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Frequency rhythmic electrical modulation system (FREMS) on H-reflex amplitudes in healthy subjects

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Aim. **Changes in the amplitude of Hoffmann reflex (H-reflex) may reflect variations in the characteristics of the largely monosynaptic circuitry that is explored and are a possible target for diagnostic and physical therapeutic intervention. However, previous attempts to induce predictable changes in the H-reflex amplitude by transcutaneous electrical nervous stimulation (TENS) have generally failed. Previous workers applied fixed frequency in the low- (2-5 Hz) or in the high- (100 Hz) field, but they did not attempt to vary frequency and/or impulse duration in time.**

Methods. **We evaluated the effect of a new type of painless electric stimulation, *i.e.* frequency rhythmic electrical modulation system (FREMS). FREMS is characterized by the use of transcutaneous electric pulses with sequentially modulated frequency (f: 1-39 Hz) and width (w: 10-40 μs) at constant, perceptive threshold voltage (~150 V). FREMS was applied at the abductor hallucis muscle (AHM), as conditioning stimulus of the H-reflex which was recorded ipsilaterally at the soleus muscle, according to the classic method, in 10 normal volunteers (age range 21-40 years).**

Results. **H-reflex amplitude was substantially decreased (-50%) during FREMS and H-reflex amplitude variations were influenced by w/f variation in time during FREMS subphase C in a predictable way (r^2=0.43; P<0.001). Our results suggest an effective ability of FREMS to modulate H reflex amplitude.**

Conclusion. **The ability to achieve large and predictable changes of the H-reflex amplitude simply by modulating both frequency and duration of a conditioning painless electrical stimulation offers new possibilities for the treatment of diseases characterized by motoneuron excitability abnormalities.**

Key words: **Reflex - Spine - Transcutaneous electrical nerve stimulation.**

Spasticity,[1, 2] dystonia,[3, 4] and fibromyalgia,[5-7] are all ominous clinical hallmarks of a heterogeneous group of diseases. In all these conditions, increased mono- or multi-segmental spinal neuron excitability has been proposed as a pathophysiological common denominator, elicited by several peripheral and central abnormal influences. [1-3, 5-13]

Peripheral reflexes may be studied *in vivo* in human beings by a careful evaluation of H-reflex. Specifically, the determination of minimal latency of the H-wave, combined with its amplitude, width and threshold, may give information about the reflex arc conduction.[14-24] The amplitude of H-reflexes mirrors the amount of synchronized α-motoneurons activation,[25] modulated by several afferents. In physical therapy, some attempts to control neuron hyperexcitability have been made by the use of transcutaneous electrical nervous stimulation (TENS). However, currently there is no consensus as regards to the effects of TENS on H-reflex. In fact, predictable modulation of H-reflex amplitude has never been observed with this approach,[26-37] practically excluding[34, 38] the therapeutical use of TENS in diseases characterized by

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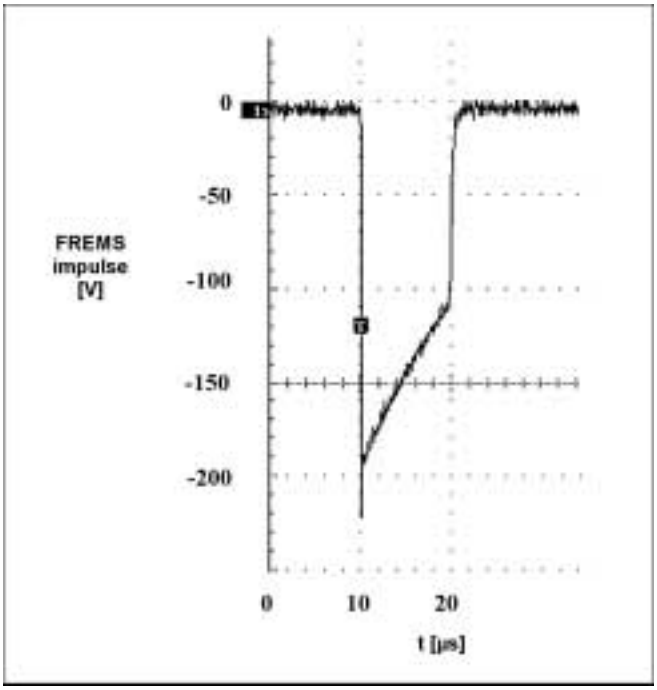

Figure 1.—Single pulse of the frequency rhythmic electrical modulation system (FREMS) sequence. This image was obtained from a high resolution oscilloscope connected with the output of the FREMS device (APTIVA,, Lorenz Biotech S.p.A., Medolla, Modena, Italy). Voltage is expressed on the ordinate (50 V per division), time on the abscissa (10 µs per division).

motoneuron hyperexcitability.[39-42] Nevertheless some interesting findings in this field have been reported by Hardy *et al*.[32] They showed that the increase in the H-reflex amplitude is inversely correlated with the stimulation intensity, that is, at the same stimulation frequency (50 Hz) with a stimulation intensity near the sensitivity threshold (ST), they were able to obtain increased H-reflex amplitude, whereas at the stimulation intensity corresponding to the motor threshold (about 50% above the ST), the effect was lost. Therefore, their results suggest that it is possible to increase the H-reflex amplitude by applying currents near to the ST. Hence the possibility to modulate spinal motoneuron excitability by some physical treatments remains a major goal of this type of therapy.

In this study we aimed at using a type of conditioning transcutaneous electrical stimulation to influence H-reflex amplitude, with a series of important variations:

a) rather than assessing the effect of conditioning stimulus by comparing H-reflex amplitude before and after stimulus application, we assessed the effect of conditioning of a stimulus applied to an ipsilateral synergistic muscle during soleus H-reflex recording;

b) instead of applying a conditioning stimulus with fixed intensity characteristics (constant current voltage, fixed impulse duration and fixed stimulation frequency), we produced an impulse sequence characterized by very brief impulses, patient/subject-regulated voltage to his/her own perception threshold (thus below motor or pain threshold) and maintained at the same identified threshold voltage during the entire stimulus administration.

This novel stimulation sequence is characterized by 3 subsequently subphases (A, B, C). The first subphase (A) is characterized by minimal width impulses at a 1 Hz frequency; the second subphase (B) is characterized by impulse width of 10 to 40 ms; in the third subphase (C) the 40 ms impulse frequency increases from 1 to 40 Hz. This sequence has been copyrighted and defined as frequency rhythmic electrical modulation system (FREMS) by Lorenz Biotech SpA, Medolla (MO), Italy. The theoretical rationale of the FREMS relies on a physiological model of voluntary temporal motor recruitment through successive discrete electrical pulse summation applied near the motor nerve.

This study consists in two steps: first, we assessed the FREMS-induced neuromuscular recruitment modality by applying FREMS on the tibial nerve and recording surface EMG of the *abductor hallucis* muscle (AHM). Subsequently we applied the same FREMS directly over the belly of the AHM, which shares with the soleus muscle the same S1 metameric innervation, to assess a possible effect of conditioning on H-reflex amplitude recorded from the soleus muscle. Our hypothesis is that we can influence the spinal motor activity by two successive patterns of stimulation, the former characterized by an increase of width of the pulses at constant frequency ("tonic" stimulation), and the latter by an increase of the frequency of the pulses at constant width ("phasic" stimulation). The method we choose was to evaluate the variations of the H-reflex amplitude obtained by conditioning H-reflex with FREMS applied over the AHM.

Materials and methods

Subjects

This study was approved by the local Ethical Committee and conducted in accordance with the

TABLE I.—*Structure of the frequency rhythmic electrical modulation system (FREMS) sequence. The first column shows the subdivision into the subphases A, B, and C, according to the criteria that during the A subphase there is no variability in width and frequency values, during the B subphase only width varies and during the C subphase only frequency varies. The W*f product was divided by 10 to allow visualization in comparisons shown in the figures.*

Subphase	Duration of subphase (s)	Width of impulse (μs)	Frequency (Hz)	w/f Ratio (10-6 s2)	W* f Product/10
A	30	10	1	10.0	1
B	5	20	1	20.0	2
B	5	40	1	40.0	4
C	1	40	2	20.0	8
C	1	40	3	13.3	12
C	1	40	4	10.0	16
C	1	40	5	8.0	20
C	1	40	6	6.7	24
C	1	40	7	5.7	28
C	1	40	8	5.0	32
C	1	40	9	4.4	36
C	1	40	19	2.1	76
C	1	40	29	1.4	116
C	1	40	39	1.0	156

Declaration of Helsinki. We recruited 10 normal subjects (mean age 31±3.5 years) out of our department employees who volunteered to participate in the study, after being carefully informed about the purpose and the procedures involved. All of them signed an informed consent. They were all thoroughly evaluated by an experienced neurologist and found free of any neurological disturbance or other diseases. Particular care was taken in decreasing interindividual variability by training the subjects with the device for 2 to 3 days before the experiments and by ensuring that all subjects were comfortably lying on their abdomen for at least 15 min prior to the experimental sessions. All the subjects abstained from using caffeine and/or psychostimulants during the previous 48 h to the experiments. The experimental environment was noise-proof and quiet and the temperature was kept constant at 22°C.

Frequency rhythmic electrical modulation system

FREMS, obtained with APTIVA® device (Lorenz Biotech S.p.A., Medolla, Modena, Italy) is characterized by a sequence of imposed voltage pulses continously cross-checked in amount of charge by a feedback circuit (Figure 1). These pulses varies both in frequency (1-39 Hz) and width (10-40 μs) (Table I) and the voltage is individually adjusted by the subjects through a remote control, in order to achieve a slight prickling sensation that in normal subjects occurs at about 150 V (perceptive threshold, PT). The pulses are ordered in sequences that are characterized by an increase in width in their early phases and by an increase in frequency in their late phases (Figure 2). The choice to increase stimulus frequency to a maximum value of 40 Hz comes from the experience of experimental studies which report that the range of responsiveness to temporal recruitment by electrical stimulation occurs when frequency varies between 20 and 80 Hz;[43-45] in our experience, with this type of electrical pulse at 150 V, the maximum value of compound muscle action potential (cMAP) obtained with temporal recruitment by increasing pulse frequency was at about 30 Hz.

Different combinations of width and frequency made up the subphases, with various durations. In accordance to these pathway, we highlighted 3 subphases (Table I): during subphase A there is no variability in width and frequency values, during subphase B only width varies, and during subphase C only frequency varies. The width by frequency product (w*f) and the ratio between width and frequency (w/f) during the whole sequence were calculated. Both rates give information about the transition from subphase B to subphase C during the sequence, the former showing an increase of slope, the latter showing an inversion from incremental to decremental of the trend (Figure 2).

However, to distinguish the different components (width and frequency) during the analysis of H-reflex modulation, we used w/f to show the diversity of the "tonic" and "phasic" subphases of the stimulation, which

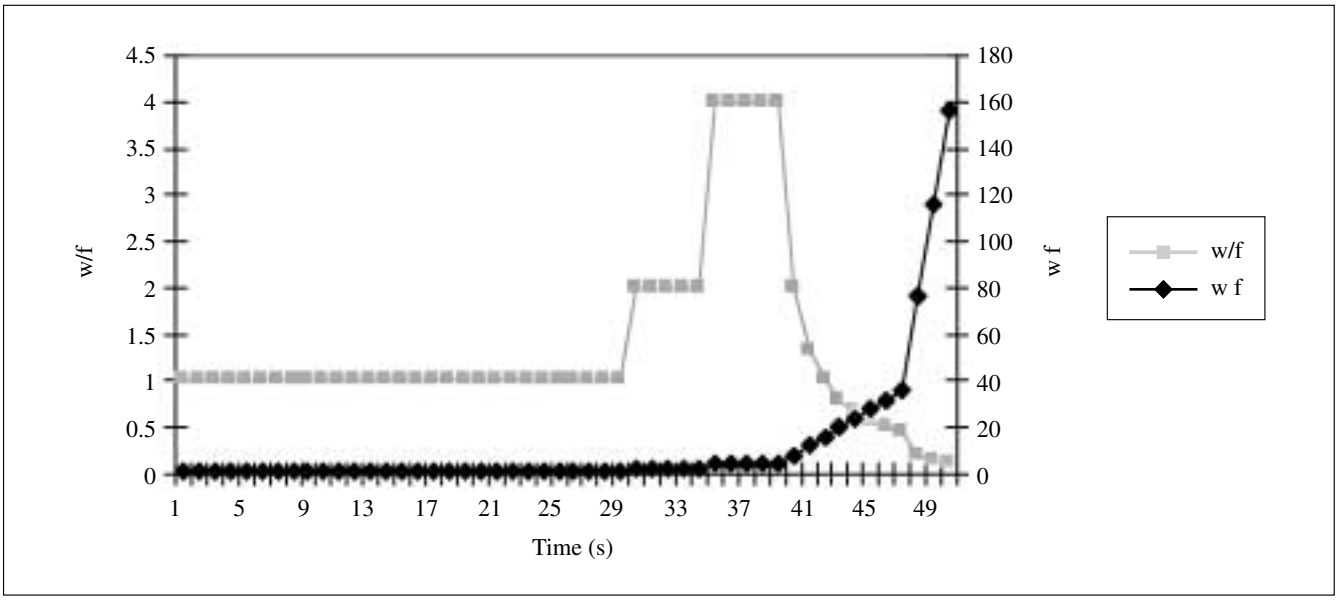

Figure 2.—Graphic view of Table I in real time. The gray line shows the w/f ratio variation [Hz /µs], the black line, the w*f variation /10 [Hz µs]. X axis: time (each FREMS sequence lasts 51 s).

allowed us to qualitatively differentiate B and C subphases. Indeed, when the stimulus frequency increases with the same width, that is in subphase C, we are able to observe the possible correlation between the variations of stimulus parameters and of H-reflex responses.

Signal recording setting

Recording and storing of cMAP and H-reflex signals were obtained through a multichannel data acquisition analogue-digital interface (1401plus®, Cambridge Electric Design, Science Park, Milton Road, Cambridge CB4 0FE, England) connected to a PC. A continuous time domain data capture and analysis software Spike 4® (Cambridge Electric Design, Science Park, Milton Road, Cambridge CB4 0FE, England) was used to record cMAPs and H-reflex was recorded by sweep-based data capture and analysis software Signal 1,94® (Cambridge Electric Design, Science Park, Milton Road, Cambridge CB4 0FE, England).

The sampling frequency rate of the device was set at 20 kHz. The electrodes used were: 1) one active surface EMG wide spaced pad electrode with 2 cm interelectrode distance for signal acquisition (Micromed, Mogliano Veneto, Treviso, Italy); 2) two stimulating cup electrodes with 3 cm interelectrode distance, each with 1 cm diameter, for nerve stimulation (Micromed, Mogliano Veneto, Treviso, Italy); 3) two 2.5 cm diameter non woven disposable electrodes with 3 cm interelectrode distance (Monitoring electrodes, Red Dot, 3M Health Care, D-41460 Neuss, Germany), for FREMS stimulation. According to the indications provided by the manufacturer, typical values of the key parameters, measured in 0.9% saline, between pairs of electrodes of the same size, DC offset voltage was 180 µV, drift 25 µV/h and noise 1 µV peak-to-peak (0.1-1000 Hz with 50/60 Hz eliminated). We used a 10-2000 Hz band pass filter and a 1902® pre-amplifier (Cambridge Electric Design, Science Park, Milton Road, Cambridge CB4 0FE, England) at 3 000 gain to acquire the analog signal, with AC couple and 50 Hz notch filter.

Compound muscle action potential summation sampling

A wide-spaced active surface EMG pad electrode with 2 cm interelectrode distance was placed on the surface of the AHM of the right foot. We then stimulated the tibial nerve at medial malleolus with FREMS sequence through another couple of surface cup electrodes; the distance between stimulation and recording electrode couple was 7 cm in all subjects. To test the consistency of our recordings, we repeatedly stimulated consecutively 3 times each subject, while recording EMG signals on a continuous track.

H-reflex sampling and frequency rhythmic electrical modulation system conditioning

The 10 subjects were comfortably lying on their abdomen; their ankle joint was maintained at an approximately 90° angle through a goniometer (Biometrics Ltd, Nine Mile Point, Gwent, NP11 7HZ, UK). FREMS was applied to the right *abductor proprius hallucis* muscle through 2 non woven electrodes. Simultaneously, we recorded the H-reflex through the triggering electrical stimulus of a Digitimer constant electrical stimulator mod. DS7A9®, (Digitimer Ltd, 37 Hydeway, Welwyn Garden City, Hertfordshire, AL7 3BE, England), with a stimulation rate of 0.5 Hz. We used a single stimulus of 1 ms width, at an intensity able to elicit the highest reproducible H-wave (H max) on the right posterior tibial nerve at the popliteal fossa, and a wide-spaced active surface EMG pad electrode with 2 cm interelectrode distance on the soleus muscle of the same limb. H-reflex amplitude was defined as the Hmax/Mmax ratio; in this way, all H-reflex variations were correlated to the constancy of stimulus effectiveness.

FREMS was administered according to the following protocol: 3 consecutive FREMS of 153 s (FREMS test), each one followed by an identical period without FREMS (control test); the same procedure was then repeated for 3 times. As a control, we recorded the H-reflex into the corresponding 6 periods (918 s) from every subject, without using FREMS, at least 3 h before and after FREMS application. No substantial Mmax variation occurred between the FREMS and control tests (P=0.62).

Signal interpretation and artifact removal

cMAP signal: once sampled, we selected a window starting at sample M of length N of the signal $x[n]$

$$x[M],\ x[M+1],\ldots,\ x[M+N-1]$$

Over sampled signals we calculated the peak amplitude, which is simply the greatest sample (in absolute value) over the window:

$$A_{peak}\{x[n]\} = max|x[n]|,\ n=M,\ldots,\ M+N-1$$

and the root mean square (RMS) amplitude:

$$A_{RMS}\{x[n]\} = \div\ P\{x[n]\}$$

where $P\{x[n]\}$ is the mean power, defined as:

$$P\{x[n]\} = \frac{1}{N}\left(|x[M]|^2 + \ldots + |x[M+N-1]|^2\right),$$

to reduce the noise/signal ratio.

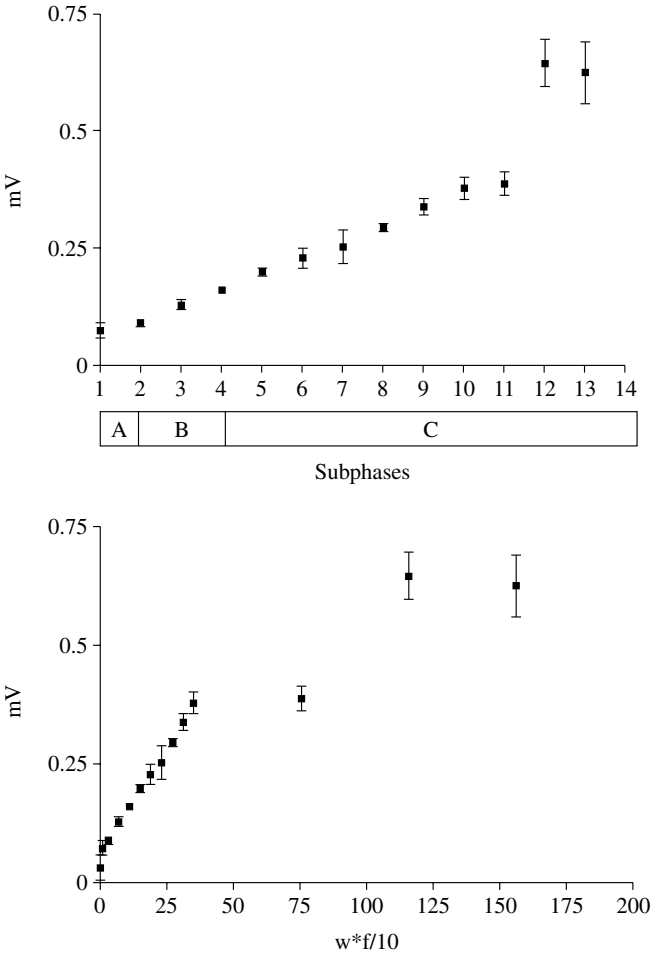

Figure 3.—Increase in the root mean square (RMS) compound muscle action potentials (cMAPs) amplitude (histogram) during the FREMS stimulation expressed in terms of the w*f ratio/10. The upper diagram shows the temporal relationship between the subsequent subphases (A, B, C) and RMS cMAPs increase, the above diagram shows the linear correlation between RMS cMAPs amplitude values and w*f ratio/10 (r^2=0.87, P <0.001). Mean data and standard deviation of the entire sample.

We calculated the RMS amplitude of the whole wave complex, removing from each wave the RMS amplitude corresponding to stimulus artefact.

H-reflex signal: Oh's guidelines[46] were employed to identify the H-reflex. Particularly, we analyzed the peak to peak amplitude of the H-reflex response main wave, by means of an automatic device that is able to recognize the maximum value between 2 static cursors, the former placed at the first deflection with respect to the isoelectric line, the later at the end of the

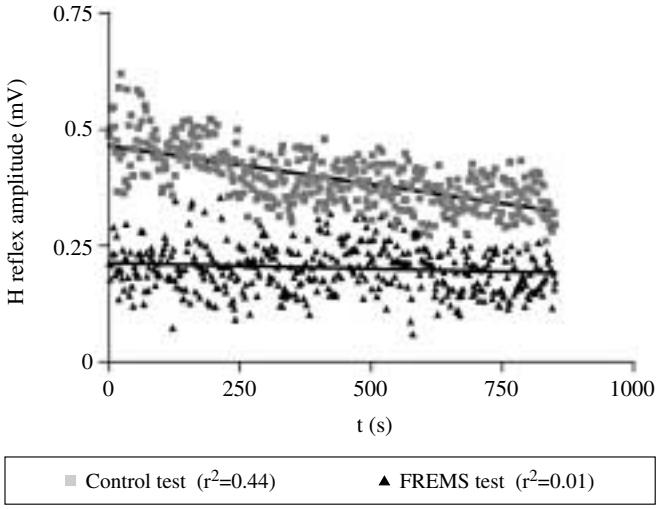

Figure 4.—Comparison between the H-reflex amplitude variation during the FREMS test and during the control test. During the FREMS test, the H-reflex amplitude appears to be about half that of the control test (FREMS test, minimum=0.06065; median=0.2017; maximum=0.3881; mean=0.2026±0.05; control test, minimum=0.2495; median=0.3896; maximum=0.6206; mean=0.3881± 0.06130). During the control test, a linear regression (R2=0.44) showed the H reflex adaptation. During the FREMS test, this adaptation and the linear regression were lost (R^2=0.01).

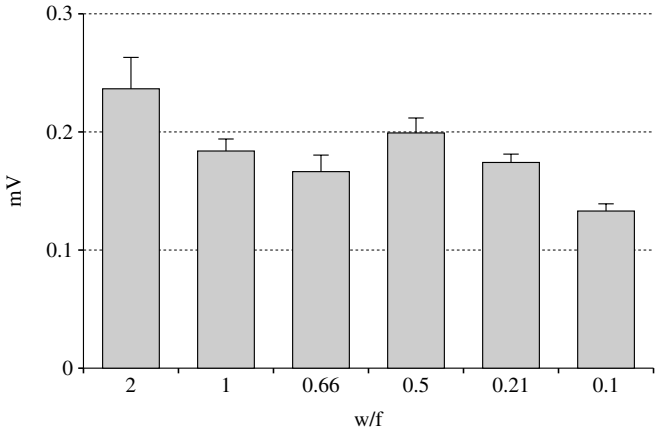

Figure 5.—H amplitude values during C subphase of the entire population. Mean values of 9 C subphases (3 subphases per 3 times in each FREMS test).

wave according to the manufacturer's suggestions of the data capture and analysis software. Neither signal smoothing nor rectification were performed. With a similar method we identified and recorded the Mmax value corresponding to Hmax. The value of H-reflex amplitude was the Hmax/Mmax ratio of every acquisition. Since the Hmax/Mmax ratio has a wide normal range, we took into account the change between baseline *vs* every acquisition rather than absolute values, so to maintain standard deviation (SD) <0.5 among subjects. A possible influence of the FREMS on the H-reflex recording device was excluded, since electrical field interference elicited by FREMS on the soleus muscle EMG track was easily recognized. When this interference occurred, we were able to detract it from the signal through mathematical elaborations.

Statistical analysis

Data are presented as means ± SD. The differences among cMAP increases were analyzed with one-way analysis of variance (ANOVA). The sequence of the H-reflex amplitude in time was analyzed by linear regression. The relationship between the H-reflex amplitude and the w/f ratio was analyzed by Pearson's linear correlation. Statistical significance was considered for P values below 0.05.

Results

Compound muscle action potential recruitment

We obtained cMAPs in the AHM by stimulating the tibial posterior nerve with FREMS. The highest cMAP amplitude, measured in terms of whole signal RMS amplitude (0.60±0.02 mV), was approximately 15 times lower than the cMAP amplitude obtained by the commonly used standard clinical neurophysiological devices (9±0.6 mV with 200-1 000 μs width stimuli). As shown in Figure 3, we found a significant positive correlation between cMAP and w*f, which means that an effector with a constant voltage is able to intensify the response increasing the impulse width and its frequency in time. A first increment of cMAP during the B subphase may be observed, when only w is changing, and a subsequent, more pronounced increment, at the end of the C subphase, when f is near to its maximum value. With a further increase of the stimulation frequency, reaching 39 Hz, RMS cMAP amplitude slightly decreased. The use of RMS amplitude measurement, which takes into account also cMAP conformation, allowed us to exclude that the observed increase could be due to pseudofacilitation phenomena which are secondary to subcutaneous ionization.[47]

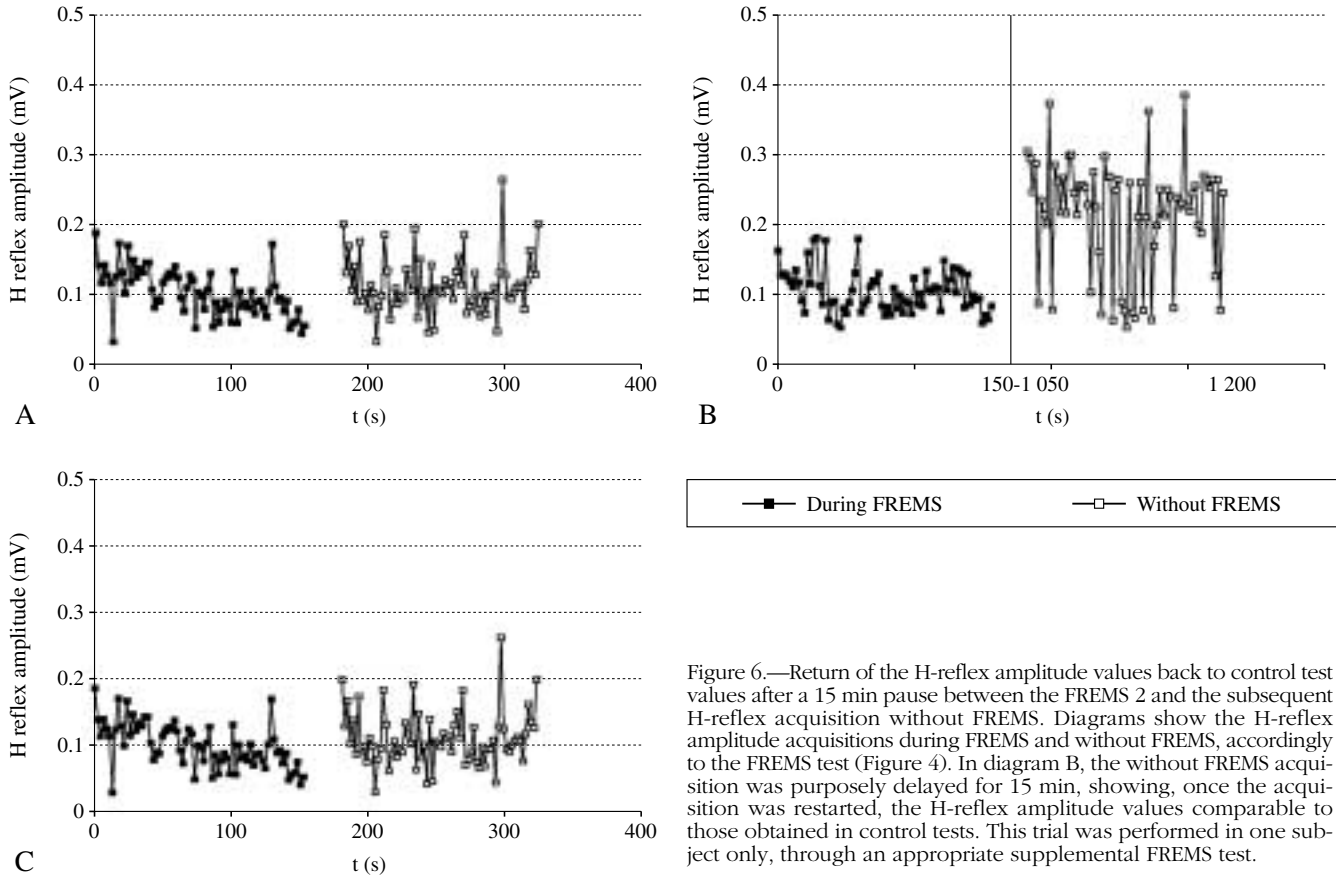

Figure 6.—Return of the H-reflex amplitude values back to control test values after a 15 min pause between the FREMS 2 and the subsequent H-reflex acquisition without FREMS. Diagrams show the H-reflex amplitude acquisitions during FREMS and without FREMS, accordingly to the FREMS test (Figure 4). In diagram B, the without FREMS acquisition was purposely delayed for 15 min, showing, once the acquisition was restarted, the H-reflex amplitude values comparable to those obtained in control tests. This trial was performed in one subject only, through an appropriate supplemental FREMS test.

H-reflex modulation

The H-reflex was elicited in all subjects without FREMS during sessions that were held not less than 3 h before or 3 h after FREMS application and was characterized by similar latency. Mean latency in our sample was 33.1±2.37 ms and mean Hmax/Mmax amplitude was 0.39 mV±0.06 mV. The recording of control H-reflex amplitude was carried-out for 918 s without FREMS stimulation, with a 0.5 Hz stimulation rate (459 samples). The H-reflex amplitude recording during FREMS conditioning (3 phases with 153 s of stimulation with the respective 3 phases of recovery, each one lasting other 153 s) was performed for 918 s. Figure 4 shows the H-reflex amplitude values of the whole population (mean values for each H-reflex sample of the 10 subjects) with and without FREMS. In the absence of FREMS, the H-reflex amplitude progressively decreased, with a negatively significant linear correlation with time (r^2=0.44): the longer the time, the lower the H-reflex amplitude. In the presence of FREMS, the H-reflex amplitude value immediately decreased and remained low, without following a linear relation (r^2=0.01).

The SD of the amplitude was in the same range with or without FREMS (0.061 *vs* 0.052), despite the fact that, with FREMS, the overall amplitude was considerably lower.

Subphases analysis

The relationships between the w/f ratio and the H-reflex amplitude were analyzed separately in the 3 subphases A, B and C. As the w/f ratio consistently varies only during the C subphase, we focused on this subphase. In fact, during each C subphase, there is a significant linear correlation between H-reflex amplitude and w/f ratio: during FREMS 1 r^2=0.37, during FREMS 2 r^2=0.78, during FREMS 3 r^2=0.41. Furthermore, all the data were taken together and

the linear correlation remained significant (r^2=0.43; P<0.001). Moreover, during the C subphase, the H-reflex amplitude values (6 values within 12 s of acquisition, from the end of B subphase till the end of C subphase) showed a trend that was identical in all subjects during the 3 times replicated FREMS (Figure 5). As shown in Figure 5, two consecutive decreasing trends appear in relationship with two sequential "halving" of w/f ratio (2-1-0.66 and 0.5-0.21-0.1). This suggests a stronger dependence of H-reflex amplitude modulation by the temporal sequence of conditioning stimulation than by the pure value of intensity of the stimulus. Instead, no significant trend was found in H-reflex amplitude values during A and B subphases.

Interestingly, during the FREMS recovery periods, the H-reflex amplitude oscillations lost the pattern shown during FREMS periods, but they remained within the same range, at least within its 153 s period. This suggests that: 1) the C subphase has the property to induce specific and reproducible H-reflex modulation despite maintaining mean H-reflex value low with respect to the control test; 2) FREMS is able to influence the H-reflex amplitude modulation for some time after its administration. Regarding this latter aspect, to assess the persistence of the FREMS effect on the H-reflex amplitude modulation, in one subject we prolonged the FREMS recovery period until the H-reflex amplitude values returned back to control test values. As shown in Figure 6 (A, B and C), this occurred 5-10 min after the end of FREMS administration (Figure 6 B).

Discussion

Spinal motoneuron excitability is under the influence of central descending pathways, systemic influences (*i.e.* endocrine and circulating neurotransmitters), propriospinal projections and peripheral reflex arcs. The peripheral reflex arcs (mono or multisynaptic) are integrated at a segmental level. The peripheral afferent is conducted through the central branch of spinal ganglia. Ganglionar neurons are connected to several peripheral receptors: spindles, tendon receptors, articular receptors and cutaneous receptors. Among the peripheral receptors, spindles are best connected to the α-motoneurons, intervening in the monosynaptic Sherrington's stretch reflex. The fusimotor system improves the spindles' ability to accurately encode wide ranges of velocity and length that occur in various natural tasks by shifting its relative importance and sensitivity to the range of length and velocity that the central nervous system (CNS) expects to occur during voluntary motor behaviour.

Although the deterministic model of the classical Sherrington's reflex is currently debated,[48, 49] the general mechanism seems still valid: the stretch afferent signal of the spindles facilitates the activity of α-motoneurons simultaneously to the elongation of the whole muscle. A series of studies, initiated by Capaday,[50] showed the H-reflex to be significantly smaller during walking than during running, suggesting regulated spinal reflex gains specific for the different functional requirements of the different motor behaviours. This phenomenon was explained in the context of presynaptic inhibition effected through interneurons. The interneurons are activated by primary afferent depolarization (PAD) mediated by high conduction velocity sensitive fibers.[51, 52] However, this explanation has been debated since its formulation. In Fung's study[53] a low intensity conditioning stimulus (2.5-3 x sensory threshold) was applied to the footplant of both healthy subjects and patients with spasticity and spinal damage showed this stimulation to reduce soleus H-reflex amplitude in both groups, but significantly so only during walking. This reduction was less pronounced in patients with spasticity, but only in certain motion stages. The author hypothesized that this phenomenon is mediated by Ia afferences, through both pre and postsynaptic inhibition.

Elicitation of compound muscle action potential by frequency rhythmic electrical modulation system

FREMS, applied along tibial nerve, induces the recruitment of cMAPs in the AHM, mainly during the frequency incremental subphase C, suggesting a functional pattern similar to the recruitment of neuromuscular junctions through incremental spike firing. The cMAP we obtained is smaller than cMAP obtainable through classical neurophysiological modalities, with pulse width >100 ms. We believe that this may depend on the narrow pulse width we used (10-40 μs), which implies that during the highest recruitment of skeletal muscle fibers by temporal summation only some of the nervous fibers contingent were recruited. The other important finding about cMAP recruitment by FREMS consists in the linear trend of cMAP incre-

ment, according to the incremental trend of both width and frequency of FREMS.

Effects of frequency rhytmic electrical modulation system on H-reflex

In the second part of the study, we applied the same stimulation sequence directly over the belly of AHM and assessed the conditioning effect of this recruitment on the H-reflex amplitude of soleus muscle. Considering that the stimulation near the muscle is not the same as the direct motor nerve stimulation, we investigated whether this modality of administration of motor subthreshold, but with sequentially ordered pulses, is able to influence the excitability of spinal motoneurons. We are as well reasonably certain that in our experimental setting (normal subjects, same posture, relaxing environment) the involvement of other peripheral pathways (afferents mediated by III ad IV type fibres) is marginal, due to a threshold which is higher than the FREMS intensity (FREMS intensity of individual pulses is below motor threshold). We could hypothesize a functional role for type II spindle afferents, but this experimental design can not specifically distinguish whether type Ia or II spindle afferents are involved. Thus, these results suggest that this modality of administration elicits direct and reproducible modulation of the excitability of the involved spinal motoneurons. Therefore, we propose that a determinant of H-reflex amplitude is not only the stimulus intensity, but that the temporal sequence of stimulations is as well fundamental at this respect.

During the C subphase of all FREMS cycles sampled, an increasingly stronger linear correlation is shown between the H-reflex amplitude and FREMS, expressed in terms of w/f ratio, in all subjects (r^2=0.43; P<0.001). This means that during the FREMS phasic subphase C we observe a precise and reproducible correlation between the H-reflex amplitude and the w/f variation in all subjects.

Relationship between spindles and H-reflex

We know that one of the most important regulatory systems of spinal excitability is the reflex pathway that originates from the muscle spindles and influences the excitability of the α-motoneuron pool directly or by means of inhibitory interneurons. We hypothesized that the electrical recruitment of muscular activity may be, at low stimulation intensities, more effective in muscle spindle activation rather than of the entire striated muscle due to the lower threshold of the spindles[54] independently from the fact that cutaneous fibres were anyway activated first. In our data H-reflex depression becomes evident just at the beginning of the FREMS conditioning, that is, before FREMS applied on the nerve have elicited cMAP. In our experimental setting we cannot well discriminate whether the contingent muscle spindle stimulation derives from electrical activation rather than the mechanical strain of the whole muscle. One important limitation derives from the recorded artifacts that arise when the FREMS muscle stimulation is produced through its nerve; furthermore, nerve stimulation by FREMS induces recurrent pulses that collide against efferent activity. Nevertheless, by directly stimulating the muscle, we obtained an immediate, dramatic drop in mean H-reflex amplitude values since the beginning of the FREMS test with respect to the control test. This might suggest that FREMS would be able to control α-motoneuron activity independently from its mechanical effects. In fact, a possible minimum contractile effect of FREMS on the neuromuscular system is apparent only during the last subphases of FREMS administration (subphase C), which are those where cMAP is well evident.

In control experiments (no FREMS) H-reflex amplitude showed a progressive spontaneous depression apparently due to an accommodation mechanism. By contrast, during FREMS the H-reflex amplitude remained constantly depressed in time (Figure 4). During the B phase FREMS is characterized by an increase of the width of the pulses (that are generated at a fixed frequency): the course of the H-reflex amplitude remains at low values with respect to those observed during the control test, but does not show significant tendencies. This inhibition is currently related to the activation of presynaptic inhibition mediated by cutaneous sensitive afferences,[33, 55-58] but is also compatible with the hypothesis that, during B phase, the afferent stimulation is carried on also by fast- and slow-nuclear bags, which are responsive to tonic stimulation. Instead, during C-phase, we have seen rapid oscillations of H-reflex just in correspondence to rapid changes in the frequency of stimulation, so we cannot exclude that chain nuclear intrafusal fibers might be activated by progressive frequency increase of FREMS.

The rapid variation of the w/f ratio during this phase could indicate that it is possible to change the H-

reflex amplitude in strict correspondence to FREMS frequency and pulse width modifications. This correlation, which indicates receptor involvement, prompts us to hypothesise that, beyond the possible H-reflex modulation through presynaptic inhibition through cutaneous afferent stimulation, the control of the excitability of the motoneurons involved occurred by means of the neuromuscular spindle contraction of the AHM similarly to what takes place during voluntary movement carried out through α-motoneurons.

Another physiological implication of this study is that the effect we observed was the persistence of the depression of mean H-reflex amplitude during 153-s recovery periods without FREMS and, mostly, during the about 5-min expectation from the end of the second FREMS cycle and initiation of the third cycle, as shown in Figure 6. This could have some relevance in terms of adaptive facilitation of spinal inhibitory activity. *Vice versa*, but presumably with a similar functional meaning, Kitago *et al*.[59] obtained a strong increase of the H-reflex amplitude (24%) after 30 tetanic stimulation of the tibialis nerve (10 s at 300 Hz; 10 s rest). The effect lasted 4-16 min. These data point to the possibility to obtain persistent changes of H-reflex by specific conditioning temporal patterns of transcutaneous electrical stimulation. Interestingly, the same group[60] has recently reported the possibility to achieve an adaptive acquisition of locomotor skills associated to a transient depression of H-reflex. It seems that, by noninvasive techniques, it is possible to induce plastic short-lasting effects over the spinal neuromotor activity. Further studies, involving patient populations, for example spastic or dystonic, may help clarify the clinical implications of this approach.

Conclusions

We demonstrate the possibility to modulate the excitability of motoneurons by applying non-painful stimuli sequences on skin surface. This may be the basis for new therapeutical modalities for the treatment of movement disorders, such as those characterized by abnormal motoneuron excitability.

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