

Conditioning effect in single human motoneurons: a new method using the unitary H reflex

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1. A new method for the study of spinal reflexes using single motor units is described.
2. The excitability of a motoneurone is assessed as the 'critical firing stimulus' (CFS), which is the difference between the test stimulus intensity needed to reach the threshold for the lowest threshold Ia fibres and the intensity which evokes firing of a motor unit with the probability of 50% (FP_{50%}). The intensity with FP_{50%} is obtained by modulating stimulator output. When the motor unit is fired by a stimulus, the next intensity is decreased, and vice versa. The Ia threshold is defined as the threshold for homonymous monosynaptic peaks in PSTHs during contraction of the muscle examined.
3. A conditioning effect is represented as a change in CFS, the extent being expressed as a percentage of the unconditioned CFS.
4. Effects obtained by conditioning stimulation with the new and conventional H reflex methods are compared. The sensitivities are almost the same and the extents of the effects have highly correlated linear relations for the two methods.
5. The advantages of the new method are (1) that it shows reflex activities on a single motoneurone, (2) that it is applicable both to muscles at rest and during contraction, and (3) that it quantifies conditioning effects as percentages of the size of test Ia EPSPs.

In experiments to analyse human spinal circuit systems the H reflex has been used extensively as the test reflex, its size being assumed to be an indicator of the excitability of the motoneuronal pool investigated. Conditioning stimulation to a nerve which, directly or indirectly via interneurons, has a neuronal connection to the motoneurons evokes postsynaptic potentials in the motoneurons. Postsynaptic potentials, excitatory or inhibitory, are assessed in terms of change in the size of the test H reflex produced by the conditioning stimuli. Supraspinal and segmental control of the excitability of the reflex pathways are studied by comparing conditioning effects in various situations, e.g. in the resting state and during (or at the onset of) the voluntary contraction of a particular muscle.

Assessment naturally depends on the physiological characteristics of the motoneuronal pool. An important factor is the dependence of the extent of the conditioning effect on the size of the test H reflex (Crone, Hultborn, Mazières, Morin, Nielsen & Pierrot-Deseilligny, 1990). When the test reflex sizes are different among comparable situations (e.g. at rest and during voluntary contraction), even with a fixed intensity for test stimuli, it would often be very difficult to compare quantitatively the extent of the conditioning effects for each situation. For this reason, equilibration of the sizes of test reflexes in order to obtain comparable situations is widely recommended (Crone,

Hultborn & Jespersen, 1985; Fournier, Meunier, Pierrot-Deseilligny & Shindo, 1986; Crone, Hultborn, Jespersen & Nielsen, 1987). This is done by adjusting the test stimulus intensity so that the test reflex sizes become the same. This manoeuvre is used in order to sample the same motoneuronal population for the test reflex among all the situations compared. Equilibration is thus based on the assumption that distributions of motoneurons sampled as test reflexes are identical when the test reflex size is constant. However, the sampled motoneurons do not necessarily give the same distribution during the resting state as during tonic contraction of the antagonists (T. Hashimoto, M. Shindo, S. Yanagawa, N. Yanagisawa, unpublished observation). Another important factor is the change in the recruitment gain of the input-output relationships of the motoneuronal pools (Kernell & Hultborn, 1990; Nielsen & Kagamihara, 1993). These 'pool problems' affect the correct evaluation of changes in conditioning effects.

To overcome 'pool problems', a single motor unit firing in response to a conditioning stimulus can be analysed as a post-(peri-)stimulus time histogram (PSTH; Ashby & Labelle, 1977; Ashby & Zilm, 1982; Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984). Although the PSTH is a powerful tool with which to explore postsynaptic potentials in a single motoneurone, its use is confined to the tonic voluntary contraction of the muscle examined.

We present here a new method that uses single motor units and which permits exploration of synaptic events during the rest stage and during voluntary contraction of the homonymous, synergistic and antagonistic muscles. The validity of this method is assessed by comparing the results obtained with those obtained by the conventional compound H reflex method.

METHODS

Twelve normal subjects, including three of the authors (aged 26–43), participated in the study. Informed consent was obtained from all of them. The experiments were approved by the Ethics Committee of Shinshu University (Matsumoto). All the procedures used followed the guidelines set forth in the Declaration of Helsinki.

The subject was seated comfortably in a reclining armchair. In the experiments on the leg the foot was bound to an immobile plate, the knee joint being kept at 160–170 deg and the ankle at 100 deg. In the experiments on the arm the forearm was held prone on an arm-rest, and the hand was gently bound to it, with a cushion underneath. The elbow joint was kept at about 120 deg.

Stimulation and recording

Stimulation. The test stimulus was delivered to the tibial nerve through a monopolar half-ball electrode located in the popliteal fossa, a reference surface electrode being placed on the skin covering the patella, to elicit the H reflex in the soleus muscle. Alternatively the stimulus was delivered to the median nerve at the medial part of the arm via a bipolar electrode to obtain the H reflexes of the flexor carpi radialis muscle. Prior to giving the test stimuli, conditioning stimuli were applied to the femoral or radial nerve, depending on the reflex pathway to be examined. The conditioning stimulus to the femoral nerve was applied with a bipolar two-needle electrode at the inguinal ligament; that to the radial nerve was applied at the radial groove in the arm using a pair of surface electrodes. Electromyograms (EMGs) of the muscles innervated by the nerve to which the conditioning stimuli had been applied were recorded to monitor the stimulus intensity with reference to the threshold for α -fibres. The conditioning intensity was expressed as a multiple of the threshold for the direct M-response (\times MT). Test and conditioning stimuli were combined randomly in the experiments. The duration of each stimulus pulse was 1 ms. The stimulator was of constant voltage type (Nihon-Kohden SEN-7203, Tokyo). Stimuli were delivered at 1.5 s intervals.

Recording. EMG recordings of the H reflex were made from the soleus or flexor carpi radialis muscle with two silver–silver chloride surface electrodes. For the soleus muscle, one electrode was placed on the soleus muscle belly 3–4 cm below the insertion of the gastrocnemius muscles, and the other was attached to the Achilles' tendon. For the flexor carpi radialis the two electrodes were placed on the skin over the muscle belly.

A motor unit within the H reflex was recorded together with the H reflex. This constituted a 'unitary' H reflex, a single motor unit potential contributing to the 'compound' H reflex. In the early experiments the motor unit potential was recorded using a monopolar active microelectrode made of tungsten, with a tip about 1 μ m in diameter and an impedance of about 10 M Ω (FHC, Brunswick, ME, USA), the reference surface electrode being attached to the skin near the active

needle electrode. In later experiments, a concentric bipolar needle electrode (Medelec E/ND1, Old Woking, UK) was used to record motor unit potentials.

The potentials were amplified with conventional biological amplifiers at a time constant of 3 ms (low cut-off frequency –3 dB at 53 Hz) and with a high cut-off filter at 3 kHz. The compound H reflex and the motor unit were recorded with a magnetic DAT recorder (Sony PC-108M, Tokyo) and a thermal rectifier (NEC-Sanei RECTI-HORIZ-8K, Tokyo) through a memory device (delay circuit, Kawasaki-Electronica TM-1410, Tokyo). After the EMG had been rectified and integrated, the size of the compound H reflex was measured as the area and expressed as a percentage of the size of the maximal direct M-response (M_{\max}).

Identification of the motor unit

The single motor unit was identified by its constant shape and latency, as well as by its all-or-none occurrence. The recording needle electrode in the leg was usually inserted in the medial–dorsal part of the lower leg to avoid the possibility of recording the motor unit potentials of the peroneal muscles in the lateral part of the leg. The position of the electrode moved only slightly and was fixed carefully with a piece of adhesive tape.

The firing threshold of a motor unit was determined from the size of the compound H reflex for a firing probability of 50% (FP_{50%}). This threshold, here called the critical firing level (CFL), is presumed to be analogous to the CFL determined from the monosynaptic reflex obtained in an experiment in the cat (Henneman, Clamann, Gillies & Skinner, 1974).

Estimation of the conditioning effect

Assessment of the excitability of a single motoneurone.

The excitability of a single motoneurone was assessed as the stimulus, relative to the Ia threshold intensity, that activated the motor unit with 50% probability. This is referred to as the critical firing stimulus (CFS). It is assumed that the Ia threshold also represents the threshold for the test excitatory postsynaptic potential (EPSP) in the motoneurone under study. The CFS will then parallel the test EPSP size in that motoneurone (see Discussion). The CFS expresses the excitability of the motoneurone and was measured in volts.

Stimulus intensity to obtain the FP_{50%}. The protocol for obtaining the intensity at FP_{50%} was an adaptation of the method by Raymond (1979). To determine the intensity at FP_{50%}, the test stimulus intensity was controlled automatically with a 16-bit laboratory computer (NEC PC-9801 VM2, Tokyo) which delivered a constant DC voltage which determined the strength of the output from the stimulator. The intensity of the test stimuli was controlled as follows. For the first stimulus, the test intensity was set manually at about the threshold for the target motor unit. From the second stimulus, the intensity was determined automatically such that it was increased by a single step when the motor unit was not fired by the preceding stimulus but decreased when the motor unit was fired. Repeated automatic adjustment of the test stimuli made the firing probability of this motor unit converge to 50%. Fifty to one hundred stimuli were needed to obtain the probability of 50%. The single step adjustment was usually 1–2 V. The experimenter was able to change the amount of voltage step during the experimental session, depending on the variability of the firing and the effect. When the intensity of the first stimulus was not appropriate, a continual increase or decrease in intensity was

needed to change the firing of the motor unit (for example, see Fig. 2*B*). In such cases, in the calculation of the intensity with $FP_{50\%}$ the first few intensities were discarded. Generally, the early intensities in a sequence of stimuli were excluded up to the stimulus with the intensity at which the firing of the motor unit began to change.

Determination of the threshold for Ia fibres. The absolute intensity obtained with $FP_{50\%}$ depended on factors other than the activity of the nervous system, such as the location of the stimulating electrode, electrode impedance and the distance between the nerve and electrode. To exclude these factors, the threshold of the Ia fibres was determined and subtracted from the absolute intensity with $FP_{50\%}$. This was done using the post-stimulus time histogram (PSTH) method as modified by Fournier *et al.* (1986). This modified PSTH method was a very sensitive way of determining the threshold of the Ia fibres when the timing for triggering the stimuli was properly set. PSTHs were constructed for a specific motor unit during weak tonic voluntary contraction of the muscle while stimulating the innervating homonymous nerve. Stimuli were triggered by a preceding motor unit potential with a specific delay so that the timing of the stimulus-induced firing would correspond roughly to that of the next spontaneous firing of the motor unit. Because each Ia fibre connects to almost all the homonymous motoneurons (Mendell & Henneman, 1971), the threshold for the homonymous monosynaptic peaks in the PSTH for a given motor unit would be nearly identical to the Ia fibre threshold. To determine the threshold of the monosynaptic peak, PSTHs were constructed using various stimulus intensities, from subliminal to supraliminal for monosynaptic peaks with steps of 0.4 V. Three PSTHs with different stimulus intensities could be constructed simultaneously during tonic voluntary contraction in a single experimental session. One to three sessions were usually required to recognize the threshold for monosynaptic peaks.

Assessment of conditioning effects. Conditioning effects on a single motoneurone were assessed in terms of change in the CFS. For example, if conditioning stimuli produced EPSPs in the motoneurone, thereby increasing its excitability, a weaker test stimulus would be sufficient to fire the motoneurone with $FP_{50\%}$, the CFS thus being decreased. The conditioning effects were expressed as percentages of the control CFSs without conditioning stimuli. In the figures showing CFSs, the ordinate is numbered in reverse of the usual in order to give an image similar to that found with the H reflex method, i.e. the decrease in CFS representing facilitation is plotted upwards and the increase downwards.

Procedures

Experiments were done in the resting state unless otherwise stated. The reflex pathways examined were heteronymous Ia facilitation from the quadriceps (innervated by the femoral nerve) to the soleus muscle and reciprocal Ia inhibition from the extensor carpi radialis muscle (the radial nerve) to the flexor carpi radialis. The time course and intensity curve were obtained. In plotting the time course, the conditioning effect was assessed for various conditioning-test stimulus intervals, with a fixed intensity for conditioning stimulation, whereas in plotting the intensity curve the effect was evaluated for various conditioning stimulus intensities at fixed interstimulus intervals. When a test stimulus was applied prior to a conditioning stimulus, the interstimulus interval was expressed as a negative value. In constructing the time courses and intensity curves, five to twelve intervals or

conditioning intensities were varied randomly in a single experimental session.

To determine the quantitative relationship between the results of the CFS and compound H reflex methods, the conditioning effects obtained with both methods were compared. Because effects obtained with the H reflex method depended on the size of the test reflex and most CFLs of the motor units were less than 15% of the M_{max} , in all the subjects the size of the test reflexes in the H reflex method was adjusted to about 10% of the M_{max} . In the comparison experiment, the sessions in which the CFS and H reflex methods were used were alternated.

Student's *t* test and correlation testing were used for the statistical analysis of the results.

RESULTS

A total of thirty-eight motor units were isolated successfully. Figure 1*A* shows sample records for a single motor unit along with compound H reflexes at rest and during voluntary contraction of the pretibial muscles for constant stimulus intensities, the shape of the motor unit being constant and its firing pattern being all-or-none. For the compound H reflex, the reflex size varied spontaneously, even for identical stimuli, reflecting spontaneous fluctuation of the excitability of the motoneuronal pool. Motor unit firing generally (Fig. 1*A*, lower traces), but not always (upper traces), paralleled the size of the H reflex, firing probability being higher the larger the H reflexes. The same unit could be evoked during voluntary contraction of the antagonist muscles and gave the same shape as when at rest, even though the stimulus intensity was somewhat stronger (Fig. 1*A*, lower traces). When the stimulus intensity was increased gradually, the motor unit did not fire with relatively weak stimuli, but firing probability increased as the stimulus intensity increased, and the unit always fired with strong stimuli (Fig. 1*B*). The CFL of this motor unit was 7.4% of M_{max} .

Sequential change in the test stimulus intensities is shown in Fig. 2, the intensity having been automatically adjusted as described in Methods. The ordinate shows test stimulus intensity, intensification being presented downwards and the threshold intensity for Ia fibres (43.4 V in this subject) having been subtracted from the absolute intensity. When the motor unit fired (●) the intensity of the next stimulus decreased (moved upwards), but if it did not fire (○) the intensity increased (moved downwards) (Fig. 2*A*). When conditioning stimulation was applied to the femoral nerve in the same experimental session, the test intensity was also adjusted automatically, but the stimulus intensity was lower on average (Fig. 2*B*). In both cases, when the first few stimuli in each condition were excluded, the firing probability of the motor unit converged to about 50%. The CFS was 32.4 V in *A* and 30.2 V in *B*, the difference being due to the conditioning stimuli. When the firing of the motor units was very

stable, the stimuli fired the motor unit alternately – an increase in intensity of one step always fired the unit, but a decrease of one step never fired it. In most units, however, a continuous increase or decrease in intensity of a few steps was often required before the stimuli changed the firing pattern (Fig. 2). This mode of intensity change was represented as variability in the CFS data instead of using only two levels of intensity, with and without firing.

Comparison of conditioning effects found with the H reflex and CFS methods

Time courses of the effects on one subject of femoral nerve stimulation at $1.5 \times MT$ on soleus motoneurons are compared in Fig. 3A for the compound H reflex and CFS methods. The CFL of the motor unit was 4.3% of the M_{max} . The curves are roughly parallel. A similar effect is seen for the intensity curves when conditioning intensities were varied systematically with a fixed conditioning–test stimulus interval of -6.8 ms (Fig. 3B). Parallel effects were recognized for all the motor units examined as long as the effect on the units existed. This was also true for inhibition of the flexor carpi radialis motoneurons produced by stimulation of the radial nerve.

A quantitative comparison of the effects of the compound H reflex and CFS methods is presented in Fig. 4, in which the correlation is analysed. For this

purpose the data obtained in intensity curves were analysed, *A* and *B* respectively giving data on facilitation of the soleus motoneurons produced by femoral nerve stimulation and on inhibition of a flexor carpi radialis motoneuron by radial nerve stimulation. The correlation coefficients are significantly high: $r = 0.967$ ($n = 10$, $P < 0.001$) for facilitation (Fig. 4A) and $r = 0.961$ ($n = 10$, $P < 0.001$) for inhibition (Fig. 4B). In all eight motor units examined, the correlation coefficients are highly significant ($P < 0.01$). Although the extent of the conditioning effects found in the H reflex study depended on the size of the test reflex (and possibly on the motor unit), that found by the CFS method was between 27.0 and 63.5% (mean, 40.2%) of the values obtained by the H reflex method when the test reflex size was maintained at about 10% of the M_{max} .

Heteronymous Ia facilitation of the soleus motoneurons produced by femoral nerve stimulation

Figure 4C shows the case when no effect on a motor unit was found using the CFS method even though significant facilitation was found using the compound H reflex method. This indicates that the afferent fibres in the femoral nerve evoked EPSPs in the soleus motoneuronal pool, but that the connection was not diffuse – it depended

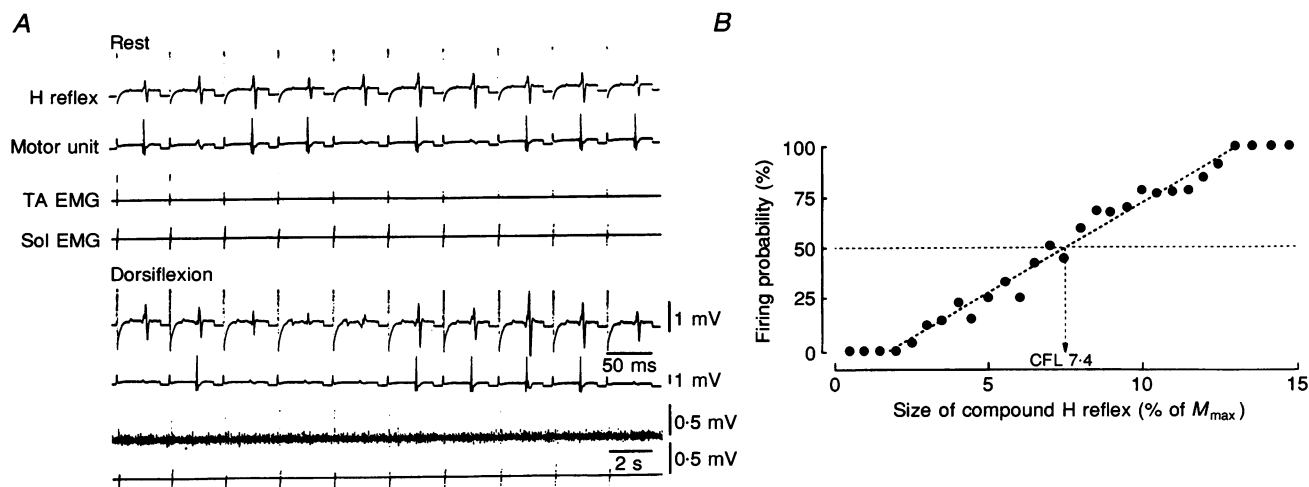


Figure 1. Sample records of a motor unit and compound H reflexes and determination of the threshold of the motor unit

A, sample records of the H reflex (uppermost trace), a motor unit (second trace), and pretibial and soleus EMGs (third and fourth traces). The records of the H reflexes and motor units were expanded through a memory device for 50 ms after each stimulus. The timing of the stimuli in all the traces is the same. The upper traces show the potentials at rest, and the lower half of the figure potentials during weak tonic contraction of the pretibial muscles. During contraction the stimuli were increased from 82.3 to 85.6 V (producing the slightly larger M-responses). H reflexes fluctuated randomly in amplitude. The probability of motor unit firing roughly paralleled H reflex size. The shapes of the motor units were identical, and firing occurred in an all-or-none fashion. *B*, the firing probability of the motor unit (ordinate) is plotted against the sizes of H reflexes with a bin width of 0.5% of the M_{max} (abscissa) at rest. As reflex size increased, firing probability increased. The threshold of the motor unit was the size of the H reflex with the firing probability of 50% (critical firing level, CFL). It was 7.4% of the M_{max} on this motor unit.

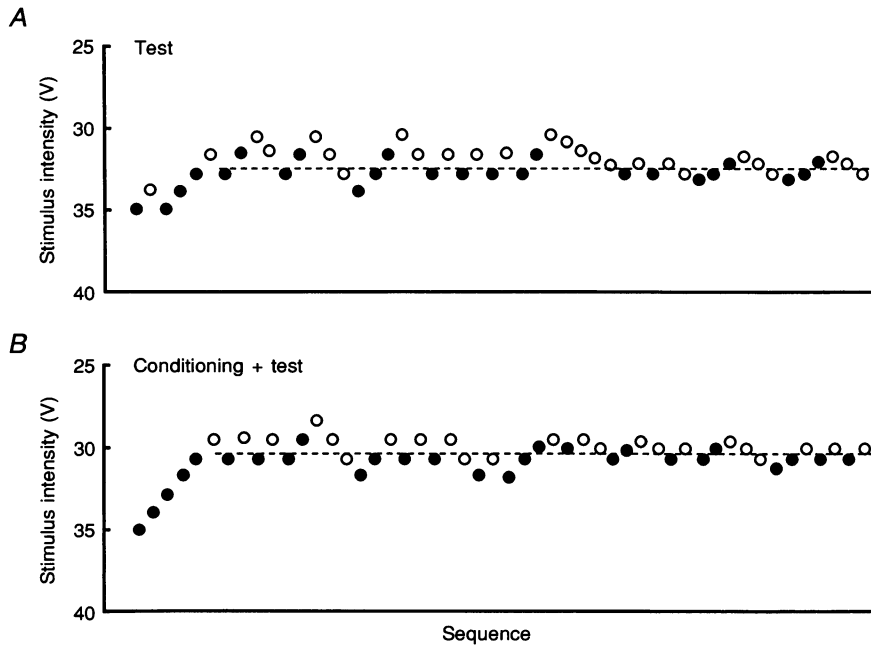


Figure 2. CFS change produced by conditioning stimuli

Test stimulus intensities (the threshold intensity for Ia fibres subtracted) are plotted for a stimulation sequence. When a motor unit fired (●), the intensity decreased (represented upward), but when not fired (○) it increased (downward). After the first few stimuli, the intensities reached the level for a firing probability of 50%. This intensity was designated the critical firing stimulus (CFS). The CFS for the test stimuli alone was 32.4 V (A), but when conditioning stimuli were added to the femoral nerve the value decreased to 30.2 V (B).

on the motoneurons. The connectivity of the femoral Ia fibres to the soleus motoneurons was determined by studying heteronymous Ia facilitation of thirty-three soleus motor units from eight subjects at a conditioning intensity of $1.5 \times MT$, which excited all the Ia fibres in the

femoral nerve. Significant Ia facilitation was found in all subjects using the conventional H reflex method, starting at interstimulus intervals of -7.5 to -5.5 ms during the time course and depending on the height of the subjects. In the motor units the effect was examined at several

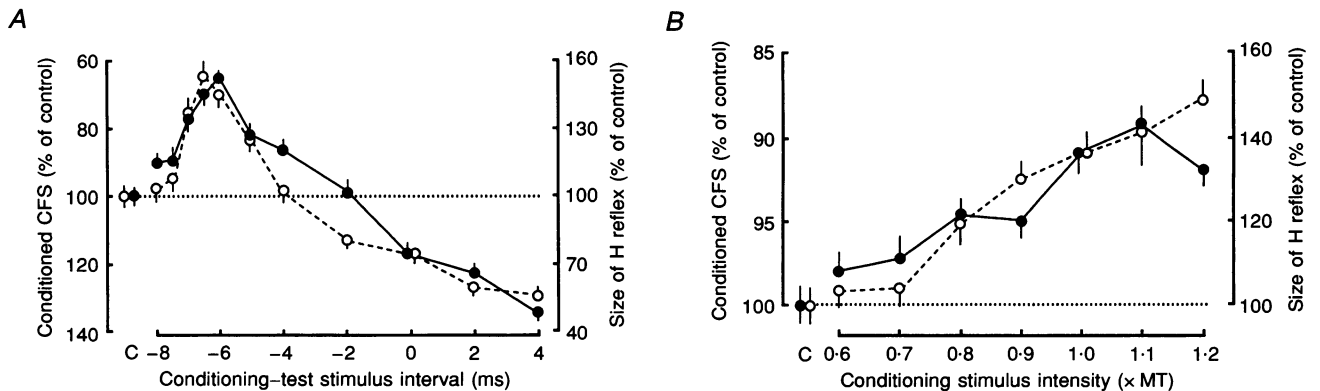


Figure 3. Comparison of heteronymous Ia facilitation for the CFS and H reflex methods

Time courses (A) and intensity curves (B) of heteronymous Ia facilitation of the soleus motoneurone(s) produced by stimulation of the femoral nerve. ○, data for H reflexes; ●, data for the CFS on a single motor unit. For the time course plot, the conditioning intensity was set at $1.5 \times MT$ in order to excite all the Ia fibres in the femoral nerve, and for the intensity curve the interstimulus interval was set at -6.8 ms. The size of the control H reflex was 10.5% of the M_{max} . The data for the two methods are roughly parallel for both the graph of the time course and the intensity curve. The circles and bars are the means \pm s.e.m. C, control.

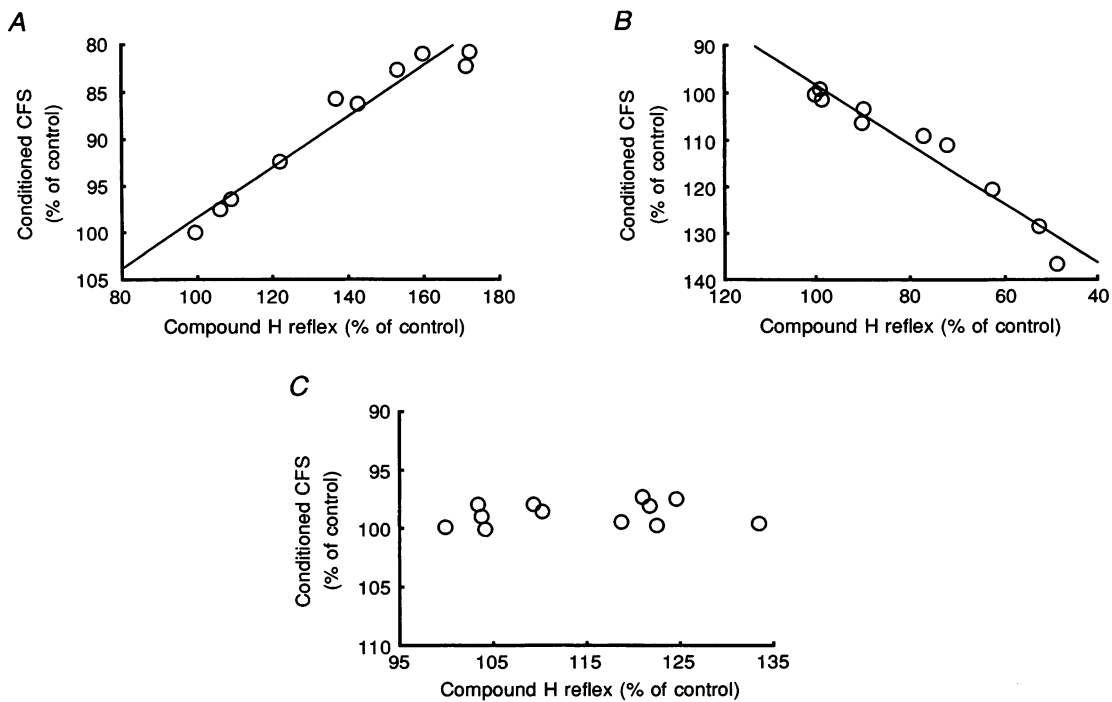


Figure 4. Correlation of the conditioning effects using the H reflex and CFS methods

Data for both methods were obtained from the intensity curves. The extents of the conditioning effect with the CFS (ordinates) and H reflex (abscissae) methods are expressed as percentages of the unconditioned control values. *A* and *C*, heteronymous Ia facilitation from the femoral nerve to a soleus motoneurone; *B*, reciprocal Ia inhibition from the radial nerve to a flexor carpi radialis motoneurone. Significant facilitation (*A* and *C*) or inhibition (*B*) is observed for the H reflex. For facilitation or inhibition of single motor unit firing (*A* and *B*) the correlation coefficients are remarkably high ($r = 0.967$, $n = 10$, $P < 0.001$, $Y = -0.270X + 125.6$ for *A*; $r = 0.961$, $n = 10$, $P < 0.001$, $Y = -0.635X + 161.7$ for *B*). Some soleus motor units in the same muscle received no facilitatory input from femoral nerve stimulation (*C*).

intervals up to maximal facilitation, with bins of 0.2 ms, and the intervals selected for evaluation of the extent of the effect were at maximal facilitation within 1 ms after the onset of facilitation. The relationship between the CFLs of the motor units and the extent of facilitation as a

percentage of the control CFS is shown in Fig. 5. Although the CFLs of the motor units were less than 15% of the M_{max} , seventeen of thirty-three soleus motor units (51.5%) received significant Ia facilitatory input from the femoral nerve.

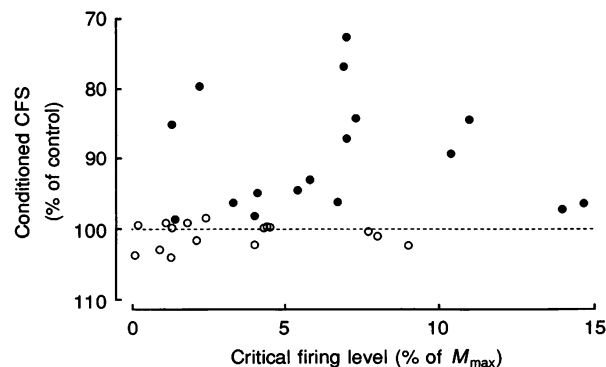


Figure 5. Frequency and extent of heteronymous Ia facilitation of the soleus motoneurons produced by stimulation of the femoral nerve

The extent of facilitation (ordinate) is plotted against the CFL (abscissa) of the 33 motor units examined. ●, significant effects ($P < 0.05$); ○, non-significant effects. There is no significant correlation between the existence or extent of facilitation and the thresholds of the motor units.

DISCUSSION

We have developed a new method for the indirect exploration of postsynaptic potentials on a single motoneurone. To assess its validity, we compared the conditioning effects found using the proposed CFS method with those found using the conventional H reflex method. Because our study was done on muscles in the resting state and because the size of the test reflexes was kept constant at 10% of the M_{max} , the populations of sampled motoneurones used for the test reflex are assumed to have been similar in all the subjects. The compound H reflex thus could be freed of 'pool problems' (see Introduction), and the conditioning effects obtained with the compound H reflex could therefore be used as the standard for evaluating the effects obtained with the CFS method.

Valid conditioning effects could be obtained using the CFS method that were similar for both facilitation and inhibition to those found with the compound H reflex method, the correlation coefficients being remarkably high. The sensitivity of the new method was nearly the same as that of the H reflex method, as seen in the intensity curves (Fig. 2B).

Heteronymous Ia facilitation of soleus motoneurones by stimulation of the femoral nerve

The connectivity of heteronymous Ia facilitation was analysed using the proposed CFS method. The frequency of the occurrence of facilitation was 51.5% (17/33) of the

soleus motoneurones by the femoral nerve stimulation in the resting state. This value is lower than those obtained by others: 92.3% for lightly anaesthetized cat (Eccles, Eccles & Lundberg, 1957), 81.8% for anaesthetized baboon (Hongo, Lundberg, Phillips & Thompson, 1984) and 70% for man during voluntary contraction of the soleus muscle (Meunier, Pierrot-Deseilligny & Simonetta, 1993). In the human experiment Meunier *et al.* (1993) used the PSTH method which required voluntary contraction of the soleus muscle, and therefore in the present study decreased presynaptic inhibition on Ia afferents from the femoral nerve during contraction of the muscle examined (Hultborn, Meunier, Pierrot-Deseilligny & Shindo, 1987) could account for the lower connectivity observed. The lower frequency obtained may also be the result of uneven sampling of the motor units because the CFLs were limited to those of less than 15% of the M_{max} . There was, however, a tendency for the low threshold motor units to have less connectivity than the higher ones (compare occurrences on motor units with CFLs of less than 5% and on those with CFLs of 5–10% of the M_{max} in Fig. 5). Connectivity on the motor units with the much higher thresholds has now to be clarified.

Significance of CFS change by conditioning stimuli

We used the CFS, which was the difference between the intensity needed to reach the threshold for Ia fibres and the intensity which evoked firing of a motor unit with 50% probability, as an indicator for assessing the excitability of a single motoneurone. The CFS is defined as

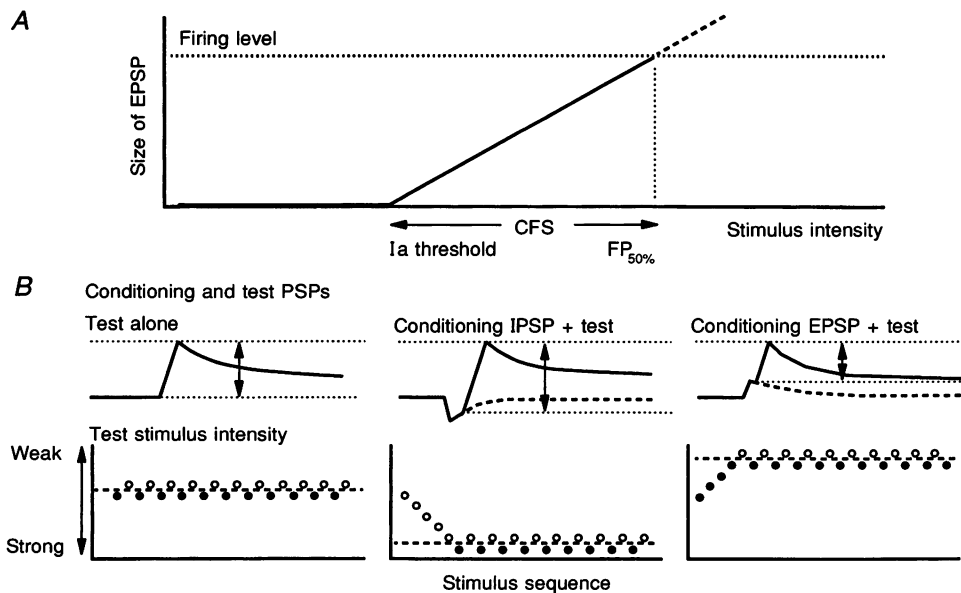


Figure 6. Diagrams showing the principle of the CFS method

The size of the test EPSP increases almost linearly with the increase in stimulus intensity (A). The range of intensities from the threshold for Ia fibres to the intensity for a firing probability of 50% (FP_{50%}) is termed the CFS. The sizes of the test EPSPs (arrows in B) correspond to the CFS. Conditioning effects, inhibition or facilitation, are shown as increases or decreases in the CFS (middle and right, B). For details see text.

the range of stimulus intensities from the threshold for Ia fibres to the intensity that gives a firing probability of 50%. Figure 6A shows diagrammatically the relationship between the intensities of the test stimuli and EPSP size within a motoneurone. The threshold intensity for Ia fibres indicates an intensity that triggers a test Ia EPSP in the motoneurone, whereas the intensity that produces $FP_{50\%}$ corresponds to the peak of the mean EPSP produced by test stimuli coinciding with the firing level of the motoneurone (Fig. 6B), regardless of fluctuation in its resting membrane potential. The CFS for a motor unit should therefore be a function of the size of the test Ia EPSPs in the corresponding motoneurone, measured as the voltage excursion between the resting membrane potential and the firing threshold of the motoneurone. Because EPSP size has an almost linear relationship to stimulus strength in the cat (Eccles, Eccles & Lundberg, 1958), the relationship between the CFS and the size of the test EPSPs can be considered almost linear. We therefore substituted the CFS for the size of the averaged test Ia EPSPs.

When conditioning stimuli produce an IPSP or EPSP within a motoneurone, stronger or weaker test stimuli are required for the motoneurone to fire with the probability of 50%, and the CFS changes, as in the sequence of stimulation shown in Fig. 6B. The extent of change naturally depends on the timing of the conditioning and test stimuli as well as on the size of the conditioning IPSPs or EPSPs (presumably on the conditioning intensity). Thus when conditioning stimuli have changed the CFS and that change is expressed as a percentage of the unconditioned CFS, the percentage of the size of the test EPSPs that is facilitated or inhibited under a given condition is indicated. For example, if the voltage excursion from the resting membrane potential to the firing threshold of a motoneurone is 10 mV (see Eccles, 1957) and if the conditioning stimulus decreases a CFS by 10% of the unconditioned CFS value, the size of the conditioning EPSP would be 1 mV (10×0.1). With the present method it is thus possible to obtain a rough estimate of the size of the conditioning EPSPs and IPSPs in an individual motoneurone.

Advantages and disadvantages of the CFS method

In studies of human spinal reflex mechanisms, the compound H reflex and conventional PSTH for single motor units are the methods commonly used, but both have limitations. The proposed CFS method has several advantages over the compound H reflex. (1) Effects on a single motoneurone can be examined apart from various 'pool problems'. (2) This new method enables us to analyse conditioning effects that depend on the motor unit,

identified most readily as the firing threshold related to the CFL. This is particularly important when studying the activity of reflex pathways during voluntary contractions executed by different motor strategies.

The CFS method also has advantages over the PSTH method, which is applicable only during tonic contraction of the muscle. (1) Use is not limited to the weak tonic contraction of a muscle; it is applicable in the resting state, during agonist contraction and even during antagonist contraction. The effect at rest usually provides a standard measurement with which to assess change in the excitability of the reflex circuitry during voluntary contraction. Similarly, supraspinal control of the activities of spinal pathways is a major interest, the analysis of which often requires antagonist contraction as an experimental protocol. (2) This new method can show the entire time course of the net postsynaptic potentials when appropriate conditioning-test stimulus intervals are chosen. In the PSTH method during voluntary contraction, the interpretation of a trough or peak after a respective peak or trough is often very difficult because of the secondary effect following facilitation or inhibition (see Ashby & Zilm, 1982; Fetz & Gustafsson, 1983). Change in the CFS shows the instantaneous postsynaptic potentials produced by the conditioning input which is separate from the previous activation. The way of thinking about conditioning effects in the CFS method therefore is similar to that in the compound H reflex method. The latency of the effects and the relationship between the conditioning and test stimuli, as well as the net effect of facilitation and inhibition represented as the decrease or increase of data (i.e. CFS), are all interpreted as in the compound H reflex method.

The new CFS method does, however, have some disadvantages. It can be applied only to muscle in which the H reflex is elicitable. This is its greatest limitation when compared to the use of the PSTH method during tonic voluntary contraction. Another problem is the technical difficulty encountered in recording high-threshold motor unit potentials. The firing of motor unit potentials with the new method is more synchronized than with the PSTH method during tonic contraction. Therefore the motor units examined are limited to those with relatively low firing thresholds. The third limitation is exploration of inhibition. If the inhibitory effect on a particular motor unit is very strong, increasing the test stimuli may evoke this motor unit as an M-response with the consequence that no firing occurs at the H reflex latencies. This is not the case for facilitation.

Even given the disadvantages of the CFS method, it provides a powerful tool with which to study neuronal connection and changes in activity in the reflex pathways of the human spinal cord.

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