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Cyclic time course of motor excitability modulation during the observation of a cyclic hand movement

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Abstract

The observation of a sinusoidal flexion–extension of the wrist was utilized to determine the continuous time course and phase relation between observed movement and its effects on the observer's motor pathways. While observing movements performed by others, the observers' cortical motor areas and spinal circuits were activated, reflecting the specific temporal and muscular pattern of the actual movement (motor resonance). H-reflexes and motor-evoked potentials (MEPs) were elicited, respectively, by electrical stimulation of the median nerve and magnetic stimulation of the appropriate cortical area, in the right forearm muscle Flexor Carpi Radialis (FCR) of subjects who were observing a 1-Hz cyclic oscillation of the right prone hand executed by a different person. Observation elicited a parallel cyclic excitability modulation of the observer's H-reflex and MEP responses with identical period as the observed movement. Modulation was phase advanced, as is muscle activation with respect to the real movement. The same results were obtained when the observed hand oscillation was executed with different frequency (1.6 Hz) and when the hands of mover and observer were supine. No motor resonance was elicited by observing the oscillation of a metal platform. The excitability modulation of MEPs simultaneously monitored in both antagonists of the observer's forearm (FCR and Extensor Carpi Radialis, ECR) was in almost perfect phase opposition, reflecting their natural reciprocal activation during the execution of a hand oscillation. These findings suggest that during observation, motor pathways are modulated subliminally reproducing with high temporal fidelity the motor commands needed to execute the observed movement.

Theme: Motor systems and sensimotor integration
Topic: Control of posture and movement

Keywords: Mirror neuron; Motor resonance; H-reflex; Motor-evoked potential; TMS; Excitability modulation

1. Introduction

It has recently emerged from a number of studies that motor pathways are activated not only during the obvious task of producing voluntary movement but also during motor imagery [33] and during action observation [43,44]. In particular, it has been proposed that during action observation, a "motor resonance", so termed to emphasize the concord of neural activity between mover and observer, may be an important mechanism underlying the automatic understanding of actions performed by others and/or the imitative learning of some motor skills [18,32,42].

Single unit recordings from a cortical premotor area in the macaque monkey (F5) [13,19] have disclosed neurons that produce a similar firing pattern when the monkey performs a goal-directed hand action and when it observes another monkey or a human experimenter perform a similar action ("mirror" neurons). Further experiments have revealed that the response of the mirror system is very robust, demonstrating that it concerns also goal-oriented actions performed with the mouth or foot [6,17,39], and that it is activated also in response to observed actions that are not completed in front of the monkey but have to be partly

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imagined by the animal [47] or that could just be inferred listening to recognizable sounds, causally linked to specific actions [36].

A corresponding observation/execution system has also been described in human observers by various techniques such as electro- and magnetoencephalography [10,26], functional magnetic resonance [6,7,29], positron emission tomography [12,24,25,42], all of which have documented the activation of cortical premotor and/or motor areas, during action observation tasks. These studies have confirmed that observation of a specific action can excite in the observer the same neural substrate necessary for the execution of that action. Differences between human and monkey observers have also emerged, such as, for example, the fact that for human subjects intransitive (not goal-directed) movements are also effective stimuli in evoking a motor resonant response [30,37]. Utilizing transcranial magnetic stimulation (TMS), several authors have been able to investigate motor resonance in human subjects with higher spatial resolution, describing a specific subliminal activation in those muscles that the observer would have used if enacting the observed movement [16,20,21,45]. With the H-reflex technique, Baldissera et al. [2] demonstrated that resonance phenomena are not confined to cortical structures but spread to modulate the excitability of spinal motoneurones. This study however showed that the excitability of the H-reflex evoked in a finger flexor muscle (Flexor Digitorum Superficialis, FDS) was depressed during the observation of fingers closing on an object, an unexpected result, given that all other evidence shows a consistent “mirror” activation of neural pathways in movers and observers. The apparent contradiction has been recently resolved, showing that, during the grasping movement, the FDS muscle, despite being a finger flexor, reaches its maximal activation during the hand opening phase [38], i.e., in accordance with maximal excitability of the H-reflex in observers. Indeed, a problem common to motor resonance studies to date has been the lack of an accurate temporal resolution of the correspondence between the observation of complex and prolonged movements and its modulatory effects in the observer.

In the present study, aimed at solving this problem, the hypothesis that a subliminal temporal pattern of muscle activation similar to that responsible for the actual movement might be produced during action observation was tested by using a simple, intransitive movement: the cyclic flexion–extension of the wrist. The excitability modulation induced in the observer’s spinal and cortical motor pathways of two wrist muscles (FCR and ECR) was sampled with both H-reflexes and MEPs. By using the same sinusoidal function to fit both observed wrist oscillation and resonance effects on the observer’s wrist motor circuits, we could describe a continuous time course of the two events and determine their precise phase relation.

2. Material and methods

Subjects gave informed written consent to all experiments, which were performed according to the Declaration of Helsinki and approved by the local ethics committee. Each experimental session involved two individuals sitting comfortably in front of each other, one performing a cyclic flexion–extension movement of the right hand around the wrist (mover) and the other observing such movement while not moving (observer, the true subject of the experiment).

2.1. Experimental procedure

Movers followed a general experimental protocol performing sequences of 4–5 flexion–extension cycles of the hand about the wrist, while observers were instructed to look at the movement of the other subject. Movers kept a tempo of ~1 Hz (for all experiments, but one control experiment at ~1.6 Hz), by listening via headphones to a metronome, audible only to them. During each oscillation, the angular position of the metal platform upon which the mover’s hand rested was recorded by a Spectrol 534 1 kΩ potentiometer coaxial with its pivot and digitized at 250 Hz. On each movement cycle, transit of the mover’s right hand across a specific position generated a trigger signal that was fed into a PC. When receiving the third signal in the sequence, the PC triggered the stimulator to elicit a response *in the observer’s* right forearm muscles (H-reflex or MEPs) at one of 5 different delays (0, 200, 400, 600 and 800 ms) corresponding to 5 different hand angular positions. The delays divided the imposed oscillation period (1000 ms) in even parts and were ordered in a random sequence. At the end of each flexion–extension sequence, 8 s elapsed before a beeping signal prompted the mover to start a new sequence. Each group of 5 delays was repeated 15 times (for a total of 75 movement sequences). All relevant signals, H-reflex or MEP traces in the observer, and potentiometric signal of the mover’s hand angular position, were recorded during the third cycle of each sequence and stored for further elaboration.

A total of 12 healthy adult right-handed volunteers of either sex (5 females and 7 males), aged 20 to 45 participated as observers, while three of the authors (PB, MM and GC) rotated as movers. Movers were seated in an armchair, with the right arm bent at the elbow and the hand fixed in either prone or supine position to a platform that could oscillate around the wrist axis. Observers were also seated in an armchair, directly facing the mover, with their right arm comfortably fixed to an armrest either in prone or supine position. Bipolar surface electrodes were placed on the two main movers of the wrist, FCR and ECR, for two purposes: recording H-reflexes, evoked in the FCR muscle by stimulation of the median nerve at the elbow with bipolar external electrodes (square pulse duration 0.8 ms), and continuously monitoring the lack of any EMG activity in FCR and ECR muscles during the movement observation. H-reflex amplitude was maintained between 5 and 15% of the maximum M response; signals were amplified, filtered (10–1000 Hz) and A/D converted (5 kHz).

In different experiments, using the same general protocol, MEPs were evoked simultaneously in both FCR and ECR muscles of observers, by supraliminal TMS. In these experiments, the observer's head was restrained by a fitted support, and an 8-shaped coil was held over the left cortical focus for activation of forearm muscles by a stereotactic apparatus. Stimulator output (Magstim 200, maximal power 2.2 T) was set at about 110% of the Motor Threshold (MT at rest, the minimum intensity evoking a visible MEP in 3 over 6 stimuli, as obtained in each subject) determined in the muscle giving the smaller MEP, MEPs rarely being exactly of the same amplitude in the two muscles. Location and efficacy of the stimulation coil were monitored throughout the experiment. Electrodes were carefully placed to eliminate cross registration of EMGs between the two muscles. MEPs were recorded and processed as described for H-reflexes.

2.2. Experimental conditions

This protocol was utilized in 4 different experiments; in both movers (hand moving) and observers (responses recorded), the right side was utilized. In experiment 1, FCR H-reflexes were recorded in observers holding their right hand prone and observing the oscillating prone right hand of the mover sitting in front of them. In experiment 2, FCR H-reflexes were recorded in observers holding their right hand supine and observing the oscillating supine right hand of the mover. In experiment 3, both FCR and ECR MEPs, simultaneously elicited by supraliminal TMS over the left cortical focus for activation of right forearm muscles, were recorded in observers holding their right hand prone and observing the oscillating prone right hand of the mover. In experiment 4, FCR H-reflexes were recorded in observers' right forearm while they sat in front of the mover's empty armchair, observing the oscillation of the sole platform. The platform was connected to the hand of a mover hidden behind a screen, by a long rod attached to its pivot, so as to produce an oscillating movement with the same kinematic characteristics as that observed during the flexion–extension of the mover's hand.

In a fifth experiment, rectified and integrated ($\tau = 20$ ms) EMGs from the FCR and ECR muscles were obtained in volunteers that were actually performing 20 cyclic flexion–extensions of the right wrist. Hand position (prone or supine) and oscillation frequency (1 or 1.6 Hz) were changed in order to establish whether a temporal correspondence exists between the onset of muscular activity, when the movement is actually performed, and the rising phase of reflex modulation, when the same movement is only observed.

2.3. Data analysis

H-reflex or MEP responses and potentiometric signals relative to the moving hand angular position were analyzed as previously described [3]. Briefly, in each subject, the ensemble-average of the hand cyclic movement during the third oscillation of all sequences was calculated and fitted by a four-parameter (period, offset, amplitude and phase) sine wave function. Parameters of the best-fit equation were calculated by minimizing the sum of the squared differences between the observed and predicted values of the hand angular position (Marquardt-Levenberg algorithm, SigmaPlot®). These records were then normalized to their calculated average cycle period. Since cycle period varied among trials and among subjects by about 5% of its average value (average period of all experiments at 1 Hz = 1021 ms ± 52 SD), normalization was necessary in order to bring all movement records, from different trials and different subjects, back to unity (1000 ms). Subsequently, to maintain the temporal correlation between the time courses of observed movement and response modulation in observers, the same normalization was performed on the 5 delays at which the H-reflex or MEP was recorded (0, 200, 400, 600 and 800 ms). Finally, changes in either H-reflex or MEP amplitude were displayed together with the correspondent average movement trace in a one-cycle diagram (360°).

The same period normalization procedure was also applied in the fifth experiment to the ensemble-average of 20 hand flexion–extension movements and their relative EMG records. The onset of EMG bursts was then determined by visual inspection of the averaged records [8].

Peak-to-peak amplitude of H-reflexes and MEPs were measured. Within each observer, in order to attenuate any long-term variability independent of the stimulus position in the cycle [3], the deviation (in μV) from the mean of the 5 responses recorded at the 5 delays during the cycle was calculated. All deviations from the mean obtained at the same delay were then averaged and averages fitted with a two parameter (amplitude and phase) sine-wave function (period = 1 and offset = 0 for all). The determination coefficient, R^2, was estimated and significance of the regression (significance limit $P < 0.05$) was ascertained by standard ANOVA procedure.

Mean H-reflex or MEP values from all different observers in each experiment were also pooled together. For each observer, mean values were normalized in size to the amplitude of their individual best-fit sine wave. This normalization equilibrated the weight of different individuals in the estimate of the common phase, by removing the between-observer differences in the amplitude of the response modulation. In addition, records from different experiments had to be aligned in time as well, given the inevitable variability in the position-triggered data acquisition onset (which had to be set anew for each mover–observer couple). To do this, the normalized movement cycles from each mover were shifted and aligned on the midpoint of the raising phase (ϕ = 0°). Again, the same procedure was applied to the 5 delays at which H-reflex or MEPs were recorded, in order to maintain the temporal correlation between observed movement and response modulation in observers. After normalization and alignment, all data were plotted together, fitted by a two-parameter sine-wave function and analyzed statistically as above.

Phase opposition in the modulation of the FCR and ECR MEPs was confirmed with a Student's t test comparing the phase values obtained from the sinusoidal best fit of each data set, after 180° had been added to one of the phase values [5]. Significance in H-reflex and EMG phase differences between the prone and supine hand positions or 1 and 1.6 Hz oscillation frequencies were also evaluated with a standard t test.

Results of all statistical analyses are given in figure legends.

3. Results

The first 2 series of experiments describe the changes of the H-reflex excitability in the resting FCR muscle induced by the observation of a cyclic wrist movement performed by a different subject. The time course of the H-reflex modulation in the observer's flexor muscle closely reproduces that of the mover's hand flexion (maximum H-reflex amplitude) and extension (minimum amplitude); this subliminar modulation is maintained irrespective of whether the hands are kept prone (downward flexion) or supine (upward flexion), or oscillated at different frequencies.

Fig. 1 illustrates the H-reflex modulation in the resting FCR muscle of a single subject (A and B) and all subjects pooled together (C and D) holding their right forearm in prone position while carefully observing cycles of flexion–extension of the mover's prone right hand. Data points are the average deviation of the reflex amplitude from the mean value at each of the 5 delays during the movement cycle. In Fig. 1A, the reflex modulation (filled circles) is plotted on the same normalized abscissa as the average observed movement (Fig. 1B, continuous line). The actual period of the movement was estimated by fitting the average movement record with a sine wave function (Fig. 1B, dashed line). The H-reflex data were then fitted by a sine-wave function with the same period as that of the movement (Fig. 1A, dashed line). This allowed for immediate phase matching between the functions fitting the observed movement and the excitability changes occurring in the FCR H-reflex. In this subject, the rising phase modulation of the FCR H-reflex anticipated the flexion phase of the movement best-fit function by 72°.

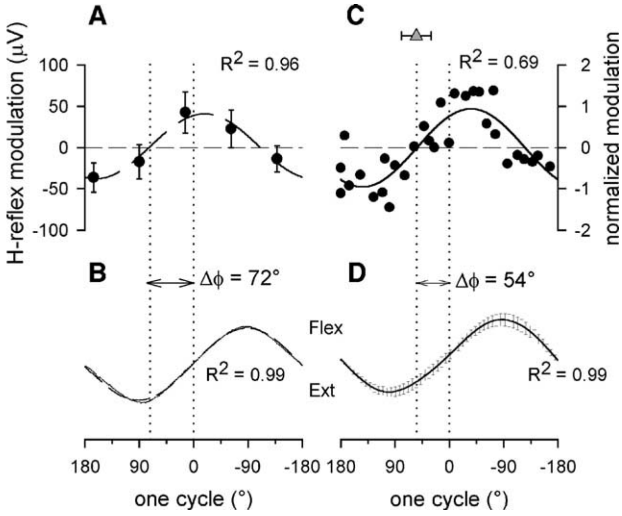

Fig. 1. Modulation of FCR H-reflex during observation of cyclic wrist flexion–extensions. Hands prone. (A) Absolute deviations of the H-reflex size from its mean value, occurring at five delays during observation of one movement cycle. Each point represents the average (±SEM) of 15 responses evoked in one subject at that delay. Data were fitted with a sine-wave function (dashed line; $R^2 = 0.96$, $P < 0.004$) with the same period (948 ms) as that of the average movement (B, continuous line), estimated by fitting with a sine-wave function (dashed line; $R^2 = 0.99$, $P < 0.0001$). $\Delta\phi$ = phase difference between reflex modulation in flexor muscle of the observer and wrist oscillation of the mover, both holding their hands in prone position (Flex = downward direction with prone hand). (C) Reflex modulation in 6 subjects. Data of each subject (filled circles) were normalized in size to the amplitude of the respective best-fit sine wave and phase-aligned. Overall data were then fitted with a common sine-wave function ($R^2 = 0.69$, $P < 0.0001$; $\Delta\phi$ = 54°). The gray triangle on top of panel C indicates the average $\Delta\phi$ (55° ± 22 SD) obtained from fitting the individual data points in each subject. (D) Average movement trace of all subjects ± SEM (sine-wave fitting $R^2 = 0.99$, $P < 0.0001$; average π = 1004 ms ± 43 SD).

Data from 6 different observers are plotted and fitted together (Fig. 1C, filled circles and dashed line) after having been normalized to the amplitude of each subject's best-fit sine wave and aligned on each subject's movement zero (midpoint of the sine-wave). Sinusoidal fitting of the movement and reflex data (Figs. 1C and D respectively) shows that the pattern of FCR excitability modulation remains consistent in different observers and continues to be linked to the flexion phase of the observed movement, with a common phase advance of 54° (average of all subjects 55° ± 22 SD).

Fig. 2 shows the H-reflex modulation in the resting FCR muscle of a single subject (A and B), and all subjects pooled together (C and D) when both observer and mover are holding their hands in supine position. As in Fig. 1, the experimental points are fitted by a sine-wave function (Fig. 2A, dashed line) with the same period as that of the observed movement (Fig. 2B, continuous line). In this subject, the rising phase of the FCR H-reflex modulation led by 117° the flexion phase of the best-fit function of the movement, now executed in the upward direction.

Data from 6 different observers with supine hands are plotted and fitted together (Fig. 2C, filled circles and dashed line) after having been normalized and aligned as described above. The phase relationship between the sinusoidal best fit functions of the reflex and of movement data remains similar across different observers ($\Delta\phi$ = 112°, average of all subjects 113° ± 17 SD). Note that this phase

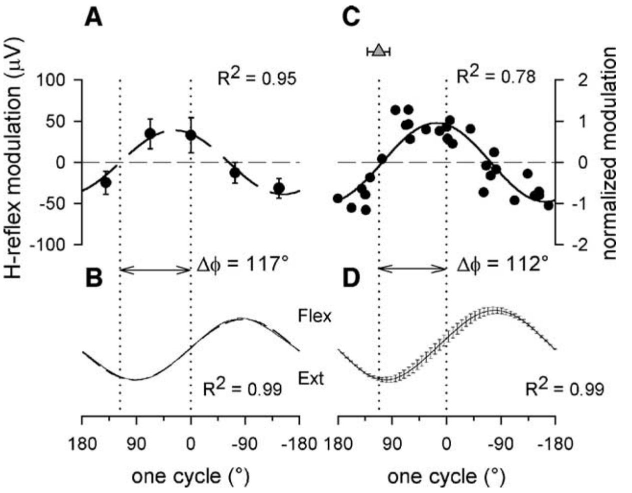

Fig. 2. Modulation of FCR H-reflex during observation of cyclic wrist flexion–extensions. Hands supine. (A) Absolute deviations of the H-reflex size from its mean value, occurring at five delays during observation of one movement cycle. Each point represents the average (±SEM) of 15 responses evoked in one subject at that delay. Data were fitted with a sine-wave function (dashed line; R^2 = 0.95, $P < 0.005$) with the same period (984 ms) as that of the average movement (B, continuous line), estimated by fitting with a sine-wave function (dashed line; R^2 = 0.99, $P <$ 0.0001). $\Delta\phi$ = phase difference between reflex modulation in observer flexor muscle and mover wrist oscillation, both holding their hands in supine position (Flex = upward direction with supine hand). (C) Reflex modulation in 6 subjects. Data of each subject (filled circles) were normalized in size to the amplitude of the respective best-fit sine wave and phase-aligned. Overall data were then fitted with a common sine-wave function (R^2 = 0.78, $P < 0.0001$; $\Delta\phi$ = 112°). The gray triangle on top of panel C indicates the average $\Delta\phi$ (113° ± 17 SD) obtained from fitting the individual data points in each subject. (D) Average movement trace of all subjects ± SEM (sine-wave fitting R^2 = 0.99, $P < 0.0001$; average π = 1010 ms ± 50 SD).

value is significantly in advance by 58° ($P < 0.001$) over that of observers with prone hands ($\Delta\phi$ = 54°).

Since this greater phase advance of the FCR H-reflex modulation was consistently measured in all observers with supine hand, we hypothesized that it might reflect a consistent difference in activation pattern of this muscle when a hand is oscillated in the supine versus the prone position. By postulating that the motoneuronal drive has a sinusoidal course, the phase relation between the motoneurone activation and the subsequent hand oscillation may be evaluated from the EMG onset of the two antagonists FCR and ECR [15]. EMG activity of the FCR and ECR muscles was therefore recorded in 5 subjects performing cyclic hand flexion–extensions of the wrist at 1 Hz with their hands in either prone or supine position. In Fig. 3, referring to one representative subject, all traces are normalized to 1 cycle and aligned to movement zero. In both A and B, the FCR rectified EMG is displayed on a positive ordinate, while the ECR EMG is reversed, to show the precise alternation between the antagonists. Note that the onset in either muscle is more advanced with respect to movement (Fig. 3C) in the supine than in the prone position. The sine-wave function that fits the series of sequential onsets has a phase advance of 47° for prone and 95° for supine hand in this subject and significantly different ($P < 0.006$) average values of 64° ± 11 SD and 91° ± 12 SD respectively, in all subjects. The larger advance in muscular activation of subjects executing a cyclic flexion–extension with the hand supine thus corresponds to the larger phase advance of the H-reflex modulation in subjects observing the flexion–extension movement with their hands in supine than in prone position.

In the next experiment, we used TMS-elicited MEPs to measure the excitability changes of two wrist antagonists (FCR and ECR) simultaneously. Responses were evoked and recorded at the usual 5 time intervals, in 5 subjects holding their right hand prone and observing flexion–extension movements of the mover's prone right hand. Samples of average MEP traces in one subject are illustrated in Fig. 4A; in this, and in most subjects, the average recordings from the ECR muscle were slightly larger than those from the FCR (all 5 delays combined =

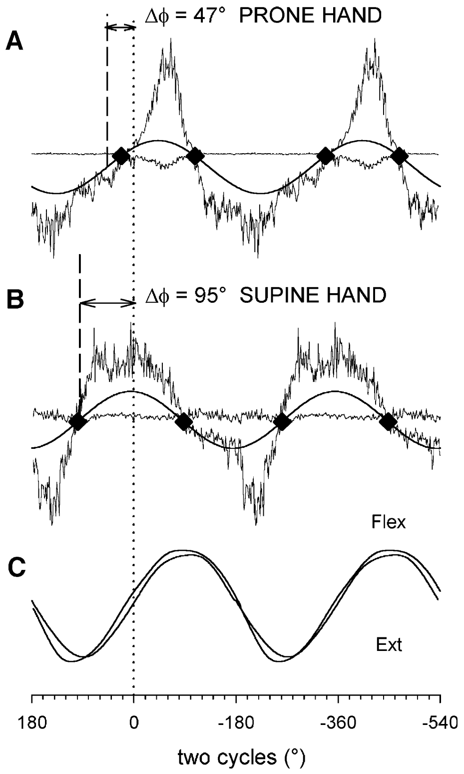

Fig. 3. EMG recordings from forearm muscles of one subject oscillating the right hand in prone or supine position. Average records of 20 rectified EMG traces of FCR and ECR (inverted) muscles simultaneously recorded during hand oscillations at 1 Hz in prone (A) or supine (B) position. Abscissa was normalized to the period of the average movement cycle (C) and aligned to the cycle zero (vertical dotted line). Both average movement traces of prone and supine hands are shown (π = 1000 ms prone; 990 ms supine). The sine-wave functions with the same period as their relative movement, passing through the EMG onsets (black diamonds) were reconstructed (thin continuous lines overlaid on the EMG traces) and phases calculated (vertical dashed lines). $\Delta\phi$ = phase difference between functions fitting EMG onsets and movement.

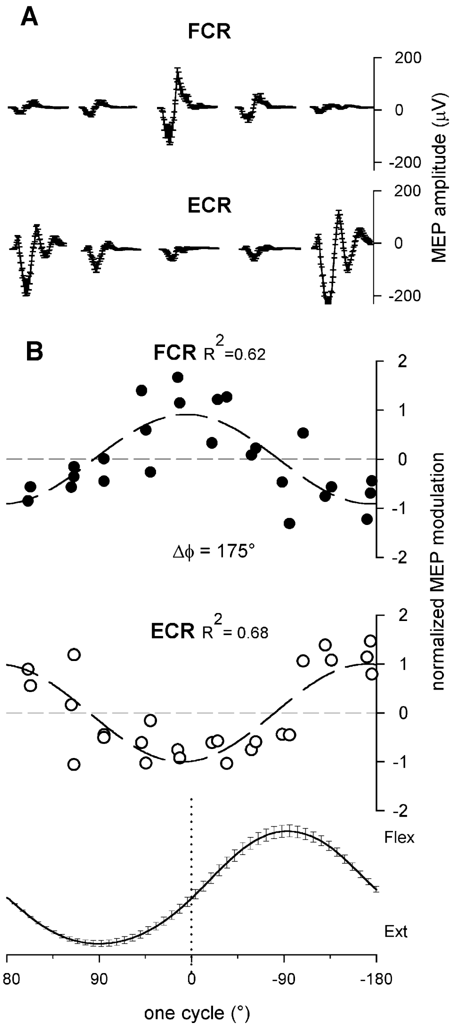

Fig. 4. Cyclic modulation of MEPs in forearm muscles during observation of cyclic wrist flexion–extensions. Hands prone. (A) Samples of TMS-evoked MEP traces, simultaneously recorded in FCR and ECR muscles of one subject at 5 delays during observation of a movement cycle (average of 25 responses ± SEM). (B) Absolute deviations of the MEP amplitude from its mean value, occurring at 5 delays during observation of one movement cycle. Five different subjects. In each subject, MEP size was normalized to the amplitude of the respective best-fit sine wave and phase-aligned. Overall data were then fitted with a common sine-wave function (dashed line; FCR filled circles, $R^2 = 0.62$, ECR empty circles, $R^2 = 0.68$; $P < 0.0001$ for both fits) with the same period as that of the average movement (bottom panel, continuous line ± SEM; $\pi = 1005$ ms ± 32 SD; $R^2 = 0.99$, $P < 0.0001$). $\Delta\phi$ = phase difference between FCR and ECR MEP modulations.

177 µV ± 117 SD and 109 µV ± 87 SD respectively). Note the reciprocal time course of amplitude modulation in the two muscles. Fig. 4B shows the mean changes in average modulation across the 5 subjects in FCR (filled circles) and ECR (empty circles) and their relative best fits (dashed lines). Once again, in each subject, the experimental data points were fitted by a sine-wave function with the same period as that of the movement observed by that subject (individual data not shown), normalized to the amplitude of that best-fit function, aligned on the movement zero and then plotted and fitted altogether. The phase opposition between the sine-waves fitting all the FCR and ECR MEP points is almost perfect ($\Delta\phi$ = 175°). Phase opposition between the two best-fit functions was confirmed by the absence of statistical difference between them, when one was shifted by 180° ($P > 0.5$).

It is worth noting that the modulation of the MEPs was between 20 and 100% of mean MEP amplitude, i.e., much larger than the modulation of the H-reflexes that was between 10 and 20% of mean reflex amplitude.

Two control experiments are illustrated in Fig. 5: the first control experiment (Figs. 5A, B) aimed at distinguishing the observation of a movement performed by a human hand from that of the mechanical oscillation of an object, at the same frequency (1 Hz). Subjects (5) were chosen among those who had already shown a good modulation in experiment 1. In this experiment, they sat in the usual set up and observed the platform, upon which the mover's hand normally rested, oscillating by itself. The movement of the empty platform was kept as similar as possible to that of the platform hosting the mover's hand. The random variability of all data points plotted together could not be fitted by a sine equation with the period of the observed movement (Fig. 5B).

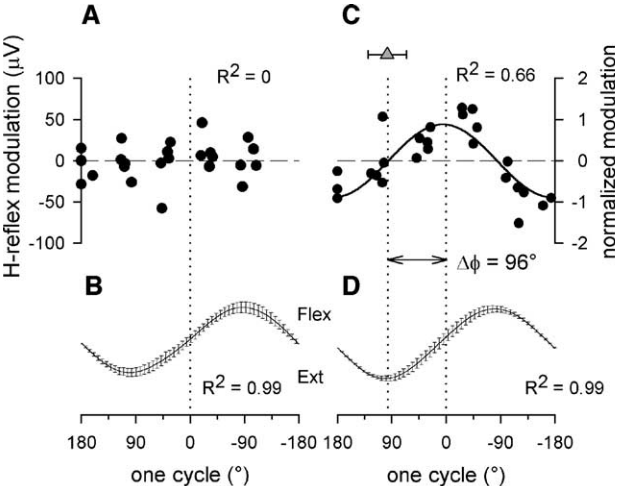

Fig. 5. Control experiments: observing a metal platform and changing the movement frequency. Hands prone. (A) H-reflex modulation (filled circles) in 5 subjects, recorded at 5 delays during observation of cyclic oscillations at 1 Hz of the empty metal platform upon which the mover's hand rested in previous experiments. The random variability of all data points plotted together could not be fitted with a sine-wave function ($R^2 = 0$). Data points were not normalized because the individual data points could not be fitted. (B) The movement of the empty platform ($\pi = 1058$ ms) produced by a human mover hidden behind a screen was identical to that produced by the mover's hand and equally well fitted by a sine-wave function ($R^2 = 0.99$, $P < 0.0001$). (C) H-reflex modulation (filled circles) in 5 subjects, recorded at 5 delays during observation of hand oscillations at 1.6 Hz. $\Delta\phi$ = 96°, phase difference between reflex modulation in observer FCR and mover wrist oscillation. The gray triangle indicates the average $\Delta\phi$ (97° ± 32 SD) obtained from fitting the individual data points in each subject. Data points were fitted with a sine-wave function (dashed line; $R^2 = 0.66$, $P < 0.0001$) with the same period as that of the average movement shown in panel D (continuous line ± SEM). Average movement period (570 ms) estimated by fitting with a sine-wave function (dashed line; $R^2 = 0.99$, $P < 0.0001$).

In the second experiment (Figs. 5C, D), the frequency of the observed flexion–extension movement was changed to 1.6 Hz to eliminate an even remote possibility that the period of the observed movement and that of the H-reflex modulation happen to coincide at 1 Hz, without really being causally related. The 5 delays at which the FCR H-reflex was recorded were consequently changed, to divide the new period of 600 ms in equal intervals. In 5 observers, the experimental data points could be fitted by a sine-wave function with the same period as that of the observed movement. These data are plotted in Fig. 5C (filled circles), where they are also fitted together (dashed line) after the usual normalization and alignment. The phase of the sinusoidal best-fit function of the FCR H-reflex anticipates the average observed movement (Fig. 5D) by 96° (average of all subjects 97° ± 32 SD). To further pursue the analysis of the temporal relation between resonant response and actual muscular activity, this experiment was complemented by the recording of FCR and ECR EMGs during actual movement, just as described above for the 1 Hz hand oscillation. The phase of the sinusoidal best-fit function of the FCR/ECR onsets during actual oscillation anticipates that of the average movement by 92° ± 23 SD. Table 1 summarizes the data obtained at the two frequencies.

4. Discussion

Observation of movement executed by others elicits in specific motor pathways of the observer a “resonant” response with the same time course and muscular activation pattern as in the observed movement. This statement is supported by several converging results obtained in this study with different experimental approaches. Watching a sinusoidal flexion–extension of the wrist elicited a sinusoidal excitability modulation in the motor pathways of the observer’s wrist muscles, measured as changes in amplitude of either the H-reflex or MEPs (evoked by TMS). The time course of observed movement and subliminal motoneurone activation were tightly correlated, and in fact, they could both be fitted by sine functions with the same period. This remained true also when the frequency of the observed oscillation was increased from 1 Hz to 1.6 Hz. The facilitation of the H-reflex in the observer’s FCR muscle mirrored the activation of the same muscle to produce the actual movement, irrespective of movement direction in space: maximal facilitation of this flexor remained linked to the flexing phase of the observed hand oscillation whether the hand was held in prone or supine position. Simultaneous recordings of MEPs from FCR and ECR also showed that modulation in the observer’s forearm muscles is reciprocal, corresponding to the natural reciprocal activation of these antagonists during the execution of the wrist sinusoidal movement. Finally, the observation of the sinusoidal oscillation of a metal platform did not elicit any coherent H-reflex modulation. Altogether, these findings suggest that during observation, motor pathways are modulated subliminally in a way that reproduces with high temporal fidelity the motor command needed to actually execute the observed movement.

Table 1
Comparison of phase advance of H-reflex modulation and muscle activation at two different movement frequencies

	1 Hz	1.6 Hz	*t* test
H-reflex modulation	55° ± 22	97° ± 32	$P < 0.03$
muscle activation	64° ± 11	92° ± 23	$P < 0.02$

Average phase advance of the sinusoidal best fit functions of H-reflex modulation during observation and of the motor command derived from the FCR and ECR onsets during actual movement. Data obtained at the two different frequencies (1 and 1.6Hz) are significantly different.

Using both H-reflex and TMS techniques, this paper shows that, in a given muscle, the excitability modulation induced by motor resonance in both primary motor cortex (M1) and spinal motoneurones has the same time course. Earlier results [2,16] had instead suggested that H-reflexes and MEPs were modulated in opposite directions by action viewing. To solve this contradiction, it had been suggested that different mechanisms may be responsible for motor resonance in M1 and spinal cord. In fact, the premotor cortex, which represents the most likely site of origin of the mirror system, projects both to the primary motor cortex [22] and, in parallel, to spinal motoneurones [14] and could therefore provide distinct effects at the two levels. Though this possibility cannot be completely excluded, in a recent study Montagna et al. [38] also showed a complete congruence between the MEPs and H-reflex response modulations induced by motor resonance and demonstrated that the spinal effect observed in motoneurons is due mainly to the excitability modulation of M1 corticospinal neurons. The congruence between the time course of MEP and of H-reflex modulation reported here supports the same conclusion also for the observation of intransitive movements.

The present experiments allowed to estimate the temporal (phase) relations between the subliminal motor command (sinusoidal FCR H-reflex modulation) recorded when the hand oscillation was observed and the overt command for execution, derived from the EMG onset in the two antagonists (FCR and ECR). The phase advance of both the subliminal and overt commands was consistently and significantly greater with the hand oscillating in supine than in prone position. This suggests that changing hand position and oscillation frequency produced the same effects on both the timing of the muscular contractions during actual movement and the subliminal “resonance” excitability modulation, presumably by partitioning of the motor command to the appropriate muscles depending on hand afferent postural input. Similar results were obtained when the hand was oscillated in prone position at two different frequencies.

These results strengthen the view that moving and observing movement activates the same motor pathways, with congruent time courses. Moreover, they indicate that also the precise timing of the onset of overt and subliminal activations is the same. This means that the resonant excitability modulation precedes the movement and does not immediately depend on its visual perception. In fact, in these experiments, the resonant response always anticipated the observed movement, in a way consistent with hand position and oscillation frequency, i.e., the H-reflex excitability anticipated movement with a phase difference which reflects the natural temporal relationship between muscle contraction (FCR and ECR) and deriving hand oscillation [1,15]. This temporal relationship may have been evidenced by the cyclic nature of the movement observed here, but it should be noted that a similar anticipation probably occurs during viewing of non-cyclic movements, as in all other action observation studies, since movements are always repeated many times and could easily be predicted by observers. Similarly, it has been shown that when the nature and onset time of an observed action is predictable, an EEG "readiness potential" in the controlateral sensori-motor cortex precedes the observed movement onset, suggesting that knowledge of a coming action automatically activates the motor system [35,48]. Another recent report [21] suggests that when an anomalous course of a reaching–grasping movement is presented, the TMS-evoked MEP modulation does not follow moment by moment what is being viewed but is either interrupted or continues to match the modulation that would have been obtained with observation of the normal movement, albeit only for a short while.

In these and other previous studies, however, the precise temporal relation between resonance effects, observed movements and/or the underlying motor commands was not attended. Thus, the anticipation that so clearly emerged here with a sinusoidal movement might be a regular property of motor resonance. This makes the distinction between motor resonance and motor imagery more uncertain. As a matter of fact, brain imaging studies have shown that the neural substrates activated during action observation and motor imagery coincide anatomically [11,31], and TMS studies have also shown that MEPs evoked during action observation or imagination tasks undergo similar modulation [9,40]. On the other hand, influence of motor imagery on spinal mechanisms is still controversial. Several authors report the absence of modulation in the FCR H-reflex during mental imagery of hand flexion–extension [27–29], even when a modulation of FCR and ECR MEPs was found [27], while others who describe a spinal modulation during motor imagery [4,31] also recorded a small muscular activation during the imagined task. In these cases, the presence of an overt muscular contraction must derive from a voluntary not imagined motor command, thus confusing the interpretation of the results.

In all studies cited above, the observed or inferred action was the reaching for and grasping of an object, i.e., a goal-oriented action. From the original experiments on mirror neurons in the monkey, it was clear that these neurons were not activated either by an object alone or by the action without the object, unless the object was hidden after the animal had seen it [47]. In subsequent experiments on human subjects, the distinction was made between actions that were object-related (object present and action directed to it) or non-object related (object not present, but action still mimicked being directed to it) and it was shown that, contrary to what had been described for monkeys, mimicking actions could activate the human premotor cortex [6]. With the present experiments, as well as in previous studies [16,30,34,37,45], the human mirror system was also shown to respond to the observation of intransitive movements, i.e., those lacking a goal, intention or meaning, a further step toward what could be viewed as a more abstract human ability to resonate with the movement of others, even in the absence of an explicit goal. The new finding shown by the present experiments is that even if the observed movement does not have a goal or meaning, it is correctly reproduced in the observer's motor pathways as a "real" movement, with all its natural motor constraints. In fact, when the MEP modulations of the FCR and ECR muscles were recorded simultaneously during the observation of the hand oscillation, they showed the expected phase opposition of antagonists contracting with an alternating pattern, as necessary to produce the oscillation of the wrist. Furthermore, in all observers, the motor resonant response maintained a phase relation with the observed sinusoidal movement that was consistent with the position and frequency-dependent muscular activation (hand prone or supine; oscillation at 1 or 1.6 Hz).

A final consideration regarding the observed movement: in order to induce a motor resonance in the observer, it must be executed by hands or other biological effectors, while tools and mechanical devices are ineffective [41,42,46]. Our results show that the observation of the oscillatory movement of the metal platform, without the mover's hand that normally rested upon it, did not evoke a resonant response, despite the fact that the platform was oscillated by a human mover hidden behind a screen, so that the movement developed with a profile indistinguishable from the flexion–extension of the mover's hand. The latter is an important detail, since kinematic properties appear to be among the key features utilized by observers to identify human movement [23].

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