Journal of Physiology (2002), **539.3**, *pp.* 903–911
DOI: 10.1013/jphysiol.2001.013282

www.jphysiol.org

Excitability changes in human corticospinal projections to forearm muscles during voluntary movement of ipsilateral foot

Fausto Baldissera, Paola Borroni *, Paolo Cavallari † and Gabriella Cerri

*Istituto di Fisiologia Umana II, * Dipartimento di Medicina, Chirugia e Odontoiatria and † Dipartimento di Scienze Precliniche-LITA Vialba, Università degli Studi di Milano, Italy*

Excitability of the H-reflex in the relaxed flexor carpi radialis (FCR) muscle was tested during voluntary oscillations of the ipsilateral foot at five evenly spaced delays during a 600 ms cycle. In some experiments the H-reflex was conditioned by transcranial magnetic stimulation (TMS). With the hand prone, the amplitude of the FCR H-reflex was modulated sinusoidally with the same period as the foot oscillation, the modulation peak occurring in coincidence with contraction of the foot plantar-flexor soleus and the trough during contraction of the extensor tibialis anterior. When the H-reflex was facilitated by TMS at short latency (conditioning–test interval: −2 to −3.5 ms), the modulation was larger than that occurring with an unconditioned reflex of comparable size. This suggests that both the peripheral and the corticospinal components of the facilitated response were modulated in parallel. When the H-reflex was tested 40–60 ms after conditioning, i.e. during the cortical 'silent period' induced by TMS, no direct effect was produced on the reflex size but the foot-associated modulation was deeply depressed. These results suggest that the reflex modulation may depend on activity fluctuations in the cortical motor area innervating the forearm motoneurones. It is proposed that when the foot is rhythmically oscillated, along with the full activation of the foot cortical area a simultaneous lesser co-activation of the forearm area produces a subliminal cyclic modulation of cervical motoneurones excitability. Should the two limbs be moved together, the time course of this modulation would favour isodirectional movements of the prone hand and foot, indeed the preferential coupling observed when hand and foot are voluntarily oscillated.

(Received 21 September 2001; accepted after revision 19 December 2001)
Corresponding author F. Baldissera: Istituto di Fisiologia Umana II, Via Mangiagalli 32, I-20133 Milano, Italy.
Email: fausto.baldissera@unimi.it

A number of constraints are experienced when trying to move two segments of the body at the same time. For instance, several coplanar movements of the upper and lower limbs of one side (e.g. axial rotation of arm and leg, flexion–extension of hand and foot) are easily performed when the segments rotate in the same direction (in-phase) whereas their association is difficult when they move in opposite directions (anti-phase) (Baldissera *et al.* 1982, 1991, 2000; Kelso & Jeka, 1992; Carson *et al.* 1995; Jeka & Kelso, 1995; Swinnen *et al.* 1995; Serrien & Swinnen, 1998).

In this context, the term nervous constraint usually refers to factors, or situations, which limit the coupling repertoire, such as for instance those factors hindering or impeding non-isodirectional coupling of ipsilateral limbs. The term constraint, however, may be understood not as a limit but rather as an obligation to produce a certain behaviour. In this view, the existence of a clear-cut preference for isodirectional (in-phase) coupling of ipsilateral limbs may be regarded as the expression of a nervous arrangement that binds the limbs to 'imitate' each other whenever they are moved simultaneously. This same nervous arrangement would discourage other types of coupling, for instance in phase opposition.

Along these lines, it was recently reported that during the voluntary rhythmic flexion–extension movement of the foot the H-reflex excitability in the resting forearm undergoes cyclic modulation (Baldissera *et al.* 1998). With the forearm in prone position, the phase of increased excitability in the flexor carpi radialis (FCR) muscle coincided with the foot plantar flexion. To account for these findings, it might be postulated that afferent signals generated by the foot movement influence the reflex excitability in the cervical spinal segments. However, it was also recently demonstrated that the cyclic modulation of the H-reflex in the resting forearm is not related to movement, but temporally bound to the activation of foot movers (Baldissera *et al.* 2001). This makes a kinaesthetic origin of the modulation unlikely and points to a central origin. In this light, one could envision that when the foot is moved in isolation, central motor areas send supraliminal commands to the foot and subliminal collateral influences in the motor pathways directed to the non-moving hand.

If this hypothesis, which proposes a neural substrate for the isodirectional coupling of hand and foot, is correct, it should be possible to monitor excitability changes in the cortical motor areas projecting to the resting hand during voluntary movement of the foot.

On this basis, we explored the excitability of the corticospinal projection to FCR muscle during cyclic flexion–extensions of the ipsilateral foot, combining transcranial magnetic stimulation (TMS) with H-reflex testing.

METHODS

Subjects gave informed written consent to the experiments, which were performed according to the Declaration of Helsinki and approved by Comitato Etico dell'Università degli Studi di Milano. Healthy adult volunteers of either sex, aged 20–60 (36.5 ± 11 years, mean ± S.D.) were seated in an armchair with the hand resting in prone position and the right foot fixed to a platform oscillating around the axis of the ankle. EMGs were recorded from the two main ankle movers, the tibialis anterior (TA) and the soleus (Sol) muscles. H-reflexes were evoked in the flexor carpi radialis (FCR) muscle using a standard technique: stimulation of the median nerve at the elbow (square pulses, 0.8 ms duration) and recording from bipolar surface electrodes over the muscle belly. Figure 1 shows the general protocol for all experiments, during which subjects were asked to perform sequences of four flexion–extension foot oscillation cycles, starting when they chose and following a tempo of 1.66 Hz (600 ms period), imposed by a metronome. Transit of the foot in front of a photocell generated a signal that was fed into a PC; at the third photocell signal, the PC triggered a stimulator to evoke an H-reflex in the FCR muscle and started acquisition of the relevant parameters (see below) for 1 s. After the fourth cycle the subject was free to stop and 8 s later a beeping signal allowed a new oscillation sequence to start.

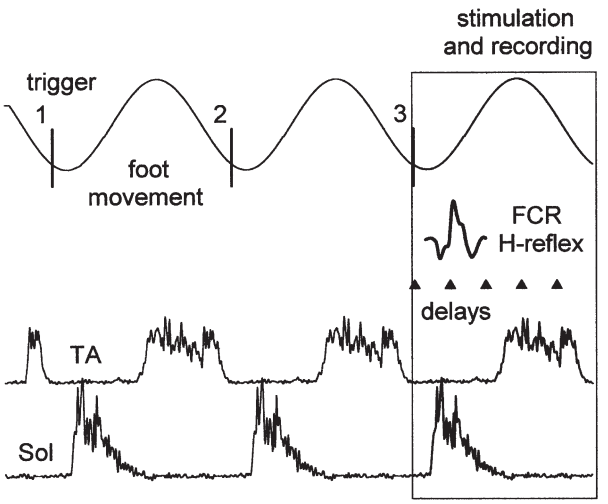

Figure 1. Schematic description of experimental procedure

Voluntary oscillations of the foot (uppermost trace) triggered a photocell at a fixed point of the movement cycle. After the third trigger, a PC-driven stimulator delivered an electric pulse to the median nerve, which evoked an H-reflex in the flexor xarpi radialis (FCR) muscle. The stimulus was timed, in random alternation, at one of five different delays (▲) dividing the cycle in equal fractions. In part of the trials, the H-reflex was conditioned by transcranial magnetic stimulation of the motor cortex. Rectified EMGs from tibialis anterior (TA) and soleus (Sol) muscles were also recorded (two lowermost traces). Box outlines the stimulation and recording period.

Modulation of FCR H-reflex excitability

The H-reflex was evoked at one of five delays regularly dividing the 600 ms metronome period (0, 120, 240, 360 and 480 ms from the photocell trigger). Its amplitude was maintained between 5 and 15 % of the maximum direct motor response response (M_{max}). The complete cluster of five delays was tested 15 or 20 times, randomly changing the order of the delays. The reflex responses were amplified, filtered and digitally converted. In order to reduce the inter-cluster variability, the deviation (in μV) of each H-reflex from the mean of its own cluster was calculated and averaged with those obtained at the same delay in the other clusters. To establish a correlation between the time courses of foot movement and arm reflex modulation, the best-fit function of the average recorded foot movement was described by a four-parameter sine-wave equation, whose parameters were calculated by minimising the sum of the squared differences between the observed and predicted values (Marquardt-Levenberg algorithm, SigmaPlot) A four-parameter sine-wave equation with the same period was then applied to fit the mean H-reflex amplitudes measured at the five points of the cycle.

Modulation of corticospinal effects

In order to explore whether the motor cortex plays a role in forearm excitability modulation during foot movements, the above experiments were repeated using transcranial magnetic stimulation (TMS). The experimental set-up and procedures differed from previous ones in the following details. The subject's head was restrained by a fitted support and a stereotactic apparatus held an 8-shaped coil, connected to a magnetic stimulator (Magstim 200, maximal power 2.2 T) over the cortical focus for TMS activation of forearm muscles. TMS was used by itself to induce compound muscle action potentials (CMAPs) in the FCR muscle, or associated with medial nerve stimulation to induce facilitation of the H-reflex in FCR (Baldissera & Cavallari, 1993). For this second purpose, the TMS was delivered 2–3.5 ms after median nerve stimulation, i.e. during the facilitation rising-phase, as determined in each subject by testing 3–4 conditioning–test intervals. The TMS intensity was just lower (80–95 %) of the threshold for evoking CMAPs at rest (usually 50–60 % of maximal output). Threshold intensity was that evoking a visible CMAP in 5 of 10 stimuli. A correlation between the time courses of foot movement and the modulation of either the CMAP or the TMS-facilitated H-reflex was established using the sinusoidal function of the averaged foot movements to fit the amplitude modulation of the responses.

Distinguishing between reflex and corticospinal modulation

To this aim, we verified the influence of foot oscillations on alternate series of unconditioned and TMS-facilitated H-reflexes (see above). The intensity of the peripheral nerve stimulation was adjusted between the series in such a way as to equalise the reflex amplitudes.

For practical purposes, H-reflex excitability was tested only at the peak (DEL1) and trough (DEL2) of the modulation cycle, as measured in every subject. Each cluster of two delays was tested 30–40 times, randomly alternating the order of DEL1 and DEL2.

The reflex responses were amplified, filtered and digitally converted. In order to reduce the inter-cluster variability, the deviation (in μV) of each H-reflex from the mean of its own cluster was calculated and averaged with those obtained at the same delay in the other clusters.

In each subject, the mean amplitudes of the unconditioned and TMS-conditioned reflexes were normalised to the mean amplitude of all H-reflexes of that subject. The difference between the two conditions was ascertained by a paired-sample *t* test.

The H-reflex was also tested during the cortical 'silent period' induced by magnetic stimulation. In order to obtain the largest cortical inhibitory effect without any spinal component, we used the highest TMS intensity that did not produce CMAPs at rest. When given during voluntary contraction, this same intensity produced an excitation followed by a prolonged silent period. Before to each experiment, the SP duration was determined in each subject following a TMS of the above-mentioned intensity, given during a voluntary contraction of hand flexors. The conditioning–test delay was then established so that the H-reflex fell before the re-appearance of EMG activity. Data analysis was performed as described above.

RESULTS

Modulation of FCR H-reflex during voluntary oscillations of the ipsilateral foot

Figure 2 illustrates, on a one-cycle diagram, the modulation of the H-reflex occurring in the resting FCR muscle during oscillations of the ipsilateral foot in one out of six experimental subjects. The reflex modulation is plotted on the same normalised abscissa as the grand average of both the foot position during the movement (Fig. 2*B*, continuous line, 'pos') and of the integrated EMGs from the foot movers ('TA' and 'Sol'). The actual movement period was estimated by fitting the average record of the movement with a sine wave function (Fig. 2*B*, dotted line; determination coefficient, $R^2 = 0.96$). Thereafter, all records (movement and integrated EMGs) were normalised to the estimated cycle period, as were the five H-reflex delays. The experimental points (mean changes of the H-reflex amplitude) were also fitted by a sine-wave function (Fig. 2*A*, dotted line) with the same period as that of the movement. This allowed for immediate phase matching between the functions fitting the foot movement and the excitability changes occurring in the FCR H-reflex. In this subject, modulation of the FCR H-reflex was fitted almost perfectly by a sinusoidal function (determination coefficient, $R^2 = 0.96$), whose rising phase led by 74 deg the plantar-flexion phase of the movement best-fit function. The modulation peak coincided in time with the EMG burst in Sol while the modulation through occurred during the EMG burst in TA.

In the other five subjects, sinusoidal fitting of the experimental points was also good. For the averaged movement R^2 was always higher than 0.96, while it ranged between 0.69 and 0.99 for the reflex data. In all subjects, the increase in H-reflex size preceded the foot flexion phase, the advance ranging between 45 and 115 deg (mean ± S.E.M., 77 ± 25 deg).

After normalising the reflex data of each subject to the amplitude of the respective best-fit sine waves, data points from all subjects were plotted together in Fig. 3 (open circles), showing the common course of their sinusoidal modulation (dotted line, $R^2 = 0.58$, a value lower than in the single subjects because of individual differences in amplitude, period and phase).

In conclusion, in all subjects the response of FCR motoneurones to Ia monosynaptic activation was facilitated

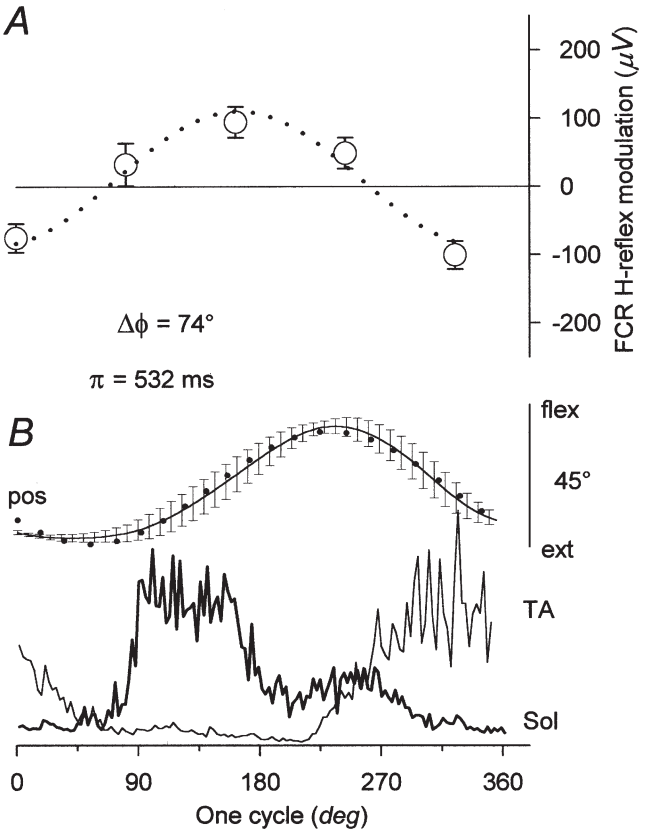

Figure 2. Cyclic modulation of FCR H-reflex during voluntary oscillation of ipsilateral foot

A, absolute deviations of the H-reflex size from its mean value (428 μV = 8 % M_{max}; see Methods), occurring at five delays during voluntary oscillations of the foot. Each point represents the average (± S.E.M.) of 15 responses evoked at that delay. *B* illustrates the average record of the foot angular position (pos, uppermost continuous line, ± S.E.M.) and the rectified EMGs from Tibialis Anterior (TA, thick continuous trace) and Soleus (Sol, thin continuous trace) muscles. Dotted lines in *A* and *B* describe the sinusoidal functions that fitted the experimental data best ($R^2 = 0.96$ and 0.99, respectively). Period of the sine-wave function fitting the position record ($\pi = 532$ ms) was utilised both for fitting the reflex data and for normalising to 1 cycle the time scale of all parameters. The phase of the best-fit sinusoids for the movement and the H-reflex modulation was measured and their difference ($\Delta\Phi$) calculated. Positive values of $\Delta\Phi$ indicate that the modulation sine wave advanced the movement sinusoid (plantar flexion, flex, upward).

during foot plantar flexion and dis-facilitated during foot extension. Should this occur during coupled movements of the hand and foot, with the hand prone, it would favour isodirectional coupling of the limbs and hinder other, e.g., anti-phase, types of coupling.

Excitability changes in cortical structures projecting to forearm motoneurones during oscillations of the foot

In a different study (Baldissera *et al.* 2001) we observed that the timing of the H-reflex modulation in the resting FCR was linked to that of muscular activation of foot movers and not to the mechanical parameters of the movement. This would suggest that the modulation might be caused by excitability changes in corticospinal neurones projecting to the resting forearm rather than to feedback kinaesthetic information from the moving foot. It was therefore of interest to investigate whether, during voluntary foot oscillations, corticospinal neurones projecting to the forearm undergo an excitability modulation parallel to that occurring in the cervical cord. With this aim, corticospinal excitability was tested by means of transcranial magnetic stimulation (TMS).

Modulation of CMAPs in forearm muscles. The most widely used method to test the excitability of corticospinal neurones by transcranial magnetic stimulation is to elicit CMAPs. Thus, we recorded in three subjects the CMAPs from the FCR muscle during foot oscillations and observed that they were modulated in a similar way to the H-reflex.

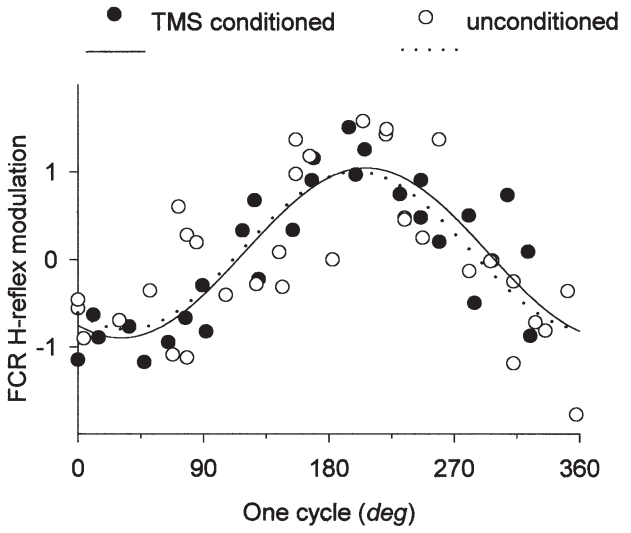

Figure 3. Cyclic modulation of H-reflex, either unconditioned or conditioned by TMS, during voluntary foot oscillations

During foot oscillation, modulation of the unconditioned (○) and TMS-facilitated (●) H-reflexes follows a virtually identical course. The best-fit functions for the two sets of data (continuous and dotted lines) show a similar phase advance ($\Delta\Phi$) with respect to movement. Besides the period normalisation to one cycle, in each subject (6), data were normalised in size to the amplitude of the respective best-fit sine wave.

An example of CMAP modulation is given in Fig. 4. Data points were well fitted ($R^2 = 0.94$) by a sine wave function with the same period as the foot movement and the modulation peak coincided in time with the rising phase of the plantar flexion, leading the movement by 102 deg. This result, however, is not sufficient to settle the question of whether a cortical excitability modulation had occurred, given that CMAP variations may also reflect excitability changes in spinal neurones. Moreover, since it is quite difficult to obtain sizeable responses in FCR muscle without co-activating its neighbours and/or antagonists, electrical 'cross-talk' may confound the measurements of CMAP

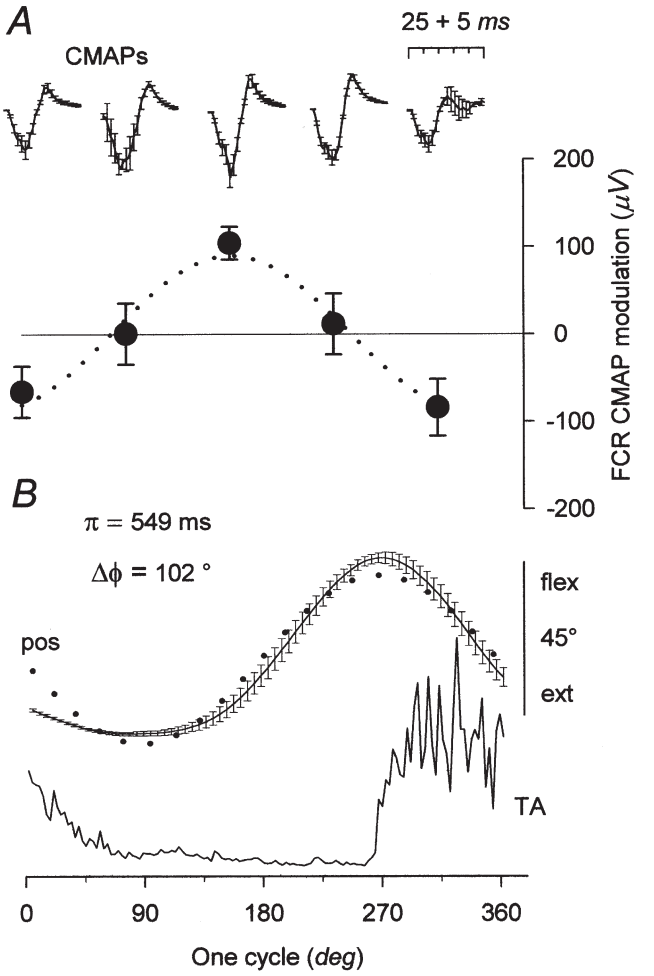

Figure 4. Size of CMAP evoked in FCR muscle by TMS stimulation of contralateral motor cortex is modulated during voluntary foot oscillations

A, the uppermost insets show the CMAPs evoked in one subject by TMS at 5 delays during the foot cycle (average of 15 responses ± S.E.M.). The lower graph shows modulation of the CMAP amplitude (●) and its best-fit sine wave (dotted line). Mean amplitude of CMAP = 423 μV. *B*, average records of the foot angular position (pos, upper continuous line, ± S.E.M.) and of the TA rectified EMG (lower continuous trace). Dotted line describes the best-fit function for movement ($R^2 = 0.95$) and $\Delta\Phi$ is the phase difference between the best-fit sinusoids for the movement and the CMAP modulation. Cycle period (π) = 549 ms. Time calibration: major ticks = 25 ms, minor ticks = 5 ms.

amplitude. To overcome these shortcomings, we evaluated cortical excitability by testing the facilitatory effect induced by TMS on the FCR H-reflex and by measuring the extent to which this facilitation was modified during cyclic movements of the foot.

Modulation of corticospinal facilitation of FCR H-reflex. A short-latency facilitation of the H-reflex is obtained by TMS when the Ia and the earliest corticospinal excitatory volleys simultaneously reach the motoneurone pool. This occurs in the FCR when the TM stimulus follows by about 0–3.5 ms the peripheral stimulus delivered to the median nerve (Baldissera & Cavallari, 1993).

In each subject, the conditioning–test interval was chosen so that it corresponded to the rising phase of the facilitation (between −2 and −3.5 ms in the different individuals). In all six subjects, the amplitude of the TMS-facilitated H-reflex was modulated sinusoidally during foot oscillations, just as for the unconditioned reflex. A time course comparison of the two modulations was performed by adding the TMS-conditioned data, after normalisation in the amplitude and time domains, to the graph of Fig. 3. Note how the conditioned and unconditioned points mingle completely and that their best-fit sine waves are practically superimposed ($\Delta\Phi$ = 58 and 52 deg, respectively).

However, since the data of Fig. 3 were obtained during different experimental sessions and with H-reflexes of different sizes, their amplitudes could not be compared. They were therefore not useful for sorting whether the modulation was confined to α-motoneurones or whether a parallel increase in excitability had occurred in the motor cortex as well. Should the modulation be confined to motoneurones, one would expect its amplitude to be identical in the two conditions, given that the size of the conditioned and unconditioned H-reflex was adjusted to be the same. If, instead, a parallel increase in excitability occurs in the motor cortex as well then TMS would elicit a larger corticospinal volley and therefore a stronger excitation of α-motoneurones. The combined increase in the spinal and corticospinal components of the response would then make the modulation larger for the conditioned than for the unconditioned H-reflex. To distinguish between these two possibilities, the modulation of conditioned and unconditioned reflexes was measured in a single experimental session in five subjects. For each of them, two or three series of unconditioned H-reflexes (10 reflexes for each delay) were alternated with an equal number of series in which the reflex was conditioned by TMS. The intensity of the peripheral stimulation was adjusted before each trial so as to match the size of the two reflex types at rest (see Methods). In order to reduce the long-term variability of both H-reflex and cortical excitability, the trial duration was shortened by testing only two delays in the cycle, i.e. those corresponding to the peak and trough of the modulation in each subject. Results are shown in Fig. 5. On the dimensionless abscissa, circles show the percentage changes in the H-reflex size occurring at the peak (DEL1) and trough (DEL2) of the modulation cycle, symbolised by the continuous line. In each cluster, open symbols refer to the unconditioned reflex, filled symbols to the TMS-facilitated H-reflex. Each couple of points vertically aligned identifies one subject. Note that in each case the peak-to-peak amplitude of the modulation is larger for the conditioned than for the unconditioned reflexes. Large triangles indicate the population means (unconditioned *vs.* conditioned) at the two cycle positions. A paired sample *t* test showed that data of the two groups differ significantly ($P < 0.01$).

In conclusion, both the peripheral and the corticospinal components of the TMS-facilitated H-reflex seem to undergo parallel modulation during voluntary oscillation of the ipsilateral foot.

Modulation of the H-reflex during cortical silent period. From the findings described above, it may by argued that, during the foot movements, parallel subliminal commands are forwarded by the corticospinal system to the forearm muscles which produce hand movements isodirectional to those actually occurring in the foot. This may be achieved either by collaterals of (some) cortico-lumbar fibres that induce the foot movement, or by co-activation of the

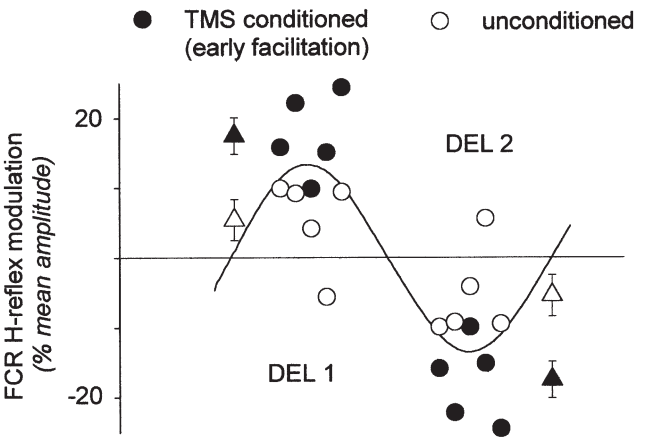

Figure 5. Cyclic modulation of corticospinal facilitation of FCR H-reflex during voluntary foot oscillations

Amplitude of the H-reflex modulation at the delays corresponding to the peak (DEL1) and trough (DEL2) of the modulation cycle (symbolised by the continuous line) was larger when the reflex was facilitated by TMS (●) then when it was unconditioned (○). Conditioning–test interval ranged between −2 and −3.5 ms in the 5 subjects. Conditioned and unconditioned reflexes were tested in separate trials and their amplitude equalised between trials. The mean H-reflex size was around 5 % M_{max} and not significantly different (paired *t* test) in the two conditions (174 μV for the unconditioned and 218 μV for the conditioned H reflex). Each couple of points, slightly shifted with respect to the others, refers to one subject. Mean amplitude of the modulation (▲ and △ ± S.E.M.) was significantly different in the two situations ($P < 0.01$, paired *t* test).

primary motor area projecting to the forearm. In any case, the modulated activity in the corticospinal tract may indeed produce the H-reflex modulation. Accordingly, an inhibition (or dis-facilation) of the corticospinal neurones should reduce or eliminate the reflex modulation.

To verify this hypothesis, the movement-induced modulation of the FCR H-reflex was tested during the cortical depression corresponding to the so-called 'silent period', i.e. the silencing of voluntary EMG activity which follows a TM stimulus (see Mills, 1999), possibly due to after-hyperpolarisation refractoriness and postsynaptic inhibition. This cortical inhibitory process is present even when the cortical stimulation is kept subthreshold for inducing a CMAP, i.e. when no motoneuronal silent period is produced (Davey *et al.* 1994).

On these premises, the TMS conditioning experiment was repeated in the same five subjects after increasing the conditioning–test interval to 40–60 ms. TMS was always subthreshold for evoking any CMAP. Since at these delays TMS was substantially ineffective at changing the amplitude of the H-reflex in the resting condition, no adjustment of the reflex size was necessary between trials. Results from all subjects are summarised in Fig. 6, which shows, with the same display criteria as in Fig. 5, the percentage changes of the unconditioned and TMS-conditioned H-reflexes (open and filled circles, respectively), occurring at the peak (DEL1) and trough (DEL2) of the modulation cycle. Note

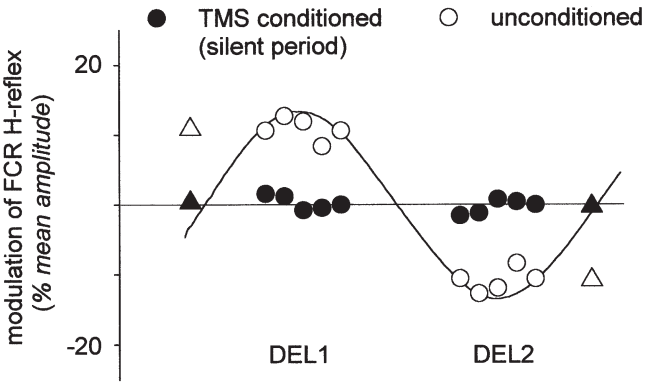

Figure 6. Cortical depression (silent period) suppresses cyclic H-reflex modulation associated with voluntary foot oscillations

H-reflex modulation at the peak (DEL1) and trough (DEL2) of the modulation cycle (○) was virtually suppressed when the reflex was evoked during the silent period induced by TMS delivered 40–60 ms in advance (●) with an intensity subliminal for evoking a CMAP. At these delays and intensities, TMS did not affect the H-reflex excitability; it was therefore unnecessary to correct the reflex size between trials. Mean values of the conditioned and unconditioned reflexes were not significantly different (paired *t* test) from each other (unconditioned = 231 μV; conditioned = 233 μV). Mean amplitude of the modulation was instead significantly different ($P < 0.001$, paired *t* test) in the two conditions (▲ and △ ± S.E.M.; S.E.M. bars are hidden by the symbols).

that, in each subject, the unconditioned H-reflex undergoes a consistent reduction when passing from the peak to the trough of the modulation cycle, whereas the TMS-conditioned H-reflex remains practically identical at the two cycle positions. The triangles indicate the means of the respective populations (unconditioned *vs.* conditioned), which were significantly different from each other ($P < 0.001$, paired *t* test). It should also be noted that during the foot movements TMS did not apparently produce any direct effect on the H-reflex, since in each subject the overall mean amplitude of the reflex at both delays was substantially the same (no significant difference, *t* test) in the presence and in the absence of the conditioning cortical stimulation.

In conclusion, when the reflex was evoked during the cortical 'silent period' only the amplitude of its modulation, but not its mean size, was strongly reduced, suggesting that the modulation depended on fluctuations of the activity level in the cortical motor area innervating the forearm muscles. Once such fluctuations were removed by the 'silent period', motoneuronal excitability remained unaffected by the foot movements.

DISCUSSION

The reported cyclic excitability changes in H-reflex, occurring in a resting forearm muscle during voluntary oscillation of the foot, may depend either on cyclic changes of motoneurone membrane potential or on modulation of presynaptic Ia terminals. At this stage we were not directly interested in the spinal mechanism responsible for the modulation and did not try any more direct investigation on presynaptic inhibition. However, some inferences concerning this problem may be derived from the following observations. If the modulation were exclusively operated by means of primary afferent depolarisation (PAD) in Ia terminals, then the facilitated H-reflex, which results from the sum of the descending and afferent-components, should be less influenced than the pure H-response, which is exclusively due to Ia EPSPs. The present data, instead, show that the TMS-facilitated H-reflex undergoes a deeper modulation than the unconditioned response. In addition, the CMAPs directly evoked by TMS underwent a clear-cut modulation (parallel to that of the H-reflex), despite the fact that changes in PAD of Ia terminals should not influence the corticospinal EPSPs. Thus, since the presynaptic terminals of cortico-motoneuronal fibres do not apparently undergo presynaptic inhibition (Nielsen & Petersen, 1994; Rudomin & Schmidt, 1999), it should follow that during foot voluntary movements motoneurone excitability is indeed modulated post-synaptically. It is worth mentioning, however, that postsynaptic effects may be induced in motoneurones if a steady afferent inflow to them is modulated by cyclic variations of PAD.

A more urgent question, from our present perspective, concerns the origin of the modulation, in particular whether it is a feedback action, generated by the kinaesthetic afferences from the foot movements, or a central action directed in parallel both to the moving lower limb and, with subthreshold intensity, to the neurones innervating the resting upper limb.

The aim of the present experiments was to explore whether the corticospinal neurones projecting to the forearm muscles undergo excitability changes parallel to those occurring at the spinal level during foot oscillations. In this regard, the corticospinal excitability was probed by TMS conditioning of the H-reflex at two conditioning–test intervals. Short-interval conditioning, which induces facilitation of the H-response, demonstrated a combined increase in both the motoneuronal reflex excitability and the descending action, the latter suggesting a cyclic variation in the excitability of corticospinal neurones. Admittedly, a modulation of the corticospinal effect might also result from segmental facilitation of propriospinal neurones, which are presumed to mediate disynaptic EPSPs from the corticospinal tract to motoneurones (Burke *et al.* 1994; Gracies *et al.* 1994). This possibility, however, should be ruled out, since we tested the H-reflex facilitation at intervals at which only the monosynaptic cortico-motoneuronal excitation is acting. Thus, the increased corticospinal effects indeed appear to depend on changes in cortical excitability. In turn, at long conditioning–test intervals ('silent period') TMS evoked a cortical depression that effectively reduced the H-reflex modulation, but it did not produce substantial effects on the H-reflex itself. Such a finding strongly suggests that the motoneuronal excitability changes associated with foot oscillations were produced by a corticospinal influence, since they disappeared during the periods of post-stimulus cortical inhibition. Thus, beyond confirming the occurrence of excitability changes in the hand motor area during cyclic voluntary movements of the foot, this added evidence favours the idea that the H-reflex modulation is actually induced by descending activities from the motor cortex.

A few considerations seem worthwhile regarding the cortical depression associated with the 'silent period'. Most studies (Davey *et al.* 1994; Classen & Benecke, 1995; Mills, 1999) agree that the silent period after TMS is mainly a cortical phenomenon; nevertheless, we kept TMS intensity subliminal for motor responses (but sufficient to excite corticospinal neurones, as witnessed by the short-latency facilitation of the H-reflex) in order to avoid any interference due to peripheral post-spiking refractoriness. At this intensity TMS still evokes suppression of voluntary EMG (Davey *et al.* 1994). Recent results suggest that the inhibitory phenomena during the silent period not only affect the corticospinal neurones but also suppress the motor drive to them (Tergau *et al.* 1999). This would fit well with the working hypothesis inspiring this investigation, i.e. that when the foot is rhythmically oscillated the hand cortical area is co-activated, producing a modulation of its descending output to cervical motoneurones. Suppression of the co-activation drive during the silent period may thus contribute to the flattening of the motoneuronal excitability modulation. In this regard, it is also worth mentioning that the existence of strong functional interactions between the foot and hand areas of the motor cortex during coupled movements of the two limbs is suggested by the recent finding (Liepert *et al.* 1999) that the output map for the thumb movers shifts apart during and after coupled synchronous movements of the thumb and ipsilateral foot. In light of this, the present results, showing the occurrence of excitability changes in the hand area when only the foot is moved, could be taken as evidence that such interactions, though weakened, are also present when one limb is moved in isolation. Further support to the hypothesis that the spinal motor structures innervating hand and foot are activated in parallel also comes from the recent observation that the cyclic modulation of the H-reflex in the resting forearm is temporally bound to the muscular activation of foot movers, not to movement (Baldissera *et al.* 2001).

Altogether, this evidence leads us to propose that, during oscillations of the foot, parallel excitability changes occur in both foot and hand cortical areas. Conversely, this same evidence suggests that the H-reflex modulation should not be caused directly by kinaesthetic signals from the movement of the foot. An afferent origin of the cortical modulation cannot, however, be ruled out. It might in fact be hypothesised that afferent signals generated by cyclic muscle contraction (not by movement) may reach the hand cortical area and periodically affect its excitability.

With regard to the functional implications, these results should first be discussed in the context of the co-ordination of coupled limb movements. As already suggested, the time course of the FCR excitability changes would favour isodirectional coupling when the hand and foot are oscillated together. It is open to question, however, whether the relatively small effect disclosed by our experiments is sufficient to determine the well-assessed preferential coupling between isodirectional hand and foot oscillations. In this regard, two aspects should be recalled. First, the strong interaction between the hand and foot cortical areas observed when both limbs move together (Liepert *et al.* 1999) is in keeping with the idea that, during coupling, the influences that bind the two areas may increase sufficiently to sustain the isodirectional preference. Second, it should also be remembered that the coupling between hand and foot during in-phase oscillations is rather loose, as witnessed by the possibility of voluntarily performing anti-phase oscillations as well as by the compensatory reaction that occurs after inertial

loading of one segment (Baldissera & Cavallari, 2001), which mainly consists of anticipating the activation of the muscles that move the loaded segment.

It might finally be argued that preferential coupling of isodirectional movements of the hand and foot has hardly any obvious purpose in ordinary behaviour. This is probably the reason why such a coupling is usually described as a constraint, rather than as an organisation pattern, as happens, for example, for the various types of limb coupling related to locomotion (cf. Orlovsky *et al.* 1999). An economy principle would predict that any motor organisation should not have developed without some functional pressure. Thus it seems interesting to speculate how to categorise hand–foot coupling within the context of motor control. In this regard, the 'anticipatory postural activities' (APAs; Marsden *et al.* 1978; Marsden *et al.* 1981; Cordo & Nashner, 1982; Bouisset & Zattara, 1987; Zattara & Bouisset, 1988) show appealing similarities to the co-activation described here. For instance, they are characterised by the parallel activation of muscles in different body segments, they are scaled with the intensity of the prime movement (Aruin & Latash, 1996) and can be reduced or abolished when the biomechanical context is modified (Aruin *et al.* 1998). Their scope is either to prepare a fixation chain connecting the moving segment to a firm support, or to produce a motor action that contrasts the postural unbalance produced by the main body action. It is of interest that when performing wrist flexion–extension while trying to maintain a constant limb posture, APAs develop in the upper limb which are characterised by directional postural synergies (Chabran *et al.* 1999). Moreover, it has been repeatedly demonstrated that both the timing and the spatial distribution of the APAs may vary when the surround conditions or some feature of the movement (e.g. direction) are changed (Nashner & Forssberg, 1986; Aruin & Latash, 1995). This allows us to postulate that even when a manifest intervention of the APA is not required, as in our experimental condition (a sitting subject with the foot supported by the oscillating platform), subthreshold effects may nevertheless take place. According to this view, the positive and negative constraints characterising ipsilateral limb coupling might indeed be an expression of some underlying postural mechanism.

REFERENCES

Aruin, A. S., Forrest, W. R. & Latash, M. L. (1998). Anticipatory postural adjustments in conditions of postural instability. *Electroencephalography & Clinical Neurophysiology* **109**, 350–359.

Aruin, A. S. & Latash, M. L. (1995). Directional specificity of postural muscles in feed-forward postural reactions during fast voluntary arm movements. *Experimental Brain Research* **103**, 323–332.

Aruin, A. S. & Latash, M. L. (1996). Anticipatory postural adjustments during self-initiated perturbations of different magnitude triggered by a standard motor action. *Electroencephalography & Clinical Neurophysiology* **101**, 497–503.

Baldissera, F., Borroni, P. & Cavallari, P. (2000). Neural compensation for mechanical differences between hand and foot during coupled oscillations of the two segments. *Experimental Brain Research* **133**, 165–177.

Baldissera, F., Borroni, P., Cavallari, P. & Cerri, G.(2001). Cyclic modulation of the excitability of resting forearm muscles is related to cyclic contraction of foot movers, not to movement. *Pflügers Archiv* **442**, R7(4).

Baldissera, F. & Cavallari, P. (1993). Short-latency subliminal effects of transcranial magnetic stimulation on forearm motoneurones. *Experimental Brain Research* **96**, 513–518.

Baldissera, F. & Cavallari, P. (2001). Neural compensation for mechanical loading of the hand during coupled oscillations of the hand and foot. *Experimental Brain Research* **139**, 18–29.

Baldissera, F., Cavallari, P. & Civaschi, P. (1982). Preferential coupling between voluntary movements of ipsilateral limbs. *Neuroscience Letters* **34**, 95–100.

Baldissera, F., Cavallari, P. & Leocani, L. (1998). Cyclic modulation of the H-reflex in a wrist flexor during rhythmic flexion-extension movements of the ipsilateral foot. *Experimental Brain Research* **118**, 427–430.

Baldissera, F., Cavallari, P., Marini, G. & Tassone, G. (1991). Differential control of in-phase and anti-phase coupling of rhythmic movements of ipsilateral hand and foot. *Experimental Brain Research* **83**, 375–380.

Bouisset, S. & Zattara, M. (1987). Biomechanical study of the programming of anticipatory postural adjustments associated with voluntary movement. *Journal of Biomechanics*, **20**, 735–742.

Burke, D., Gracies, J. M., Mazevet, D., Meunier, S. & Pierrot-Deseilligny, E. (1994). Non-monosynaptic transmission of the cortical command for voluntary movement in man. *Journal of Physiology* **480**, 191–202.

Carson, R. G., Goodman, D., Kelso, J. A. S. & Elliott, D. (1995). Phase transitions and critical fluctuations in rhythmic coordination of ipsilateral hand and foot. *Journal of Motor Behavior* **27**, 211–224.

Chabran, E., Fourment, A., Maton, B. & Ribreau, C. (1999). Chronology of upper limb anticipatory postural adjustments associated with voluntary wrist flexions and extensions in humans. *Neuroscience Letters* **268**, 13–16.

Classen, J. & Benecke, R. (1995). Inhibitory phenomena in individual motor units induced by transcranial magnetic stimulation. *Electroencephalography & Clinical Neurophysiology* **97**, 264–274.

Cordo, P. J. & Nashner, L. M. (1982). Properties of postural adjustments associated with rapid arm movements. *Journal of Neurophysiology* **47**, 287–302.

Davey, N. J., Romaiguere, P., Maskill, D. W. & Ellaway, P. H. (1994). Suppression of voluntary motor activity revealed using transcranial magnetic stimulation of the motor cortex in man. *Journal of Physiology* **477**, 223–235.

Gracies, J. M., Meunier, S. & Pierrot-Deseilligny, E. (1994). Evidence for corticospinal excitation of presumed propriospinal neurones in man. *Journal of Physiology* **475**, 509–518.

Jeka, J. J. & Kelso, J. A. S. (1995). Manipulating symmetry in the coordination dynamics of human movement. *Journal of Experimental Psychology: Human Perception & Performance* **21**, 360–374.

Kelso, J. A. & Jeka, J. J. (1992). Symmetry breaking dynamics of human multilimb coordination. *Journal of Experimental Psychology: Human Perception & Performance* **18**, 645–668.

Liepert, J., Terborg, C. & Weiller, C. (1999). Motor plasticity induced by synchronized thumb and foot movements. *Experimental Brain Research* **125**, 435–439.

Marsden, C. D., Merton, P. A. & Morton, H. B. (1978). Anticipatory postural responses in the human subject. *Journal of Physiology* **275**, 47–48*P.*

Marsden, C. D., Merton, P. A. & Morton, H. B. (1981). Human postural responses. *Brain* **104**, 513–534.

Mills, K. R. (1999). *Magnetic Stimulation of the Human Nervous System*. Oxford University Press, Oxford, New York.

Nashner, L. M. & Forssberg, H. (1986). Phase-dependent organization of postural adjustments associated with arm movements while walking. *Journal of Neurophysiology* **55**, 1382–1394.

Nielsen, J. & Petersen, N. (1994). Is presynaptic inhibition distributed to corticospinal fibres in man? *Journal of Physiology* **477**, 47–58.

Orlovsky, G. N., Deliagina, T. G. & Grillner S. (1999). *Neuronal Control of Locomotion.* Oxford University Press, Oxford, New York.

Rudomin, P. & Schmidt, R. F. (1999). Presynaptic inhibition in the vertebrate spinal cord revisited. *Experimental Brain Research* **129**, 1–37.

Serrien, D. J. & Swinnen, S. P. (1998). Load compensation during homologous and non-homologous coordination. *Experimental Brain Research* **121**, 223–229.

Swinnen, S. P., Dounskaia, N., Verschueren, S., Serrien, D. J. & Daelman, A. (1995). Relative phase destabilization during interlimb coordination: the disruptive role of kinesthetic afferences induced by passive movement. *Experimental Brain Research* **105**, 439–454.

Tergau, F., Wanschura, V., Canelo, M., Wischer, S., Wassermann, E. M., Ziemann, U. & Paulus, W. (1999). Complete suppression of voluntary motor drive during the silent period after transcranial magnetic stimulation. *Experimental Brain Research* **124**, 447–454.

Zattara, M. & Bouisset, S. (1988). Posturo-kinetic organisation during the early phase of voluntary upper limb movement. 1. Normal subjects. *Journal of Neurology, Neurosurgery & Psychiatry* **51**, 956–965.

Acknowledgements

This study was supported by grants from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica and by the Università degli Studi di Milano.