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EVIDENCE FOR INTERNEURONALLY MEDIATED Ia EXCITATORY EFFECTS TO HUMAN QUADRICEPS MOTONEURONES

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SUMMARY

1. The possibility was investigated that interneuronal pathways contribute to Ia excitation of quadriceps motoneurones in normal man. Two techniques were used: (1) the indirect spatial facilitation technique for investigating summation of Ia excitatory effects in interneurones which may be interposed in pathways to quadriceps motoneurones; (2) the post-stimulus time histogram method for time course measurement of the firing probability of voluntarily activated motor units following femoral nerve stimulation.

2. The spatial facilitation technique was applied while using the quadriceps H reflex to assess the excitability of the whole motoneurone pool: the comparison was made between the excitatory effects of two conditioning stimuli applied either separately or together. Summation of effects at a premotoneuronal level is suggested if facilitation of the reflex evoked on combined conditioning stimulation is larger than the algebraic sum of facilitations evoked by separate stimuli.

3. Quadriceps tendon tap and electrical stimulations applied to either the femoral nerve or to two of its branches, the nerves to the vastus lateralis and vastus medialis muscles, were used as conditioning stimuli. Since these stimuli were very weak (their strength being about at the threshold for facilitation of the test reflex), it can be assumed that they activated predominantly Ia fibres.

4. The facilitation of the quadriceps H reflex evoked on combined stimulation was significantly larger than the algebraic sum of facilitations evoked by separate stimuli. In many experiments, although conditioning stimuli did not evoke any reflex facilitation when applied alone, a significant facilitation appeared on combined stimulation.

5. This 'extra' facilitation of the reflex on combined stimulation appeared with a central latency of 4–5 ms. It is argued that the only mechanism compatible with such a latency is summation at a premotoneuronal level.

6. Post-stimulus time histograms (p.s.t.h.s) of voluntarily activated quadriceps motor units were made following femoral nerve stimulation. Stimulation was triggered at a fixed delay time after the activation of the motor unit. A special attempt was made to set this delay so that the motoneuronal after-hyperpolarization

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following the spike would partially prevent the discharge evoked by a monosynaptic excitatory post-synaptic potential (e.p.s.p.).

7. At stimulus strengths near motor threshold, femoral nerve stimulation regularly evoked an early increase in firing probability of motor units with the same latency as the H reflex. A decrease in the stimulation strength resulted in the disappearance of this early peak and in the appearance of a second peak of increased firing probability, whose latency was about 5 ms longer than that of the early peak. It is argued that the second peak is caused by an interneuronally mediated Ia e.p.s.p.

8. Because both the 'extra' facilitation of the reflex on combined stimulation and the late peak in the p.s.t.h. of individual motor units have the same latency and are evoked by similarly weak stimuli, it is suggested that they are mediated through the same interneurones. Because of their long latency it appears unlikely that they are brought about by oligosynaptic (di- or trisynaptic) pathways located close to motoneurones. It is proposed that these effects are mediated by propriospinal neurones located several segments above motoneurones.

9. It is argued that the interneuronally mediated Ia effects described in this paper do not significantly contribute to the unconditioned H reflex.

INTRODUCTION

After the demonstration by Lloyd (1943) that the tendon jerk reflex is mediated through a monosynaptic pathway it was generally assumed that muscle spindle Ia fibres have exclusively monosynaptic connexions to homonymous motoneurones. Increasing evidence for the existence of interneuronally mediated Ia excitatory effects has, however, progressively emerged from experiments performed in the decerebrate cat (Alvord & Fuortes, 1953; Tsukahara & Ohye, 1964; Kanda, 1972; Homma, Mizote & Watanabe, 1975) and, more recently, very good evidence for the existence of oligosynaptic (di- or trisynaptic) Ia excitation of lumbosacral and intercostal motoneurones has been presented (Jankowska, McCrea & Mackel, 1981; Kirkwood & Sears, 1982*a*).

The experiments reported here were undertaken to explore whether in man interneuronal pathways contribute to homonymous Ia excitation. They concern polysynaptic pathways to quadriceps motoneurones; these were easier to demonstrate than polysynaptic pathways to soleus motoneurones. Two techniques were used to provide independent evidence for interneuronal Ia excitatory pathways: (1) the spatial facilitation technique (see Lundberg, 1975) was used to show summation at a premotoneuronal level of excitatory actions elicited by two conditioning stimuli applied to Ia fibres; (2) the post-stimulus time histogram (p.s.t.h.) method was used for time course measurement of the firing probability of voluntarily activated motor units following femoral nerve stimulation.

METHODS

The experiments were carried out on seven healthy subjects aged 24–50 years, all of whom gave informed consent to the experimental procedure. The experiments were repeated many times on four of the subjects to test the reproducibility of the results.

H reflex and spatial facilitation technique

The subjects were comfortably seated in an armchair and the examined leg was loosely fixed with the hip semi-flexed (120 deg), the knee slightly flexed (160 deg) and the ankle in 100 deg plantar flexion.

Test reflex. Surface electrodes were used both for stimulation and for recording. The test reflex was the quadriceps H reflex obtained by stimulating the femoral nerve with rectangular pulses of 0·5 ms duration delivered through a unipolar electrode every 4 s. The active electrode in the femoral triangle consisted of a half-ball (2·5 cm diameter). The reference electrode was placed on the posterior and upper aspect of the thigh. The reflex responses were measured as the peak-to-peak amplitude of muscle action potentials recorded by two non-polarizable disk electrodes (0·9 cm diameter) placed 1·5 cm apart on the vasto-crureus muscle (6–8 cm above the patella, anterior aspect of the thigh). After amplification, the reflex responses were computer-analysed 'on line', and the results stored on disk for further analysis.

Conditioning stimuli. Four different kinds of conditioning stimulation were used: the femoral nerve and two of its branches, the nerves to the vastus lateralis and vastus medialis muscles, were stimulated with pulses of 0·5 ms duration through a unipolar electrode, and a slight tap was applied to the quadriceps tendon. The conditioning stimulus applied to the femoral nerve was delivered through the same electrode as the test stimulus. The femoral nerve branches were stimulated by a similar active electrode (half-ball, 2·5 cm diameter), which was placed either 6–12 cm below the femoral arch and 2 cm lateral to the mid line (vastus lateralis nerve) or 18 cm below the femoral arch and 3 cm medial to the mid line (vastus medialis nerve). The site of stimulation was chosen such that increasing the stimulation strength above the motor threshold resulted in a steep increase of the motor response in the corresponding muscle. The current delivered by the stimulators for conditioning stimuli was measured by a current probe (Tektronix 6021). Since it is very difficult to record ascending afferent volleys in man, stimulus strength was expressed in multiples of the threshold strength of the motor wave (x motor threshold).

The quadriceps tendon was percussed using a vibrator (Brüel and Kjaer, model 4809) driven by monophasic rectangular pulses of 1·5–2 ms duration. It was possible to grade the strength of the tap using a power amplifer. Because the efficacy of the tap depended very much on the position of the hammer with respect to the tendon, the strength of the tendon tap was expressed in multiples of the strength of the threshold for the tendon jerk reflex (x tendon threshold). Here the strength used for the conditioning stimulus was always below 0·1, and usually between 0·01 and 0·04 times the strength of the tendon jerk threshold.

Conditioning stimuli strength. Two different stimuli (X and Y) were used to condition the quadriceps H reflex. In most cases the strength of the two conditioning stimuli was adjusted so that separate stimulation X or Y did not elicit any facilitation of the test reflex. However, in some experiments it was convenient to use conditioning stimuli which evoked a test reflex facilitation when applied alone.

Spatial facilitation technique. In animal experiments the indirect technique of recording post-synaptic potentials (p.s.p.s) in motoneurones has been used to demonstrate both the existence of interneurones interposed in a given pathway (Eccles & Lundberg, 1957) and convergence from two different fibre systems (X and Y) onto common interneurones (see Lundberg, 1975). The principle of the spatial facilitation technique is simple: the intensity of the stimuli is so adjusted that separate stimulation of either X or Y does not elicit any excitatory post-synaptic potential (e.p.s.p.) in the motoneurone. If combined stimulation of X and Y then gives an e.p.s.p., the explanation must be excitatory convergence onto common interneurones projecting onto the recorded motoneurone. In order to be sure that stimuli are sufficient to create p.s.p.s in the interneurones, it proved convenient to use stimuli which separately excited at least a few interneurones, thus evoking small p.s.p.s in the motoneurone. When two individual e.p.s.p.s from different sources are evoked simultaneously in one motoneurone the resulting e.p.s.p. can, at the most, be equal to their algebraic sum (see Eccles, 1964). Thus, excitatory convergence onto common interneurones can be inferred when the e.p.s.p. on combined stimulation is larger than the algebraic sum of the e.p.s.p.s evoked by separate stimuli.

The principle of the spatial facilitation technique can also be applied while using a monosynaptic reflex to assess motoneurone pool excitability: the excitatory effects of two conditioning stimuli (X and Y) are measured when applied separately and together. As seen above, summation of excitatory effects elicited by X and Y in common interneurones can be considered when facilitation of the reflex on combined stimulation (X + Y) is larger than the algebraic sum of the facilitations

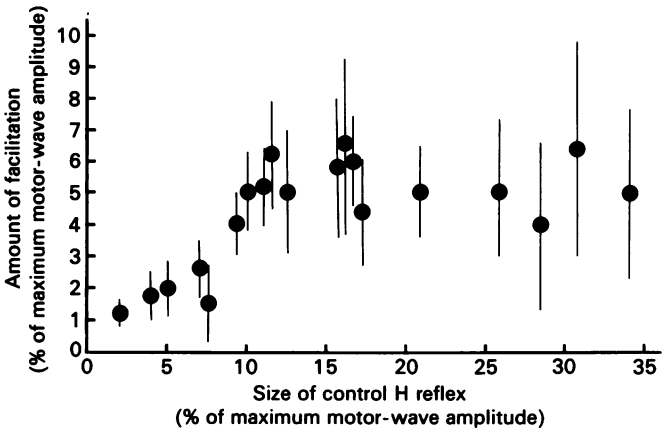

Fig. 1. Susceptibility to facilitation of quadriceps H reflexes of different sizes. Conditioning stimulation applied to the femoral nerve was constant (0·65 × motor threshold, conditioning test interval 6 ms). At such a weak intensity the conditioning stimulus alone did not evoke any reflex response. The amount of reflex facilitation (difference between the sizes of the conditioned and the control reflexes expressed as a percentage of the maximum motor-wave amplitude) is plotted against the control reflex size (expressed as a percentage of the maximum motor-wave amplitude). Each symbol represents the mean of fifteen measurements. Vertical bars, 1 S.E. of mean.

evoked by separate stimuli. However, since here the test response assesses the changes in excitability of the whole motoneurone pool, this extra facilitation on combined stimulation may have its origin within this pool and be due to: a non-linear input–output relation for the motoneurone pool (see below); and/or a non-linear summation of conditioning e.p.s.p.s at motoneuronal level (see Discussion). Thus, in human experiments, both 'motoneuronal' possibilities must be ruled out before spatial facilitation in interneurones can be inferred from extra facilitation of the H reflex on combined stimulation.

Changed susceptibility to facilitation (and inhibition) of H reflexes of different sizes. This has already been shown in the cat (Hunt, 1955; Kuno, 1959) and in man (Meinck, 1980; Mazières, Morin & Pierrot-Deseilligny, 1984) using the soleus monosynaptic reflex. Thus susceptibility to facilitation of quadriceps H reflexes of different size was tested when conditioned by a constant excitatory input: e.g. in the results illustrated in Fig. 1 the constant conditioning stimulus was a femoral nerve stimulation (conditioning test interval 6 ms, intensity 0·65 × motor threshold) and the size of the control test reflex was systematically varied by changing the strength of the test stimulus within the range at which it did not evoke any motor response. In Fig. 1 the amount of reflex facilitation (the difference between the absolute sizes of the conditioned and the control reflexes) is plotted against the control reflex size, which results in a two-part curve: (1) at low reflex amplitudes (below 10 % of the maximum motor-wave amplitude) the susceptibility to facilitation increases with the increase in size of the unconditioned reflex; (2) with further increases in the control reflex size, the amount of facilitation remains constant, the curve exhibiting a plateau. Similar curves were obtained for all subjects: with the moderate facilitations used here, the onset of the plateau always occurred when the control reflex amplitudes were 8–12 % of the maximum motor-wave amplitude. Increasing susceptibility to facilitation of small H reflexes reflects a non-linear input–output relation within the motoneurone pool, which could, by itself, be the cause of the extra facilitation evoked on combined stimulation (X + Y): an excitatory conditioning stimulus (X) increases the size of the test reflex and thus, if it is small, its further suceptibility to facilitation evoked by the second stimulus (Y). This cause of extra facilitation on combined stimulation was excluded by: (1) choosing a test reflex size large enough to be within the range where its susceptibility to facilitation no longer increased along with the test reflex size (which was possible in all subjects but one); (2) adjusting the strength of the conditioning stimuli so that at least one of them, and in most cases both of them, did not evoke any H reflex facilitation when applied alone.

Organization of the experiment and analysis of results. Four kinds of stimulation were used: A, test stimulus alone; B, first conditioning (X) stimulus + test stimulus; C, second conditioning (Y) stimulus + test stimulus (in order to assess the effects of conditioning stimulations when applied alone); and D, combined conditioning (X + Y) stimuli + test stimulus. In each sequence, twenty of each group of stimuli were randomly presented (Fournier, Katz & Pierrot-Deseilligny, 1984). To ensure the reproducibility of the results, at least five sequences were repeated using the same parameters of stimulation. The amount of facilitation (or inhibition) evoked by separate conditioning stimuli (B − A) and (C − A) and by the combined stimuli (D − A) was calculated from the size of the different kinds of reflexes. The difference between the effect of combined stimulation (D − A) and the algebraic sum of the effects caused by separate stimuli (B − A) + (C − A)) was compared to zero by an F test.

Study of single motor units

P.s.t.h.s of the discharge of voluntarily activated motor units were constructed for the period following femoral nerve stimulation. This stimulation was delivered via the same electrode, and with the same current duration as in the reflex studies (see above).

Recording of motor unit potentials. The electromyogram (e.m.g.) of the vastus lateralis was recorded by surface electrodes placed 1·5 cm apart on this muscle: 25–30 cm above the patella, lateral aspect of the thigh. While the subject performed a very weak but steady quadriceps contraction the electrode was moved on the skin (previously rubbed with abrasive paste) until it was possible to isolate one motor unit, either because it was the only one active or because it was significantly larger than the others. After several training sessions this was achieved rather easily in all the subjects thus examined. The e.m.g. potentials of single motor units were converted into standard pulses by a discriminator with variable trigger levels and were used to trigger first a computer (Apple II) and then the stimulator delivering femoral nerve stimulation. The motor unit potential and the trigger pulse were continuously monitored to detect pulse triggers due to other active units and to ensure that the motor unit shape and trigger position remained constant within and between sequences. Furthermore, a special test (see below) was used to ensure that the discriminated pulse did not originate from different motor units. Correction was made for the trigger delay to confirm the monosynaptic origin of the onset of the early peak in the p.s.t.h. (see Results).

Experimental design. If the femoral nerve stimulation elicits a monosynaptic discharge in the recorded motor unit, the subsequent motoneurone membrane trajectory prevents any polysynaptic e.p.s.p. from firing it. This post-spike motoneurone membrane trajectory is hereafter referred to as after-hyperpolarization even if other factors (such as recurrent inhibition) contribute to it. For the purpose of this study it was therefore necessary to prevent the occurrence of a monosynaptic reflex discharge in the recorded units as far as possible. This was achieved by applying the stimulation at an optimal delay *vis-à-vis* the previous motoneurone discharge, when the resulting after-hyperpolarization would reduce the probability of firing due to the monosynaptic e.p.s.p. evoked by the stimulation, but would have less effect on any polysynaptic e.p.s.p.s since they would occur later when the after-hyperpolarization had decayed. It will be shown (see Discussion) that this procedure may actually contribute to favouring the increase in firing probability caused by polysynaptic Ia e.p.s.p.s.

The experimental design is schematically represented in Fig. 2. The spontaneous voluntary discharge of the motoneurone produced, after the peripheral efferent conduction time (p.e.t.), a motor unit potential which was discriminated and converted into a standard pulse A. This pulse fed the computer and after a variable delay (D) (see above) the stimulator was triggered and the afferent volley evoked. Thus the delay which separated the previous spike from the arrival of the afferent volley at the motoneurone was the sum of the peripheral (efferent + afferent) conduction time and the delay D introduced by the experimenter. After conversion into a standard pulse (B) of the same motor unit potential following stimulation, its latency after stimulation was computed. Sometimes, as represented in Fig. 2, the e.p.s.p. evoked by the afferent volley was large enough to cause the motoneurone to fire in spite of the after-hyperpolarization, and the action potential was then advanced.

Method for ensuring that the trigger pulse was from only one motor unit. This was built into the experimental design described above. After satisfactory results had been obtained (see below in Results) with a motor unit U the experiment was repeated while using a delay D equal to 0. Thus the delay separating the previous spike from the arrival at the motoneurone of the afferent volley

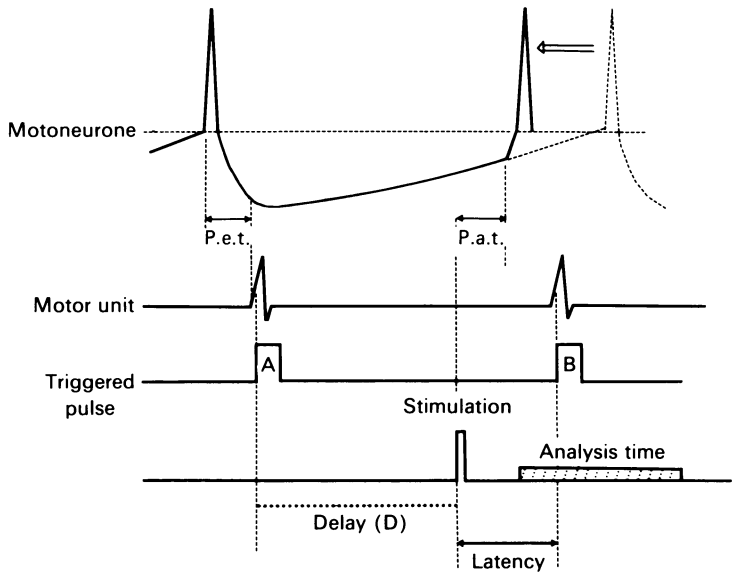

Fig. 2. Schematic diagram representing the experimental design used to construct p.s.t.h.s, the stimulation being triggered at a fixed delay after the preceding spike. The delay separating this spike from the arrival of the afferent volley evoked by the stimulation to the motoneurone was the sum of the peripheral efferent conduction time (p.e.t.) and the delay D introduced by the experimenter plus the peripheral afferent conduction time (p.a.t.). The top trace shows motoneurone discharges and the membrane trajectory during the interspike interval. Interrupted spike: 'spontaneous' firing; double arrow aiming at continuous spike: a stimulation-induced e.p.s.p. advances the spike. The second trace shows the corresponding motor unit potentials and the third trace their conversion into standard pulses. The bottom trace shows the delay D introduced by the experimenter. The analysis was not made immediately on stimulation in order to avoid false triggers caused by the stimulus artifact. The latency of the first motor unit potential following stimulation was measured.

was reduced to the peripheral (efferent + afferent) conduction time (i.e. about 30 ms). With such a short delay the after-hyperpolarization was still deep and prevented the motoneurone from discharging even though the conditioning stimulation was strong enough (1 × motor threshold) regularly to evoke a monosynaptic discharge of this motor unit after longer delay times. Under these conditions a significant peak in the p.s.t.h. within the 20–50 ms post-stimulus interval was considered to originate not from unit U but from other units that were also discriminated. Results obtained from such non-selective recordings were discarded.

E.p.s.p.s in single motor units. Subjects were given an auditory and visual feed-back of the e.m.g. potential. The firing interval, which varied from 80 to 180 ms, was chosen such that it could easily be maintained more or less constant (i.e. ±10 ms) during a long sequence. Femoral nerve stimulation was always set to be subthreshold for the compound H reflex. Stimuli were delivered at a mean rate of 0·7/s. P.s.t.h.s of the voluntarily activated motor unit discharge were constructed 20–50 ms following stimulation, using a bin width of 1 ms. In all experiments, the contraction strength was well below 5% of the maximal voluntary power. The motor units studied were, therefore, all low-threshold units within the vastus lateralis pool.

When stimulation is delivered at a fixed interval after the previous discharge, the probability of discharge depends not only on the e.p.s.p.s evoked by the stimulation but also on the after-hyperpolarization. Without any stimulation, there is a progressive increase in the probability of discharge with increasing time intervals, reflecting the motoneurone membrane trajectory following the previous spike (e.g. filled columns in Fig. 6). To take account of the changes in firing

probability reflecting only the motoneurone membrane trajectory during the interspike interval a histogram of firing probability was also constructed in a control situation without stimulation. The control situation (without stimulation) and the situation with femoral nerve stimulation were randomly alternated (same number of triggers) within a sequence. Thus two histograms were constructed, the equivalent bins in both cases being obtained at the same moment on the motoneurone membrane trajectory. The control histogram represented the background firing probability to which the results following stimulation were compared.

Statistical analysis. Within different time interval windows a χ^2 test was used to determine to what extent the distribution of firing probability after stimulation differed from that obtained in the control situation. This was examined not only for individual bins but also for the peaks within which several consecutive bins exhibited an increased firing probability. The results were considered as providing evidence for a change in the firing probability of the motor unit induced by femoral nerve stimulation if the statistical significance was high enough ($P < 0{\cdot}01$).

RESULTS

Responses of motoneurone population

Effect of conditioning stimuli when applied separately. As described in Methods, four different stimulations were used to evoke a Ia conditioning volley: (1) a quadriceps tendon tap; (2) femoral nerve stimulation; (3) vastus lateralis nerve stimulation; and (4) vastus medialis nerve stimulation.

Fig. 3*A* shows the time course of the changes in the quadriceps H reflex when it is preceded by a quadriceps tendon tap (0·75 × tendon threshold). The facilitation of the test reflex started at a conditioning–test stimulus interval of 6 ms, was maximal at an interval of 12–14 ms and then declined to control levels at intervals of approximately 25 ms, with inhibition of the test reflex at longer intervals. Since a similar facilitation (same latency and duration) of the soleus H reflex after an Achilles tendon tap has been demonstrated to be Ia in origin (Burke, Gandevia & McKeon, 1983, 1984), this quadriceps H reflex facilitation can be attributed to the tendon tap-evoked Ia volley. As shown in Fig. 3*B* (conditioning–test stimulus interval constant at 16 ms), this facilitation of the test reflex appeared when the strength of the tap was as low as 0·02 × tendon threshold.

The facilitation following the femoral nerve stimulation (0·65 × motor threshold) was maximal at conditioning–test stimulus intervals of 3–4 ms (Fig. 3*C*). Since both conditioning and test stimuli were applied to the same nerve the real onset of facilitation was obscured by refractoriness of the responding afferent fibres (Fukushima, Yamashita & Shimada, 1982). Fig. 3*D* shows that, with the conditioning–test stimulus interval constant at 5 ms, the test reflex facilitation appeared when the conditioning stimulus strength was equal to 0·6 × motor threshold. Such an H reflex facilitation due to subliminal stimulation of the homonymous nerve has been demonstrated to be Ia in origin (Pierrot-Deseilligny, Morin, Bergego & Tankov, 1981; Fukushima *et al.* 1982).

Stimulation of either the vastus lateralis nerve or the vastus medialis nerve, both branches of the femoral nerve, produced effects similar to those following femoral nerve stimulation. This is shown in Fig. 3*E* for conditioning stimulation applied to the vastus lateralis nerve (0·5 × motor threshold) but essentially the same results were obtained by stimulating the vastus medialis nerve. There was first a decrease in the test reflex amplitude at a conditioning–test interval of 4 ms (refractoriness of Ia fibres) and then a facilitation, which peaked at 5 ms and progressively decreased

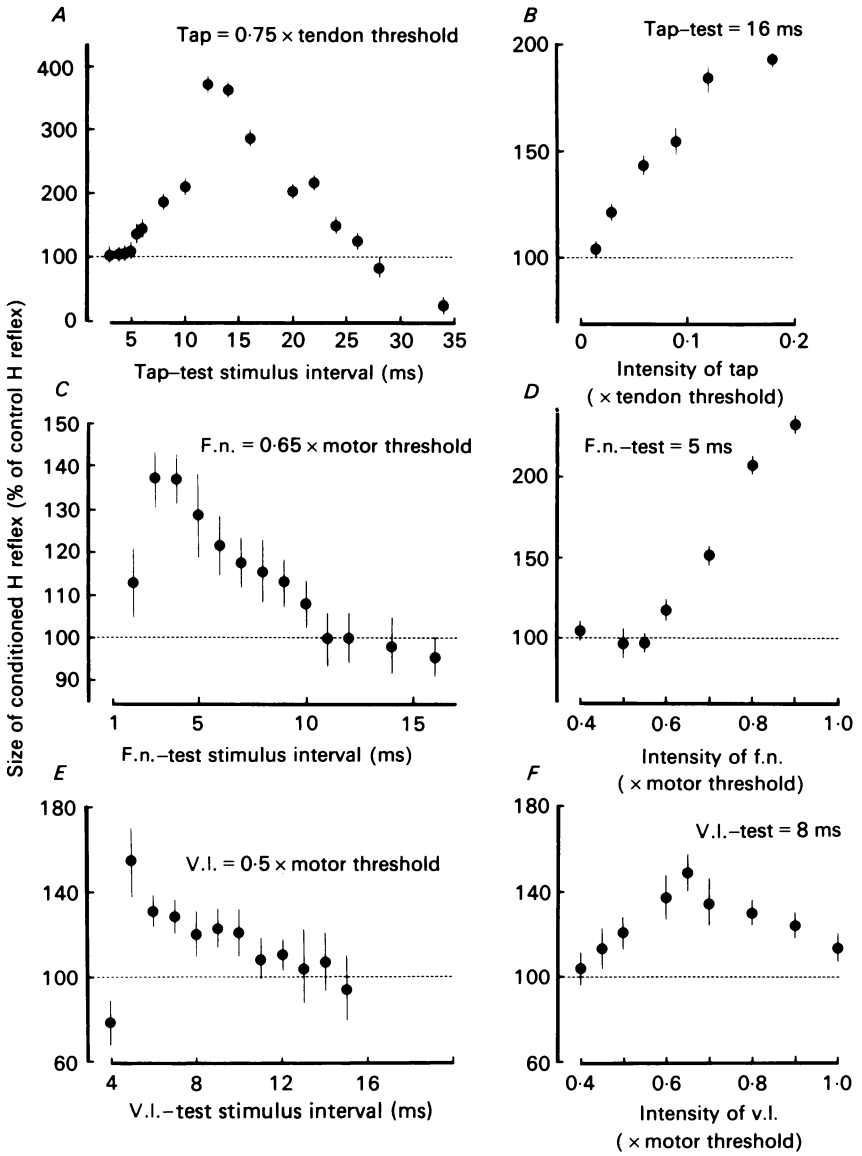

Fig. 3. Effect of different homonymous conditioning stimuli on the quadriceps H reflex. The size of the test reflex expressed as a percentage of its unconditioned value is plotted against either the conditioning–test stimulus interval (*A*, *C* and *E*) or the conditioning stimulus strength (*B*, *D* and *F*). Conditioning stimuli were quadriceps tendon tap (*A* and *B*), femoral nerve (f.n.) stimulation (*C* and *D*) and vastus lateralis (v.l.) nerve stimulation (*E* and *F*). Each symbol represents the mean of 20 measurements. Vertical bars, 1 S.E. of mean. These results were obtained in the same subject as in Fig. 1.

afterwards (Fig. 3*E*). The very low conditioning stimulus strength (0·5 × motor threshold) evoking this facilitation strongly suggests a Ia origin, a view which is supported by the time course: when the conduction velocity of 65 m/s for the fastest

Ia fibres (Magladery & McDougal, 1950; Burke *et al.* 1983) and of the distance of 12 cm between the sites of vastus lateralis nerve and femoral nerve stimulation are taken into account, 2 ms are necessary for Ia impulses to go from the former to the latter. It is therefore of interest that the time courses of the facilitations evoked by femoral nerve (Fig. 3*C*) and vastus lateralis nerve (Fig. 3*E*) stimulation are similar, the latter being delayed by 2 ms with regard to the former.

Fig. 3*F* shows the variations in the test reflex when the conditioning stimulus strength (applied to the vastus lateralis nerve) was varied while the conditioning–test interval remained constant at 8 ms. The facilitation appeared at 0·45 × motor threshold. Increasing the conditioning stimulus strength first resulted in an increase, which reached a peak at 0·65 × motor threshold. Upon further increases in the conditioning stimulus strength, facilitation declined, an effect which has been interpreted as being due to the activation of Ib inhibitory pathways (Pierrot-Deseilligny *et al.* 1981). The lower facilitation threshold on stimulation of the vastus lateralis nerve (0·45 × motor threshold) than on stimulation of the femoral nerve (0·6 × motor threshold, Fig. 3*D*) can be related to the mode of expression of this threshold as a multiple of the motor threshold (Pierrot-Deseilligny *et al.* 1981): the difference between the excitability of Ia fibres and that of α-motor fibres is larger distally at the stimulation site of the vastus lateralis nerve than proximally at the stimulation site of the femoral nerve since the motor axons are known to branch on their way to the muscle they supply (Eccles & Sherrington, 1930) whereas there is almost no branching of the Ia fibres (Barker, 1974).

Extra facilitation of the quadriceps H reflex on combined stimulation. In this section, the comparison is drawn between the effects on the quadriceps test reflex of two conditioning stimuli applied either separately or together. The amount of facilitation (or inhibition) of the test reflex, i.e. the difference (conditioned reflex − unconditioned reflex) is expressed as a percentage of the size of the unconditioned reflex. Conditioning stimuli were those described above; they have been shown to facilitate the quadriceps H reflex because they elicit a Ia volley from the quadriceps muscle or one of its heads (Fig. 3). Conditioning–test intervals were chosen long enough to allow polysynaptic Ia excitatory effects to manifest themselves (see below).

A typical example of the central finding of the present experiments is shown in Fig. 4, A–E. Conditioning stimuli were a femoral nerve stimulation (0·6 × motor threshold, conditioning–test interval 5 ms) and a quadriceps tendon tap (0·02 × tendon threshold, conditioning–test interval 17 ms). The size of the control test reflex was 18% of the maximum motor-wave amplitude, i.e. largely within the range where the susceptibility of the reflex to facilitation does not increase along with increasing reflex size (Fig. 1). When applied alone, femoral nerve stimulation (Fig. 4, A) and tendon tap (Fig. 4, B) evoked a small Ia facilitation of the test reflex. The algebraic sum of these two facilitatory effects evoked by separate stimuli was calculated (Fig. 4, C), and was found to be significantly smaller than the actual facilitation of the reflex evoked on combined stimulation (Fig. 4, D). To display this 'extra' facilitation evoked on combined stimulation the difference between these two results (facilitation evoked on combined stimulation − algebraic sum of effects evoked by separate stimuli) was calculated (Fig. 4, E). This latter result differed significantly from zero ($P < 0·05$).

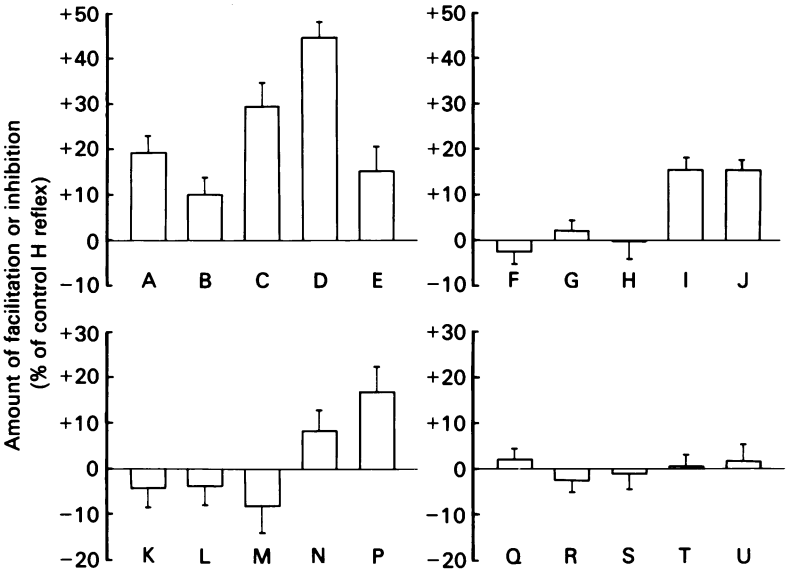

Fig. 4. Comparison between the effects on the quadriceps H reflex of two conditioning stimuli applied separately and together. The amount of facilitation (or inhibition) of the test reflex (conditioned reflex – unconditioned reflex) is expressed as a percentage of the size of the control reflex. A and B, F and G, K and L, Q and R: effects of conditioning stimuli when applied alone. C, H, M and S: algebraic sum of effects evoked by separate stimuli. D, I, N and T: effect obtained on combined stimulation E, J, P and U: difference between the effect obtained on combined stimulation and the algebraic sum of effects evoked by separate stimuli. Conditioning stimuli were tendon tap (0·02 × tendon threshold, conditioning–test stimulus interval 17 ms) and femoral nerve stimulation (0·6 × motor threshold, conditioning–test stimulus interval 5 ms) in A–E, tendon tap (0·015 × tendon threshold, conditioning–test stimulus interval 17 ms) and femoral nerve stimulation (0·55 × motor threshold, conditioning–test stimulus interval 5 ms) in F–J, vastus lateralis and medialis nerve stimulation (0·4 × motor threshold, conditioning–test stimulus interval 8 and 9 ms) in K–P, or vastus lateralis nerve (0·4 × motor threshold) and cutaneous stimulation (see text) in Q–U. Each column represents the mean of 300 measurements (A–E, K–P and Q–U) or 600 measurements (F–J). Vertical bars, 1 S.E. of mean.

This 'extra' facilitation evoked on combined stimulation seemed even more dramatic when compared with the absence of facilitation by separate stimuli, as illustrated in Fig. 4, F–J. Conditioning stimuli were the same as in Fig. 4, A–E but their strength was adjusted to be just below the threshold of reflex facilitation when applied alone. This absence of reflex facilitation after both femoral nerve stimulation (Fig. 4, F) and tendon tap (Fig. 4, G) must not be taken as reflecting an absence of Ia fibre stimulation, since it was possible to discern the facilitation at earlier conditioning–test intervals. The difference between the facilitation evoked on combined stimulation (Fig. 4, I) and the algebraic sum of the effects evoked by separate stimuli (Fig. 4, H) is shown in Fig. 4, J. This result was highly significant ($P < 0{\cdot}001$).

The results obtained from the six subjects examined in this way are presented in Table 1. For all six subjects the difference between the facilitation seen on combined

TABLE 1. Comparison between the effects on the quadriceps H reflex of femoral nerve stimulation (f.n.) and tendon tap (conditioning–test stimulus intervals 5 and 17 ms respectively) in six subjects when applied separately and together

Subjects	Control reflex	F.n. alone	Tendon tap alone	F.n. + tendon tap	Extra facilitation	Statistical significance (P)
A.R.	12	95±4	101±4	123±4	+27±8	< 0·05
E.P.-D.	25	98±2	107±3	127±2	+22±3	< 0·01
H.H.	13	175±6	98±3	198±7	+25±11	< 0·05
M.D.	22	99±2	100±2	115±1	+16±3	< 0·01
M.S.	2	104±2	106±2	130±2	+20±4	< 0·01
S.M.	10	98±4	101±4	123±4	+23±8	< 0·05

The size of the control reflex (expressed as a percentage of M_{max} (maximum motor-wave) is given in the second column. Note that in all subjects but one (M.S.) it was within the range where its susceptibility to facilitation no longer increased along with the test reflex size. The size of the test reflex when conditioned by f.n., tendon tap or combined stimulation (f.n. + tendon tap) is expressed as a percentage of its unconditioned value (mean + S.E. of mean). Extra facilitation on combined stimulation is the difference between facilitation on combined stimulation and the algebraic sum of the effects evoked by separate stimuli. The last column shows the statistical significance of this result.

stimulation and the algebraic sum of the effects evoked by separate stimuli was positive and significant. Although this result (extra facilitation on combined stimulation) usually emerged from each sequence, i.e. twenty reflexes of each kind (see Methods), repetition of many sequences was always necessary to get a significant result.

Similar results were also found when using stimulation of the vastus lateralis nerve (conditioning–test stimulus interval 8 ms) and the vastus medialis nerve (conditioning–test stimulus interval 9 ms) (Fig. 4, K–R). The strength of these two conditioning stimuli was adjusted to be just below the facilitation threshold, when applied alone (see Fig. 4, K and I, where there is even a slight but non-significant inhibition). By contrast, a facilitation appeared on combined stimulation (Fig. 4, N), and the difference (Fig. 4, P) between it and the algebraic sum of the effects evoked by separate stimuli (Fig. 4, M) was significant ($P < 0·05$). A similar result was found for all four subjects examined in this way.

These results indicate an interaction between the two conditioning volleys in spinal pathways. One must then ask which afferent fibres are responsible for it. Since the conditioning stimuli were very weak (close to the threshold of Ia facilitation) it can be assumed that group I fibres were the only muscle afferents to be activated. However, cutaneous afferents contained in the femoral nerve or its branches and the skin beneath the electrodes were also stimulated. A possible action of cutaneous stimulation was therefore tested (Fig. 4, Q–U). The electrode stimulating the vastus medialis nerve was moved 3 cm lower and 3 cm more medial than previously. At this new site the same cutaneous sensation was obtained without stimulation of muscular afferents contained in the vastus medialis nerve (the current passage was below 0·2 × motor threshold). Such a pure cutaneous stimulation did not evoke any change in the quadriceps H reflex size when applied separately (Fig. 4, R), and combined stimulation of the vastus lateralis nerve and of this pure cutaneous branch did not

evoke any facilitation (Fig. 4, T). This result was also found when replacing any of the conditioning stimuli used in Fig. 4, A–P by a pure cutaneous stimulation evoking the same sensation. This indicates that the extra facilitation on combined stimulation is due to the spinal effects of group I afferents activated by the conditioning stimuli. Since the conditioning stimuli applied to the femoral nerve and its branches were extremely weak (their strength being about at the threshold for Ia facilitation of the test reflex), it can be assumed that these stimuli predominantly activated Ia fibres. Similarly, it can be assumed that the extremely weak tendon tap used in relaxed subjects activated muscle spindles only (Lundberg & Winsbury, 1960).

It is shown in the Discussion that non-linear summation of conditioning effects at the motoneuronal level cannot account for the extra facilitation observed on combined stimulation. Thus the results shown in Fig. 4 suggest a spatial facilitation in interneurones interposed in homonymous Ia excitatory pathways to quadriceps motoneurones. Further support for this interpretation is provided by the particular time course of this extra facilitation (see below).

Time course of extra facilitation obtained on combined stimulation. In the experiments whose results are illustrated in Fig. 5 *A* and *B*, the two conditioning stimuli were a quadriceps tendon tap and a femoral nerve stimulation. The time interval between them was constant (12 ms) whereas the interval between femoral nerve stimulation and the test stimulation was varied in 1 ms steps from 2 to 12 ms, which caused the interval between the tendon tap and the test stimulation to vary from 14 to 24 ms. It must be pointed out that, within these respective intervals, both femoral nerve (Fig. 3*C*) and quadriceps tendon tap (Fig. 3*A*) stimulations were able to elicit a quadriceps H reflex facilitation, provided the conditioning stimulus was strong enough. Here the strength of the quadriceps tendon tap was 0·015 × tendon threshold, which did not elicit any H reflex facilitation whatever the conditioning–test stimulus interval (Fig. 5*A*, triangles). The strength of the femoral nerve stimulation was 0·55 × motor threshold, which did not evoke any reflex facilitation except for the early (3–4 ms) conditioning–test stimulus intervals (Fig. 5*A*, open circles). As previously seen (Fig. 4), facilitation appeared or became larger on combined stimulation (Fig. 5*A*, filled circles) but only when the interval between femoral nerve stimulation and the test stimulation was 4–7 ms. This is most easily seen in Fig. 5*B*, where the difference between the facilitation evoked on combined stimulation and the algebraic sum of the effects evoked by separate conditioning stimuli is plotted against the conditioning–test stimulus interval. This difference was positive within a narrow range (4–7 ms femoral nerve–test stimulus interval, i.e. 16–19 ms tap–test stimulus interval) where it differed significantly from zero ($P < 0·01$).

(In the experiments whose results are shown in Fig. 5*A* and *B*, the time interval separating the test stimulus from the two conditioning stimuli was varied. Thus the absence of extra facilitation on combined stimulation seen at short conditioning–test stimulus intervals could have been caused by the lessening of the interval between the test stimulation and either the femoral nerve stimulation or the tendon tap. The former appears more likely since: (1) the variations of the interval between femoral nerve stimulation and the test stimulation explored the onset of the corresponding Ia facilitation (see Fig. 3*C*); (2) the shortest tap–test stimulus interval explored (14 ms) corresponded to the peak of the tendon tap-evoked facilitation occurring 8 ms

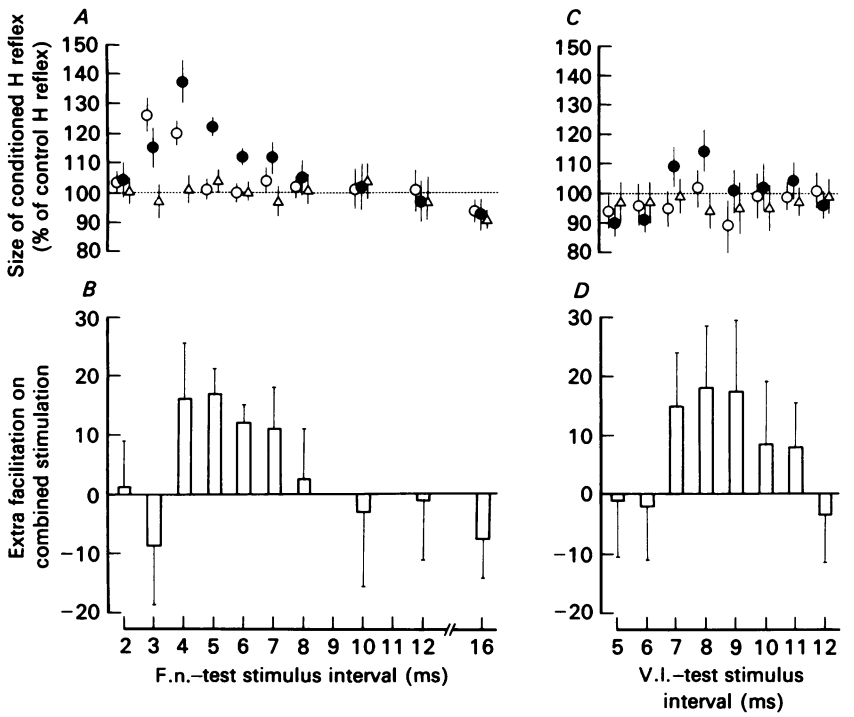

Fig. 5. Time course of the extra facilitation of the reflex on combined stimulation. *A* and *C*, the size of the test reflex (expressed as a percentage of its unconditioned value) plotted against the conditioning–test stimulus interval when conditioning was by separate stimuli: femoral nerve stimulation (open circles) and tendon tap (triangles) in *A*, vastus lateralis nerve stimulation (open circles) and vastus medialis nerve stimulation (triangles) in *C*, and by combined stimulation (filled circles) in *A* and *C*. *B* and *D*, the extra facilitation evoked on combined stimulation (i.e. the difference between the facilitation obtained on combined stimulation and the algebraic sum of the effects of separate stimuli) plotted against conditioning–test stimulus interval. *A* and *B*, the abscissa shows the interval between femoral nerve stimulation and the test stimulation. *C* and *D*, the abscissa shows the interval between vastus lateralis nerve stimulation and the test stimulation. Each symbol and each column in *A–D* represents the mean of 200 measurements. Vertical bars, 1 S.E. of mean.

after its onset (see Fig. 3*A*). Further evidence against variations of the tap–test interval being the cause of the initial part of the time course of Fig. 5*B* is provided by the results of reducing this interval while keeping constant the femoral nerve–test stimulus interval (at 5 ms): extra facilitation on combined stimulation continued to appear at tap–test stimulus intervals shorter than 16 ms (the interval below which it disappeared in Fig. 5*B*).)

Thus the relevant time factor in Fig. 5*A* and *B* is the interval between femoral nerve stimulation and the test stimulation. Since the test and conditioning volleys were obtained through the same electrode, the abscissa in Fig. 5*A* and *B* actually represents the time interval between the arrival at the spinal level of the conditioning and test volleys.

Similar results were obtained when the conditioning stimuli were applied to the vastus lateralis and medialis nerves respectively. The time interval between them was constant and equal to 1 ms, thus allowing the Ia impulses elicited beneath the two conditioning electrodes (separated by 6 cm, see Methods) to pass beneath the test electrode at the same time. The conditioning–test stimulus interval was varied from 5 to 12 ms (vastus lateralis nerve, i.e. from 6 to 13 ms for the vastus medialis nerve). The strength of both conditioning stimuli was adjusted to be just below the facilitation threshold when they were applied alone (Fig. 5*C*, open circles, triangles). The difference between facilitation on combined stimulation and the algebraic sum of the effects evoked by separate stimuli (Fig. 5*D*) disclosed a significant ($P < 0{\cdot}05$) extra facilitation between 7 and 11 ms. To estimate the time intervals at the spinal level, the conditioning–test stimulus intervals have to be reduced by the peripheral conduction time necessary for Ia impulses to pass from the conditioning electrode site to the test electrode site: i.e. 2 and 3 ms for impulses elicited beneath the vastus lateralis and vastus medialis nerve stimulation sites respectively, as seen above. Thus extra facilitation on combined stimulation occurred when the conditioning–test stimulus intervals at the spinal level ranged from 5 to 9 ms.

(It is conceivable that the real onset of the extra facilitation on combined stimulation is obscured, like that of homonymous Ia facilitation, by the refractoriness of Ia fibres after conditioning stimulation. In fact, in Fig. 5, the absence of extra facilitation on combined stimulation is seen at short conditioning–test stimulus intervals where the refractoriness of Ia fibres is no longer dominating the facilitatory effect due to conditioning e.p.s.p.s: at 3 ms in Fig. 5*A* and *B* femoral nerve stimulation elicited a significant reflex facilitation; and at 5–6 ms in Fig. 5*C* and *D* (i.e. 3–4 ms at the test electrode site) vastus lateralis stimulation facilitated the test reflex for intervals above 4 ms (2 ms at the test electrode site) when stronger conditioning stimuli were used (Fig. 3*E*).)

Similar results were obtained for all three subjects examined in this way: the extra facilitation on combined stimulation was not noticed immediately on arrival of the conditioning volley at the spinal cord and a minimum delay of 4–5 ms was necessary to disclose it. Since refractoriness of Ia fibres cannot account for this delay (see above), it represents the central latency of the extra facilitation on combined stimulation. This delay is of crucial importance since, as examined in the Discussion, the only mechanism compatible with it is the summation at an interneuronal level of Ia impulses evoked by the two conditioning stimuli.

Responses of individual motoneurones

Changes in firing probability elicited by femoral nerve stimulation. A typical example of the central finding of the experiments dealing with single motor units is shown in Fig. 6. As shown by the filled columns in Fig. 6, after the previous spike the spontaneous firing in the control situation was first interrupted and then progressively increased with increasing time intervals, reflecting the post-spike motoneurone membrane trajectory (see Methods). The delay D (40 ms) was set such that the early increase in firing probability elicited by a femoral nerve stimulation of 1 × motor threshold occurred just before the reappearance of the spontaneous firing in the control situation. On the left of Fig. 6 the comparison is drawn between the discharge

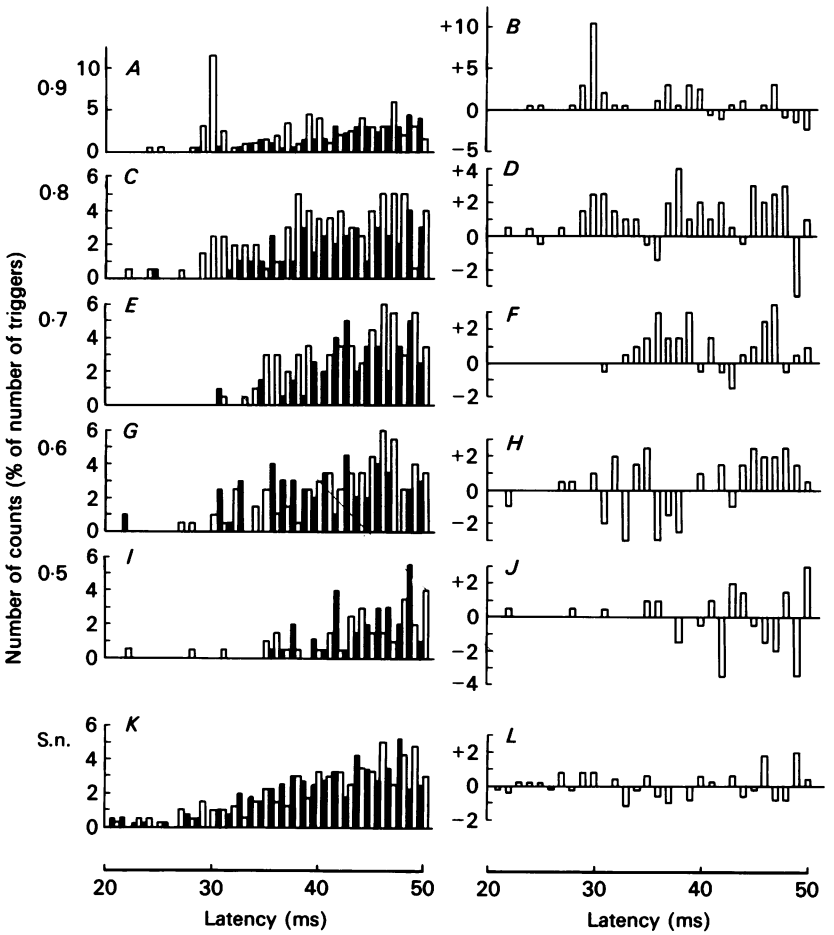

Fig. 6. Left: time histograms of the discharge of a voluntarily activated motor unit in response to femoral nerve stimulation (open columns) and in control conditions (filled columns). The spontaneous firing interval was on average 85 ms. The delay D between the previous spike and the stimulation was 40 ms. Right: the difference between the two histograms shown on the left. The number of counts per 100 triggered is plotted against the latency. The femoral nerve stimulus strength was decreased from top (*A* and *B*) to bottom (*I* and *J*) (figures on the far left indicate the stimulation strength in multiples of the motor threshold). Bottom row (*K* and *L*): stimulation was applied to the saphenous nerve (s.n.). Number of triggers used: 200. Note that the scale of the ordinate is smaller in *C–L* than in *A* and *B*.

of this motor unit after femoral nerve stimulation (open columns) and the control situation (filled columns). In addition, to display the differences between the two histograms in each bin better, the value obtained in the control situation was subtracted from that obtained after stimulation and the results of these subtractions are shown on the right of Fig. 6.

The pattern of increasing firing probability of this motor unit after femoral nerve

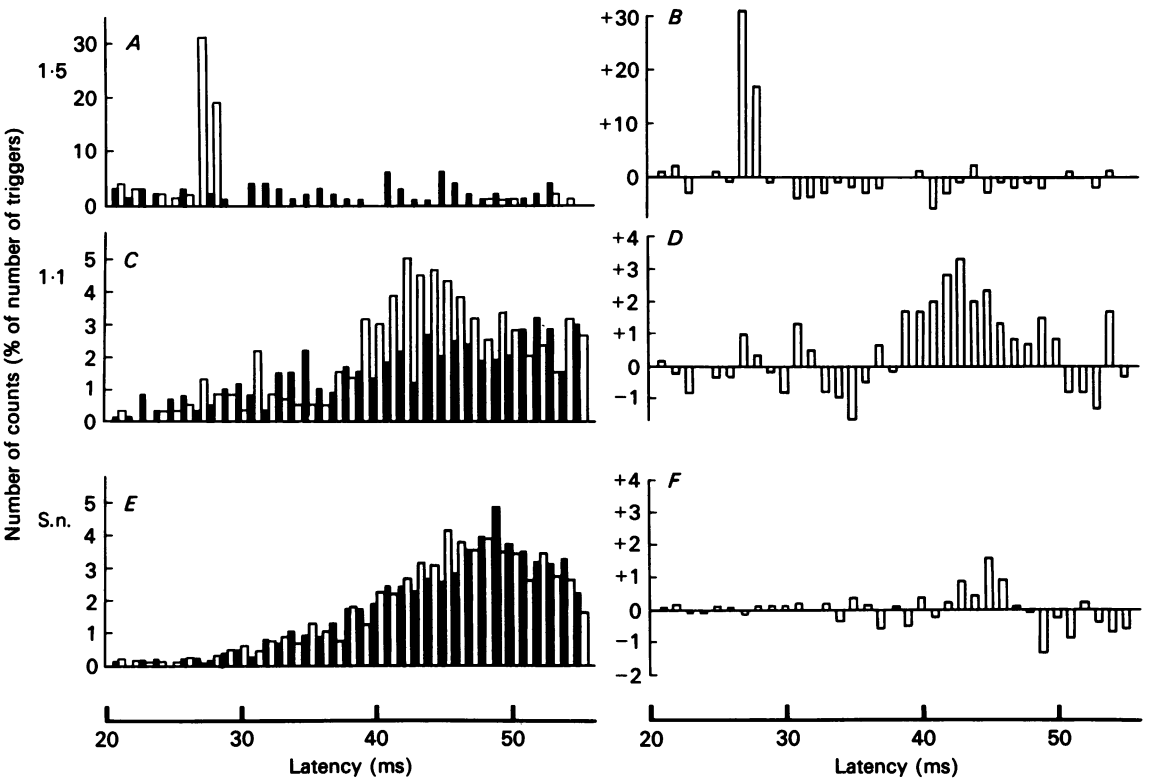

Fig. 7. Time histograms of the discharge of another voluntarily activated motor unit, obtained as in Fig. 6. Spontaneous firing interval, 120 ms; delay, D, 70 ms; number of triggers, 600. The femoral nerve stimulation strength was 1·5 × motor threshold in *A* and *B* and 1·1 × motor threshold in *C* and *D*. *E* and *F*, stimulation was applied to the saphenous nerve (s.n.).

stimulation displays three peaks: an early peak at 29–31 ms, a second peak between 33 and 42 ms and a late peak after 44 ms. Fig. 6 shows that the pattern of increasing firing probability changed when the strength of the femoral nerve stimulation was decreased: (1) At 0·9 × motor threshold (*A* and *B*) the early peak (29–31 ms) was very clear ($P < 0·001$, as examined by a χ^2 test, see Methods); (2) at 0·8 × motor threshold (*C* and *D*) the first peak was smaller and the second and late peaks appeared; (3) at 0·7 × motor threshold (*E* and *F*) the early peak disappeared completely, the second peak (33–39 ms) was very clear and highly significant ($P < 0·001$) and the late peak (44–47 ms) was barely significant ($P < 0·05$); (4) at 0·6 × motor threshold (*G* and *H*) only the late peak (44–50 ms) was still significant ($P < 0·01$); (5) at 0·5 × motor threshold (*I* and *J*) whatever the latency, femoral nerve stimulation did not produce any significant increase in the firing probability of this unit.

In almost half of the cases, the pattern of the increased probability of discharge on femoral nerve stimulation could be clearly divided into the three peaks described above. However, as shown in Fig. 7, the analysis of the results was sometimes more

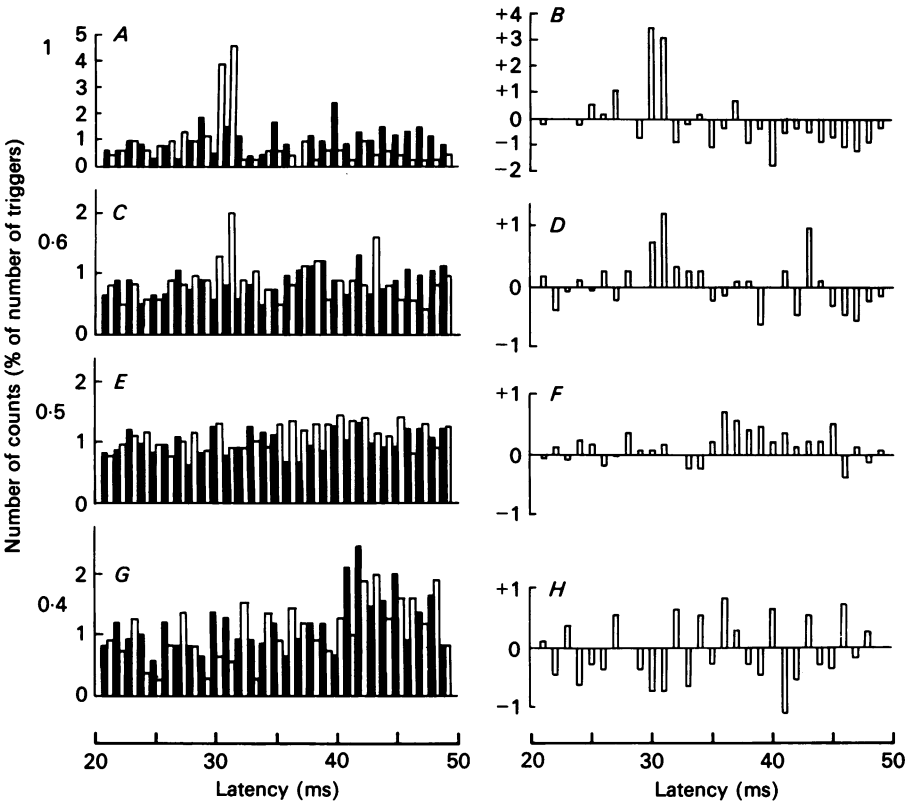

Fig. 8. Time histograms of the discharge of another voluntarily activated motor unit, obtained as in Fig. 6. Spontaneous firing interval, 140 ms; delay D, 110 ms; number of triggers, 1500. The femoral nerve stimulation strength was decreased from top (*A* and *B*) to bottom (*G* and *H*) (figures on the far left indicate the stimulation strength in multiples of the motor threshold).

difficult. At 1·5 × motor threshold (Fig. 7 *A* and *B*) femoral nerve stimulation elicited an early (27–28 ms) and highly significant ($P < 0·001$) increase in firing probability in this high threshold motor unit. At 1·1 × motor threshold, this early peak did not appear but there was a very clear and highly significant ($P < 0·001$) increase in the firing probability of this unit between 39 and 50 ms. It must be pointed out that in this unit the latency of the second peak occurred 12 ms after that of the early peak, i.e. much later than in the case of Fig. 6 (4 ms). This is possibly due to the period (33–36 ms) of the decrease in firing probability which preceded it and which could hide its real onset.

In records like those of Fig. 6 there was no spontaneous firing during the period preceding the monosynaptic latency. Thus it was not possible to check whether there was any significant difference between the two p.s.t.h.s in this period. Other records were therefore obtained in which the delay D and the spontaneous firing interval were longer. The filled columns in Fig. 8 show that there was a significant spontaneous firing in the early intervals. Neither in this nor in the twenty-five others obtained

under similar conditions was the difference between the two p.s.t.h.s significant in the period preceding the monosynaptic latency. For the most part, the results illustrated in Fig. 8 look like those obtained in Fig. 6: at high intensities there was an early peak (30–31 ms) and the second peak (35–45 ms) was clear and highly significant ($P < 0{\cdot}001$) at 0·5 × motor threshold. The thresholds of the early and the second peaks, 0·6 and 0·5 × motor threshold respectively, were, however, lower than in the case of Fig. 6 (0·8 and 0·7 × motor threshold). This can be explained by the longer delay after the previous spike in Fig. 8 (D = 110 ms) compared to that in Fig. 6 (D = 40 ms). Thus the after-hyperpolarization had diminished more and was more easily overcome by the different e.p.s.p.s elicited by femoral nerve stimulation.

Six subjects were examined in this way and forty-four motor unit recordings were considered as satisfactory since: (1) in each run the unit's spike train had been recorded without contamination during at least 400 triggers (200 controls and 200 with stimulation); (2) it had been possible to construct the p.s.t.h. with various femoral nerve stimulation strengths above and below the threshold of the early peak. The results from these forty-four recordings are discussed below.

Afferent fibres responsible for the different increases in firing probability. Since the stimulation applied to the femoral nerve was below the motor threshold, it can be assumed that group I fibres were the only muscle afferents to be activated. However, cutaneous afferents contained in the femoral nerve were also stimulated. Thus to estimate the role of cutaneous afferents in the production of the increased motoneurone firing probability, the p.s.t.h. was constructed after stimulation of a pure cutaneous branch of the femoral nerve, the saphenous nerve. This stimulation was applied to the upper part of the leg, 45 cm lower than that of the femoral nerve, and was experienced by the subject (like that evoked by the nerve trunk stimulation) as a slight tactile sensation. Taking into account a conduction velocity of about 50 m/s for the cutaneous fibres activated by such a weak stimulus (Burke *et al.* 1983), it was necessary to allow 9 ms for cutaneous impulses to pass beneath the femoral nerve electrode. The femoral and saphenous nerve stimulations and the control situation were randomly alternated within the same sequence, with cutaneous stimulation being advanced 9 ms with regard to femoral stimulation so that the latency of the effects they evoked could be approximately compared. Cutaneous stimulation did not evoke any increase in the motor unit firing probability at latencies corresponding to those of the early and second peaks evoked by femoral nerve stimulation, which can therefore be attributed to the action of group I fibres. By contrast, cutaneous stimulation evoked in some cases an increasing probability of discharge at a later latency (43 ms in Fig. 7*E* and *F*, i.e. 16 ms after the early peak onset), so it is likely that this contributes to the late peak elicited by femoral nerve stimulation at the same latency.

Monosynaptic e.p.s.p. In all forty-four satisfactory recordings there was a significant early increase in the motor unit firing probability, whose actual latency (after correction for the trigger delay) corresponded to that of the H reflex. A similar early homonymous facilitation arising from low threshold afferents and of short latency has already been observed in various muscles (Ashby & LaBelle, 1977; Ashby & Zilm, 1982), including the vastus medialis (Mao, Ashby, Wang & McCrea, 1984), and has been attributed to monosynaptic connexions of muscle spindle Ia afferents, although a contribution of oligosynaptic group I excitatory pathways (Jankowska *et al.* 1981)

to the early e.p.s.p. appears possible (Mao *et al.* 1984) or even probable (Burke *et al.* 1984). At 1 × motor threshold, this early increase in firing probability was lodged within two (Fig. 8), or at the maximum three (Fig. 6) consecutive bins, which fits the 2·4 ms mean duration found by Burke *et al.* (1984).

Polysynaptic Ia e.p.s.p.s. In thirty-one out of the forty-four satisfactory recordings there was a second peak with a significant increase in firing probability which occurred after the monosynaptic peak but before the late peak.

In seventeen of these thirty-one units the evidence for a polysynaptic Ia e.p.s.p. is, however, questionable, since the second peak might be attributable to either the falling phase of a monosynaptic e.p.s.p. or to a rebound after a trough. This is because contrary to the case for large e.p.s.p.s, which produce motor unit discharge only on their rising phase, small e.p.s.p.s have their falling phase sampled and represented in the p.s.t.h. profile (Kirkwood & Sears, 1982*b*; Gustafsson & McCrea, 1984). In four motor units the increase in firing probability remained maximal at the monosynaptic latency, even for low stimulus strengths. Thus in such cases one cannot eliminate the possibility of the 7–8 ms prolongation of the early increase in firing probability being due to the falling phase of a small monosynaptic e.p.s.p. In seven other cases, as in Fig. 7*C* and *D*, the facilitation occurring at the polysynaptic latency was clear but was preceded by a 4–6 ms decrease in the firing probability, which was likely caused by a Ib inhibitory post-synaptic potential (i.p.s.p.). Since it has been shown in animal experiments that the decrease in motoneurone firing probability through the peak of an i.p.s.p. is followed by a rebound increase in firing (Fetz & Gustafason, 1983), these seven cases were not considered evidence for polysynaptic Ia e.p.s.p.s. Finally, in six motor units it was impossible to evoke the second peak in isolation, this peak being separated from the early peak by a trough of 3–5 ms. In such a case it cannot be ruled out that the trough was due to a Ib i.p.s.p., the second peak being then just a rebound increase in firing.

Thus, several requirements were necessary before it was possible to infer the existence of polysynaptic Ia e.p.s.p.s from an increase in the motor unit firing probability: (1) the increase had to occur after the latency of the monosynaptic e.p.s.p. but not too late, since cutaneous effects can evoke a facilitation 15 ms later than the monosynaptic peak; (2) its threshold had to be lower than that of the monosynaptic e.p.s.p. (cf. Figs. 6 and 8); (3) it had not to be preceded by any decrease in the firing probability. These requirements were fulfilled for fourteen motor units. First, at 1 × motor threshold, the first bin of the uninterrupted sequence of 2–3 bins characterizing the monosynaptic facilitation was located and considered to be the monosynaptic latency. Then the existence of a polysynaptic peak was sought at a stimulus strength low enough not to evoke any monosynaptic facilitation, i.e. no increase in firing probability at the monosynaptic latency. A polysynaptic peak was accepted if there was an increase in firing probability in three or more adjacent bins and if the significance of the peak as a whole was high ($P < 0{\cdot}01$; in nine cases $P < 0{\cdot}001$). The first bin of this peak was used to locate its latency, and its duration was measured as the interval between this bin and the last bin of an uninterrupted sequence with increasing firing probability. Thus the latency of this polysynaptic e.p.s.p. was on average 5·1 ms ± 1·3 (S.D.) longer than the monosynaptic latency, and the peak lasted on average 7·6 ms ± 2·6 (S.D.).

DISCUSSION

Evidence for non-monosynaptic Ia excitation of quadriceps motoneurones

Independent evidence for spinal non-monosynaptic Ia excitatory pathways has emerged from two kinds of experiment: reflex studies using the spatial facilitation technique and construction of p.s.t.h.s in voluntarily activated motor units. The results illustrated in Figs. 4 and 5 can be summarized as follows: the quadriceps H reflex facilitation evoked on combined stimulation of quadriceps group I fibres is either larger than the algebraic sum of the facilitation evoked by separate stimuli or contrasts with the absence of facilitation by separate stimuli. It has been shown that most probably only stimulation of Ia fibres is responsible for this result, hereafter referred to as 'extra facilitation' on combined stimulation. As argued in Methods, a spatial facilitation in interneurones projecting onto quadriceps motoneurones might account for this extra facilitation on combined stimulation.

A non-linear summation at motoneuronal level must, however, be considered before reaching this conclusion. This could occur if the distribution of the conditioning e.p.s.p.s within the pool were different from that of the test Ia e.p.s.p.s, with the former giving most excitation on 'fast' motoneurones recruited only by larger reflexes. Since the test reflex preferentially recruits slow motoneurones (see Henneman & Mendell, 1981; Burke, 1981), one could then imagine that the conditioning e.p.s.p.s in these fast motoneurones are not large enough to allow them to be recruited by the test reflex, thus giving no demonstrable effect with separate stimuli. On combined stimulation, summation of conditioning e.p.s.p.s in the fast motoneurones would increase their excitability enough to fire them in the test reflex, thus producing an extra facilitation on combined stimulation. However, since the conditioning stimuli used here involve the very same Ia afferents as those recruited by the test volley, the excitatory effects of the conditioning and test volleys must have the same distribution within the motoneurone pool. Hence any sizeable conditioning Ia e.p.s.p.s should fire those motoneurones of the test stimulus-created subliminal fringe which are just below threshold, and facilitate the test reflex. Absence of reflex facilitation with separate stimuli therefore reflects the absence of sizeable conditioning Ia e.p.s.p.s in motoneurones, and extra facilitation on combined stimulation cannot be due to summation of e.p.s.p.s in motoneurones but indicates an interaction between the two conditioning volleys in interneurones.

In fact the situation is more complex, since on paired nerve stimulation the second Ia volley becomes less efficient on account of 'transmitter depletion' (Curtis & Eccles, 1960; Táboříková & Sax, 1969; Katz, Morin, Pierrot-Deseilligny & Hibino, 1977): e.g. the absence of H reflex facilitation by separate femoral nerve stimulation at intervals longer than 4 ms in Fig. 5*A* indicates that the facilitatory action of the conditioning Ia e.p.s.p.s (seen at earlier intervals) is counteracted by this depressive effect which decreases the test Ia e.p.s.p.s. That a homonymous conditioning stimulation has no demonstrable effect on the test reflex does not exclude, therefore, a residual decaying e.p.s.p. It remains, however, that the absence of reflex facilitation caused by separate stimuli indicates that the composite e.p.s.p. due to summation of the conditioning and the diminished (because of transmitter depletion) test e.p.s.p.s has the same efficiency as the test e.p.s.p. when elicited alone. Since these conditioning and test e.p.s.p.s have the same distribution within the pool, the subliminal fringe created by the test stimulus alone or by the ensemble due to each separate conditioning stimulus + test stimulus is the same (i.e. with no large e.p.s.p. 'hidden' in fast motoneurones). Under these conditions, extra facilitation on combined stimulation cannot be due to summation of e.p.s.p.s in motoneurones.

Further evidence against a non-linear summation at motoneurone level is provided by the time course of extra facilitation on combined stimulation. If the facilitation were caused by summation of conditioning monosynaptic Ia e.p.s.p.s within the motoneurone pool, extra facilitation should appear with a latency as short as that of the homonymous Ia facilitation. It is therefore of importance that extra facilitation is disclosed at a long latency (4–5 ms, Fig. 5) whereas homonymous monosynaptic (and oligosynaptic) Ia facilitation of the H reflex can be demonstrated at a conditioning–test stimulus interval of 1 ms despite the refractoriness of Ia fibres (Fukushima *et al.* 1982) and, in any event, becomes evident at 3 ms (Fig. 3*C*).

Summation of the effects evoked by the two conditioning volleys in common interneurones interposed in Ia excitatory pathways to quadriceps motoneurones seems therefore to be the explanation for the extra facilitation of the reflex on combined stimulation: separate stimulation gives only a subliminal effect in interneurones but summation of these effects on combined stimulation will discharge a number of interneurones, causing motoneurone e.p.s.p.s and thus H reflex facilitation. When conditioning stimuli are applied to different nerves (vastus lateralis and vastus medialis) this summation is spatial, indicating that Ia fibres from these two heads of the quadriceps muscle converge onto common excitatory interneurones. On the other hand, it is probable that a slight tendon tap and a weak femoral nerve stimulation activate the same largest Ia fibres, which have both the largest dynamic index and the lowest electrical threshold (see Matthews, 1972). Extra facilitation in this latter case could therefore be due to temporal summation in these excitatory Ia interneurones as well.

Further evidence for polysynaptic Ia pathways

Further evidence for polysynaptic Ia pathways emerges from the study of p.s.t.h.s in individual motor units. The results illustrated in Figs. 6 and 8 can be summarized as follows: femoral nerve stimulation is able to evoke an increase in the motor unit firing probability occurring both at a longer latency (5 ms) and with a lower threshold than the firing elicited by a monosynaptic Ia e.p.s.p. At such a latency a cutaneous contribution is eliminated, and the fact that the increased firing probability was obtained with a stimulation strength below the threshold of monosynaptic Ia facilitation strongly suggests that it was purely Ia in origin. A decrease in the size of monosynaptic e.p.s.p.s is not responsible for a 5 ms shift in the latency of the increase in firing probability since it has been demonstrated in the cat that decreasing the size of the e.p.s.p. increases the latency of the corresponding increase in firing probability by 0·35 ms at the most (Fetz & Gustafsson, 1983). The increase in firing probability occurring at a latency 5 ms longer than the monosynaptic latency is therefore very likely caused by a polysynaptic Ia e.p.s.p.

Several papers have already been concerned with p.s.t.h.s constructed after stimulation of the homonymous nerve in various human muscles (Ashby & LaBelle, 1977; Ashby & Zilm, 1982; Burke *et al.* 1983, 1984; Mao *et al.* 1984). It might therefore seem surprising that the late increase in firing probability, as illustrated in Figs. 6*E* and *F* and 8*E* and *F*, has never been reported, if one excepts the mention by Ashby & Zilm (1982) of a possible polysynaptic component in tibialis anterior motor units. This discrepancy with our results can be explained as follows:

(1) In all these papers but one (Mao *et al.* 1984), p.s.t.h.s were constructed only with stimuli strong enough to elicit a clear increase in firing probability at the monosynaptic latency. As illustrated in Figs. 7*A* and *B* and 8*A* and *B*, such a clear peak is generally followed by a long-lasting decrease in firing probability to which several causes contribute: after-hyperpolarization of the motoneurone, recurrent inhibition, Ib inhibition. Whatever the origin of this depression, it prevents polysynaptic e.p.s.p.s from firing motoneurones.

(2) In our experiments not only was the stimulus strength very low, but a special attempt was made to set the delay between the previous spike and the stimulus so that the following after-hyperpolarization would obstruct the discharge evoked by a monosynaptic Ia e.p.s.p.

(3) In most papers, special (if not unique) attention has been paid to p.s.t.h.s in soleus motor units. Parallel experiments (M. Shindo & E. Pierrot-Deseilligny, unpublished results), including both p.s.t.h.s and reflex studies with the spatial facilitation technique, show that it is much more difficult to demonstrate the existence of polysynaptic Ia pathways to soleus (if they exist at all) than to quadriceps motoneurones.

Characteristics of the involved polysynaptic pathways

The existence of non-monosynaptic excitatory Ia pathways in man can be inferred from two independent findings: extra facilitation of the H reflex on combined stimulation and a late increase in the firing probability of individual motor units. From the present results, it is impossible to ascertain whether or not those polysynaptic Ia e.p.s.p.s found in individual motoneurones are mediated by the interneurones in which summation of Ia impulses produces extra facilitation of the reflex on combined stimulation. On the basis of similarities between these two findings, it can, however, be assumed that they are mediated through the same pathway: (1) both extra facilitation of the reflex evoked on combined stimulation and the increase in firing probability of individual units occurred at the same long latency (5 ms longer than the monosynaptic facilitation); (2) both were obtained with a very low stimulus strength below the threshold of monosynaptic Ia facilitation, indicating that both were caused by activation of the first-recruited Ia fibres.

However, the apparent absence of a monosynaptic Ia effect at stimulus strengths where a polysynaptic one was detected is a little puzzling, since it seems to indicate a different organization of Ia excitatory pathways in man and in the cat. In fact, this result can, at least partly, be explained by technical artifacts, since the threshold of monosynaptic Ia e.p.s.p.s, as assessed by both H reflex facilitation and p.s.t.h.s, was raised on account of the methods used. (1) On paired nerve stimulation the second Ia volley becomes less efficient because of transmitter depletion (see small print section p. 162). Transmitter depletion at Ia terminals is very likely to reduce both monosynaptic and polysynaptic Ia e.p.s.p.s. Reduction of polysynaptic Ia e.p.s.p.s elicited by the test volley would, however, reduce the facilitation of the test reflex induced by conditioning stimuli only if they significantly contributed to the H reflex, which is not the case (see below). (2) It has been argued (see Methods) that in p.s.t.h.s the after-hyperpolarization following the previous spike would reduce the probability of firing due to monosynaptic e.p.s.p.s but would have less effect on any polysynaptic

e.p.s.p.s occurring 5 ms later. It was demonstrated that this factor played such a role in cases where the delay D (between the stimulation and the previous spike) was so set that the monosynaptic latency occurred before the reappearance of the spontaneous firing in control conditions. In such cases, it was sometimes possible to obtain an isolated polysynaptic increase in firing probability (Fig. 6*E* and *F*, 0·7 × motor threshold), but increasing the delay D by 10 ms produced a dramatic change in the result: a monosynaptic peak appeared whereas the polysynaptic peak was significantly reduced. However, that the threshold of polysynaptic Ia effects is lower than that of the monosynaptic ones is not entirely explained by the waning after-hyperpolarization: it was sometimes possible (Fig. 8*E* and *F*, 0·5 × motor threshold) to obtain an isolated polysynaptic increase in firing probability when the after-hyperpolarization had completely waned (as shown by the stable spontaneous firing throughout the 20–50 ms following the trigger). This indicates that for low stimulus strengths the polysynaptic Ia e.p.s.p.s are bigger than the monosynaptic ones. In a forthcoming paper (H. Hultborn, S. Meunier, E. Pierrot-Deseilligny & M. Shindo, unpublished) it will be shown that, during a one-motor-unit voluntary contraction (as is required when using the p.s.t.h. method), interneurones mediating polysynaptic Ia effects receive a strong excitation from descending tracts, which results in a significant increase in the H reflex Ia facilitation at the polysynaptic latency. Thus one can imagine that, because of this interneuronal facilitation, stimulation of very few Ia fibres produces a detectable polysynaptic Ia e.p.s.p. whereas the monosynaptic Ia e.p.s.p. elicited by the same stimulation is too small relative to noise transients to produce any increase in firing probability (probably because the number of triggers used, 1500, was too small). An alternative possibility would be that the very weak stimulation applied to the femoral nerve activates Ia fibres which have no monosynaptic projection onto the vastus lateralis motoneurone studied in the p.s.t.h. but feed the interneurones mediating polysynaptic Ia effects. This absence of monosynaptic projection onto individual vastus lateralis motoneurones from a proportion of Ia fibres could fit animal data if these fibres were heteronymous, i.e. originating from other heads of the quadriceps (for references see Matthews, 1972).

Although all results were obtained while recording from the quadriceps muscle and stimulating Ia fibres from this muscle it is difficult to affirm that the non-monosynaptic Ia excitatory pathways are (strictly speaking) homonymous: e.g. when conditioning stimuli were applied to the vastus lateralis and vastus medialis nerves while the reflex was recorded in the vasto-crureus muscle there is evidence for convergence of Ia fibres from two close synergists (vastus lateralis and vastus medialis) onto common interneurones projecting onto motoneurones of another close synergist (vasto-crureus muscle). Even when conditioning stimuli activate Ia fibres from the whole muscle (tendon tap, femoral nerve stimulation) it cannot be ruled out that extra facilitation on combined stimulation is due to the action of Ia fibres from close synergists. One can wonder, however, to what extent such a distinction between homonymous and heteronymous pathways is important when considering three muscles which have the same insertions and the same (purely knee extensor) function.

Which pathways are responsible for these non-monosynaptic Ia excitatory effects? In the cat the existence of oligosynaptic (di- or trisynaptic) homonymous (or from close synergists) Ia e.p.s.p.s has been suggested by several authors (Tsukahara &

Ohye, 1964; Pacheco & Guzman-Flores, 1969; Watt, Stauffer, Taylor, Reinking & Stuart, 1976), and has now been established with certitude (Jankowska *et al.* 1981; Kirkwood & Sears, 1982*a*). The latency of these e.p.s.p.s is at the very most 2 ms longer than that of monosynaptic e.p.s.p.s, a value which does not fit the 5 ms central latency found here. This would imply that the non-monosynaptic Ia excitatory pathways described in this paper contain more interneurones and/or are located at a different segment from motoneurones (the conduction time between different segments explaining then the long central latency). In this connexion, propriospinal interneurones may be considered: in the cat cervical spinal cord, they have been shown to be located several segments above motoneurones, to receive extensive excitatory input from descending tracts and to be also excited, although to a much lesser extent, by primary afferents (including group I fibres) (see Lundberg, 1979). A similar system would seem to exist in the hind limb (Kozhanov & Shapovalov, 1977), though it is much less investigated. This propriospinal system might be a good candidate for mediation of the polysynaptic effects described here, especially as it will be shown in a forthcoming paper (H. Hultborn, S. Meunier, E. Pierrot-Deseilligny & M. Shindo, unpublished) that the interneurones mediating polysynaptic Ia excitation receive strong excitation from descending tracts.

Contribution of polysynaptic Ia pathways to the quadriceps H reflex

After the demonstration by Magladery, Porter, Park & Teasdall (1951) that the onset of the soleus H reflex in man has a monosynaptic latency, it was taken for granted that the whole reflex was mediated through a monosynaptic pathway. Recently Burke *et al.* (1984) have shown that the rising phase of the composite e.p.s.p. responsible for the H reflex is long enough to allow possible oligosynaptic Ia pathways to affect the motoneurone discharge. Even though they have not established that oligosynaptic Ia pathways actually exist, their paper raises an important question: to what extent can one continue to consider the H reflex as a tool for assessing the excitability of the monosynaptic reflex arc exclusively?

It is therefore of importance to consider whether the polysynaptic Ia pathways described in this paper contribute to the quadriceps H reflex. It is known that the H reflex discharge at spinal level is not synchronized and that there is a difference between the latencies of the first spikes (likely monosynaptic) and the last spikes (possibly evoked through polysynaptic pathways). The problem is therefore to know whether the duration of the test reflex discharge is long enough to allow polysynaptic Ia e.p.s.p.s to contribute, despite their long latency (5 ms), to the firing of those motoneurones recruited in the last part of the reflex discharge. An estimate of the duration of the test reflex discharge at spinal level was therefore attempted. It is based on the finding by Araki, Eccles & Ito (1960) that the earliest conditioning–test stimulus interval at which reciprocal Ia inhibition can inhibit the test reflex corresponds to the interval at which the conditioning volley suppresses only the last individual spikes of the reflex discharge. In man, the time course of Ia excitatory effects from the inferior soleus nerve to the quadriceps H reflex was studied; the conditioning–test stimulus interval corresponding to a simultaneous arrival at the spinal level of the conditioning and test volleys was estimated from calculations based on the distance between electrodes and the conduction velocity in Ia fibres. It was

found that the earliest Ia excitatory effects, very likely monosynaptic in origin, occurred when the conditioning volley entered the spinal cord 1·5 ms after the test volley (Pierrot-Deseilligny *et al.* 1981). According to Araki *et al.* (1960), this is likely to be due to the fact that these earliest Ia conditioning effects manifest themselves when reaching the tested motoneurone pool at the end of the test reflex discharge. This would indicate that the last spikes contributing to the test reflex discharge are elicited 1·5 ms after the arrival of the test volley (at the spinal level). This is far too short a time to allow polysynaptic Ia e.p.s.p.s, whose latency is 5 ms, to contribute to the H reflex.

Theoretically the possibility remains that the test volley, after having evoked in 'slow' motoneurones the early monosynaptic discharge (lasting 1·5 ms at the spinal level), elicits in 'fast' motoneurones a polysynaptic discharge (occurring with a latency of 4–5 ms or more). Because the axons of the latter have a faster conduction velocity, this polysynaptic component could reach the muscle at approximately the same time as the monosynaptic one. Thus, using the method described above based on the time course of Ia facilitation from soleus to quadriceps motoneurones, we explored conditioning–test stimulus intervals corresponding to an arrival of the conditioning volley 3–6 ms after the test volley. There was no facilitation of the quadriceps H reflex at such conditioning–test stimulus intervals, and thus no evidence for a late polysynaptic discharge in the H reflex.

Thus the unconditioned H reflex is not contaminated by the interneuronally mediated Ia excitatory effects described in this paper, and the H reflex remains a good tool for analysing inhibitory effects to motoneurones. However, in the case of a facilitatory conditioning volley it cannot be excluded that a summation of conditioning and test e.p.s.p.s in interneurones produces a polysynaptic discharge in 'fast' motoneurones (see above) which would contribute to an increase in the H reflex.

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