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Technical note

A method to estimate EMG crosstalk between two muscles based on the silent period following an H-reflex

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ABSTRACT

The crosstalk phenomenon consists in recording the volume-conducted electromyographic activity of muscles other than that under study. This interference may impair the correct interpretation of the results in a variety of experiments. A new protocol is presented here for crosstalk assessment between two muscles based on changes in their electrical activity following a reflex discharge in one of the muscles in response to nerve stimulation. A reflex compound muscle action potential (H-reflex) was used to induce a silent period in the muscle that causes the crosstalk, called here the remote muscle. The rationale is that if the activity recorded in the target muscle is influenced by a distant source (the remote muscle) a silent period observed in the electromyogram (EMG) of the remote muscle would coincide with a decrease in the EMG activity of the target muscle. The new crosstalk index is evaluated based on the root mean square (RMS) values of the EMGs obtained in two distinct periods (background EMG and silent period) of both the remote and the target muscles.

In the present work the application focused on the estimation of the degree of crosstalk from the soleus muscle to the tibialis anterior muscle during quiet stance. However, the technique may be extended to other pairs of muscles provided a silent period may be evoked in one of them.

1. Introduction

Surface electromyography is a powerful tool extensively used to provide insights into muscle physiology and motor control, as well as for the evaluation and diagnosis of neuromuscular diseases.

Although the surface electromyogram (EMG) provides an easy assessment of muscle activity, caution should be taken since the signal recorded from a given muscle may not mirror exclusively its intrinsic activity. One may record at the same time the activity from neighboring and underlying muscles. The electric potential generated by a remote muscle propagates through the volume conductor to the electrodes placed over the target muscle. This unwanted interference could lead to false interpretations of neurophysiologic phenomena (e.g. [1–3]).

In many applications, surface EMG is preferable to invasive recording because it provides a more global measurement of muscle activity. For example, in most studies of posture and gait it is more interesting to analyze muscle activation patterns rather than the activity of individual motor units. However, the researcher must always be wary of the possibility that a part of what he/she is recording actually comes from another muscle.

During the gait cycle, for example, the recorded EMG reflects a forceful and alternated activity of the soleus (SO) and tibialis anterior (TA) muscles [4] and, usually, the crosstalk between both muscles may be considered as negligible [5]. Nevertheless, in other studies of the same muscles [6] their activity may be relatively low, e.g., during quiet stance, and the crosstalk may lead to wrong interpretations of the data.

Ideally, one could estimate the level of crosstalk by asking the subject to contract a given remote (or source) muscle (e.g., the SO) and recording the volume-conducted activity on the target muscle at rest. The drawback of this approach is that one cannot assure the voluntary activation of a given single muscle without the activation of synergists, or even antagonists acting around a joint [2,7–9].

The compound muscle action potential (CMAP) elicited by electrical stimulation can be used as a tool to evaluate how much the remote muscle activation contaminates the EMG signal of the target muscle [8–10]. However, in most situations the aim would be to estimate the crosstalk between two muscles during their natural contraction as occurs during upright stance, during gait or during any other postural task. Instead of the electrically elicited synchronized motor unit potentials, the EMG interference pattern is characterized by the asynchronous discharges of the recruited motor units. Consequently, the crosstalk estimation based on the CMAP experiments may not be extrapolated to voluntary contraction [11] since the volume conductor properties may affect both

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signals (CMAP and EMG asynchronous activity) in different ways, resulting in different patterns and levels of crosstalk.

We focused on an approach in which it is possible to briefly abolish muscle activity involuntarily (e.g., of a postural muscle during upright stance). This can be achieved by applying an electrical percutaneous stimulus that activates the Ia afferent axons of a mixed nerve (that supplies the remote muscle) to elicit a reflex response (H-reflex). The H-reflex is the electric homologue of the muscle stretch reflex and is observed in the EMG as a CMAP resulting from the synchronous reflex discharge of the motoneurones. During a sustained muscle contraction, the H-reflex is followed by a brief period of absence of EMG activity ("silent period") due to spinal mechanisms, such as motoneuronal refractoriness and recurrent inhibition [12,13].

This possibility of "turning off" (briefly) the electrical activity of a given muscle was the motivation behind the approach proposed in this paper to quantify the level of crosstalk from that muscle – called here the *remote* muscle – to the muscle whose EMG one is actually interested in studying and analysing—called here the *target* muscle. The remote muscle is "turned off" briefly (silent period) and the resultant effect on the target muscle is measured. This is compared with the ongoing background EMG levels of both muscles.

For the crosstalk estimation, the root mean square (RMS) values of the EMGs of both the remote (R) and target (T) muscles are calculated at two periods: *before the stimulus delivery* (background EMG activity) and *during the silent period* of the remote muscle.

The present work illustrates the new protocol to estimate the crosstalk level between two leg muscles during a specific task, namely, the control of upright posture. However, the proposed technique is potentially useful for crosstalk assessment between different muscle pairs provided a silent period is obtainable from one of the muscles.

2. Methods

2.1. Mathematical formulation

All EMGs were assumed to be stationary stochastic processes with zero mean during the ongoing background activity and during the silent period which follows an H-reflex. Under these assumptions the square root of the variance is equal to the RMS value.

The remote muscle is supposed to have an ongoing background EMG activity Rb (Fig. 1a and c) with RMS value represented by $\underline{Rb}$. This activity causes a crosstalk signal Rb_c on the target muscle with an RMS value indicated by $\underline{Rb_c}$. It is a fraction γ of $\underline{Rb}$, where γ is a non-negative real number, smaller than 1:

$$\underline{Rb_c} = \gamma \underline{Rb} \quad \text{(I)}$$

If $\underline{Tb}$ indicates the RMS level of the EMG recorded from the target muscle (Tb) during background activity (see Fig. 1b and d), then the proposed crosstalk index (CI) is defined as the fraction of the total muscle activity that is attributable to crosstalk due to the remote muscle:

$$CI = \frac{\underline{Rb_c}}{\underline{Tb}} \quad \text{(II)}$$

The CI defined in Eq. (II) is similar to the inverse of a signal-to-noise ratio, the noise being the crosstalk from the remote muscle.

From the equations above:

$$CI = \gamma \frac{\underline{Rb}}{\underline{Tb}} \quad \text{(III)}$$

The challenge is then to evaluate γ. This can be achieved with the help of the measurements of the activity levels of the remote and target muscles during the silent period.

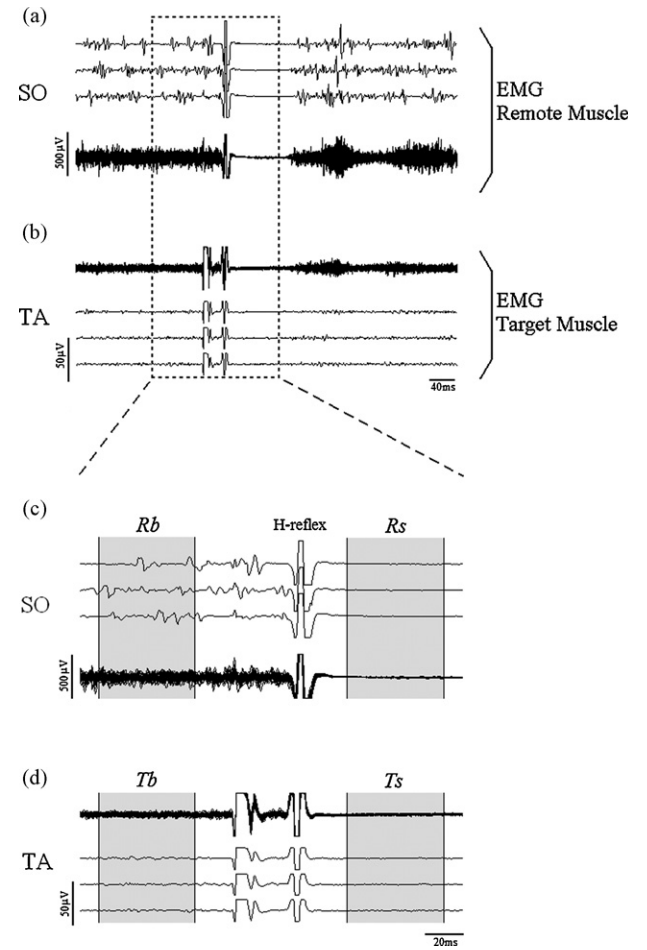

Fig. 1. EMG recordings obtained from a subject during upright stance. (a) EMG recordings from the remote muscle (SO). The three uppermost traces exemplify individual recordings obtained in response to each electrical stimulus. These are followed below by a superposition of 50 individual traces. (b) EMG recordings from the target muscle (TA). The equivalent superposition of 50 individual EMG recordings followed by three examples of individual recordings. Note the SO H-reflex recorded at a distance from the TA electrodes. (c and d) Expansions of the same recordings from (a) and (b) (SO and TA, respectively). The shadowed regions represent the 50 ms periods (*Rb*, *Rs*, *Tb* and *Ts*) used to evaluate the crosstalk index *CI* (see text).

The background EMG activity recorded at the target muscle (Tb) is the sum of the intrinsic activity of the target muscle itself (Ti), the crosstalk from the remote muscle (Rb_c) and the crosstalk from other muscles (Ob_c). These random signals, Ti, Rb_c and Ob_c are assumed uncorrelated and hence, the variance of Tb is the sum of the variances of Ti, Rb_c and Ob_c:

$$\underline{Tb}^2 = \underline{Rb_c}^2 + \underline{Ti}^2 + \underline{Ob_c}^2 \quad \text{(IV)}$$

During the silent period, the target muscle EMG (Ts) is equal to the sum of the crosstalk from the remote muscle during the silent period (Rs_c), the intrinsic activity of the muscle itself (Ti) and the crosstalk from other muscles (Ob_c). Note that here the assumption is that the activities of both the target muscle and the other muscles that cause crosstalk (except for the remote muscle) are not affected by the stimulus that causes the silent period on the remote muscle. Again, the random processes are assumed uncorrelated, resulting in

$$\underline{Ts}^2 = \underline{Rs_c}^2 + \underline{Ti}^2 + \underline{Ob_c}^2 \quad \text{(V)}$$

Subtracting (V) from (IV) yields:

$$\underline{Tb}^2 - \underline{Ts}^2 = \underline{Rb_c}^2 - \underline{Rs_c}^2 \quad \text{(VI)}$$

The remote muscle activity has its activity reduced by a factor α during the silent period with respect to the background level, i.e.:

$$\underline{Rs} = \alpha \underline{Rb} \qquad \text{(VII)}$$

As a first approximation, a linearity hypothesis (see ahead) gives the relation

$$\underline{Rs_c} = \gamma \underline{Rs} \qquad \text{(VIII)}$$

Combining (VII) with (VIII) gives

$$\underline{Rs_c} = \alpha\gamma \underline{Rb} \qquad \text{(IX)}$$

Squaring (I) and (IX) and substituting in (VI) gives

$$\underline{Tb}^2 - \underline{Ts}^2 = \gamma^2 \underline{Rb}^2 - \alpha^2\gamma^2 \underline{Rb}^2 \qquad \text{(X)}$$

which together with (VII) results in

$$\gamma^2 = \frac{\underline{Tb}^2 - \underline{Ts}^2}{\underline{Rb}^2 - \underline{Rs}^2} \qquad \text{(XI)}$$

Finally, (XI) in (III) gives the expression for *CI* which depends on the measured RMS levels during the background and silent periods of both the remote and the target muscle:

$$CI = \sqrt{\frac{1 - (\underline{Ts}/\underline{Tb})^2}{1 - (\underline{Rs}/\underline{Rb})^2}} \qquad \text{(XII)}$$

2.2. Experimental set up and acquisition

Six volunteers aging 28.7 ± 5.6 years (mean ± STD), five males and one female, without any neurological impairment were tested in the present experiments. The protocol was approved by a local ethics committee. The experimental paradigm was tested with the subjects in upright stance. During sitting, the isometric ankle torque was measured under voluntary activation of the TA.

Two disc electrodes with a diameter of 0.8 cm were placed medially over the SO belly, 4 cm and 6 cm beneath the junction of the two gastrocnemii heads [14]. The TA disc electrodes (0.8 cm diameter) were situated at the first proximal quarter of the fibula with 2 cm inter-electrode distance.

All the electrodes were fixed with an anti-allergic tape. The skin was prepared with a special-purpose sandpaper in order to obtain an impedance lower than 20 kΩ (in some instances, impedances below 5 kΩ were attained). The ground electrode was located around the most distal portion of the tibia. The bandwidth of the filter was from 10 Hz to 1 kHz, and the sampling rate was 2.5 kHz.

The electrical stimuli with 1 ms duration were applied by surface electrodes located at the popliteal fossa (diameter 7.5 mm, inter-electrode distance 2 cm), to evoke the soleus H-reflex. The stimulus intensity was adjusted to elicit an H-reflex with a peak-to-peak amplitude within 20–30% of the maximum direct response (20–30%Mmax). An EMG time window of 600 ms duration was acquired (200 ms before and 400 ms after the stimulus delivery).

The stimuli were applied by a MEB 4200 (Nihon-Kohden), triggered in synchronism with the signal acquisition. The signals were converted into ASCII and processed using MATLAB.

2.3. Procedures

One hundred electrical stimuli at 0.3 Hz were delivered to the tibial nerve of the subjects. The SO was defined as the remote muscle, i.e., the source of crosstalk. The TA was defined as the target muscle, i.e., where the crosstalk level is to be evaluated.

The RMS values were evaluated for the muscles involved in two different periods of 50 ms: background EMG activity and silent period (following the H-reflex of the SO) (Fig. 1). The waveform observed in the TA's EMG at the same latency of the SO H-reflex was due to the volume-conducted potentials originated from the SO [1].

The RMS values $\underline{Rb}$ and $\underline{Tb}$ corresponding to the background EMGs *Rb* and *Tb* of equal duration within windows of 50–100 ms before stimulus delivery were computed (Fig. 1c and d). The RMS values corresponding to the silent period for the remote and the target muscles ($\underline{Rs}$ and $\underline{Ts}$, respectively) were also computed for windows of 50–100 ms duration. The EMG epoch to evaluate $\underline{Rs}$ and $\underline{Ts}$ was specified for each subject, since the silent period duration and the latency of the H-response in the remote muscle may vary among the subjects. The RMS values associated with all these periods were calculated after the subtraction of a best-fit straight line (*detrending*) to the samples in a given EMG section (indicated by the shaded region in Fig. 1c and d).

The formula presented in Eq. (XII) was applied to each EMG epoch within each record of a given subject. The 100 values of *CI* obtained from the records in a trial were averaged resulting in an overall average index.

For the purpose of verifying the validity of the linearity assumption, i.e., a constant value for γ, in the mathematical derivations presented above, values of γ were estimated using expression (XI) for different RMS values $\underline{Rb}$ of the remote muscle (SO) EMG. The $\underline{Rb}$ fluctuated along each trial associated with the postural oscillations that occur naturally during the standing position [6] covering a range of values of physiological significance. Thus, if the proportion of crosstalk signal is constant, one would expect a horizontal regression line in the plot of γ as a function of $\underline{Rb}$. A *t*-test was used to detect differences in the slope α of the fitted regression line from zero [15] (significance level set at $P < 0.05$):

$$t = \frac{\alpha * STD(\underline{Rb}) * \sqrt{n-1}}{STD(\gamma) * \sqrt{(1-r^2) * (n-1)/(n-2)}},$$

where n is the number of observations, r is the correlation coefficient, $STD(\underline{Rb})$ and $STD(\gamma)$ are, respectively, the standard deviation of the RMS values of the background remote muscle activity and the standard deviation of the γ values (obtained from each of the 50–100 sweeps). Some outlier points were discarded from this analysis.

As an additional tool for the verification of linearity, the linear regression between the cross-talked H-reflex waveform amplitudes measured from TA (H_TA) and the SO H-reflex amplitudes (H_SO), for each subject, was analyzed visually. Thereafter, the *t*-test described above was used to test the null hypothesis that the slope of the regression line between the ratios of cross-talked H-reflex waveform amplitudes measured from TA to SO H-reflex amplitudes (H_TA/H_SO) as a function of H_SO was zero.

3. Results

During the silent period of the SO there was a simultaneous decrease in EMG activity of the TA (Fig. 1a–d). Also, at the end of the silent period, an increase in EMG activity with a spindle-shaped envelope in the SO EMG was observed simultaneously with a similar spindle-shaped EMG activity in the TA (see the superimposed traces in Fig. 1a and b). The silent period of the target muscle was not associated with a refractoriness of its motoneurons, since no H-reflex was elicited in the TA (the cross-talked H-reflex from the SO is seen in the TA EMG).

Fig. 2b and d shows a typical EMG record of the TA muscle during a weak isometric activation (at 0.75% of the maximum voluntary contraction—MVC; Fig. 2a) in a seated position, as compared with the EMG of the same muscle during quiet stance (Fig. 2c and e). The low level of background noise observed in the muscle during quiet

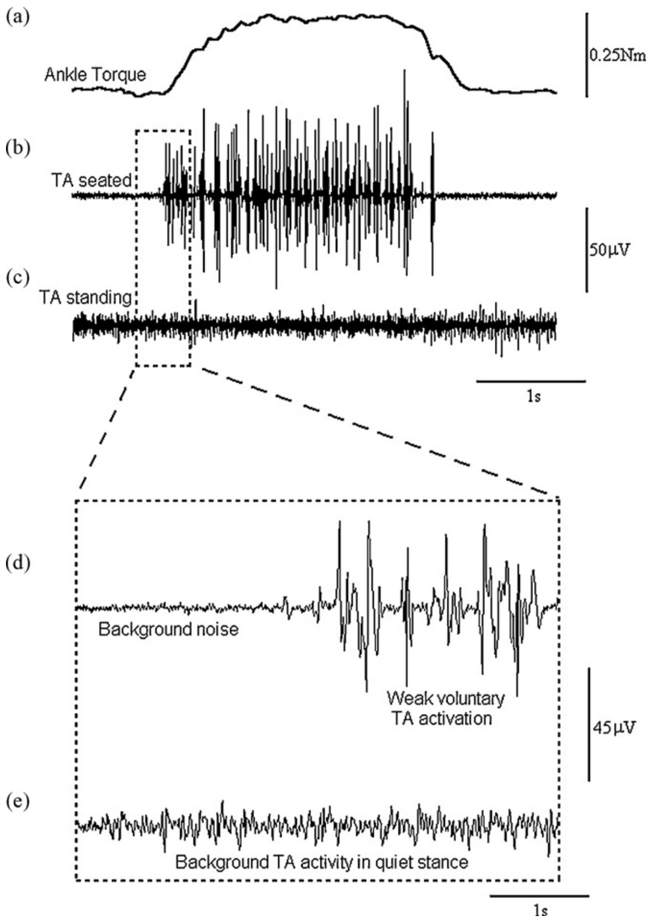

Fig. 2. EMG signals from the TA in seated position exerting a small torque and in the upright position. (a) Torque recorded during a ramp-and-hold isometric ankle dorsi-flexion of one subject in a seated position. (b) The corresponding EMG activity of the TA. Note the bursts of motor unit firings occurring during this gentle muscle contraction (0.75% MVC). (c) EMG activity recorded in the TA during upright stance. (d and e) Expanded views of the EMG signals in (b and c), respectively. Compare the background noise recorded from the TA in both conditions at rest (d) and during upright stance (e), as well as, the actual TA activity during a mild contraction (second half of the trace showed in d) with the background activity during stance (e).

stance in this subject suggests that the TA EMG activity recorded in upright stance is not intrinsically generated in the TA, but is indeed a crosstalk signal from the SO and, perhaps, other muscles.

The γ values plotted against the background remote (SO) muscle activity level in RMS ($\underline{Rb}$) are shown in Fig. 3a for a single subject. It is clear that for the depicted case the γ values remained relatively constant for different values of $\underline{Rb}$. The slope of the regression line was not significantly different from zero ($P > 0.05$). The regression lines obtained from all the six subjects are presented in Fig. 3b. The *t*-test detected significant difference from zero slope ($P < 0.05$) for the three steepest lines.

Therefore, the null hypothesis that the regression line relating γ and $\underline{Rb}$ had zero slope was not rejected ($P > 0.05$) for three of the subjects. Hence, for these subjects, the linearity assumption adopted in the mathematical derivations seems to be a reasonable approximation. This means that a single value for *CI* may be representative for the recordings obtained from the muscle pair. For the remainder 3 subjects the test indicated that the regression line was better represented by non-constant values of γ as a function of $\underline{Rb}$. For these subjects, the *CI* value would depend on the level of voluntary contraction of the remote muscle.

The linear regression relating the peak-to-peak H-reflexes from SO (these reflex amplitudes change randomly every time they are elicited [16]) and the cross-talked waveform peak-to-peak amplitude recorded from the TA showed (visual analysis) that in five out of six subjects the line intercepted the axes almost at the origin, as illustrated in Fig. 3c for one of the subjects. The statistical test for the slope of the regression line relating H_TA/H_SO and H_SO indicated differences from zero ($P < 0.05$) in three of the six subjects tested. Notwithstanding the overall results being similar to those obtained for the γ values, the slopes of the lines were different (Fig. 3b versus d) (see Section 4).

To illustrate the estimation of the levels of crosstalk, the *CI* (computed by Eq. (XII)) was evaluated for the three subjects that passed the linearity criterion. The corresponding *CI* values resulted: 84.8 ± 1.72%; 74.7 ± 1.98% and 74.2 ± 2.88% (mean ± SEM).

4. Discussion

In the present work we approached the problem of estimating the crosstalk caused by a specific remote muscle on a target muscle by taking advantage of the generation of a silent period in the remote muscle. The protocol consisted in measuring the EMG signals from both muscles during a maintained activity and also during an evoked silent period in the remote muscle. This silent period followed the occurrence of a reflex compound action potential caused by the stimulation of the afferents of the nerve that activate the remote muscle.

Some approaches have been proposed in the literature to evaluate crosstalk between two muscles, such as cross-correlation [17–19] and protocols based on the activation of the remote muscle and analysis of the EMG acquired from the target muscle [5,10,20,21]. The limitations of these approaches have also been pointed out in the literature [7–9,11]. Concerning the voluntary recruitment of a single muscle, it is extremely difficult to activate just one muscle, i.e., it is not easy to voluntarily separate the neural drive from different muscles [2,7–9]. These biophysical and functional restrictions may result in misleading crosstalk evaluations. In addition, the use of intra muscular electrodes to check for the presence of crosstalk [10,22] may lead to errors, because the recordings are rather selective and absence of activity is not a proof of absence of crosstalk or muscular activation [2,9].

To overcome the problem raised by the voluntary contraction approach, some authors have adopted the use of electrical stimulus to selectively activate the muscle of interest [8,23]. However, the estimated crosstalk may be in error due to the different time-spectral characteristics of CMAPs and the EMG interference pattern.

The advantage of the present proposed protocol for crosstalk assessment is that it is based on the EMG interference pattern itself (physiological patterns of motor unit discharge) instead of the use of the CMAPs (synchronous motor unit firing). Therefore, the present *CI* is evaluated from EMG signals of physiologically relevant amplitudes and frequencies [23], while the subject is performing a natural motor task (upright posture maintenance in our example). The approach relies on an electrophysiologically generated muscle silence (see Fig. 1) in opposition to techniques that involve asking subjects to contract a given muscle.

The mathematical derivations adopted in the present study were based on two assumptions. First, the target and the other muscles that may cause crosstalk (besides the remote muscle) are not responsive to the nerve stimulation to the remote muscle. This may be verified in practice by visual observation and palpation besides the reliance on neuroanatomical knowledge. Second, the crosstalk ratios (γ) during background activity and during the silent period from the remote to the target muscle were assumed equal, which implies a linearity condition (actually, the homogeneity or proportionality part of the linearity condition). The data from Fig. 3 indicated that the proportionality assumption was a valid approximation for half of the subjects tested. Therefore, for those subjects,

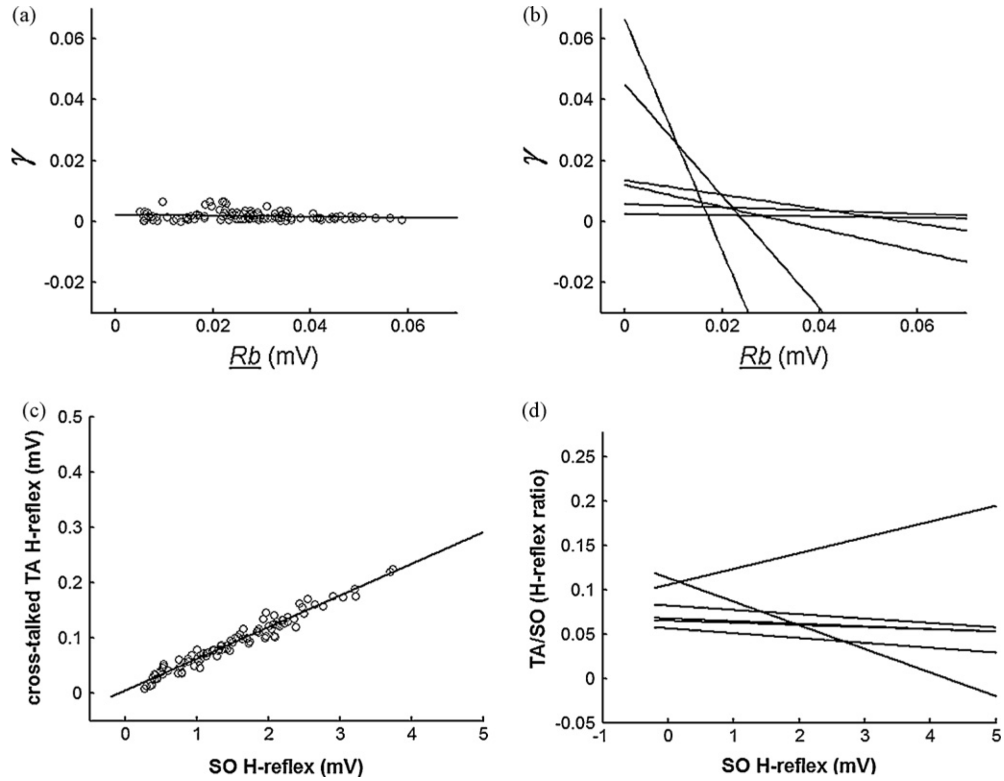

Fig. 3. Regression lines for the relations between the γ values and $\underline{Rb}$ and also between the crosstalk waveforms from H-reflexes. (a) Regression line fitting the γ values and $\underline{Rb}$ from one subject. The *t*-test failed to reject the hypothesis of zero slope ($P > 0.05$). (b) The regression lines from all subjects, with the 3 steepest lines having slopes significantly different from zero ($P < 0.05$). In (c) one has the scatter plot of amplitudes of H-reflexes from the SO muscle (H_SO) in the abscissa and the cross-talked waveform recorded in the TA muscle (H_TA) in the ordinate, from one subject. (d) Regression lines evaluated from the ratios H_TA/H_SO as a function of the H_SO values for all subjects. Again, 3 out of the 6 subjects showed significant difference from zero in the regression line slope ($P < 0.05$).

a *CI* can be readily evaluated irrespective of the level of muscle contraction. For the remainder subjects, the *CI* should be estimated for each different muscle activation level.

We also used CMAP signals to study the proportionality condition, with similar statistical results to those obtained before using the γ. Even though the reflex CMAPs could be an additional approach to check the proportionality condition, a measurement based on the interference pattern of the EMG (as used here in studying the relation between γ and $\underline{Rb}$) seems more representative of the physiological characteristics of the crosstalk associated with ongoing EMG activity.

The subjects showed a considerable degree of crosstalk from SO to TA under the experimental condition of very low background TA activity. High values of *CI* might be related to the very low $\underline{Ti}$ values (see Eqs. (II) and (IV)). Indeed, Fig. 2 shows that the TA muscle was not active during quiet upright stance. Fig. 2b and c clearly show that for a seated subject even a very low TA activity (producing an isometric ankle dorsi-flexion torque of 0.75% of MVC; Fig. 2a) was characterized by sparse motor unit potentials that had higher amplitudes than the background EMG activity observed during quiet stance. However, the background activity recorded at the TA during upright stance (Fig. 2e) is higher than the background noise observed in the first half of Fig. 2d. This suggests that the low level EMG background activity recorded in TA during upright stance is primarily due to crosstalk from the soleus and with a secondary contribution from other leg muscles and artificial electrical noise (amplifier electronic noise, electromagnetic interference, etc.).

In the particular case of quiet upright posture, or during forward body inclination, or over a downward inclined surface [6], the SO seems to have a great importance in the control of balance [24] while the TA is practically always silent. Therefore, in most subjects the SO should have a strong effect on the crosstalk to the TA. However, other extensor muscles that generate ankle torque to counteract the forward fall [25] might also contribute to the crosstalk observed in the TA in different experimental situations.

In the case of no TA intrinsic muscle activity during stance (tested as in Fig. 2, for example) the expressions derived before may be useful to estimate the relative contribution from the SO and from the other sources to the crosstalk recorded at the TA muscle. The results obtained from one of the records (out of the 100) of one subject were: $\underline{Ti} = 0$, $CI = 0.87$, $\underline{Tb} = 2.1\,\mu\text{V}$ and $\underline{Rb} = 50.8\,\mu\text{V}$. Then, from (III) $\gamma = 0.87{*}2.1/50.8 = 0.036$ and from (I) $(\underline{Rb_c})^2 = 3.34\,\mu\text{V}^2$. From (IV) one gets $4.41\,\mu\text{V}^2 = 3.34\,\mu\text{V}^2 + (\underline{Ob_c})^2$. From this, the RMS value of the crosstalk recorded at the TA originating from muscles other than the SO (plus possible contributions from artificial electrical noise) is equal to $1.07\,\mu\text{V}$. Thus, for the specific record of this subject, the SO contributed with about 76% of the crosstalk signal variance recorded in the TA while other sources (leg muscles such as peroneus longus and peroneus brevis and artificial electrical noise) contributed with 24% altogether in signal variance. Note that, these values (for simplicity reasons) refer to RMS measurements in only one record out of the one hundred.

The present protocol could also be useful to estimate crosstalk during co-contractions of both remote and target muscles, provided the hypotheses of the mathematical derivations are satisfied. In this case, the relative contribution of the remote muscle to the EMG recorded at the target muscle would be lower than that found in the present experiments, hence decreasing the *CI* value.

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Conflict of interest statement

There is no conflict of interest.

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