

Startle Stimuli Exert Opposite Effects on Human Cortical and Spinal Motor System Excitability in Leg Muscles

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Summary

Increased excitability of the spinal motor system has been observed after loud and unexpected acoustic stimuli (AS) preceding H-reflexes. The paradigm has been proposed as an electrophysiological marker of reticulospinal tract activity in humans. The brainstem reticular formation also maintains dense anatomical interconnections with the cortical motor system. When a startling AS is delivered, prior to transcranial magnetic stimulation (TMS), the AS produces a suppression of motor evoked potential (MEP) amplitude in hand and arm muscles of healthy subjects. Here we analyzed the conditioning effect of a startling AS on MEP amplitude evoked by TMS to the primary motor leg area. Ten healthy volunteers participated in two experiments that used a conditioning-test paradigm. In the first experiment, a startling AS preceded a suprathreshold transcranial test stimulus. The interstimulus interval (ISI) varied between 20 to 160 ms. When given alone, the test stimulus evoked a MEP amplitude of approximately 0.5 mV in the slightly preinervated soleus muscle (SOL). In the second experiment, the startling AS was used to condition the size of the H-reflex in SOL muscle. Mean MEP amplitude was calculated for each ISI. The conditioning AS suppressed MEP amplitude at ISIs of 30-80 ms. By contrast, H-reflex amplitude was augmented at ISIs of 100-200 ms. In conclusions, acoustic stimulation exerts opposite and ISI-specific effects on the amplitude of MEPs and H-reflex in the SOL muscle, indicating different mechanism of auditory-to-motor interactions at cortical and spinal level of motor system.

Key words

Transcranial magnetic stimulation • Startling acoustic stimulus • H-reflex • Motor cortex

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Introduction

Sudden, unexpected and very loud acoustic stimulus (AS) can activate startle motor response, which is characterized by a generalized cranio-caudal sequence of motor activation, affecting different muscle groups, distinctive on electromyographic testing (Brown *et al.* 1991). This effect has been attributed mainly to subcortical activity of the neurones in the nucleus reticularis pontis caudalis of the reticular formation (Davis *et al.* 1982).

Previous studies on healthy humans used startling AS to condition the size of the H-reflex in leg muscles and measured the relative change of the mean H-reflex amplitude of various inter-stimulus intervals (ISI) in a conditioning-test paradigm. The H-reflex is a reflex consistently obtained after electrical stimulation of sensory fibers and considers equivalent of the stretch reflex, predominantly characterized by the monosynaptic and oligosynaptic projections of group Ia afferents onto

homonymous motoneurons. However, in addition to extensive use in clinical neurophysiology, H-reflex can be used as an unambiguous measure of the excitability of the motoneuron pool (Ilic and Deuschl 2005).

The strongest auditory H reflex facilitation was achieved for ISIs around 75-125 msec, suggesting activation of slow conducting descending pathways (Delwaide *et al.* 1995). Along with the data from lesioning experiments in animals, it was assumed that the auditory startle response is mediated through reticulo-spinal pathways (Yeomans *et al.* 1996). Reticular nuclei involved in the startle response maintain also dense interconnections with the cortical motor system. Recently, two independent research groups have demonstrated suppression of the contralateral motor evoked response (MEP) amplitude in hand and arm muscles, if slightly suprathreshold magnetic test stimulus was preceded by a sudden, surprise AS in humans (Furubayashi *et al.* 2000, Kuhn *et al.* 2004). This effect was not present when transcranial magnetic stimulation (TMS) was substituted by transcranial electric stimulation, implicating the cortical origin of MEP amplitude inhibition. The reciprocal action of startle stimuli on reticulospinal and corticospinal system may serve to suppress voluntary motor action in potentially dangerous situations that require fast reflexive motor responses.

Since these potentially complex effects of acoustic stimulation at different levels of the motor system have not been explored for the leg muscles, we analyzed the conditioning effect of a startling AS on the MEP amplitude evoked by TMS to the primary motor leg area and compared the effect to startle conditioned H-reflexes of the soleus muscle.

Methods

Subjects

Neurophysiological examinations were performed in 10 healthy volunteers (mean age 33.18 ± 3.63 years, range 29-39; 2 women). All subjects were right-handed as tested with the Edinburgh inventory (Oldfield 1971). Written informed consent was obtained from all subjects. The study was performed according to the declaration of Helsinki and was approved by the ethics committee of Christian-Albrechts University of Kiel, Germany.

EMG responses

Subjects were seated comfortably in a reclining

chair. Surface EMG was recorded from the soleus (SOL) and tibialis anterior (TA) muscle of the right leg, using surface electrodes in belly-tendon montage. After amplification with filters set at 10 Hz - 2 kHz the EMG signal was passed through a CED micro 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) and fed into a personal computer (sampling rate 5 kHz), using customized data collection and conditional averaging software (Signal for Windows, ver. 2.13, Cambridge Electronic Design, Cambridge, UK) for off-line analysis.

Transcranial magnetic stimulation

TMS was applied over the vertex through a figure-of-eight coil (outer diameter of each loop, 90 mm; peak magnetic field ~ 1.5 T) using Mag Pro X100 magnetic stimulator (Medtronic, Skovlunde, Denmark) throughout all measurements. The stimulating coil was placed flat on the skull in medio-lateral orientation with the handle pointing to the right. Thus, the current induced in the brain was directed approximately perpendicular towards the assumed line of the interhemispheric sulcus (biphasic reversal mode). This is the optimal orientation for a predominantly trans-synaptic activation of the corticospinal neurone (Di Lazzaro *et al.* 2001, Kaneko *et al.* 1996). The optimal coil position for activating the right SOL was determined as the site where stimulation produced consistently the largest MEP at a slightly supra-threshold stimulus intensity. This site was marked with a pen in order to assure a constant placement of the coil throughout the experiment which was fixed on the holder. TMS intensity was adjusted to produce a target response amplitude of 0.5 mV (0.3-0.7 mV), peak-to-peak in the slightly preinervated SOL muscle. During experimental sessions subjects were encouraged to maintain a slight level of contraction in the right SOL muscle.

H-reflex

In order to evoke the Soleus H reflex, the stimulating electrodes were placed in the upper part of the popliteal fossa over the posterior tibial nerve. The leg was fixed with the knee flexed at 120 degrees and the ankle at 100 degrees. A single rectangular shock (1 ms) was given at an intensity that induced an H reflex with an amplitude of 30 % of the Soleus maximal motor response (8.51 ± 5.04 /SD/ mV). To record the H reflex EMG surface, electrodes were inserted over the Achilles tendon and 3-5 cm proximally. The ground was placed over the calf. The signals were amplified (gain 1000), filtered

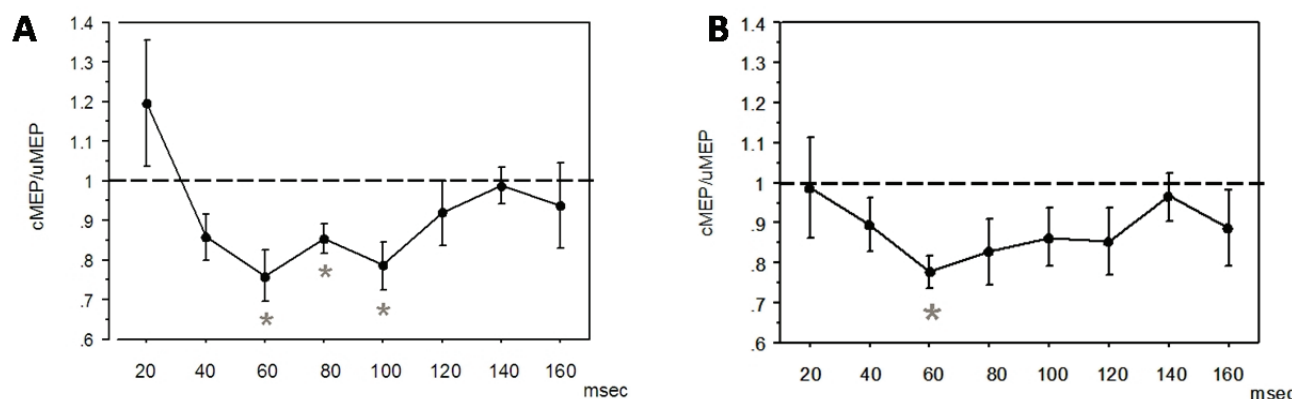


Fig. 1. Mean conditioned/unconditioned (c/u) MEP amplitude in slightly preinnervated SOL muscle (**A**) and resting TA muscle (**B**). Conditioning AS preceded TMS by 8 different ISI, which are plotted on the x-axis. All data are means \pm SE ($N=10$). Significant suppression was evoked at ISIs of 60-100 ms in SOL muscle, and at ISI of 60 ms in TA muscle. The asterisk marks a significant difference ($P < 0.05$; ANOVA test, post-hoc analysis).

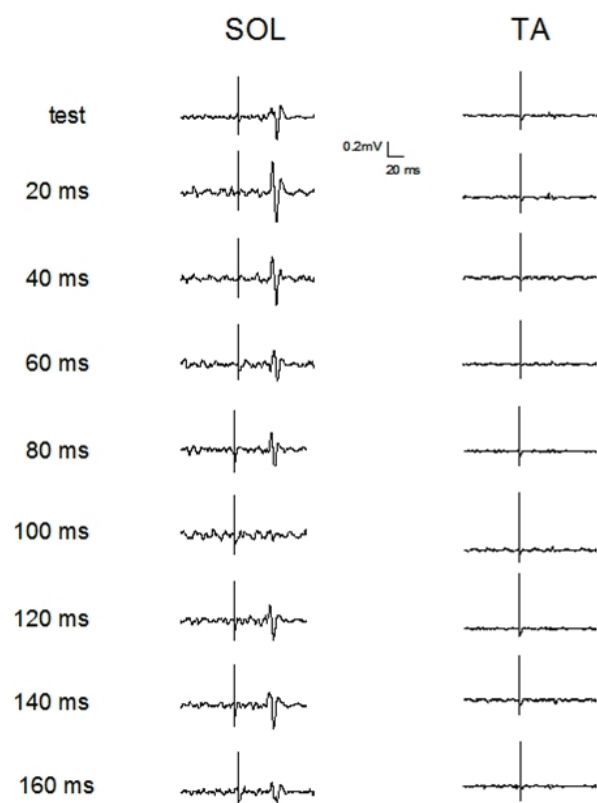


Fig. 2. Averaged data traces from one representative subject showing the effect of conditioning AS on MEP amplitudes in SOL and TA. Each trace is the average of fifteen responses, except for test response where 30 MEPs were averaged.

(bandpass filter between 80 Hz - 2.5 kHz) and digitised (sampling frequency 5 kHz).

Conditioning auditory stimulation

The test H-reflex was conditioned by preceding binaural AS (100 ms, 100 dB, 500 Hz) delivered through headphones at 9 different randomly alternated ISI (0, 50,

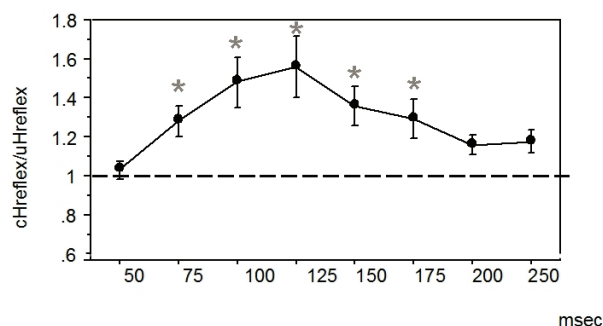


Fig. 3. Average ratio change in H-reflex amplitude for right SOL muscle. Each point is the mean (\pm SE) of the data from ten subjects, and each value is the average of ten responses. Responses at ISIs 75-175 ms are significantly larger compared to test values ($P < 0.05$; ANOVA test).

75, 100, 125, 150, 175, 200, 250 ms). Stimulation was repeated randomly at intertrial intervals (ITI) of 20-30 seconds. For each ISI, 10 H-reflexes were recorded and compared to 30 randomly intermixed unconditioned reflexes.

In the second experiment, startling AS with the same characteristics are used to condition the size of MEPs in SOL muscle. The ISIs varied between 20-160 (eight steps of 20 ms increments). For each ISI condition 15 MEPs were recorded and compared to 30 randomly intermixed unconditioned responses.

The amplitude change of the conditioned reflexes was expressed as a percentage of unconditioned control values.

Statistical analysis

The normalized mean values of MEPs or H-reflexes were tested in an one-factor ANOVA model with ISI as the main effect (8 levels). Based on the

significance of the F-value, the different time intervals were compared with the control responses by Bonferroni-corrected multiple paired t-tests. The significance level for all tests was set to $p < 0.05$ (two-sided).

Results

Conditioned MEP amplitude following TMS of the cortical leg area

The test MEP amplitude in right SOL muscle could be maintained at the target level of approximately 0.5 mV (0.64 ± 0.29 mV) with a TMS intensity of 64.67 ± 12.64 % maximal stimulator output. Conditioning of the MEP amplitude by preceding sudden loud AS evoked a MEP inhibition curve (Fig. 1a with a slight to moderate conditioning effects at ISIs of 60, 80 and 100 ms ($p < 0.05$). The inhibitory effect was observed in the resting right TA muscle (MEP amplitude 0.23 ± 0.08 mV), but exclusively at the ISI of 60 ms (Fig. 1b, $p < 0.05$). Fig. 2. shows averaged MEPs in a single illustrative case.

In contrast to these findings, the inhibitory effect was saturated when the test MEP amplitude was adjusted to 1 mV.

Conditioned H-reflex

Conditioning the H-reflex in SOL muscle with a preceding auditory stimulation yielded a consistent pattern of responses across the various subjects. Figure 3 shows average H-reflex responses in the right SOL muscle with clear facilitation. In the intervals 75-175 ms marked increase in H-reflex amplitude was observed ($p < 0.05$). The maximum of the averaged conditioned response was 153.2 % of the control response size at the ISI of 125 msec.

Discussion

The main finding to emerge from this study was that auditory preconditioned MEP has revealed significant amplitude decrease, in contrast to H-reflex facilitation in leg muscles under the similar circumstances.

Loud and sudden auditory stimuli elicit strong electromyographic (EMG) responses in various muscles throughout the length of the body, representing the so called startle reflex. Animal models have implicated the ventrocaudal part of the nucleus reticularis pontis caudalis of the reticular formation to generating the startle reflex. Studies using electrical stimulation of the

medullary reticular formation have suggested that generalized motor inhibition can be induced *via* strong connections which activate spinal cord motoneurons through reticulospinal projections (Drew and Rosignol 1990, Jankowska *et al.* 1968, Takakusaki *et al.* 2001, Takakusaki *et al.* 1989, Takakusaki *et al.* 1994).

In healthy subjects, auditory stimulus at 90 dB induced not only SOL H reflex facilitation with a peak after 75-125 msec but also in the antagonistic TA muscle, and in hand muscles (Delwaide and Schepens 1989). Taking into account the latency differences between arm and leg muscles and the distance over the spine, the conduction velocity of the descending facilitatory wave was calculated to be around 20 m/sec, that corresponds to a very slow conducting descending pathway other than the corticospinal tract. The same conduction velocity was also estimated from animal experiments (Iwamoto *et al.* 1990, Wu *et al.* 1988).

In recent studies auditory preconditioning TMS has induced clear inhibitory effects, on TMS to arm (Kuhn *et al.* 2004) and hand muscles (Furubayashi *et al.* 2000). By contrast, transcranial electric stimulation (TES) in first dorsal *interosseous* muscle of healthy volunteers didn't change MEP amplitude at ISIs of 30-60 ms, while late facilitation (ISI of 80 ms) was observed (Furubayashi *et al.* 2000). In line with those findings, subcortical electrical stimulation through deep electrodes implanted in subthalamic nucleus of five implanted Parkinsonians evoked facilitation (Kuhn *et al.* 2004). Both approaches promote evidence against a possible interaction of acoustic stimulation at the subcortical level, with motor evoked potentials. In agreement with those observations, it has been speculated that the startle brainstem system can exert opposite effects on spinal and cortical motoneurons (Valls-Sole *et al.* 1999). It was speculated that loud and sudden auditory stimulus activate synchronously reticulocortical and reticulospinal projections.

So far, studies of auditory preconditioned MEP have been performed exclusively in upper limb muscles. In this study we decided to combine auditory H-reflex modulation in SOL muscle in line with auditory preconditioned TMS. Spinal reflex mechanisms are preferentially evaluated in human leg muscles due to the phylogenically distinct spinal mechanisms subserving stance and walking. It might be assumed that a strong spinal level facilitation mediated *via* descendent reticulospinal projections at least partially cancelled an inhibition of the cortical motor pathway following

audiogenic startle stimuli, which could explain the relatively modest modulation of auditory preconditioned MEP compared to the strong facilitation of auditory preconditioned H-reflexes.

Studies of the interaction between cortical and spinal brainstem motor systems in patients with various neurological diseases (e.g. gait improvement in Parkinsonian where patients underwent deep brain stimulation) can provide better understanding of pathophysiological mechanisms.

In summary, here we show for the first time motor cortical inhibition following auditory stimulation

in a leg muscle, indicating similar mechanisms also confined to the motor cortex area representing hand and arm muscles.

Conflict of Interest

There is no conflict of interest.

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