Modulation of Transmission in the Corticospinal and Group Ia Afferent Pathways to Soleus Motoneurons During Bicycling

H. S. PYNĐT AND J. B. NIELSEN
Division of Neurophysiology, Department of Medical Physiology, The Panum Institute, University of Copenhagen, 2200 Copenhagen, Denmark
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Pyndt, H. S. and J. B. Nielsen. Modulation of transmission in the corticospinal and group Ia afferent pathways to soleus motoneurons during bicycling. *J Neurophysiol* 89: 304–314, 2003. 10.1152/jn.00386.2002. Transmission in the corticospinal and Ia pathways to soleus motoneurons was investigated in healthy human subjects during bicycling. Soleus H reflexes and motor evoked potentials (MEPs) after transcranial magnetic stimulation (TMS) were modulated similarly during the crank cycle being large during downstroke (concomitant with soleus background electromyographic (EMG) activity) and small during upstroke. Tibialis anterior MEPs were in contrast large during upstroke and small during downstroke. The soleus H reflexes and MEPs were also recorded during tonic plantarflexion at a similar ankle angle, and matched background EMG activity as during tonic plantarflexion at a similar ankle joint position, corresponding ankle angle, and matched background EMG activity as during the different phases of bicycling. Relative to their size during tonic plantarflexion, the MEPs were found to be facilitated in the early part of downstroke during bicycling, whereas the H reflexes were depressed in the late part of downstroke. The intensity of TMS was decreased below MEP threshold and used to condition the soleus H reflex. At short intervals (conditioning-test intervals of −3 to −1 ms), TMS produced a facilitation of the H reflex that is in all likelihood caused by activation of the fast monosynaptic corticospinal pathway. This facilitation was significantly larger in the early part of downstroke during bicycling than during tonic plantarflexion. This suggests that the increased MEP during downstroke was caused by changes in transmission in the fast monosynaptic corticospinal pathway. To investigate whether the depression of H reflexes in the late part of downstroke was caused by increased presynaptic inhibition of Ia afferents, the soleus H reflex was conditioned by stimulation of the femoral nerve. At a short interval (conditioning-test interval: −7 to −5 ms), the femoral nerve stimulation produced a facilitation of the H reflex that is mediated by the heteronymous monosynaptic Ia pathway from the femoral nerve to soleus motoneurons. Within the initial 0.5 ms after its onset, the size of this facilitation depends on the level of presynaptic inhibition of the Ia afferents, which mediate the facilitation. The size of the facilitation was strongly depressed in the late part of downstroke, compared with the early part of downstroke, suggesting that increased presynaptic inhibition was indeed responsible for the depression of the H reflex. These findings suggest that there is a selectively increased transmission in the fast monosynaptic corticospinal pathway to soleus motoneurons in early downstroke during bicycling. It would seem likely that one cause of this is increased excitability of the involved cortical neurons. The increased presynaptic inhibition of Ia afferents in late downstroke may be of importance for depression of stretch reflex activity before and during upstroke.

INTRODUCTION

Locomotion is a highly complex but automated movement consisting of alternating rhythmic extension and flexion of the limbs. Animal experiments have documented that the basic alternating rhythmic activity is predominantly generated at the spinal level (for review see Grillner 1981). Peripheral sensory feedback from muscle, skin, and joints plays a significant role in the maintenance and timing of this activity (Forssberg et al. 1977; Pearson et al. 1998). Although corticospinal activity contributes to the muscle activity during uncomplicated over-ground locomotion, it has its main role in the visual guidance of locomotion and in gait modifications in response to environmental and motivational influences (Armstrong 1988, Armstrong and Marple-Horvat 1996; Drew 1991).

Experiments in human subjects are beginning to reveal whether human bipedal walking is controlled in a similar way. Observations in patients with spinal cord lesions suggest that there is a network in the human spinal cord that has the capacity of generating rhythmic alternating muscle activity similar to that seen during walking (Calancie et al. 1994; Dimitrijevic et al. 1998), but there is still no strong evidence regarding the potential role of this network during walking in intact human subjects. Several groups have studied the modulation of cutaneous and muscular reflexes during the gait cycle (reviewed in Dietz 1996; Zehr and Stein 1999), and recently Sinkjaer et al. (2000) have provided evidence that feedback in muscle afferents via spinal interneurons contributes to the activation of soleus motoneurons in the stance phase of walking. Corticospinal function has been investigated by transcranial magnetic stimulation (TMS) of the motor cortex during treadmill walking by several groups (Capaday et al. 1999; Petersen et al. 1998, 2001; Schubert et al. 1997, 1999). One of the main findings from these studies is that the corticospinal tract appears to contribute to the muscle activity during uncomplicated treadmill walking (Petersen et al. 1998, 2001) but, as in the cat, may have its main role in relation to visually guided walking (Schubert et al. 1999).

Whereas there is thus an emerging understanding of the
central control of walking in human subjects, it is less investigated whether similar control paradigms also exist in relation to other rhythmic alternating movements, such as bicycling. Although the biomechanics of bicycling has been investigated thoroughly (Ericson 1986; Hull and Jorge 1985), only few studies have addressed the central control mechanisms (Boorman et al. 1992; Brooke et al. 1992; Zehr et al. 2001). There are some obvious methodological advantages of studying bicycling. The task mechanics can be easily controlled and manipulated, and the patterns of electromyographic (EMG) activity are well defined and remain relatively constant (Jorge and Hull 1986). The upper part of the body remains relatively constant in space, which makes the application of, for instance TMS, very easy. An understanding of how rhythmic movements are generated and controlled during bicycling will help to understand how far general principles underlie the control of rhythmic activity across specific motor tasks in human subjects.

The purpose of the present study was to obtain evidence of possible changes in the transmission in the corticospinal and group Ia afferent pathways during ergometer bicycling. In the first part of the study, soleus H reflexes and motor-evoked potentials (MEPs) following TMS were compared in different phases of the crank cycle. Evidence suggesting a relative increase of MEPs during the rising phase of soleus muscle activity (early downstroke) and a relative decrease of the H-reflex during the falling phase of the activity (late downstroke) was obtained. In the second part of the study, the mechanisms underlying these observations were investigated.

**METHODS**

Twenty-four subjects aged 22–38 yr (13 females, 11 males) participated in the study. Not all subjects participated in the different parts of the study. The subjects received written and oral information about the procedures of the experiments before giving their written consent. The experiments were conducted in accordance with the Helsinki declaration and approved by the local ethics committee.

**General setup**

The subjects pedaled at a constant speed, 60 rpm, at an external load of 0.5 or 1.0 kg with their feet fastened to the pedals with straps, on a bicycle-ergometer (Monarch 834E) modified to monitor the position of the crank continuously.

**Recordings**

EMG recordings were made from the right mm. tibialis anterior et soleus with bipolar (1 cm² recording area; 2 cm between poles) Ag-AgCl electrodes placed over the belly of the muscles. The EMG signals were sampled at 2,000 Hz, amplified (2,000–5,000 times), band-pass-filtered 25–1000 Hz before being stored on a PC for later analysis. The signals were recorded in a window from 50 ms before until 200 ms after stimuli.

**Stimulations**

The H reflex was evoked by monopolar stimulation of the right posterior tibial nerve (PTN; Fig. 1A). The anode was placed above the patella and the cathode in the popliteal fossa. The stimulation electrodes were secured with adhesive tape to prevent them from moving during the experiment. MEPs were evoked by TMS of the contralateral motor cortical leg area using a figure-eight coil (loop diameter, 9 cm) and a MagStim 200 stimulator (Magstim, Dyfed, UK) with the capability to deliver a magnetic field of 2 T for 100 µs (Fig. 1A). The position of the coil in respect to the head was secured by mounting the

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**FIG. 1.** A: the electrical stimulation of the posterior tibial nerve (PTN) in the popliteal fossa and the transcranial magnetic stimulation (TMS) of the motor cortex. Electrical stimulation of PTN activates Ia afferents projecting monosynaptically to motoneurons in the spinal cord. Magnetic stimulation of the motor cortex evokes transmission through the corticospinal tract projecting monosynaptically to motoneurons in the spinal cord. The resulting responses [H reflexes and motor-evoked potentials (MEPs), respectively] may be recorded by surface electrodes placed over the belly of the muscle. B: the peak-peak modulation of the tibialis anterior MEP (top), the soleus MEP (middle), and the soleus H reflex (bottom) at the 8 crank angles investigated during bicycling. Each trace is an average of ≥10 recordings. The abscissas of the traces show the recordings from 10 ms before until 100 ms after stimulation.
magnetic coil on a harness (Balgrist Tec, Zurich, Switzerland) (for details, see Schubert et al. 1997), which the subjects wore throughout the experiment. Before the experiments the optimal spot for stimulation eliciting the largest MEP was found during tonic plantar flexion of the ankle joint. The optimal spot was marked on the skull and used to check that the coil did not move during the experiments.

Modulation of the H reflex and MEP during bicycling

This part of the study consisted of two different types of experiments. In the first experiment, the modulation of the soleus H reflex and MEP were studied at constant stimulation intensity. In the second experiment, the size of the responses was measured as a function of stimulation intensity.

EXPERIMENT 1: MODULATION OF H REFLEX AND MEP DURING BICYCLING WITH CONSTANT STIMULATION INTENSITY. In 10 subjects, H reflexes and MEPs were evoked during bicycling at eight different crank angles during the crank cycle (see Fig. 1B): at top dead center (TDC), at 23, 45, 67, 90, 135, 180, and 270° after TDC. A trigger signal was generated at TDC and used to activate the computer that controlled the stimulators. The stimulus was hereafter applied at the delay corresponding to the investigated crank angle. With a variation in pedaling speed of ±2 rpm this corresponds to a variation in crank-angle of ±2.3°. The order in which the eight crank angles were investigated was assigned pseudorandomly. The H reflex and MEP were related to the maximal M response (Mmax) in the soleus muscle evoked by supramaximal stimulation of PTN. Mmax may change significantly with changes in muscle length such as those during bicycling (Gerilovsky 1986; Simonsen and Dyhre-Poulsen 1999), and it may change in the course of an experiment (Crone et al. 1999). It is therefore important to measure Mmax at the same muscle length and at the same time during the experiment as the H reflexes and MEPs. In the experiments by Simonsen and Dyhre-Poulsen (1999), this was solved by evoking Mmax 60 ms after the stimulation eliciting the H reflex. We decided not to use this procedure in the present experiment because a delay of 60 ms corresponds to a change of 22° during bicycling at 60 rpm, which may represent a significant change in muscle length. Furthermore, the large number of supramaximal stimuli necessary easily disrupts the normal activation pattern during bicycling. Instead we measured the size of Mmax in independent trials just before and after H reflex and MEP measurements at the same crank angle. This ensured that the muscle length was similar during the measurements and that the potential influence of time factors was minimized. For comparison between tasks, the intensity of the stimulation, which elicited the H reflex, was adjusted to evoke an M response corresponding to 20% of Mmax throughout the experiment (measurements in which the M response deviated by more than 10% from this value were omitted from the subsequent analysis). A consequence of this was that the H reflex was measured on the descending part of the recruitment curve, where it may be less sensitive to modulation than during the ascending part. However, because all H-reflex measurements were made at the same moment of the recruitment curve throughout the study, this had little consequence for the obtained results.

The intensity of TMS was adjusted to approximately 1.2 times soleus (SOL) MEP threshold, during bicycling when the EMG activity of the SOL muscle was increasing (usually at 45–90° after TDC). The threshold of the MEP was determined as a clearly detectable response following at least 50% of the magnetic stimuli. The stimulation intensity ranged between 50 and 60% of maximal stimulator output and the same intensity of stimulation was used throughout the experiment. The position of the coil was checked throughout the experiment.

During bicycling, the average background EMG from 50 to 0 ms before stimulation was calculated at the eight different crank angles. Because the background EMG is strongly modulated during bicycling, this will either be an under- or overestimation of the actual EMG corresponding to the point of stimulation. It was necessary to measure the background EMG before stimulation to avoid the stimulation artifact. It was ensured that this made only minor differences in the estimate obtained when calculating the background EMG just prior to the MEP. During tonic contraction, the pedals were locked at each of the eight crank angles, and sitting on the bicycle, the subjects were instructed to maintain a level of SOL and tibialis anterior (TA) background EMG activity similar to that during bicycling. At 270°, corresponding to the upstroke phase, where there was no SOL activity during pedaling the subjects were requested to be at rest. The EMG of the SOL muscle was amplified, rectified, integrated, and monitored on an oscilloscope in front of the subject for visual feedback. Recordings of H reflexes and MEPs during tonic contraction were made in the same way as mentioned above at the eight different crank angles.

EXPERIMENT 2: SIZE OF THE SOL H REFLEX AND MEP AS A FUNCTION OF THE STIMULATION INTENSITY DURING BICYCLING. To further characterize the responses, we constructed input-output relations for the H reflex and MEP in 11 subjects during bicycling when the SOL EMG was increasing (early downstroke) and when the SOL EMG was decreasing (late downstroke) as well as during tonic plantarflexion in standing subjects with an EMG activity corresponding to that recorded during bicycling. We chose to investigate tonic plantarflexion in standing rather than sitting subjects because it was easier for the subjects to produce the same level of EMG as during bicycling while standing. Control experiments revealed that there was no difference in the responses during tonic plantarflexion in standing as compared with sitting subjects. It was necessary to perform plantar flexion during standing in these experiments because much more values were recorded and the measurements lasted much longer in experiment 1. The crank angles selected for stimulation during bicycling were chosen to have the same level of SOL EMG activity during the increasing and decreasing part of the EMG. The stimulation intensities for the H-reflex input-output curves were varied from below H-reflex threshold to above the intensity eliciting Mmax. The stimulation intensities for the MEP input-output curve were varied from below threshold to intensities where no further increase in the MEP was seen. The stimulation intensities were varied in pseudorandom order.

Conditioning of the SOL H reflex by TMS and femoral nerve stimulation

CONDITIONING OF THE H REFLEX BY TMS. In 10 subjects, the effect of subthreshold TMS on the SOL H reflex was investigated during the crank cycle. The crank angle was chosen on the increasing part of the SOL EMG where the EMG activity was approximately 50% of its maximum (early downstroke). The crank angle was approximately 67° after TDC. The level of background EMG activity at the selected crank angle was recorded, and the subjects were asked to match this level of contraction when performing tonic plantarflexion. For visual feedback, the EMG activity was amplified, rectified, integrated, and monitored on an oscilloscope in front of the subject.

During bicycling and tonic plantarflexion the SOL Mmax was recorded prior to each part of the experiment (bicycling and tonic contraction). The size of the control SOL H reflex was adjusted to approximately 20% of Mmax throughout the experiment. The intensity of TMS was adjusted to an intensity just below the threshold for eliciting a MEP in the early downstroke during bicycling. A time course of the effect of TMS on the H reflex was constructed with conditioning-test intervals every ms from −6 to 14 ms. A negative conditioning test interval indicates that the conditioning stimuli were elicited after the test stimuli (i.e., at a conditioning test interval of −6 ms PTN stimuli preceded magnetic stimuli by 6 ms). Conditioned and unconditioned reflexes were randomly alternated. A conditioning test interval within the initial 1 ms after the onset of facilitation of the H reflex (cf. Nielsen et al. 1993) was hereafter used to construct an
intensity curve of the effect of TMS on the H reflex during bicycling and tonic contraction. The TMS intensities were varied from approximately 50 to 100% of the MEP threshold.

**CONDITIONING THE H-REFLEX BY FEMORAL NERVE STIMULATION.**

In seven subjects, the effect of femoral nerve stimulation on the SOL H reflex was investigated during the crank cycle. Seven to 10 crank angles during the crank cycle were investigated. At each crank angle, Mmax was recorded and the H reflex was adjusted to approximately 10% of Mmax. The H reflex was kept low because it is was found not to be possible to elicit an H reflex larger than 5–10% of Mmax during late downstroke in most subjects. Because it was only possible to elicit a sufficiently large H-reflex in two of the seven subjects during upstroke, this experiment was limited to early and late downstroke. Stimulation of the femoral nerve was evoked by monopolar stimulation with the anode placed on the back of the thigh and a ball-shaped cathode pressed into the femoral triangle just below the inguinal ligament. The stimulation of the femoral nerve was adjusted throughout the experiment to evoke a small M wave (approximately 1.1 × motor threshold) in the lateral head of the quadriceps. In the beginning of the experiment, a time course was constructed at rest to find the conditioning test interval for eliciting the very first facilitation of the SOL H reflex. The time course was constructed in two steps. First, a time course was constructed with conditioning test intervals from −9 to 0 ms in steps of 0.5 ms to find the approximate time of the very first facilitation. Second, a time course from 2 ms before until 2 ms after the time of facilitation was constructed in steps of 0.2 ms. Conditioned and unconditioned reflexes were randomly alternated. A conditioning test interval within the initial 0.5 ms of the facilitation was hereafter used throughout the experiment (cf. Hultborn et al. 1987). Although this method investigates modulation of presynaptic inhibition of heteronymous Ia afferents, the findings also apply to homonymous SOL Ia afferents. Hultborn et al. (1987) demonstrated that modulation of presynaptic inhibition depends on where the Ia afferents project rather than where they come from.

**Data analysis**

In all experiments, at least 10 peak-to-peak H-reflex or MEP recordings were made in each trial. The first elicited H reflex during the trials in each part of the study was discarded to avoid any influence of post activation depression (Crone and Nielsen 1989). The H-reflex data from the first part of the study were analyzed on-line to ensure that only H reflexes with a simultaneous M wave of 10–30% of Mmax were used for later analysis. The recording software automatically rejected recordings with an M wave outside the 10–30% range of Mmax.

Differences in the size of MEPs and H reflexes during bicycling as compared with tonic plantarflexion were determined by the Student’s t-test for data from individual subjects. For population average data the two-way ANOVA test, multiple comparison procedure (Tukey’s test) was used.

Data from the input-output (I-O) relations were fitted to the Boltzmann sigmoidal function by the Levenberg-Marquard nonlinear, least-mean-squares algorithm (as previously described by Devanne et al. 1997). The Boltzmann equation relating the amplitude of the H reflex, M wave, or MEP (response) and the stimulus intensity (S) is given by the following equation

\[
\text{Response}(S) = \text{Response}_{\text{Max}} \cdot (1 + \exp(\frac{[S_{50} - S]}{K}))
\]  

(1)

Response (S) is the response (H reflex, M wave, or MEP) at a given stimulation intensity (S), ResponseMax is the maximal response, S50 is the stimulation intensity required to obtain a response 50% of maximum, and K is the slope parameter. ResponseMax, S50, and K were the parameters found by iteration. No significant differences were found between the population average ResponseMax and the population average of the exact recorded values of the H reflex, M wave, or MEP using Student’s t-test. The \(R^2\) for the iterations averaged 0.80 ± 0.03 for the MEP and 0.98 ± 0.01 for the H reflex.

The equation was differentiated and the parameters were used to find the maximal steepness of the curve at \(S_{50}\).

\[
\text{Response}'(S_{50}) = \frac{\text{Response}_{\text{Max}}}{4K}
\]  

(2)

The threshold, or the x intercept, was calculated using the steepness of the curve [Response'(S_{50})] and the points [S_{50}, Response(S_{50})].

The above-mentioned analysis was used to estimate the threshold for M wave. To compare data among subjects the input-output curve for the H reflex was measured as stimulation intensity/intensity for threshold M wave versus H reflex in percentage of Mmax. The MEP input-output curve was measured as stimulation intensity versus MEP in percentage of Mmax.

The population average of the calculated parameters was used to calculate the population average ResponseMax, S50, and K values to construct population averaged I-O curves for the H reflex and MEP during early and late downstroke during bicycling and during tonic plantarflexion. Because it is not possible to make statistical analysis of the calculated parameters for each subject, this analysis was only applied to the population average. Differences in population average of H-reflex size, MEP size, H reflex and MEP threshold, and steepness of the H reflex and MEP recovery curves between bicycling and tonic plantarflexion were tested using the Student’s paired t-test. For all tests, the level of significance was set to \(P < 0.05\).

For experiments in which the SOL H-reflex was conditioned by TMS or femoral nerve stimulation at least 10 peak-to-peak measurements were averaged at each stimulation alternative. The mean ± SE were calculated at each conditioning test interval for each subject. Statistically significant differences between conditioned and unconditioned H reflexes were determined using the Student’s paired t-test. For both single-subject data and population-averaged data two-way ANOVA tests were used to determine significant differences in the time course and intensity curve between bicycling and tonic plantarflexion.

Differences in the amount of SOL H-reflex facilitation evoked by femoral nerve facilitation in early and late downstroke during bicycling were determined using the Student’s paired t-test.

**RESULTS**

**Modulation of the H reflex and MEP during bicycling**

**EXPERIMENT 1: MODULATION OF H REFLEX AND MEP DURING BICYCLING WITH CONSTANT STIMULATION INTENSITY.** Figure 2 shows the modulation of the TA and SOL MEPs during bicycling in a single subject (Fig. 2A) and as the population average for nine subjects (Fig. 2B). It is seen that both MEPs were modulated with the background EMG activity in their respective muscles. The SOL MEP was thus absent during upstroke and largest during downstroke when the background SOL EMG activity was largest. Conversely the TA MEP was absent during downstroke and most of the early part of upstroke but appeared together with the background TA EMG activity in the late part of upstroke.

It was not possible to evoke an H reflex in the TA muscle during bicycling in any of the investigated subjects, and a comparison of the modulation of the H reflex and MEP was therefore restricted to the SOL muscle. Figure 3 shows representative data from a single subject, and Fig. 4 shows pooled data from all 10 investigated subjects.

Figure 3, E–G, shows a comparison of the SOL background EMG activity (Fig. 3E), the SOL H-reflex (Fig. 3F), and the SOL MEP (Fig. 3G) at the eight different crank positions.
FUNCTION OF THE STIMULATION INTENSITY DURING BICYCLING.

EXPERIMENT 2: SIZE OF THE SOL H-REFLEX AND MEP AS A FUNCTION OF THE STIMULATION INTENSITY DURING BICYCLING.

during bicycling (■ and ●) and during tonic plantarflexion at matched SOL background EMG activity and ankle joint positions (□ and ○). Figure 3E confirms that the background EMG activity during the two tasks was well matched for each of the eight crank angles. As is seen from Fig. 3, F and G, the SOL H reflex was modulated in the same way as the MEP being largest when the EMG was at its maximum and smallest when there was no EMG activity during upstroke.

However, significant differences were observed when comparing the responses during the two tasks more closely. Significantly smaller H reflexes were thus recorded in the late part of downstroke and during upstroke than during tonic plantarflexion at corresponding background EMG activity and crank position (P < 0.05; Fig. 3F; late downstroke and upstroke). In the same phases of the movement, there was no difference in the size of the MEPs during the two tasks.

Notice, that the crank angle at which the H reflex is seen to be depressed corresponds to the time where the position of the ankle joint changes in the dorsiflexion direction (Fig. 3B).

In the early part of downstroke, when the SOL EMG activity was still increasing, the MEPs were significantly larger than during tonic plantarflexion (P < 0.05; Fig. 3G; early downstroke). At the same positions there were no differences in the H reflexes during the two tasks.

A similar significant increase of the MEP in the early part of downstroke during bicycling as compared with plantarflexion was observed in 6 of 10 subjects. In the remaining four subjects, the P values were between 0.05 and 0.1. Only 1 of the 10 subjects had a significant increase in the H reflex observed in early downstroke. In contrast, 7 of the 10 subjects showed a significant depression of the H reflex in late downstroke, whereas a similar depression of the MEP was only observed in a single subject.

This overall picture is also evident from the population averages shown in Fig. 4. A two-way ANOVA test on the illustrated data confirmed a significant facilitation of the MEP in early downstroke and a significant depression of the H reflex in late downstroke and upstroke (P < 0.05).

EXPERIMENT 2: SIZE OF THE SOL H-REFLEX AND MEP AS A FUNCTION OF THE STIMULATION INTENSITY DURING BICYCLING.

Figure 5 shows the size of the H reflex (Fig. 5, A and C) and the MEP (Fig. 5, B and D) as a function of the stimulation intensity in a single subject. For the H reflex, a comparison was made between tonic plantarflexion and the late downstroke, whereas for the MEP, a comparison was made between tonic plantarflexion and early downstroke. The background EMG activity was the same in all three cases. Nevertheless, it is evident when comparing Fig. 5, A and C, that the threshold was higher and the maximal amplitude of the H reflex smaller in the late part of downstroke than during tonic plantarflexion. For the MEP, the threshold was lower and the maximal amplitude larger in the early part of downstroke as compared with tonic plantarflexion (compare Fig. 5, B and D). In both cases, a parallel shift (to the right and left, respectively) of the recruitment curves was thus seen during bicycling in relation to plantarflexion. Notice that there is a drop in the MEP at high stimulation intensities. This drop may be explained by activation of inhibitory indirect pathways to the SOL motoneurons (Nielsen et al. 1993).

This parallel shift was confirmed when analyzing the pooled data from all the subjects who participated in this experiment (11 subjects participated in the H reflex experiments. The MEP threshold was very high in three of these subjects (more than 60% of maximal stimulator output) and the MEP input-output curve was therefore only obtained in eight of the subjects. To pool the data, a sigmoid curve was fitted to the data by the Levenberg-Marquardt algorithm (Fig. 5, -- ). Figure 6, A and B, show the raw H reflex and MEP measurements as a function of the stimulation intensity for all subjects. Figure 6, C and D, show a comparison of the pooled average of the sigmoid curves obtained from the individual subjects. The square of the correlation coefficient between the calculated curve and the recorded plot was on average 0.91 ± 0.02 for the H reflex and MEP during bicycling and tonic contraction.

The threshold for the H reflex was significantly (P < 0.05) higher and the maximal amplitude was significantly smaller (P < 0.05) during the late part of downstroke compared with tonic plantarflexion (Fig. 6C, compare — and ··· ···). Similar measurements were also made for the early downstroke, and we found no difference in the H-reflex recruitment curve in the early part of downstroke as compared with tonic plantarflexion. We found no differences in the slopes of the recruitment curves during early downstroke, late downstroke, and tonic contraction.

The threshold of the MEP was significantly lower (P < 0.05) in the early part of downstroke as compared with plantarflexion (Fig. 6D, compare — and ··· ···). The maximal amplitude of the
MEP tended to be larger during bicycling than during tonic plantarflexion, but this did not reach a statistically significant level (P = 0.121). There was no difference in either parameter when comparing the late part of downstroke and tonic plantarflexion. The MEP recruitment curve during early downstroke was significantly steeper than during late downstroke (P < 0.05). There was no difference in slope between early downstroke and tonic plantarflexion. There were no significant differences in the amount of background EMG activity in the two phases of bicycling and during tonic plantarflexion (P = 0.25).

Conditioning of the SOL H-reflex by TMS and femoral nerve stimulation

The facilitation of the MEP in early downstroke without a similar facilitation of the H reflex suggests that there is an increased corticospinal transmission in this phase of the movement. One possible mechanism, which could explain this, is increased excitability of the corticospinal cells projecting to SOL motoneurons. The depression of the H reflex in late downstroke without a similar depression of the MEP on the other hand suggests a decreased transmission in the group Ia pathway in that phase of the movement. One possible mechanism, which could explain this is, increased presynaptic inhibition of Ia afferents. The purpose of the experiments in part II of the study was to investigate whether these mechanisms might explain the differential modulation of the H-reflex and MEP.

Conditioning of the SOL H-reflex by TMS. TMS at an intensity below MEP threshold has been shown in previous studies to produce a short-latency facilitation of the SOL H reflex, which is in all likelihood mediated by the fast conducting monosynaptic corticospinal pathway to the SOL motoneurons (Nielsen and Petersen 1995; Nielsen et al. 1993). Figure 7A demonstrates that a similar facilitation may also be produced during bicycling (● measurement in early downstroke during bicycling; 72° after TDC). The facilitation is seen to begin at a conditioning-test interval of 3 ms and to last until a conditioning-test interval of 5 ms, after which it is replaced by an inhibition. It has been
previously argued that the size of this facilitation within the initial 0.5–1.0 ms is sensitive to changes in the excitability of the cortical cells (Nielsen and Petersen 1995; Nielsen et al. 1993; Petersen et al. 1998). A comparison was therefore made between the size of the facilitation in the early part of downstroke during bicycling and tonic plantarflexion (C). It was ensured that the background SOL EMG activity, the control H reflex, and the intensity of TMS were the same in the two tasks. As can be seen from the figure, TMS also produced a facilitation of the SOL H reflex during tonic plantarflexion, but the very first facilitation, within the initial 1.0 ms, was significantly smaller than during bicycling (P < 0.05). Both the late part of the facilitation and the subsequent inhibition had the same size during the two tasks.

Essentially similar findings were obtained in all 10 subjects who participated in this experiment as evidenced from the pooled data in Fig. 7B. The data were pooled by aligning the data from each individual subject in relation to the onset of the facilitation during bicycling. Time 0 in Fig. 7B thus designates the onset of the facilitation during bicycling. It is seen that the facilitation was significantly (P < 0.05) larger during bicycling than during tonic plantarflexion within the first millisecond after its onset.

The intensity of TMS was systematically varied from well below the threshold of the facilitation up to the threshold of the MEP during the two tasks. In all subjects, a conditioning-test interval within the initial 1 ms of the facilitation was investigated. This ranged from −3 to 0 ms. As shown in Fig. 7C, the threshold of the facilitation was significantly lower in the early

![Image](http://jn.physiology.org/)

**FIG. 5.** H-reflex and MEP recruitment curves during bicycling and tonic plantarflexion. The data are from a single subject. A and C: the H reflex (●) and M wave (○) as a function of the stimulation intensity and the calculated sigmoid functions (---) during late downstroke (122/340 ms after TDC) during bicycling and during tonic plantarflexion during standing. The thresholds of the H reflexes were 0.77 and 0.75 of motor threshold and the plateau values were 20 and 51% of Mmax during bicycling and tonic plantarflexion, respectively. B and D: the MEP as a function of stimulation intensity (●) and the calculated sigmoid functions (---) during early downstroke (67/186 ms after TDC) during bicycling and during tonic plantarflexion during standing. The threshold of the MEP was 48 and 54% of maximal stimulator output and the plateau value was 35 and 26% of Mmax during bicycling and tonic plantarflexion, respectively.

**FIG. 6.** H-reflex and MEP recruitment curves (population average). The figures show the H reflex and MEP as a function of stimulation intensity during bicycling (● and ‒ ‒ ‒ ) and during tonic plantarflexion (○ and ‧ ‧ ‧ ‧). A: H-reflex data for each individual subject (n = 11). The data are aligned to the motor threshold in each subject. B: the MEP data for each individual subject (n = 8). The data are aligned to the MEP threshold during down-stroke of bicycling. C: the calculated population average H reflex as a function of stimulation intensity during late downstroke of bicycling and tonic plantarflexion during standing. On the abscissa, the data are aligned to the population average threshold for evoking an M wave. The thresholds for the H-reflex curves are 0.83 and 0.79 (P = 0.027) and the plateau values 53 and 60% of Mmax (P = 0.040) for late downstroke during bicycling and tonic plantarflexion during standing, respectively. The slopes of the curves are 4.37 ± 0.71 and 4.83 ± 0.80 [(H reflex/Mmax)/(stimulus intensity/motor threshold)] for late downstroke of bicycling and tonic plantarflexion during standing, respectively. D: the calculated population average MEP as a function of stimulation intensity during downstroke of bicycling and tonic plantarflexion during standing. On the abscissa, the data are aligned to the population average MEP threshold during bicycling. The average thresholds for the curves are 52 and 56% of maximal stimulator output (P = 0.019), and the average plateau values are 26 and 21% of Mmax (P = 0.121) during downstroke during bicycling and tonic contraction during standing, respectively. The slopes of the curves are 0.017 ± 0.005 and 0.016 ± 0.004 [stimulus intensity/(MEP/Mmax)] for early downstroke of bicycling and tonic plantarflexion during standing, respectively.

downstroke during bicycling than during tonic plantarflexion in 9 of the 10 investigated subjects. In the last subject, the threshold was the same in the two tasks.

**MODULATION OF HETERONYMOUS SOL H-REFLEX FACILITATION AFTER FEMORAL NERVE STIMULATION.** Figure 8A shows a time course of the effect of femoral nerve stimulation (1.1 × MT) on the SOL H reflex in one subject at rest. At a conditioning-test interval of −6.8 ms, the femoral nerve stimulation produced a facilitation of the reflex. Hultborn et al. (1987) have argued that the facilitation is caused exclusively by the monosynaptic group Ia pathway from the quadriceps muscle to SOL motoneurons within the initial 0.5 ms. Hultborn et al. (1987) also demonstrated that the size of the facilitation reflects the amount of presynaptic inhibition of the Ia afferents on SOL motoneurons. When presynaptic inhibition is increased, a decreased facilitation is thus observed. We consequently measured the size of the facilitation at different times during bicycling. Figure 8B shows the size of the facilitation measured at a conditioning test interval of −6.8 ms during bicy-
cycling. In early downstroke, a significant facilitation of 130% was observed \((P < 0.05)\), but in late downstroke, the femoral nerve stimulation had no effect on the H reflex at the investigated conditioning-test interval.

Similar findings were obtained in all seven investigated subjects. Pooled data from all the subjects are shown in Fig. 8C. On average, the femoral nerve stimulation produced a significant facilitation of around 131% in the early part of downstroke. However, in the late part of downstroke, the stimulation had essentially no effect on the control H reflex (nonsignificant facilitation of 104%). The effect of the femoral nerve stimulation in the late part of downstroke was significantly different \((P < 0.01)\) from the effect in the early part of downstroke. This thus provides evidence of increased presynaptic inhibition at the time of the crank cycle where the H reflex was observed to be depressed in the first part of the study.

![Facilitation of SOL H reflex by subthreshold TMS during bicycling.](Image)

**FIG. 7.** Facilitation of SOL H reflex by subthreshold TMS during bicycling. A: the time course of the TMS conditioned H reflex during early downstroke during bicycling (●) and tonic plantarflexion during standing (○) at a comparable level of background EMG for 1 representative subject. B: the population average time course during early downstroke during bicycling (●) and tonic plantarflexion during standing (○). The time course from each individual subject was aligned according to the interval at which the very first facilitation was observed (0 ms on the abscissa) before the data from the subjects was averaged \((n = 10)\). In A and B, the unconditioned H reflex was adjusted to just below threshold during downstroke of bicycling. C: the difference in threshold of the short-latency facilitation of the H reflex produced by TMS during downstroke of bicycling as compared with tonic plantarflexion for each of the investigated subjects \((n = 10)\) as well as the population average (top ●). The facilitation was measured within the initial 1.0 ms after its onset. —, no significant difference between conditioned and unconditioned H reflex during tonic plantarflexion was reached within the range of stimulation and therefore the difference between thresholds was larger than shown with the bar. *, significant difference between bicycling and tonic plantarflexion \((P < 0.05)\).

![Facilitation of SOL H reflex by femoral nerve stimulation during bicycling.](Image)

**FIG. 8.** Facilitation of SOL H reflex by femoral nerve stimulation during bicycling. A: the time course of the effect of femoral nerve stimulation \((1.1 \times MT)\) on the soleus H reflex at rest for 1 representative subject. The facilitation started at a conditioning test interval of \(-7.2\) ms. During rest, the H reflex was adjusted to approximately 20% of Mmax. B: the effect of the femoral nerve stimulation on the soleus H reflex at a conditioning-test interval within the initial 0.5 ms after the onset of the facilitation \((conditioning test interval of \(-6.8\) ms, — in A). Measurements were made at 9 different positions during the crank cycle. At least 10 measurements of conditioned and unconditioned H reflex were recorded at each crank angle. During the crank cycle, the H reflex was kept low to minimize differences in H-reflex size between early and late downstroke. In this subject, the H reflex averaged 13.3 ± 1.4% of Mmax for the 9 investigated crank angles. C: the population average \((n = 7)\) of the femoral nerve induced facilitation of the H reflex during early and late downstroke as percent of the unconditioned H reflex. In each subject, the size of the facilitation was measured within the initial 0.5 ms after its onset. During early downstroke, the conditioned H reflex was 131 ± 7% of the unconditioned H reflex, whereas during late downstroke, the conditioned H reflex was 104 ± 7% of the unconditioned H reflex. The facilitation was significantly larger during early downstroke as compared with late downstroke (*, \(P < 0.01)\).
DISCUSSION

Modulation of H reflexes and MEPs

Several previous studies have reported a similar modulation of the SOL H reflex with the background EMG activity during bicycling as we have reported here (Brooke et al. 1992; Boorman et al. 1992). A basically similar modulation has also been observed in relation to treadmill walking and running (Capaday and Stein 1986; Crenna and Frigo 1987; Simonsen and Dyhre-Poulsen 1999). The modulation of the TA and SOL MEPs has to our knowledge not been reported before, but a basically similar modulation has been observed in relation to walking (Capaday et al. 1999; Schubert et al. 1997). These findings suggest that H reflexes and MEPs in general faithfully reflect the excitability level of the motoneurons as also found in several previous studies (Capaday and Stein 1986, Morita et al. 1999, Schubert et al. 1997).

However, significant differences were found when comparing the SOL H reflex and MEP to each other and when comparing their size during bicycling and tonic plantarflexion at comparable levels of background EMG activity. In late downstroke and during upstroke, the H reflex was more depressed than the MEP, and it was also significantly depressed in relation to its size during tonic plantarflexion at a comparable level of background EMG activity. The recruitment curve of the H reflex revealed that the threshold of the reflex was higher and the maximal H reflex size smaller in late downstroke and upstroke during bicycling as compared with tonic plantarflexion. The MEP was conversely significantly larger and had a lower threshold in early downstroke than during tonic plantarflexion at a comparable level of EMG activity. A similar facilitation of the H reflex was not observed. These differential changes of the two responses—depression of the H reflex in late downstroke and facilitation of the MEP in early downstroke—are not easily explained by changes at a motoneuronal level because the measurements were made at comparable levels of background EMG activity and because the two responses would have been expected to be equally affected. These observations are thus more easily explained by changes in transmission in the corticospinal and Ia afferent pathways during bicycling (in early and late downstroke, respectively). However, this conclusion rests on the assumptions that the background EMG activity reliably reflects the level of net excitability in the motoneurons activated by the two responses and that the two responses are comparable in terms of motoneuronal activation.

Differential changes in MEPs and H reflexes at comparable background EMG activity

There are several reasons why it is not possible to exclude changes at a motoneuronal level as an explanation of changes in the H-reflex or MEP size despite a comparable background EMG activity in two tasks: 1) The H reflex and MEP do not reflect activation of the same motoneurons as those activated in the background EMG. The synaptic drive to motoneurons activated in the two responses or in the subliminal fringe may be modulated differently in the two tasks as compared with those activated in the background EMG. 2) The background EMG activity does not provide any information of possible changes in the gain with which successive motoneurons are recruited (recruitment gain) (Kernell and Hultborn 1990; Nielsen and Kagamihara 1993). However, in the present study, such changes in the recruitment gain probably did not occur because the increase of the size of the two responses with increases in stimulation intensity was the same during bicycling and tonic plantarflexion. 3) The background EMG activity does not provide any information about possible nonlinearities, such as bistability, in the integration of synaptic input in the individual motoneurons (Crone et al. 1988). If such nonlinearities are switched on in one task, significant differences in the evoked responses may occur despite a comparable level of background EMG activity. At present it is unknown whether nonlinearities present serious problems for the interpretation of H reflex and MEP experiments. 4) During bicycling, the SOL background EMG activity is rapidly increasing during early downstroke and rapidly decreasing during late downstroke. This makes it difficult to make an exact comparison to the more static EMG level during tonic plantarflexion. However, failure to match the EMG levels exactly would be expected to influence the H reflex and MEP equally and it therefore cannot explain why only the H reflex was depressed during late downstroke and only the MEP was facilitated during early downstroke.

Comparison of H reflexes and MEPs

Comparison of H reflexes and MEPs is commonly used either to provide evidence of changes in cortical excitability in relation to fatigue (Brasil-Neto et al. 1993a) and plasticity (Brasil-Neto et al. 1993b; Schieppati et al. 1996) or alternatively to provide evidence of changes in presynaptic inhibition of Ia afferents (Berardelli et al. 1987). The reasoning behind this is that the H reflex and MEP are both assumed to be mainly monosynaptic in origin and to activate the same population of motoneurons. Any change in the MEP without a concomitant change in the H reflex is therefore suggested to be caused by a change in cortical excitability, whereas a change in the H reflex without a concomitant change in the MEP is suggested to be caused by a change in presynaptic inhibition of the Ia afferents, which mediate the reflex. However, as pointed out by Nielsen et al. (1999) and Morita et al. (1999), this type of reasoning may not be valid. For both the MEP and the H reflex, there is now ample evidence that neither response can be considered fully monosynaptic and that various indirect excitatory and inhibitory pathways make an important contribution (Burke et al. 1984; Nielsen et al. 1993, 1999; Pierrot-Deseilligny 1994). Furthermore, the MEP and H reflex cannot be assumed to always activate the same population of motoneurons. Indeed, single-unit recordings from the extensor carpi radialis muscle revealed that several motor units were recruited in the MEP but not in the H reflex and that similarly sized responses generally did not reflect activation of the same motor units (Morita et al. 1999). However, we do not know whether this also applies to the SOL muscle.

Neither the background EMG activity nor a comparison of the H reflex and MEP may thus provide conclusive evidence regarding changes in transmission in the corticospinal and Ia afferent pathways. This is the reason why we also performed experiments in which we conditioned the SOL H reflex by subthreshold TMS and femoral nerve stimulation. As argued in the following text, these two techniques may provide additional evidence for differ-
ences in the corticospinal and Ia afferent transmission than experiments comparing H reflexes and MEPs.

Evidence for increased corticospinal transmission in early downstroke

In previous studies ( Nielsen and Petersen 1995; Nielsen et al. 1993; Petersen et al. 1998; see also Baldiesser et al. 1993; Mazzocchio et al. 1994), it has been argued that the short-latency facilitation of the H reflex produced by TMS reflects transmission in the fast conducting monosynaptic pathway to the spinal motoneurons. As argued by Nielsen et al. (1993), it is unlikely that any other pathway influences the size of the facilitation within the initial 0.5–1.0 ms after its onset. The arguments why changes in motoneuronal excitability do not influence this facilitation were also presented in that study. Our observation of a larger facilitation in the early part of downstroke as compared with tonic plantarflexion thus suggests that increased transmission in the monosynaptic corticospinal pathway is at least partly responsible for the larger size of the MEPs. We believe, in line with what has been found in relation to walking ( Petersen et al. 1998), that the main reason for the larger facilitation during bicycling as compared with tonic plantarflexion is that the cortical neurons increase their excitability and thereby become more susceptible to TMS. An alternative explanation is increased transmission across the spinal terminals of the corticospinal fibers. We cannot fully exclude this explanation, although Nielsen and Petersen (1994) have provided evidence that the neurons, which are responsible for presynaptic inhibition of Ia afferents, do not project to corticospinal fibers. In all likelihood, there are other systems that may modulate the corticospinal transmission at the level of the corticospinal terminals, although at present, we have no knowledge of such systems. Nevertheless, because our findings are essentially similar to those obtained by Petersen et al. (1998), we believe that increased cortical excitability is the most likely explanation of the larger short-latency facilitation in the early part of downstroke during bicycling.

This suggests that the corticospinal tract makes a contribution to the activation of the SOL muscle in this phase of bicycling. In a previous study, we have provided independent evidence that the motor cortex plays a role in the generation of the basic rhythmic activity during bicycling ( Christensen et al. 2000). During active pedaling in supine subjects, increased local cerebral blood flow is thus observed in the primary motor cortex when subtracting the changes in blood flow induced by the sensory feedback evoked by passive limb movements. We therefore suggest that the corticospinal tract participates in the initiation and generation of the SOL muscle activity in early downstroke. The larger short-latency facilitation of the H reflex as compared with tonic plantarflexion may be explained by the necessity of a larger descending drive when recruiting and accelerating the motoneuronal activity. A larger short-latency facilitation of the H reflex is also seen during the dynamic phase of voluntary isometric ramp-and- hold plantarflexion ( Nielsen and Petersen 1995).

Evidence for increased presynaptic inhibition during late downstroke and upstroke

The use of the femoral nerve-induced heteronymous monosynaptic Ia facilitation of the SOL H reflex as an estimate of the level of presynaptic inhibition of Ia afferents was introduced by Hultborn et al. (1987). Within its initial 0.5 ms, this facilitation reflects the size of the underlying monosynaptic EPSP ( Hultborn et al. 1987). A decrease in the facilitation as we observed in late downstroke during bicycling thus provides evidence that presynaptic inhibition of the Ia afferents is increased in this phase of the movement. One problem is that changes in recruitment gain of the SOL motoneuronal pool may also produce changes in the size of the femoral nerve facilitation ( K ernell and Hultborn 1990; Nielsen and Kagamihara 1993). However, this cannot explain our observations because in this case, we would also have observed a change in the slope of the H-reflex recruitment curve between early and late down-stroke during bicycling; but this was not the case.

The increase of presynaptic inhibition as evidenced from the decrease of the H reflex and the decrease of the femoral nerve-induced facilitation coincided with the time during the crank cycle where the ankle joint position changed in ankle dorsiflexion direction ( Fig. 3B). Although the velocity of the induced stretch of the ankle plantarflexors is rather low (approximately 22°/s), it is sufficient to induce significant muscle spindle afferent activity, and it seems likely that the role of the increased presynaptic inhibition is to prevent this activity from activating the ankle plantarflexors at a time when it is functionally important that they are kept silent. The decrease of the H reflex around the transition from stance into swing during walking is likely also partly caused by increased presynaptic inhibition of SOL Ia afferents ( Capaday and Stein 1986, Faist et al. 1996), and as during bicycling, this probably helps to prevent that stretch reflexes are evoked in the ankle plantarflexors. It may be argued against this interpretation that Morita et al. (1998) have demonstrated that stretch reflexes are less sensitive to presynaptic inhibition than H reflexes. However, the data from Morita et al. (1998) demonstrate that stretch reflexes are influenced by presynaptic inhibition, although not to the same extent as H reflexes, and it seems likely that the extent of presynaptic inhibition induced in late downstroke is sufficient to at least diminish the Ia afferent feedback to the motoneurons sufficiently to help prevent them from discharging. Presumably postsynaptic inhibition also contributes to this.

Concluding remarks

The findings in the present study suggest that the transmission in the fast monosynaptic corticospinal pathway is increased during early downstroke of bicycling and that the H reflex is depressed by presynaptic inhibition during late downstroke and upstroke. These findings stress that both the corticospinal and Ia afferent pathway are involved in the control of bicycling. As for walking and other complex motor tasks, this illustrates that bicycling is generated by the integrated activity of several different control systems at different levels of the CNS. Revealing the exact contribution and role of these different systems is of fundamental importance not least in relation to rehabilitation of patients with lesions of the central motor control systems. The modulation of the H reflex and MEP that we have found is basically similar to that observed in relation to walking and suggests that these two rhythmic motor tasks may be controlled in a very similar way. However, more studies are required to address this question further.
REFERENCES


