in the postactivation state there was less facilitation (Fig. 3). In one subject, there was strong disinhibition of the test MEP without any facilitation of the conditioning MEP. The disinhibition of the test pulse generally lasted about 10 s (Fig. 4). The facilitatory effect of postactivation on the conditioning MEP lasted much longer and was still present after disinhibition of the test MEP had disappeared (Fig. 4). The data on conditioning MEP amplitude in Fig. 4 are normalized to the individual subject's resting MEP amplitude. The mean resting MEP amplitude across subjects was 234.9 μV (SD 201.7)

At rest, the MEP to TES was inhibited ($P<0.05$) at the 40-ms ISI in two of three subjects and at 60 ms in one of these and the other subject. No significant inhibition of the test MEP to TES was found at longer ISIs. Postactivation had no significant effect on the pattern of inhibition of a TES test MEP at any ISI in any subject. Data for one of the subjects tested with ISIs of 40 and 60 ms are shown in Fig. 5. (Data from this subject are also shown in Figs. 2, 3.) This subject had strong inhibition of the test MEP to TMS at ISIs 40 and 60 ms during rest and activation and significant reversal of this inhibition with postactivation. In this case there may have been

some facilitation of the unconditioned MEP to TES with postactivation (Fig. 5). This was not seen in the other two subjects.

Discussion

Our findings on TMS paired pulse inhibition under conditions of rest are largely parallel to those of Valls-Solé et al. (1992). Experiments using trains of repetitive TMS (Pascual-Leone et al. 1994) also provide evidence of a period of inhibition of MEPs 50–100 ms following the stimulus. This latter study also found facilitation of stimuli at ISIs of 200–300 ms, which agrees with the present findings.

There appear to be three effects of voluntary muscle activation on this process. First, activation eliminates the initial facilitatory phase, seen at the earliest ISIs in some subjects. Pascual-Leone et al. (1994) suggested that this type of facilitation might result from summation of monosynaptic excitatory postsynaptic potentials (EPSPs) produced by the test pulse, with longer latency polysynaptic EPSPs resulting from the conditioning pulse. Voluntary activation of the spinal motoneuron pool could eliminate relative facilitation of the second pulse if both pulses were maximally facilitated by voluntary drive. This explanation, however, is not compatible with the phase of facilitation occuring during activation at ISIs of about 130–200 ms (Figs. 1, 2), which, although not significant in the grand mean, was present to some degree in all subjects. It is more likely that the inhibitory phase of the response to TMS is potentiated by voluntary activation and cancelled the augmentation of the test MEP which was apparent during rest at short ISIs.

The second effect of activation is faster decay of paired pulse inhibition (Fig. 1). The recovery from inhibition during activation began at an ISI of 100 ms, about 30 ms earlier than during rest (Fig. 1). As discussed below, at an ISI of 100 ms, spinal inhibition of the test pulse has almost certainly worn off. Therefore, the effect of activation is to overcome inhibition that is probably acting at the cortical level. Presumably, during activation the command signal acting on the corticospinal cells provides some background excitation throughout the period of inhibition. Thus, as the inhibition begins to weaken at about 100 ms, excitatory processes are able to supervene and the test pulse finds the network nearer to threshold than it would be at rest.

Third, with activation there is a striking decrease in the variability of the ratio of the test to the conditioning MEP, which is illustrative of the fact that while a subject is at "rest", excitability in the corticospinal system can vary throughout the entire range below that which produces detectable EMG. Whereas, at least some mechanisms modulating excitability may be essentially "clamped" within a smaller range when a target muscle is activated to a fixed degree.

The mechanism for the paired pulse inhibition demonstrated at ISIs greater than about 60 ms appears to be cortical. Inghilleri et al. (1993) were unable to produce inhibition of MEPs at intervals over about 50 ms using TES of the cortex and brainstem, and Fuhr et al. (1991) found that inhibition of the H-reflex, following much stronger TMS than we used, had usually worn off by about 80 ms. We (Wassermann et al. 1993) and others (Roick et al. 1993), on the basis of other evidence, have concluded that the locus of this type of inhibition is at least partially in the motor cortex. The inhibition of MEPs to TES that we observed at ISIs of up to 60 ms was probably of spinal origin (Inghilleri et al. 1993), because low-intensity TES produces primarily direct activation of corticospinal neurons (Amassian et al. 1987; Burke et al. 1990; Rothwell et al. 1994) and therefore bypasses intracortical networks. Spinal inhibition probably accounts for the fact that postactivation generally did not result in complete reversal of paired pulse inhibition at short ISIs (e.g., 60 ms in Fig. 1).

Whatever the mechanism by which one TMS pulse inhibits another, the effect can be reversed in a dramatic and long-lasting way by postactivation. This phenomenon is not a manifestation of increased cortical or segmental excitability. If it were, the test MEP would have been disinhibited by ongoing muscle activation, which strongly potentiated the conditioning MEP (Fig. 3). While postactivation caused facilitation of the conditioning MEP, this phenomenon lasted much longer than the disinhibitory effect on the test MEP (Fig. 4). We have investigated this phenomenon further in another study where we found that it could last for minutes (Samii et al., in press). This phenomenon was more variable across subjects than the reversal of paired pulse inhibition. In some subjects, postactivation caused significant disinhibition of the test MEP in subjects where it produced little and, in some cases, no facilitation of the conditioning MEP. These results demonstrate that postactivation causes specific inactivation of the mechanism(s) responsible for paired pulse inhibition at the intervals under study. Our failure to find disinhibition by postactivation of MEPs evoked by TES after conditioning TMS is evidence that the disinhibition arises from a cortical source.

In their study of various animals, Krnjevic et al. (1966) found that an electrical pulse to the cortex resulted in inhibition of surrounding cortical neurons lasting 100–300 ms, which they attributed to the activation of a horizontal network of intracortical inhibitory fibers (Asanuma and Rosén 1973). Presumably, prolonged inhibition mediated by many intracortical interneurons would involve their synchronous activation and sustained firing. Postactivation probably makes this network refractory in some way. Paired pulse inhibition at ISIs of 30–50 ms also exists between cortical pyramidal cells (Thomson and Deuchars 1994). Such direct interactions between output cells might also contribute to the paired pulse inhibition occurring with TMS.

While very brief muscle activation produces the facilitation and disinhibition of MEPs described here and elsewhere (Samii et al., in press), inhibitory effects become measurable with longer, more intense exercise

(Brasil-Neto et al. 1993, 1994; Samii et al., in press). Whether any of these effects of exercise have common physiological bases is unclear; however, their different time courses and dissociation in some disorders (Samii et al. 1994, 1995, in press) suggests that they differ.

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