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CROSS-CORRELATION ASSESSMENT OF SYNAPTIC STRENGTH OF SINGLE Ia FIBRE CONNECTIONS WITH TRICEPS SURAE MOTONEURONES IN CATS

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SUMMARY

1. The relation between excitatory post-synaptic potentials (e.p.s.p.s) produced by single Ia fibres and the resultant cross-correlograms in triceps surae motoneurones was investigated in barbiturate-anaesthetized cats. The e.p.s.p.s were documented first, using the discharge of single Ia fibres evoked by muscle stretch to compile spike-triggered averages of motoneurone membrane potential. Subsequently, Ia fibre action potentials were cross-correlated with rhythmic discharge of the same motoneurones induced by intracellular injection of current.

2. Primary correlogram peaks were statistically significant for thirty-one of forty-nine single Ia fibre–motoneurone connections. Cumulative sums of correlograms were used to identify the onset and duration of peaks. For twenty cases involving more than 2000 trigger spikes, thirteen showed significant correlogram peaks. For these thirteen, the mean percentage increase (m.p.i.) in motoneurone firing probability, defined as the mean height of the correlogram peak above base line, ranged from 29 to 138%. The k values (maximum height divided by base line) ranged from 2·1 to 5·2. Peak duration varied from 1·8 to 3·2 ms. In the remaining seven cases the Ia e.p.s.p.s produced no significant correlogram peak (i.e. $P > 0{\cdot}05$).

3. A significant positive relationship ($r = 0{\cdot}76$; $P < 0{\cdot}005$) was found between m.p.i. in motoneurone firing probability and e.p.s.p. amplitude ($n = 13$), with a mean slope of $0{\cdot}30\%/\mu V$. The k values were more weakly related to e.p.s.p. amplitude ($r = 0{\cdot}67$; $P < 0{\cdot}01$). The correlogram parameter most strongly related to e.p.s.p. amplitude ($r = 0{\cdot}80$) was correlogram peak area (number of spikes above base line per excitatory post-synaptic potential). E.p.s.p. rate of rise was not significantly related ($P > 0{\cdot}10$) to either m.p.i. in firing probability ($r = 0{\cdot}28$) or peak area ($r = 0{\cdot}36$).

4. The shapes of the primary correlogram peaks could be accounted for largely by a function proportional to the e.p.s.p. derivative (after temporal alignment). Subtracting a function proportional to the e.p.s.p. derivative from the correlogram peak

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left either a negligible remainder or a remainder term whose duration was shorter than the e.p.s.p.

5. To investigate properties of single-fibre Ia e.p.s.p.s occurring near motoneurone threshold during repetitive firing, e.p.s.p.s were selectively averaged using Ia spikes occurring near the end of the depolarizing ramp in membrane potential. These 'ramp e.p.s.p.s' tended to be somewhat smaller (by *ca.* 8%) than the 'rest e.p.s.p.s' produced at the same connections with the motoneurone at rest. Ramp-e.p.s.p. rise times were often substantially shorter or longer than rest-e.p.s.p. rise times, but did not differ in a consistent direction. Correlogram peak area and m.p.i. in firing probability were better related to ramp- than to rest-e.p.s.p. properties.

6. Properties of the correlogram peaks were not obviously related to (a) intrinsic motoneurone properties, including rheobase current and conduction velocity, (b) the type of Ia fibre–motoneurone connection, homonymous or heteronymous, or (c) the condition of the spinal cord, intact or acutely transected.

7. It is concluded that cross-correlograms provide a direct measure of synaptic strength at single Ia fibre–motoneurone connections These correlograms confirm that single Ia fibres raise motoneurone firing probability in proportion to the amplitude of the underlying e.p.s.p.s

INTRODUCTION

The effectiveness of a synaptic connection between neurones can be measured by cross-correlating their extracellularly recorded spike activity. The magnitude of primary peaks in cross-correlograms measures the effects of activity in presynaptic neurones on the firing probability of their post-synaptic target (Moore, Segundo, Perkel & Levitan, 1970; Kirkwood, 1979). The strength of synaptic connections between afferent fibres and spinal α-motoneurones has been evaluated by cross-correlating motoneurone activity with the stretch-evoked discharge of single-spindle afferent fibres (Cameron, Binder, Botterman, Reinking & Stuart, 1980; Kirkwood & Sears, 1982), and with the discharge of afferent fibres synchronously activated by electrical stimulation (Fetz & Gustafsson, 1983) or brief muscle stretch (Gustafsson & McCrea, 1984).

Another estimate of synaptic strength comes from intracellular records of excitatory post-synaptic potentials (e.p.s.p.s). The properties of e.p.s.p.s produced by single Ia fibres in motoneurones have been well documented for hind-limb motoneurone pools in the anaesthetized cat using the spike-triggered averaging technique (Mendell & Henneman, 1971; Scott & Mendell, 1976; Watt, Stauffer, Taylor, Reinking & Stuart, 1976; Nelson & Mendell, 1978; Harrison & Taylor, 1981; Fleshman, Munson & Sypert, 1981*b*; Lucas, Cope & Binder, 1984; Clamann, Henneman, Luscher & Mathis, 1985). The amplitude of those single-fibre Ia e.p.s.p.s can range from less than 10 to 600 μV and their rise time and half-width cover a 10-fold range. Consequently, the synaptic strength of individual Ia fibres as measured by their correlational efficacy could be expected to vary proportionately. Theoretical analyses of motoneurone models (Knox, 1974; Knox & Poppele, 1977; Ashby & Zilm, 1982; Fetz & Gustafsson, 1983; Homma, Musha, Nakahima & Okamoto, 1984) predict that correlogram peaks produced by large e.p.s.p.s in the absence of synaptic

noise should resemble the e.p.s.p. derivative. Indeed, the cross-correlogram peaks produced by aggregate e.p.s.p.s in motoneurones were empirically found to have shapes largely proportional to e.p.s.p. derivative, and their amplitudes were related to e.p.s.p. amplitude and rising slope (Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984). However, small aggregate e.p.s.p.s in large-amplitude background synaptic activity produced correlogram peaks wider than the derivative of the e.p.s.p.s, as predicted by theoretical considerations (Kirkwood, 1979; Fetz & Gustafsson, 1983). The effects of single afferent fibres on firing probability of intercostal motoneurones has also been documented, and compared to the shapes of single-fibre e.p.s.p.s recorded in separate experiments (Kirkwood & Sears, 1982). The present report provides the first direct comparison of single-fibre Ia e.p.s.p.s and correlograms for the same single Ia fibre–motoneurone connections.

The aim of this investigation was to explore the relation between these two measures of synaptic strength at single Ia fibre connections with hind-limb motoneurones. Single-fibre e.p.s.p.s were first obtained by compiling spike-triggered averages of membrane potential with the motoneurones at rest. Action potentials recorded from the motoneurone during repetitive firing were then cross-correlated with spikes from the same afferent fibre to determine the strength of their correlational linkage. The results provide new evidence on the correlational consequences of single-fibre e.p.s.p.s. A brief synopsis of these results has been published (Cope, Matsumura & Fetz, 1982).

METHODS

Surgical and recording preparation

Eleven adult cats of either sex ranging in weight from 2·6 to 3·9 kg were prepared for surgery by intraperitoneal injection of pentobarbitone (Nembutal; 35 mg/kg). Supplemental doses of pentobarbitone were injected intravenously throughout the experiment to maintain a deep level of anaesthesia, as judged by complete suppression of forelimb withdrawal reflexes. A cannula was placed in the trachea to facilitate respiration, and in most cases ventilation was unassisted. In cases in which respiratory movement prevented stable intracellular recording, cats were paralysed with gallamine triethiodide (Flaxedil) and artificially ventilated at volumes dictated by end-tidal P_{CO_2} (maintained between 3·7 and 4·2%). Blood pressure was monitored via a cannula placed in the carotid artery. An intravenous infusion of 10% dextran in lactated Ringer solution was given when necessary to help to maintain diastolic blood pressure above 70 mmHg.

A laminectomy was performed, exposing the lumbar enlargement of the spinal cord. In nine cases the spinal cord was completely severed at T13 in an attempt to increase the range in amplitude of the single-fibre Ia e.p.s.p.s sampled (Nelson, Collatos, Neichaj & Mendell, 1979). The left hind limb, hip and tail were completely denervated with the exception of the medial gastrocnemius and/or the lateral gastrocnemius and soleus muscles. The tendons of insertion of these muscles were dissected free of surrounding tissues and tied to a short length of suture, which permitted them to be stretched to produce maintained discharge of their Ia afferent fibres (see below). The cat was secured to a rigid frame to provide stable recording and to construct mineral-oil pools over all exposed tissues. Rectal and mineral-oil pool temperatures were monitored and maintained at 37 ± 1 °C by a servo-controlled heating pad and radiant heat.

The dura was cut and reflected, and dorsal rootlets in L7 and S1 were carefully dissected free from surrounding tissue and placed in continuity on fine bipolar platinum electrodes to obtain records of the stretch-evoked discharge of afferent fibres supplying medial gastrocnemius and/or lateral gastrocnemius–soleus muscles. Rootlets were further dissected into fine filaments until action potentials of single afferent fibres could be distinguished readily and used to trigger pulses from a window discriminator (BAK DIS-1 Time–Amplitude Window Discriminator). Group Ia

fibres were identified in part from the conduction velocity of orthodromic action potentials (> 80 m/s) evoked by electrical stimulation of the peripheral nerve via a silver bipolar hook electrode. The unique response of group Ia fibres to electrically evoked muscle contraction under isometric load was also used in their identification (see Matthews, 1972). The spikes of up to three different Ia fibres were recorded simultaneously during these experiments.

Single afferent fibres often discharged spontaneously with no stretch applied to the muscle. In the remaining cases, the muscle was stretched and held at a length that maintained steady Ia fibre firing rates, ranging from 20 to 50 impulses/s. To reduce the potential effect that discharge synchronization of stretch-activated afferent fibres might have on averaged e.p.s.p.s (see below), only those rootlets in L7 and S1 used for recording and their immediate neighbour rootlets were left intact. Dorsal roots L6 and S2 were sectioned completely. Cross-correlation of spikes from pairs of Ia fibres used in averaging (four cases) showed no evidence of synchronization.

Data acquisition

Intracellular records were obtained from triceps surae motoneurones using glass micropipettes filled with 3 M-potassium chloride and bevelled to resistances of 5–10 MΩ. Motoneurones selected for study exhibited antidromic action potentials that exceeded 55 mV in amplitude (range 55–100 mV; mean = 76 ± 13 mV (S.D.)). Following intracellular penetration of a triceps surae motoneurone, identified by antidromic stimulation, we recorded on FM tape (Honeywell 5600; band width d.c. to 5 kHz at 7·5 in/s) the motoneurone 'resting' membrane potential, both a.c.-coupled (100 Hz to 5 kHz) and d.c.-coupled (d.c. to 5 kHz), and the concomitant discharge of the single Ia fibre(s). The high-gain (× 500) a.c.-coupled record was also led into a PDP8 computer for on-line spike-triggered averaging as described below. This average revealed whether that Ia fibre produced an e.p.s.p.

When at least one Ia fibre produced an e.p.s.p., depolarizing current was injected through the micro-electrode to elicit rhythmic discharge of the motoneurone. The injected current (5–40 nA) was adjusted to maintain steady discharge; mean discharge frequency ranged between 5 and 21 impulses/s. When discharge ceased, current injection was discontinued and subsequently repeated if the antidromic action potential still exceeded 55 mV. This procedure was continued until the motoneurone either failed to fire or no longer displayed an acceptable antidromic action potential. The action potential was the primary criterion for the quality of intracellular penetration, since rectification of the micro-electrode generally precluded reliable measurement of the absolute value of motoneurone membrane potential.

In some cases we measured rheobase current, defined as the amount of depolarizing current (injected into the motoneurone as square pulses lasting 50 ms in duration at 1 impulse/s) required to achieve motoneurone discharge threshold. This measurement was made before rhythmic discharge was induced.

Data analysis

Spike-triggered averaging procedures essentially identical to those first described by Mendell & Henneman (1971) were used to study single-fibre Ia e.p.s.p.s. Off-line averages were compiled by leading the tape-recorded, a.c.-coupled intracellular records of motoneurone membrane potential into the PDP8 computer, which was triggered by pulses from action potentials of a single Ia fibre (e.g. Fig. 1*A*). For an interval beginning 5 ms before and lasting 15 ms after each trigger, the computer digitized the motoneurone membrane potential and afferent fibre records at 100 μs/bin (chosen to match correlogram bin width). Each e.p.s.p. studied with the motoneurone at rest (Fig. 1*B*) was typically averaged from at least 500 spike-triggered sweeps of motoneurone membrane potential. From these averaged e.p.s.p.s, we measured their amplitudes, rise times and rates of rise (both measured from 10–90% of peak), half-widths, and latencies from the occurrence of the afferent spike.

In some cases we also analysed single-fibre Ia e.p.s.p.s occurring on the membrane-potential ramp just before the action potentials of repetitively firing motoneurones, by selectively triggering the averager from those afferent spikes that preceded the motoneurone spikes. Tape recordings were played backward and a 4 ms gate pulse was triggered 6 ms 'after' each motoneurone action potential. The corresponding record of Ia fibre spikes was used to trigger the PDP8 computer when the Ia spike occurred during the gate pulse. The time axis of the resulting average was then reversed to restore the original sequence. This procedure yielded a spike-triggered average of those e.p.s.p.s

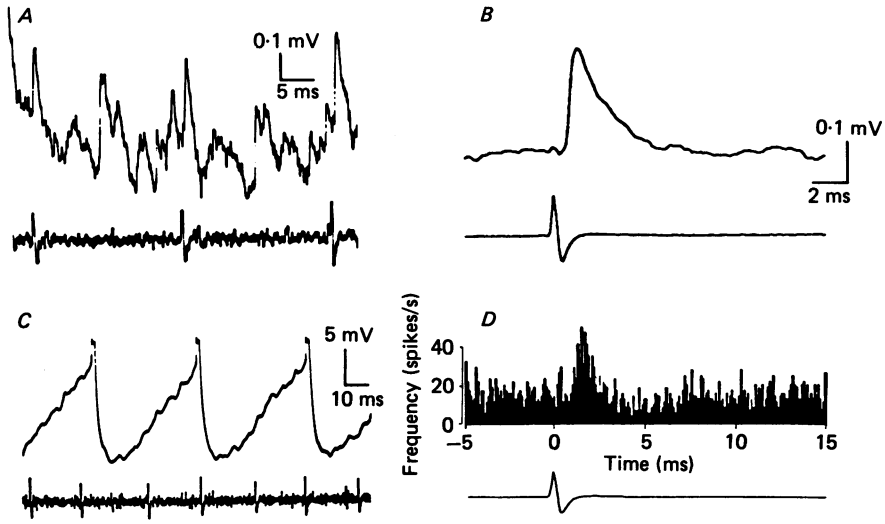

Fig. 1. Effects of single Ia afferent fibre on a single medial gastrocnemius (m.g.) α-motoneurone. *A*, top trace: a.c.-coupled recording of membrane potential of motoneurone at rest; bottom trace: simultaneous records of dorsal root filament showing spikes of a group Ia fibre from m.g. *B*, top trace: rest e.p.s.p. averaged from 1000 Ia fibre trigger spikes (bottom trace) with motoneurone at rest. *C*, top trace: motoneurone membrane potential during repetitive discharge induced by current injection; action potentials are truncated; bottom trace: simultaneous record from dorsal root filament with spikes from same Ia fibre as in *A*. *D*, top trace: cross-correlogram (case no. 5 in Table 1) constructed from 6337 Ia fibre trigger spikes (averaged in bottom trace) and 2266 motoneurone spikes. Bin width for *B* and *D* 100 μs.

(referred to as 'ramp e.p.s.p.s') produced by spikes occurring during the depolarizing membrane trajectory, 6–10 ms before the occurrence of an action potential. This 4 ms period was the shortest interval that yielded a sufficient number of triggers (at least 100) to provide an acceptable signal-to-noise ratio for measuring ramp-e.p.s.p. properties. Ia fibre spikes occurring less than 6 ms before a motoneurone spike were excluded as triggers, because the amplitude and rise time of their e.p.s.p. could be obscured by the onset of the action potential. Since the motoneurone action potentials occurred during the falling phase of these ramp e.p.s.p.s, the half-width of ramp e.p.s.p.s was not measured from these records. To assist the visual comparison of rest and ramp e.p.s.p.s, the slope of the rising membrane potential was subtracted by the computer, producing a flat base line. Subtracting this linear base-line ramp did not alter the amplitude or rise time of the ramp e.p.s.p.s.

Cross-correlograms were compiled for the same single Ia fibre–motoneurone connection by cross-correlating the spike trains of the Ia fibre and action potentials of the motoneurone, illustrated in Fig. 1*C*. These histograms were digitized at 100 μs bin width over a 20 ms analysis period, as were the e.p.s.p.s. An example of a correlogram compiled in this way is illustrated in Fig. 1*D*. Correlogram parameters and their statistical analyses were all computed using the counts in 100 μs bins.

To assist in determining the existence and onset of the correlogram peak, the computer calculated the cumulative sum (cusum) of each correlogram (Ellaway, 1978). This involved calculating the mean number of spikes per bin ($\bar{n}$) in the correlogram base line, defined as the interval preceding the Ia fibre spike. The cusum over the entire 20 ms period of the correlogram was then calculated using the formula

$$C_k = \sum_{j=0}^{k} (n_j - \bar{n}), \tag{1}$$

where C_k = content of the kth cusum bin, and n_j = content of the jth correlogram bin. With the cusum displayed in temporal register with its correlogram (Fig. 2), the onset and maximum of the

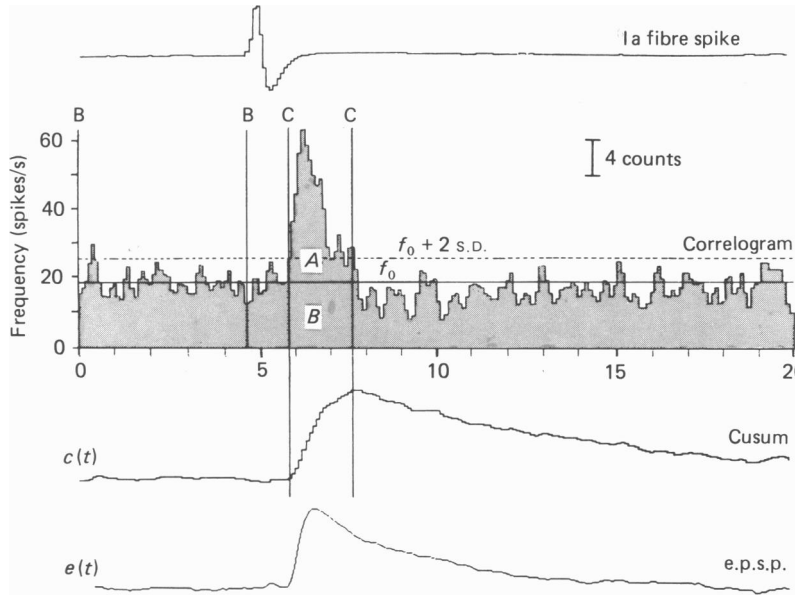

Fig. 2. Definition of base-line interval and correlogram peak parameters. This correlogram (second trace) was compiled from 4496 trigger spikes recorded from a single Ia fibre and 1639 motoneurone spikes (case no. 1 in Table 1). For illustration purposes only, histogram was smoothed, i.e. counts in nth bin replaced by sum of $\frac{1}{2}$ counts in bin $n+\frac{1}{4}$ counts in the two adjacent bins. Calibration bar provides for conversion from frequency to counts. Base-line motoneurone firing rate was calculated from the interval from the beginning of the pre-trigger period to onset of the Ia trigger spike (top trace), bounded by the vertical lines labelled B. Cumulative sum (cusum) constructed from correlogram is shown in third trace. The cusum was used to determine beginning and end of the correlogram peak, indicated by vertical lines labelled C, at onset and maximum of the cusum peak. Bin width was 110 μs. Horizontal lines represent the mean base-line frequency (f_0) and two standard deviations of the base-line values above the mean (f_0+2 S.D.). The m.p.i. in firing probability is proportional to the ratio of area A to B. Peak area is given by A. Bottom trace shows e.p.s.p. produced by the afferent fibre; for comparison, its onset was shifted to match onset of the cusum.

cusum peak were used to identify the onset (t_o) and termination (t_e), respectively, of the positive correlogram peak. This assisted in identifying the boundaries of the more obscure correlogram peaks. Correlogram peak duration (T_p), was defined as $T_p = t_e - t_o$.

The bin counts n_j in the cross-correlation histogram are proportional to the mean firing rate $[f(t_j)]$ at the time t_j of the jth bin.

$$n_j = f(t_j)\,\Delta t\,N, \tag{2}$$

where Δt = bin width and N = total number of sweeps or trigger events. With this proportionality the cusum and other measures of the correlogram peak may be expressed in terms of either bin counts or firing rates.

The magnitude of the effect of the Ia fibre on motoneurone firing probability was quantified by several measures of the correlogram peak. The maximum peak *height* relative to base line has been expressed in terms of the parameter k (Sears & Stagg, 1976; Kirkwood & Sears, 1982), defined as the ratio of the spike number in the bin with the largest count (n_p) to the mean number (n_b) in the base line bins. In terms of frequencies,

$$k = n_p/n_b = f_p/f_0, \tag{3}$$

where f_p is the maximum frequency in the correlogram peak and f_0 is the average base-line frequency. Another measure of peak height that has been used for correlograms generated by compound e.p.s.p.s (Gustafsson & McCrea, 1984) is the relative peak height above base line, defined as:

$$\text{relative peak height} = (f_p - f_0)/f_0 = k - 1. \tag{4}$$

To obtain a more representative measure of the mean increase in firing probability throughout the correlogram peak (not just its maximum value) we computed the *mean percentage increase* (m.p.i.) in firing probability, defined as:

$$\text{m.p.i. in firing probability} = 100 \times \frac{1}{T_p} \int_{t_0}^{t_e} \left(\frac{f(t) - f_0}{f_0} \right) dt, \tag{5}$$

where $f(t)$ = motoneurone firing rate at time t after the afferent spike. The m.p.i. measures the average increase above base line during the peak, expressed as a percentage of the base line value. Although the mean height of the correlogram peak is not as large as its maximum, the m.p.i. in firing probability represents the entire peak, and was better correlated with e.p.s.p. parameters (see Results).

In addition to the above measures of the *height* of the correlogram peak, we calculated the *area* between the peak and base line. The peak area was computed as the integral over the correlogram peak of the firing rate minus base line:

$$\text{peak area} = \int_{t_0}^{t_e} [f(t) - f_0]\, dt. \tag{6a}$$

The peak area represents the number of spikes above base line produced by each e.p.s.p. For a given cross-correlation histogram, the peak area can also be computed in terms of bin counts in the peak:

$$\text{peak area} = \frac{1}{N} \sum_{j_0}^{j_e} (n_j - n_b), \tag{6b}$$

where the summation extends over the bins in the peak. The peak area is equivalent to the product of (m.p.i. in firing over base-line) × (the peak duration) × (the base-line firing rate).

Documenting both the e.p.s.p. and cross-correlogram at the same single Ia fibre–motoneurone connections enabled us to evaluate relations between their properties directly. Because the detection pulses generated by motoneurone spikes occurred about 250 μs after the onset of the motoneurone action potentials, the onset latencies of the correlogram peaks were corrected for this factor by subtracting the mean delay of the Schmitt trigger pulse after the onset of the motoneurone action potential.

The statistical significance of correlogram features was assessed by treating bin counts with Poisson statistics (Moore *et al.* 1970; Sears & Stagg, 1976). Following Cox & Lewis (1966) and Garnett & Stephens (1980), we determined whether the mean counts in the correlogram peak differed significantly from the base-line mean by computing the statistic

$$z = \left(\frac{N_p}{T_p} - \frac{N_b}{T_b} \right) \sqrt{\left(\frac{N_p}{T_p^2} + \frac{N_b}{T_b^2} \right)}, \tag{7}$$

where N_p and N_b are the total counts and T_b and T_b are the durations of the peak and base line, respectively. This function can be treated as having a normal distribution and unit variance, even when the number of counts is small; it can be used, therefore, to obtain confidence intervals and significance tests for the null hypothesis that counts in the base line and peak arise from the same Poisson means. Additional statistical analyses included the use of one-way analysis of variance to test differences between group means, and the use of the Pearson correlation coefficient (r) to measure the strength of the linear fits. In each case, significance was tested using a Student's t test (one-tailed), and the P values reported designate the probability that the differences or relationships were random.

RESULTS

Intracellular records of the membrane potentials of thirty-six triceps surae motoneurones (thirty-one medial gastrocnemius and five lateral gastrocnemius–soleus motoneurones) were collected with simultaneous records of the stretch-evoked discharge of one to three Ia afferent fibres. The presence of spike-triggered averaged e.p.s.p.s verified monosynaptic connections between forty-nine different single Ia fibre–motoneurone pairs in this sample. After documenting the e.p.s.p.s, we compiled cross-correlograms for all forty-nine connections.

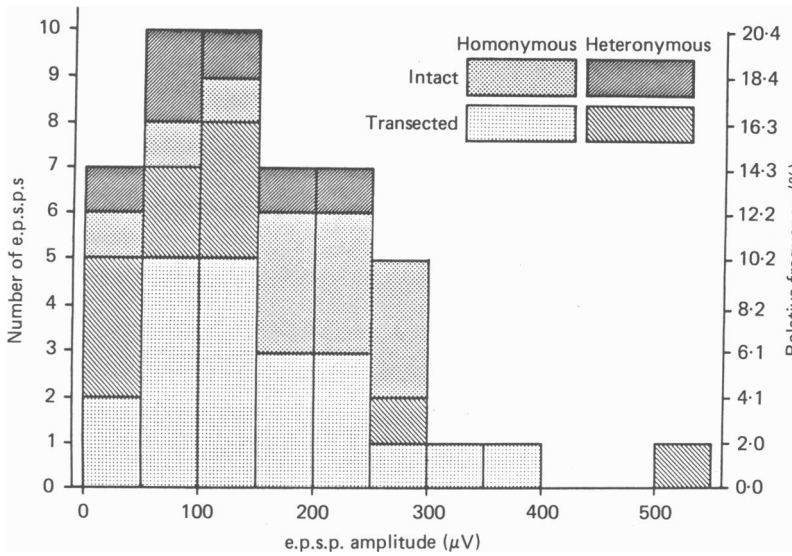

Fig. 3. Amplitude histogram for e.p.s.p.s recorded at all forty-nine single Ia fibre–motoneurone connections. Hatching identifies e.p.s.p.s from heteronymous connections and stippling represents homonymous connections. E.p.s.p.s from cats with intact spinal cords are represented by darker fills than e.p.s.p.s from acute spinal transected cats.

E.p.s.p.s at single Ia fibre–motoneurone connections

Spike-triggered averaged e.p.s.p.s were compiled for Ia fibre–motoneurone connections with the motoneurone at 'rest' (referred to as rest e.p.s.p.s) (Fig. 1*A* and *B*). The distribution of e.p.s.p. amplitude is illustrated in Fig. 3. Amplitudes of the forty-nine e.p.s.p.s ranged from 24 to 538 μV, with a mean value of 157 ± 15 μV (S.E. of mean). The wave forms of these e.p.s.p.s also varied considerably: rise time ranged from 0·2 to 2·1 ms (mean = $0{\cdot}7 \pm 0{\cdot}1$ ms (S.E. of mean)), and half-width, for the forty-four e.p.s.p.s in which it could be measured reliably, varied between 0·9 and 7·7 ms (mean = $3{\cdot}6 \pm 0{\cdot}3$ ms (S.E. of mean)). These single-fibre Ia e.p.s.p. parameters were quite similar to those previously observed under comparable conditions (Mendell & Henneman, 1971; Scott & Mendell, 1976; Watt *et al.* 1976; Nelson *et al.* 1979; Harrison & Taylor, 1981; Fleshman *et al.* 1981*b*; Lucas *et al.* 1984; Clamann *et al.* 1985).

To understand the factors affecting e.p.s.p. parameters (and the corresponding correlograms), we considered some relevant properties of the Ia fibre–motoneurone connections sampled. Two factors previously shown to influence e.p.s.p. amplitude, indicated in Fig. 3, are the relation between the Ia fibre's muscle of origin and the motoneurone, and transection of the spinal cord. Consistent with earlier findings (Scott & Mendell, 1976), we found that e.p.s.p.s generated at normal homonymous connections tended to be larger than those produced at heteronymous connections. However, the difference in mean amplitudes of twelve e.p.s.p.s at homonymous (190 ± 22 μV (S.E. of mean)) and six at heteronymous connections (124 ± 31 μV (S.E. of mean)) was not significant ($P > 0{\cdot}1$).

Acute spinal transection was performed to increase the range in e.p.s.p. amplitude beyond that observed for medial gastrocnemius Ia fibre–motoneurone connections in cats with intact spinal cords (Nelson *et al.* 1979). Our three largest e.p.s.p.s (> 300 μV) were obtained in cats with transected spinal cords. However, none of our e.p.s.p.s exceeded the normal ranges previously reported, and the mean amplitude at homonymous connections in the transected cats (156 ± 20 μV (S.E. of mean); $n = 21$) was not significantly different ($P > 0{\cdot}1$) from that for cats with intact cords (190 ± 22 μV; $n = 12$). As for homonymous–heteronymous differences in cats with intact cords, the amplitudes of ten e.p.s.p.s at heteronymous connections in cats with transected cords (139 ± 49 μV) tended to be smaller than those at homonymous connections, yet the difference in mean values for these groups was not significant ($P > 0{\cdot}1$).

Two properties intrinsic to the motoneurone were also noted, namely conduction velocity and rheobase current. Their values spanned most of the range previously reported for triceps surae motoneurones recorded under similar experimental conditions (see Sypert & Munson, 1981; Ulfhake & Kellerth, 1984): conduction velocity ranged from 67 to 100 m/s (mean = 83 ± 2 m/s (S.E. of mean); $n = 23$) and rheobase current varied between 3 and 28 nA (mean = $13{\cdot}4 \pm 1{\cdot}6$ nA (S.E. of mean); $n = 26$). These motoneurone properties have been reported to be correlated with e.p.s.p. amplitude (Henneman & Mendell, 1981; Sypert & Munson, 1981; Collins, Honig & Mendell, 1984), but such correlations were not obvious in our sample.

Correlograms at single Ia fibre–motoneurone connections

Cross-correlating the stretch-evoked spikes of single Ia fibres with current-induced rhythmic firing of triceps surae motoneurones produced cross-correlation histograms, as exemplified by Figs. 1*D*, 2, 4, 5 and 9. Fig. 4 shows correlograms for homonymous and heteronymous Ia fibre–motoneurone connections in triceps surae motoneurones of cats with intact and acutely transected spinal cords.

In some cases, correlogram peaks were apparent but not prominent. To test quantitatively for the presence of a peak, we determined the significance of the difference between the mean number of motoneurone spikes in the positive peak and in the base-line interval using the z statistic (eqn. (7)). This statistic verified correlogram peaks significantly greater than base line ($P < 0{\cdot}05$) at thirty-four of forty-nine Ia fibre–motoneurone connections; the majority (thirty-one of thirty-four) were significant at $P < 0{\cdot}01$. For six of the remaining fifteen correlograms, cusum features were evident, yet the cusum-delineated peak in each case failed to achieve statistical significance at $P < 0{\cdot}05$. An example of such a correlogram is shown in Fig. 5*A*. In the other nine cases, even the cusums did not suggest the presence of a primary peak, thereby precluding statistical analysis.

An important factor affecting the significance of correlogram features is the number of pre- and post-synaptic spikes used to construct them (Sears & Stagg, 1976; Kirkwood, 1979). In our data, correlograms with significant *vs.* non-significant peaks had comparable numbers of Ia trigger spikes (2408 ± 287 spikes (S.E. of mean) *vs.* 2363 ± 410 spikes, respectively) and comparable numbers of motoneurone spikes (673 ± 95 spikes *vs.* 686 ± 114 spikes, respectively). Furthermore, some correlogram peaks reached significance with 500 trigger spikes or less. Conversely, 7009 Ia fibre

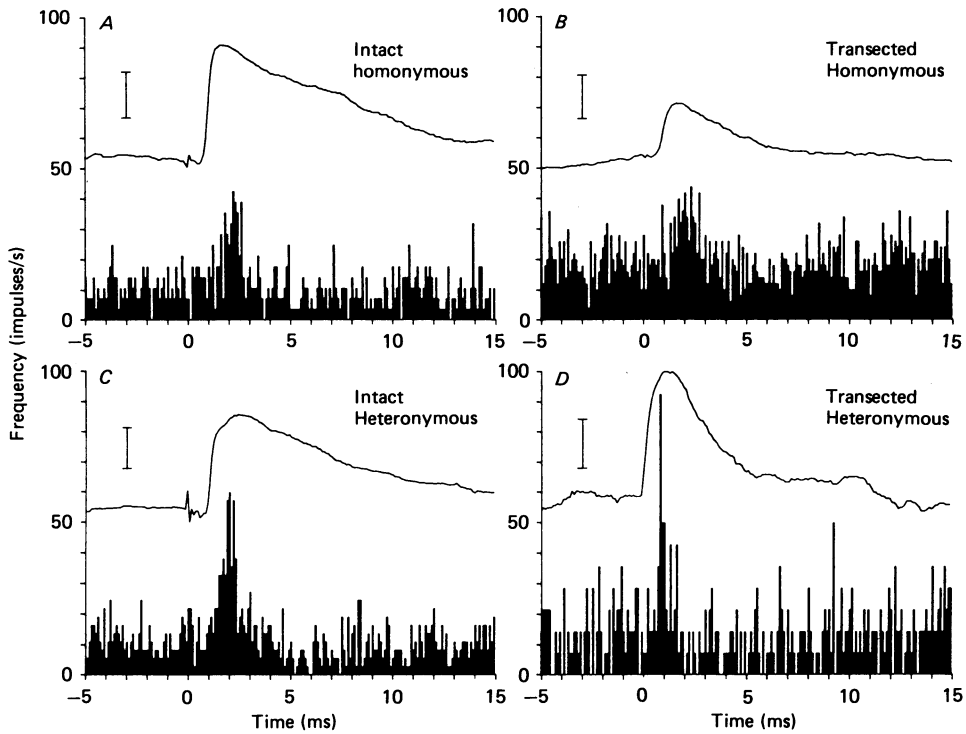

Fig. 4. Cross-correlation histograms and spike-triggered averaged e.p.s.p.s, each recorded from a single Ia fibre–motoneurone pair classified according to type of connection, i.e. homonymous (top) or heteronymous (bottom), and state of spinal cord, i.e. intact (left) or acutely transected at T13 (right). In all cases the Ia fibre supplied medial gastrocnemius muscle; both heteronymous cases involved lateral gastrocnemius–soleus motoneurones. Cases *A–C* appear in Table 1 as cases 4, 9 and 6, respectively. 1410 Ia trigger spikes and 414 motoneurone action potentials were used to produce the correlogram in *D*. Bin width for all histograms is 100 μs. Deflections preceding e.p.s.p.s in *A* and *C* were artifacts introduced by Ia fibre trigger spikes.

trigger spikes and 1407 motoneurone spikes accumulated at one connection produced a correlogram that exhibited no significant peak (Fig. 5*B*).

The magnitude of Ia fibre influence on motoneurone discharge frequency was quantified by the mean percentage increase (m.p.i.) in firing rate above base line during the correlogram peak (eqn. (5)). The value for m.p.i. ranged from 24 to 318% (mean = $89 \pm 9{\cdot}6$% (S.E. of mean)) for all thirty-four cases exhibiting significant peaks.

Detailed analysis was restricted to twenty connections (described in Table 1) where correlograms were generated by an arbitrary limit of at least 2000 trigger spikes. Fig. 6*B* illustrates the distribution of the m.p.i. in firing probability measured for these correlograms. Values for the thirteen significant peaks in this group ranged from 29 to 138% with a mean of 74 ± 9% (S.E. of mean). The height of the correlogram peaks was also measured in terms of the k value (eqn. (3)) and relative peak

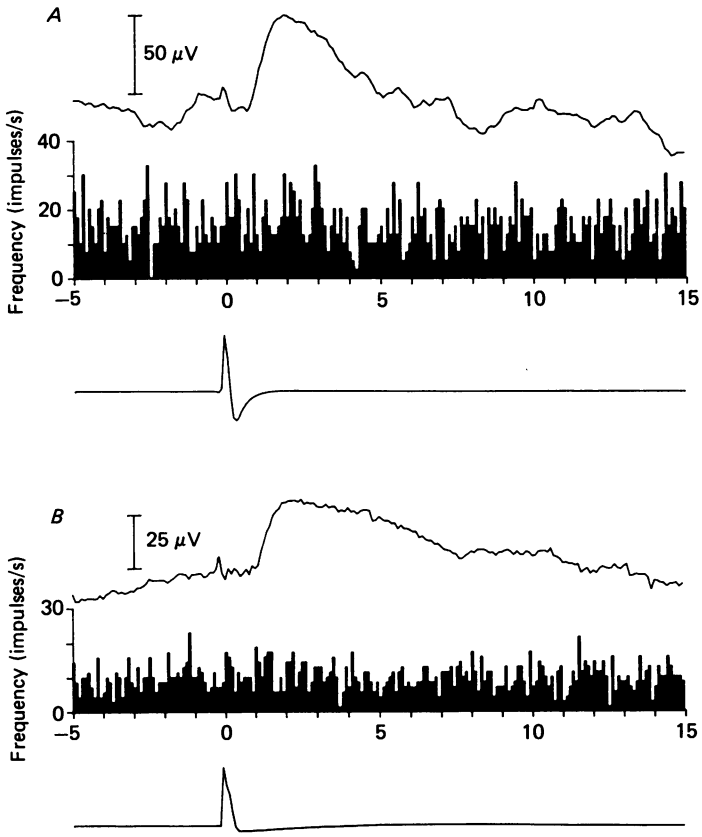

Fig. 5. Records from two Ia fibre–motoneurone pairs for which no significant correlogram peak was identified. Each set shows the Ia fibre spike and spike-triggered averaged e.p.s.p. in bottom and top traces respectively, with their corresponding correlogram in the middle. Correlograms in *A* and *B* (cases 17 and 18, respectively, in Table 1) were constructed from, respectively, 4000 and 7009 Ia trigger spikes and 1247 and 1407 motoneurone spikes. Bin width for both histograms is 100 μs.

height (equivalent to $k-1$; eqn. (4)). The mean value for k measured from thirteen significant peaks (individual values listed in Table 1) was $3{\cdot}6 \pm 0{\cdot}3$ (S.E. of mean) and that for relative peak height was $2{\cdot}6 \pm 0{\cdot}3$.

The time from onset of the cusum peak to its maximum was taken as the duration of the correlogram peak. The durations of the significant correlogram peaks are listed in Table 1. Their mean duration was $2{\cdot}5 \pm 0{\cdot}1$ ms (S.E. of mean). These durations were not significantly related to m.p.i. in firing probability ($r = 0{\cdot}03$; $P > 0{\cdot}1$).

A period of decreased firing probability was sometimes evident immediately after the correlogram peak. Although this correlogram 'trough' was shallow and difficult to resolve in many cases, it was clearly present in others (see Figs. 1, 2 and 4*A*, *B* and *C*). If the correlogram trough is produced by spikes advanced into the peak, their areas (measured from base line) should be equal, and the mean firing rate averaged over both the peak and trough together should equal the base-line rate. Consistent

TABLE 1. Values for parameters measured at each of twenty selected Ia fibre–motoneurone connections

Case no.	Spinal cord	Connection type	Rest-e.p.s.p. amp. (μV)	Rest-e.p.s.p. r.t. (ms)	Rest-e.p.s.p. deriv. (mV/ms)	Rest-e.p.s.p. h.w. (ms)	Ramp-e.p.s.p. amp. (μV)	Ramp-e.p.s.p. r.t. (ms)	Ramp-e.p.s.p. deriv. (mV/ms)	m.p.i. in firing probability (%)	k	Peak dur. (ms)	Lat. (ms)	m.n. spike no.	Trig. spike no.
1	T	Hm	310	0·3	0·63	2·4	305	0·6	0·38	105	3·6	2·1	0·3	1639	4496
2	I	Hm	292	1·4	0·17	nm	240	1·1	0·16	81	4·5	2·5	0·3	423	1997
3	I	Hm	266	0·6	0·36	2·2	247	0·9	0·23	105	4·9	2·6	0·7	789	3065
4	I	Hm	261	0·5	0·44	7·2	nm	nm	nm	84	3·9	2·8	0·2	647	2843
5	T	Hm	245	0·4	0·53	1·6	230	0·6	0·31	68	3·0	2·1	0·1	2266	6337
6	I	Ht	230	1·0	0·17	4·8	236	1·0	0·19	138	5·2	2·5	0·0	903	3686
7	I	Hm	217	0·4	0·44	2·2	188	0·7	0·23	80	4·0	2·5	0·4	780	3810
8	T	Hm	205	0·6	0·25	5·9	nm	nm	nm	83	5·6	3·0	0·0	1429	2586
9	T	Hm	124	0·5	0·19	3·0	108	1·0	0·11	30	2·2	3·2	0·1	2040	5041
10	T	Hm	120	0·9	0·11	3·2	105	1·3	0·07	50	2·5	3·0	0·3	1506	6833
11 (3)	I	Hm	117	0·2	0·43	1·4	108	0·3	0·26	69	3·4	1·8	0·5	715	3013
12	T	Hm	87	0·3	0·23	1·4	nm	nm	nm	39	2·3	1·9	0·6	1356	3815
13	T	Hm	86	0·3	0·24	0·9	77	0·4	0·16	29	2·1	2·0	0·0	1277	5280
14 (6)	I	Ht	86	0·3	0·23	nm	71	0·3	0·17	ns	nm	nm	nm	519	2682
15 (8)	T	Hm	82	0·5	0·13	1·9	nm	nm	nm	ns	nm	nm	nm	1536	3696
16	I	Hm	68	1·0	0·06	3·8	nm	nm	nm	ns	nm	nm	nm	348	1993
17 (1)	T	Hm	67	0·8	0·07	2·7	65	0·6	0·11	ns	nm	nm	nm	1247	4000
18	I	Ht	35	0·7	0·04	4·9	nm	nm	nm	ns	nm	nm	nm	1407	7009
19	T	Ht	30	0·5	0·05	2·5	nm	nm	nm	ns	nm	nm	nm	753	2007
20 (12)	T	Hm	25	0·5	0·04	4·6	nm	nm	nm	ns	nm	nm	nm	1071	2922

Symbols: spinal cord intact (I) or transected (T); connection type homonymous (Hm) or heteronymous (Ht); e.p.s.p. rise time (r.t.); e.p.s.p. rate of rise for 10–90% of peak (deriv.); e.p.s.p. half-width (h.w.); motoneurone (m.n.); Ia-fibre trigger (Trig.); value not measured (nm); m.p.i. non-significant, i.e. $P > 0·05$ (ns). In five cases the same motoneurone was studied with two different Ia fibres and the numbers in parentheses beside case number identify the other case in the pair.

with this expectation, the net mean firing rate, calculated from onset of the correlogram peak to the end of the average, was not consistently different from base line.

Relations between correlogram peaks and single-fibre e.p.s.p.s

The e.p.s.p.s and cross-correlograms obtained from the same Ia fibre–motoneurone connections were used to evaluate the relation between their properties. Systematic evaluation was based on the twenty Ia fibre–motoneurone connections involving more than 2000 correlogram trigger spikes. The mean values and range of the properties of these e.p.s.p.s (listed in Table 1) were essentially the same as those of the whole population ($n = 49$). Fig. 6*A* illustrates the distribution of e.p.s.p. amplitudes, which ranged from 25 to 310 μV, with a mean of 148 ± 21 μV (S.E. of mean).

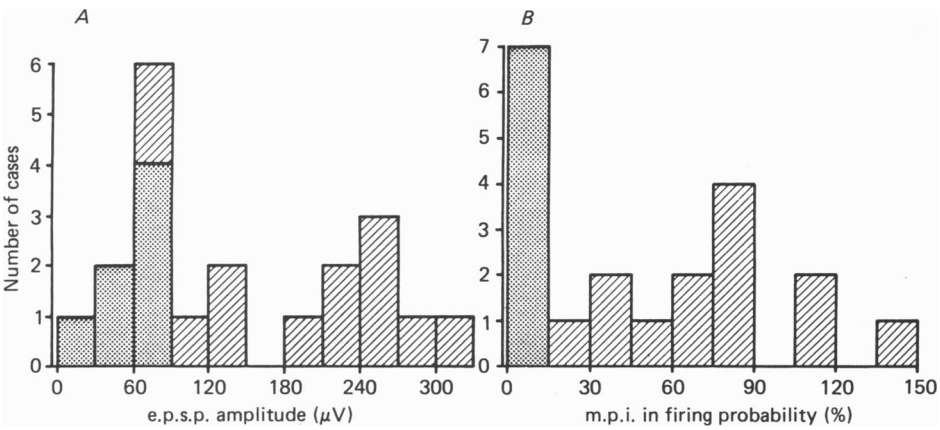

Fig. 6. Histograms of *A*, e.p.s.p. amplitude and *B*, mean percentage increase in motoneurone firing probability for twenty Ia fibre–motoneurone connections. Stippled bars indicate cases in which no significant correlogram peak was resolved.

Considerable variability was also noted for e.p.s.p. wave forms among these connections: rise time ranged from 0·2 to 1·4 ms (mean = $0{\cdot}6 \pm 0{\cdot}1$ ms (S.E. of mean)) and half-width (for eighteen cases in which it was reliably measured) ranged from 0·9 to 7·2 ms (mean = $3{\cdot}1 \pm 0{\cdot}4$ ms (S.E. of mean)).

Correlogram peak amplitude. The most obvious relation between e.p.s.p.s and their associated correlogram peaks involved their amplitudes. The histograms in Fig. 6 show that those connections that produced correlograms without a significant peak (stippled bars in Fig. 6*B*) tended also to have the smallest e.p.s.p.s (stippled bars in Fig. 6*A*). This trend can also be seen in Table 1 which ranks the cases in order of decreasing e.p.s.p. amplitude. The e.p.s.p.s. that produced no effect had a mean amplitude (56 ± 10 μV (S.E. of mean)) significantly smaller ($P < 0{\cdot}0001$) than those associated with significant peaks (197 ± 22 μV). For the entire sample of Ia fibre–motoneurone connections ($n = 49$), those that generated e.p.s.p.s less than 86 μV failed to produce correlogram peaks significant at $P < 0{\cdot}05$. This dependence on e.p.s.p. amplitude was also reflected in the five cases, identified in Table 1, in which

e.p.s.p.s and correlograms were produced by two different Ia fibres in the same motoneurone; in each case the larger e.p.s.p. of the pair was associated with the larger m.p.i. in firing probability.

The m.p.i. in firing probability had a significant positive relation with e.p.s.p. amplitude ($r = 0{\cdot}76$; $P < 0{\cdot}005$) (Fig. 7*A*). The slope of the linear regression line was $0{\cdot}30\,\%/\mu V$. The linear regression between m.p.i. in firing probability and e.p.s.p. amplitude was also significant when calculated for the entire sample of connections producing significant correlogram peaks ($n = 34$; $r = 0{\cdot}76$; $P < 0{\cdot}0005$). Moreover, the slope of this relationship ($0{\cdot}45\,\%/\mu V$) was similar to that derived from our select group.

Positive relations were also found between e.p.s.p. amplitude and the two measures of maximum correlogram peak height, namely k and relative peak height, for thirteen significant peaks. The correlation coefficient for either k or peak height *vs.* e.p.s.p. amplitude ($r = 0{\cdot}67$) (equal of necessity) was not as strong as for m.p.i. in firing probability *vs.* e.p.s.p. amplitude, but significant ($P < 0{\cdot}01$). The slope of their relationship with e.p.s.p. amplitude was $0{\cdot}010/\mu V$.

Of all the correlogram peak parameters, the one most strongly related to e.p.s.p. amplitude was the correlogram peak area (eqns. (6*a*) and (6*b*)). The correlogram peak area increased with e.p.s.p. amplitude with a slope of $1{\cdot}1 \times 10^{-4}$ impulses/μV; this relation had a correlation coefficient of $r = 0{\cdot}80$ (Fig. 7*B*).

None of the measures of correlogram amplitude (m.p.i. in firing probability, peak area, k or relative peak height) was strongly related to either e.p.s.p. rise time or half-width. The only statistically significant correlation found in these comparisons was that for k *vs.* half-width ($r = 0{\cdot}57$; $P < 0{\cdot}025$). Similarly, e.p.s.p. rate of rise was not significantly related to the measures of correlogram peak amplitude ($P > 0{\cdot}05$ in all cases). The relationship between m.p.i. in firing probability and e.p.s.p. mean rate of rise ($r = 0{\cdot}28$) for thirteen significant peaks is illustrated in Fig. 8.

Correlogram peak shape. The shapes of the correlogram peaks were compared to the e.p.s.p.s and their derivatives, after accounting for some difference in their latencies. The latency from e.p.s.p. onset to correlogram peak onset was measured for the thirteen single fibre–motoneurone connections with significant correlogram peaks (see Table 1). The onset of the increase in firing probability (measured from cusum onset and corrected for the mean delay of the Schmitt trigger pulse) occurred on average at $0{\cdot}3 \pm 0{\cdot}1$ ms (S.E. of mean) after e.p.s.p. onset. In three cases, e.p.s.p. and correlogram peak onset was simultaneous; the longest latency was 0·7 ms. The delay of the correlogram peak after onset of the e.p.s.p. was not clearly related to any of the correlogram or e.p.s.p. parameters measured in this study.

After temporal alignment, the shapes of the correlogram peaks produced by single-fibre e.p.s.p.s. could often be accounted for, in large part, by a function proportional to the derivative of the underlying e.p.s.p. However, the correlogram peaks were typically wider than the e.p.s.p. derivative. To determine the difference in time course between these two functions, the e.p.s.p. derivative was subtracted from the correlogram peak. First the e.p.s.p. derivative was temporally aligned with the correlogram in such a way that their peaks coincided, then the derivative peak was scaled to the height of the correlogram peak above base line and subtracted. Representative examples are illustrated in Fig. 9. In four cases (nos. 3, 4, 7 and 8 in Table

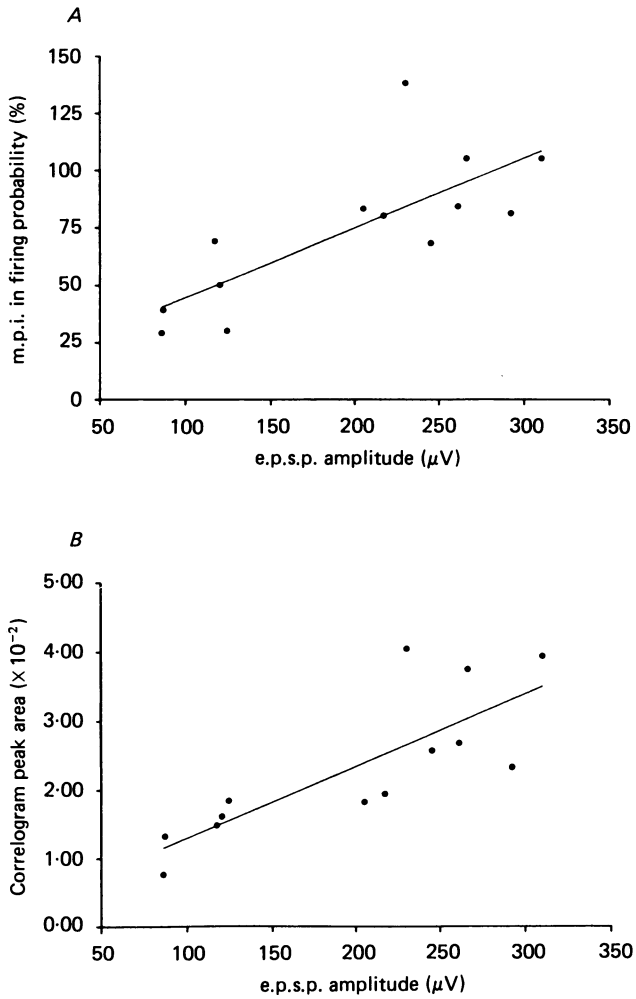

Fig. 7. Measures of correlogram peak amplitude plotted against e.p.s.p. amplitude. Each plot includes thirteen cases from Table 1 with significant correlogram peaks and shows the linear regression line. *A*, mean percentage increase (m.p.i.) in motoneurone firing probability *vs.* e.p.s.p. amplitude ($y = (0{\cdot}30\%/\mu V)\ x + 14{\cdot}10\%$; $r = 0{\cdot}76$). *B*, correlogram peak area *vs.* e.p.s.p. amplitude ($y = (1{\cdot}1 \times 10^{-4}/\mu V)\ x + 2{\cdot}5 \times 10^{-3}$; $r = 0{\cdot}80$).

1), the difference term was negligible, indicating that the e.p.s.p. derivative term accounted reasonably well for the correlogram peak, e.g. Fig. 9*A*. (Note: the peak durations in Table 1 are consistently greater than the e.p.s.p. rise times. However, this peak duration, defined as the time from onset to peak of the cusum, includes some bins at each end whose contents did not deviate from base line sufficiently to contribute visibly to the difference term.) In six cases (nos. 1, 5, 6, 9, 10 and 11) the correlogram peak was wider than the e.p.s.p. derivative; these cases revealed a difference term whose duration was shorter than the e.p.s.p. (Fig. 9*B*). In one case (no. 2) the width of the correlogram peak was distinctly less than that of the derivative.

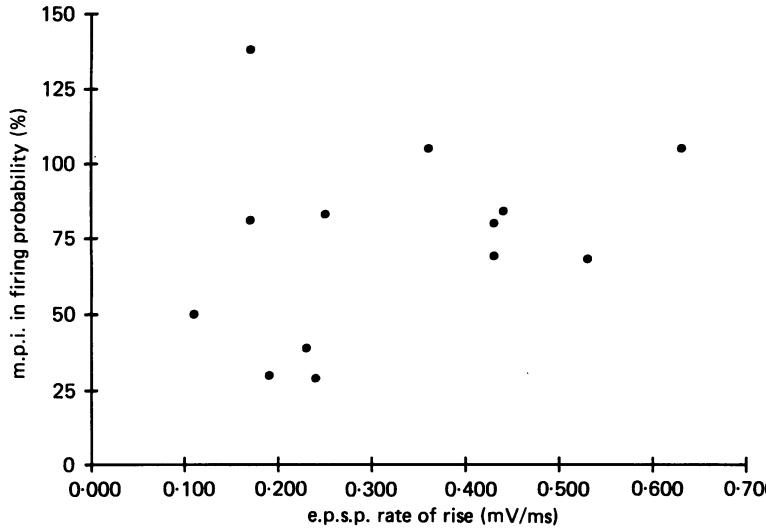

Fig. 8. Mean percentage increase (m.p.i.) in motoneurone firing probability plotted against e.p.s.p. mean rate of rise, for thirteen cases in which m.p.i. in firing probability was significant.

For the twelve largest correlograms we tested the degree to which the correlogram could be approximated by the linear transform between firing rate ($f(t)$) and the e.p.s.p. ($e(t)$) proposed by Kirkwood & Sears (1979):

$$f(t) = f_0 + ae(t) + b\mathrm{d}e/\mathrm{d}t. \tag{8}$$

The values of the coefficients a and b that would produce the best fit were computed for each case by the least-squares method. The optimal fits were obtained for various latency shifts between the correlogram and the e.p.s.p.; the latency shifts which produced the best match ranged between 0·1 and 1·3 ms (mean 0·7 ms $\pm$ 0·3 (S.D.)). At the best latency the optimal coefficients a of the e.p.s.p. terms had a mean value of 22·1 $\pm$ 12·5 impulses s^{-1} mV^{-1} (S.D.) (range: 1·7–46·3 impulses s^{-1} mV^{-1}). The coefficients b of the derivative term had a mean of 0·051 $\pm$ 0·020 impulses/mV (range: 0·008 – 0·084 impulses/mV). The mean value for the ratios of b to a coefficients was 8·2 $\pm$ 13·3 ms (range: 0·2–43·6 ms). A more comparable and direct measure of the relative contributions of the last two terms in eqn. (8) is the ratio of their magnitudes. The magnitudes of the derivative terms (b de/dt) were, on average, about 15 times larger than the amplitudes of e.p.s.p. terms (ae). This ratio ranged from 0·6 to 99·7 and was not clearly correlated with any of the e.p.s.p. or correlogram parameters. We also compared the error between the correlogram and the best derivative match (above) with the error of the best match with derivative plus e.p.s.p. (eqn. (8)). This showed that addition of the e.p.s.p. term to the derivative term improved the match with the correlogram relatively little. The size of the e.p.s.p. term was usually limited because its decay introduced a mismatch with the correlogram trough.

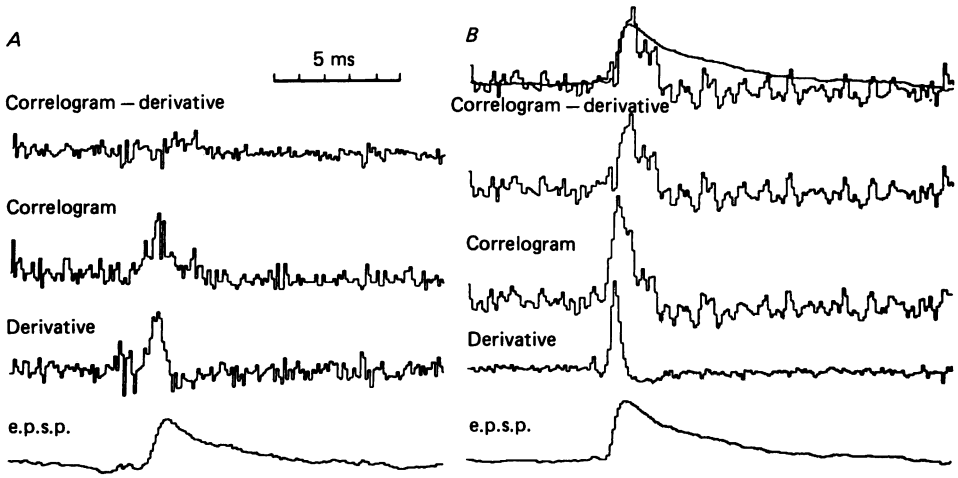

Fig. 9. Comparison of correlogram peak with e.p.s.p. derivative for two representative cases. Records show, from bottom up, e.p.s.p., e.p.s.p. derivative, correlogram peak (shifted in time to align with derivative peak), and correlogram minus derivative (after equalizing their heights). In some cases the difference between the correlogram peak and e.p.s.p. derivative was negligible (top left); in others the difference term had a time course more brief than that of the e.p.s.p., as seen by their superposition (top right).

Ramp-e.p.s.p. properties

The e.p.s.p.s which most directly influence the firing probability of a repetitively discharging motoneurone are those that occur near threshold. The above comparisons have involved properties of e.p.s.p.s measured with the motoneurone at rest. Since the amplitude and wave form of monosynaptic e.p.s.p.s have been reported to change with motoneurone membrane potential (Edwards, Redman & Walmsley, 1976; Engberg & Marshall, 1979), it seemed relevant to see whether the e.p.s.p. occurring near threshold in the repetitively discharging motoneurone differed from those recorded with the motoneurone at rest.

At thirty-one of the forty-nine connections studied it was possible to accumulate enough Ia trigger spikes with associated motoneurone membrane potential records (at least 100 sweeps) to resolve ramp e.p.s.p.s whose properties could be measured satisfactorily. Rest and ramp e.p.s.p.s obtained at two representative connections are illustrated in Fig. 10. Compared with spike-triggered averaged e.p.s.p.s generated at rest, amplitudes of ramp e.p.s.p.s were smaller on average by $7{\cdot}7 \pm 2{\cdot}0\,\%$ (S.E. of mean). However, the difference between mean amplitudes for rest and ramp e.p.s.p.s at these thirty-one connections (188 ± 19 S.E. of mean) and $173 \pm 17\ \mu$V, respectively) was not significant ($P > 0{\cdot}1$). In seven cases, the amplitude of the ramp e.p.s.p. exceeded that of the corresponding rest e.p.s.p. The percentage difference in amplitude of ramp e.p.s.p. relative to rest e.p.s.p. ranged from -23 to $+11\,\%$. Despite this difference, the amplitudes of rest and ramp e.p.s.p.s were strongly correlated ($r = 0{\cdot}98$; $P < 0{\cdot}0005$).

In addition to amplitude changes, e.p.s.p. rise time also differed during repetitive discharge compared with rest. The rise time of ramp e.p.s.p.s tended to be greater

(mean = 23 ± 9% (S.E. of mean)) than that of the corresponding rest e.p.s.p.s. However, different connections showed substantial variability: rise times of ramp e.p.s.p.s were greater than those of rest e.p.s.p.s in sixteen cases, smaller in nine cases and the same in the remaining six cases. Furthermore, the percentage difference in ramp-relative to rest-e.p.s.p. rise time was quite large for some connections, ranging from +157 to −62%. None the less, there was a significant positive correlation ($r = 0{\cdot}60$; $P < 0{\cdot}0005$) between ramp- and rest-e.p.s.p. rise time.

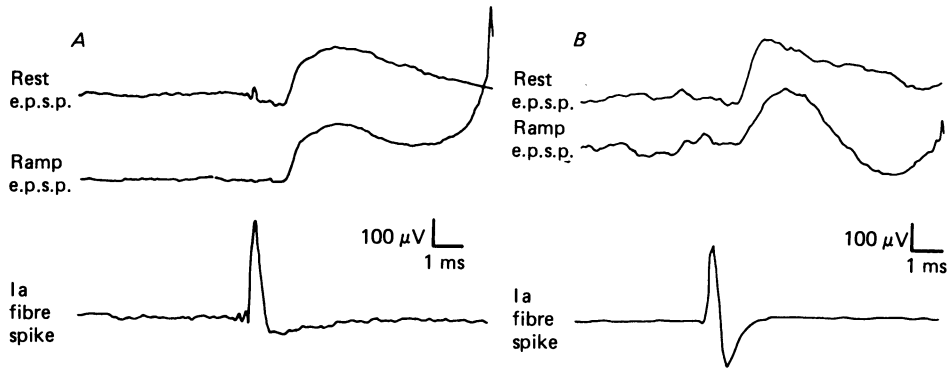

Fig. 10. Comparison of rest e.p.s.p.s with ramp e.p.s.p.s. Spike-triggered averaged e.p.s.p.s (100 sweeps each) from two Ia fibre–motoneurone connections. Top traces are e.p.s.p.s averaged with motoneurone at rest. Middle traces are spike-triggered averages of e.p.s.p.s occurring within 6–10 ms of motoneurone action potential, recorded on the depolarizing trajectory of rhythmically firing motoneurones. Slope of trajectory ramp was subtracted from base line. The distributed occurrence of motoneurone action potentials produced the increase in membrane potential toward end of ramp e.p.s.p.s. Averaged Ia spikes in bottom traces were compiled with ramp e.p.s.p.

The percentage difference in the rate of rise also varied considerably, over a range of +133 to −67%. The differences in rise times cannot, therefore, be ascribed entirely to differences in amplitude between rest and ramp e.p.s.p.s.

These differences between ramp- and rest-e.p.s.p. parameters were evaluated with respect to rest-e.p.s.p. characteristics and certain motoneurone properties. There was no obvious tendency for rest e.p.s.p.s with particular characteristics, e.g. brief rise time or large amplitude, to show systematic changes in any parameter when the e.p.s.p.s were averaged on the ramp. Similarly, the difference in ramp e.p.s.p.s relative to rest e.p.s.p.s for amplitude and rise time could not be predicted from motoneurone rheobase or conduction velocity. The differences between ramp and rest e.p.s.p.s were also unrelated to the type of synaptic connection (homonymous or heteronymous) and the state of the spinal cord (intact or transected). That the difference between ramp and rest e.p.s.p.s is unrelated to the above factors was further supported by cases in which two different Ia fibres projected to the same motoneurone. The amplitude and rise-time changes in ramp e.p.s.p.s relative to rest e.p.s.p.s were sometimes quantitatively and qualitatively different, even when recorded in the same motoneurone. For example, two different Ia fibres converging on the same motoneurone produced rest e.p.s.p.s that were 17% larger and 3% smaller than their corresponding ramp e.p.s.p.s. Similarly, the rise times for ramp and rest e.p.s.p.s differed by −25% (ramp e.p.s.p. shorter than rest e.p.s.p.) for one afferent and by +100% for another Ia fibre.

Differences between ramp and rest e.p.s.p.s may be ascribed in part to factors in measuring these events. The wave forms of ramp e.p.s.p.s tended to be noisier than rest e.p.s.p.s for two reasons. The

restricted time interval over which ramp e.p.s.p.s could be taken usually precluded averaging many more than 100 sweeps at a single Ia fibre–motoneurone connection. Additionally, the depolarizing trajectory of motoneurone membrane potential exhibited larger background fluctuations than the resting motoneurone, probably due to enhancement of inhibitory p.s.p.s. These factors introduced greater variance into the measurement of ramp-e.p.s.p. wave form.

Another factor that may have exaggerated the observed difference between ramp and rest e.p.s.p.s was the temporal resolution of the records. Since spike-triggered averages were digitized at 0·1 ms/bin, a difference in one bin could alter the rise time of a typical e.p.s.p. in this study (0·7 ms) by more than 14%. This could have contributed in part to the large differences in e.p.s.p. rise time and rate of rise observed between ramp and rest e.p.s.p.s. However, the onset times of ramp and rest e.p.s.p.s following the Ia trigger spike agreed quite well.

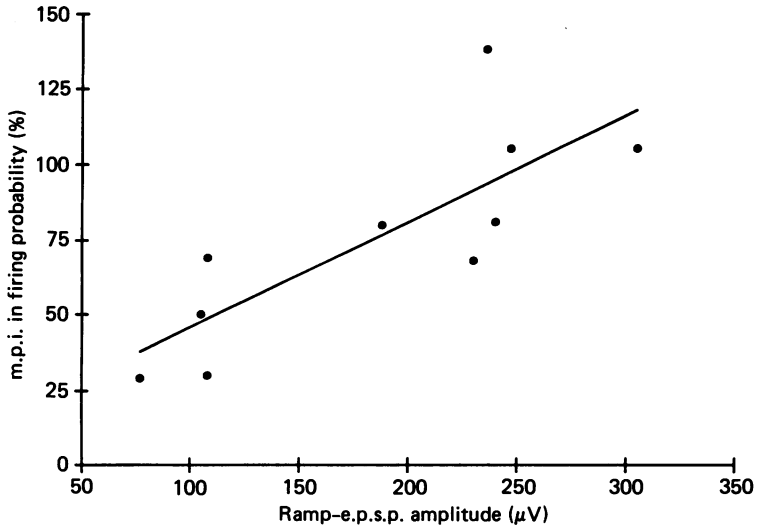

Fig. 11. Mean percentage increase (m.p.i.) in motoneurone firing probability *vs.* ramp e.p.s.p. amplitude. Linear regression line ($y = (0{\cdot}35\%/\mu V)\ x + 10{\cdot}75\%$; $r = 0{\cdot}80$) drawn for ten cases with significant correlogram peaks.

Comparison of correlogram properties with ramp e.p.s.p.s

Ramp e.p.s.p.s could be measured at twelve of the twenty selected Ia fibre–motoneurone connections (see Table 1). These e.p.s.p.s exhibited changes in properties similar to those of the entire group, reported above: the percentage reduction in amplitudes of ramp relative to rest e.p.s.p.s was $8{\cdot}8 \pm 1{\cdot}8\%$ (S.E. of mean); their rise times were greater by $38{\cdot}0 \pm 12{\cdot}3\%$. When m.p.i. in motoneurone firing probability was compared with ramp-e.p.s.p. properties at these connections, the relationship with e.p.s.p. amplitude was slightly improved (Fig. 11). The correlation coefficient of the relation between m.p.i. in firing probabilities for ten significant peaks and ramp-e.p.s.p. amplitude was $r = 0{\cdot}80$ ($P < 0{\cdot}005$) compared to $r = 0{\cdot}74$ ($P < 0{\cdot}01$) for the correlation with rest-e.p.s.p. amplitude at the same connections. Similarly, the correlogram peak area showed a stronger correlation with the amplitudes of ramp e.p.s.p.s ($r = 0{\cdot}88$) than the rest e.p.s.p.s ($r = 0{\cdot}80$) at the same ten connections. The k values had the same correlation ($r = 0{\cdot}71$) with both.

The correlation of m.p.i. in firing probability with the rate of rise of the ten ramp e.p.s.p.s was also stronger than that for the corresponding e.p.s.p.s: the correlation coefficient (r) increased from 0·24 to 0·47. However, the improvement did not yield a statistically significant relation ($P > 0·05$).

DISCUSSION

Our main objective was to examine the effect of single-fibre Ia e.p.s.p.s on motoneurone firing probability. In most cases, cross-correlograms between action potentials of individual Ia fibres and their target motoneurones revealed significant primary correlogram peaks, confirming that single Ia fibres can raise the firing probability of repetitively discharging motoneurones (Kirkwood & Sears, 1982; Cameron *et al.* 1980). Our data also indicate that the existence of a monosynaptic connection does not ensure the generation of a significant correlogram peak (cf. Lindsey & Gerstein, 1979; Kirkwood, 1979), since correlogram peaks were not produced at every connection that exhibited a single-fibre Ia e.p.s.p.

Correlogram peaks produced by different Ia fibre–motoneurone pairs varied considerably in amplitude and duration. At one extreme, the m.p.i. in motoneurone firing probability exceeded 100% (in ten of forty-nine cases), while in fifteen other cases no significant change was detected. This distribution reflects a substantial range in the correlational efficacy of group Ia fibre connections with spinal motoneurones.

Previous studies have demonstrated empirically and theoretically (Moore *et al.* 1970; Knox, 1974; Sears & Stagg, 1976; Knox & Poppele, 1977; Kirkwood & Sears, 1978, 1982; Lindsey & Gerstein, 1979; Ashby & Zilm, 1982; Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984; Surmeier & Weinberg, 1985) that certain parameters of correlogram peaks depend largely on properties of their associated post-synaptic potentials. In rhythmically firing motoneurones, e.p.s.p.s appreciably larger than the background synaptic noise produced correlogram peaks proportional to the e.p.s.p. derivative (Fetz & Gustafsson, 1983). For smaller e.p.s.p.s in synaptic noise of comparable amplitude, the correlogram peaks are often wider than the e.p.s.p. derivative (Kirkwood, 1979; Kirkwood & Sears, 1982; Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984). All direct comparisons to date have involved composite e.p.s.p.s evoked by electrical stimulation or muscle stretch, which often have a time course different from e.p.s.p.s generated by single afferent fibres. To investigate the relation between single-fibre Ia e.p.s.p.s and changes in motoneurone firing probability, we compared spike-triggered averaged e.p.s.p.s with the cross-correlograms they produced at the same single Ia fibre–motoneurone connection.

The e.p.s.p.s and motoneurones sampled in our study were comparable in range to those previously documented, so our data would be relevant to estimating correlational synaptic strength from prior description of e.p.s.p.s. Our values for amplitude, rise time and half-width were within the established range for e.p.s.p.s recorded from triceps surae motoneurones in barbiturate-anaesthetized cats. Our data were also typical in that e.p.s.p. amplitude tended to be greater, albeit insignificantly, for homonymous than for heteronymous connections.

There were notable discrepancies, however, involving mean e.p.s.p. amplitude. The mean amplitude of our e.p.s.p.s from normal homonymous medial gastrocnemius connections (190 μV) was larger than the 100 μV average amplitude typically found for the whole population of this species

of Ia fibre–motoneurone connections (Henneman & Mendell, 1981). In addition, our mean amplitude for e.p.s.p.s recorded at homonymous medial gastrocnemius connections in cats with acute spinal cord transections (156 μV) was half the value of 313 μV observed under the same conditions by Nelson *et al.* (1979). Although we do not know the basis for these discrepancies, certain properties of our motoneurones suggest an explanation.

The values for conduction velocity and rheobase current of our entire sample of motoneurones matched well the range of those previously described (Sypert & Munson, 1981; Ulfhake & Kellerth, 1984). However, separating our data into intact *vs.* spinal-transected groups revealed unequal distributions for both of these parameters. The relation between these intrinsic motoneurone properties and e.p.s.p. amplitude may explain the unequal distribution for e.p.s.p. amplitude in these groups. In the intact group, half of the e.p.s.p.s were taken from motoneurones with rheobase less than 6 nA. Below this rheobase value almost all medial gastrocnemius motoneurones belong to type S motor units (Fleshman, Munson, Sypert & Friedman, 1981*a*); these motoneurones were shown to exhibit larger e.p.s.p.s than the population mean (Fleshman *et al.* 1981*b*). This finding may explain why our mean e.p.s.p. amplitude was larger than normal. On the other hand, more than half of the e.p.s.p.s recorded from motoneurones in the group with spinal transections were taken from motoneurones with rheobase current greater than 20 nA. According to Fleshman *et al.* (1981*a*, *b*), these motoneurones would be expected to correspond to motor-unit types FF and FI, in which Ia e.p.s.p.s tend to be smaller than the population mean. A sample bias toward higher values of conduction velocity and rheobase current, which are not caused by acute spinal cord transection (Nelson *et al.* 1979; Gustafsson, Katz & Malmsted, 1982; Collins *et al.* 1984) would therefore tend to produce a lower mean e.p.s.p. amplitude. In addition, this factor may have obscured any increase in e.p.s.p. amplitude induced by transection, since transection tends to produce the greatest increases in motoneurones with the slowest conduction velocities (Nelson *et al.* 1979). Although the basis of the increase in synaptic efficacy following acute spinal cord transection remains unresolved, our data are consistent with previous demonstrations (Cope, Nelson & Mendell, 1980) that the effect of transection is unequally distributed among Ia fibre–montoneurone connections.

Of all properties measured in this study, only those of the e.p.s.p. were correlated with correlogram peak characteristics. Correlogram characteristics were not directly related to the class of Ia fibre–motoneurone connection (homonymous or heteronymous) or condition of the spinal cord (intact or acutely transected) or to the intrinsic motoneurone properties. Slight tendencies for correlogram peaks to be related to these factors can be explained simply on the basis of their relation to e.p.s.p. properties.

Relations between correlogram and e.p.s.p. parameters

The m.p.i. in motoneurone firing probability was found to be directly proportional to the amplitude of its associated single-fibre e.p.s.p. (Fig. 7*A*). This finding is in agreement with the positive correlations found for correlogram peak height and the amplitude of aggregate e.p.s.p.s produced by electrical stimulation (Fetz & Gustafsson, 1983) or muscle stretch (Gustafsson & McCrea, 1984) in spinal motoneurones. In our study, the m.p.i. in firing probability was clearly correlated with e.p.s.p. amplitude ($r = 0{\cdot}76$), suggesting that single-fibre Ia e.p.s.p. amplitude is a principal determinant of correlogram peak size. However, the scatter in the values indicates that other factors also influence the correlogram peak as discussed below.

In two previous studies, correlogram peak height was compared with e.p.s.p.s that were comparable in size to those studied here. Gustafsson & McCrea (1984) applied brief triangular stretches to triceps surae muscles to evoke e.p.s.p.s whose amplitudes and rise times could be varied independently. Many of their e.p.s.p.s were less than

300 μV and some were as small as 30 μV. As illustrated in their Fig. 5*A*, the relative peak height of the associated correlograms (i.e. the maximum firing rate during the peak relative to base line) ranged from about 4 to less than 1, which corresponds well with the range of 4·6–1·1 obtained by measuring our correlogram peaks in the same way. However, the slope of the relation between the amplitudes of correlogram peaks and the underlying e.p.s.p.s was 0·010/μV for our data, more than twice their value of 0·004/μV. This difference may reflect the lower rates of rise of stretch-evoked e.p.s.p.s compared with single-fibre e.p.s.p.s (see below). For our sample, the slope of this relationship falls between that found by Gustafsson & McCrea (1984) and the value of 0·024/μV found by Fetz & Gustafsson (1983) for electrically evoked aggregate e.p.s.p.s.

In another comparable study, Kirkwood & Sears (1982) cross-correlated spike trains of single afferent fibres with spikes of intercostal motoneurones recorded during respiration. They measured correlogram peak amplitudes in terms of k, the ratio of the maximum bin content to base-line mean; their k values ranged from 1·09 to 2·14 for the nine single fibre–motoneurone connections studied. Our k values for thirteen significant peaks ranged from 2·1 to 5·6, with a mean of 3·6 (see Table 1). We calculated k using a bin width (0·1 ms) that was half of theirs, which might make our values comparatively larger. Since Kirkwood & Sears (1982) did not measure the e.p.s.p.s associated with their single afferent fibre correlograms, it also remains possible that their correlograms were associated with e.p.s.p.s that were smaller than their assumed mean value of 171 μV. Another possible explanation for their lower k values is that the relation between e.p.s.p. and correlogram peak amplitude for synaptically activated thoracic motoneurones may differ from that for lumbosacral motoneurones induced to fire by current injection. The higher level of synaptic noise in their experiments may distribute the correlogram peaks over longer intervals, reducing the peak value.

We chose to quantify the height of the correlogram peak by the m.p.i. in firing probability, which measures the mean increase over the whole correlogram peak, rather than by the k value or relative peak height, which reflect the maximum value in a single bin. The m.p.i. in firing probability seems more representative of the total effect of the e.p.s.p., and is less susceptible to statistical fluctuations and choice of bin parameters. Indeed, for our records m.p.i. had a somewhat stronger relation to e.p.s.p. amplitude than k. The greater scatter in the k measure may reflect the greater variance of a single-bin sample than of an average of all the samples in the peak. Another advantage of m.p.i. is that it is relatively independent of bin width (as long as the peak straddles several bins). In contrast, the k value depends on the size of the histogram bins (Kirkwood, 1979). Even for a given bin size, the k value could change with a shift in the location of the bins relative to the trigger spikes; for example, changing the acceptance level on the triggering spike would shift the location of the bin boundaries, which usually modifies the bin contents. As an average over all the bins in the correlogram peak, m.p.i. is less affected by the location of individual bin boundaries.

The e.p.s.p. amplitude was most strongly related to the correlogram peak area ($r = 0·80$). Like m.p.i. the peak area is representative of the entire correlogram peak; however, it differs from m.p.i. in that it measures the absolute net increase in spikes,

rather than the mean increase, above base line. Thus, the peak area provides a direct measure of the number of spikes produced by the e.p.s.p. during the peak, independent of their temporal distribution and of base-line firing rate.

Transform between e.p.s.p. and correlogram

The mathematical relation between the shape of the e.p.s.p. ($e(t)$) and the firing rate which it produces in the cross-correlogram ($f(t)$), if known, would allow the various measures of the correlogram peak to be directly related to parameters of the e.p.s.p. A useful expression for this relation is the first-order linear transform proposed by Kirkwood & Sears (1978), which applies when the correlogram can be described by the sum of terms proportional to the e.p.s.p. and its derivative:

$$f(t) = f_0 + ae(t) + b\mathrm{d}e/\mathrm{d}t. \tag{8}$$

The coefficients a and b are constants to be determined empirically or theoretically. This transform was initially deduced to explain the totally analogous relation between cross-correlograms of intercostal motoneurones (Sears & Stagg, 1976) and the underlying 'average common excitation potential' (Kirkwood & Sears, 1978).

In the first direct comparison between e.p.s.p.s and correlograms in the same mammalian motoneurones, Fetz & Gustafsson (1983) found that large e.p.s.p.s in the absence of background synaptic noise produced correlogram peaks with the time course:

$$f(t) = f_0 + \frac{f_0}{\dot{v}}\left(\frac{\mathrm{d}e}{\mathrm{d}t}\right), \tag{9}$$

where $\dot{v}$ = rate of closure between motoneurone membrane potential and threshold during rhythmic firing. This relation is deducible from a simple threshold-crossing model and was confirmed empirically for larger e.p.s.p.s (after aligning onsets of the e.p.s.p. and correlogram peak). Indeed, eqn. (9) described the correlogram trough, as well as the peak, so long as the e.p.s.p. rate of decay did not exceed $-\dot{v}$. Under these conditions, then, the transform is a special case of eqn. (8), in which the coefficients $a = 0$ and $b = f_0/\dot{v}$, for all e.p.s.p.s.

Using small stretch-evoked e.p.s.p.s in synaptic noise, Gustafsson & McCrea (1984) found that the corresponding correlograms could be better matched by eqn. (8) than (9), when the coefficients a and b could be optimized for each case. Obtaining the best fits between correlogram peaks (and troughs) and the sum of such terms, Gustafsson & McCrea (1984) found that the relative size of the e.p.s.p. term required to yield the best fit increased as the noise became larger than the e.p.s.p. Nevertheless, the derivative term typically remained larger than the e.p.s.p. term; for example, even when the noise was twenty times greater than the e.p.s.p., the best fits involved e.p.s.p. terms about half the size of the derivative term.

Thus, with appropriate choices of parameters, eqn. (8) appears to be compatible with the results of the above two studies, as well as the empirical data of Kirkwood & Sears (1978). In circumstances described by eqn. (8), the measures of the correlogram peak can be directly related to parameters of the e.p.s.p. For example, the correlogram peak area is given by:

$$\text{peak area} = \int_{t_0}^{t_e} (f(t) - f_0)\,\mathrm{d}t = a\int_{t_0}^{t_e} e(t)\,\mathrm{d}t + be(t_e). \tag{10}$$

For the noiseless case, described by eqn. (9), the correlogram peak area is directly proportional to the e.p.s.p. amplitude:

$$\text{peak area} = \left(\frac{f_0}{\dot{v}}\right) e(t_e). \tag{11}$$

This result expresses the fact that the net increase in firing during the peak (the total number of motoneurone firings above base line in the peak) is proportional to the net probability that the e.p.s.p. intercepts threshold, which in the noiseless case, is directly proportional to its amplitude (cf. Ashby & Zilm, 1982). The fact that peak area was also strongly related to e.p.s.p. amplitude for our single-fibre e.p.s.p.s. suggests that synaptic noise may broaden the correlogram peak without drastically affecting peak area.

For the general transform (eqn. (8)), the m.p.i. in firing probability can also be related to the e.p.s.p., as follows:

$$\text{m.p.i.} = \frac{100}{f_0 T_p}\left[a \int_{t_o}^{t_e} e(t)\,dt + be(t_e)\right], \tag{12}$$

where T_p is the correlogram peak duration.

In the case of large e.p.s.p.s (eqn. (9)) the m.p.i. in firing probability is proportional to the mean rate of rise of the e.p.s.p.:

$$\text{m.p.i.} = \left(\frac{100}{\dot{v}}\right)\frac{e(t_e)}{T_p}. \tag{13}$$

The parameter k, measuring the maximum height of the correlogram peak, is related to the e.p.s.p. by an expression that for the general transform (eqn. (8)) is:

$$k = 1 + \frac{1}{f_0}\left[ae(t) + b\frac{de}{dt}\right]_{\max}. \tag{14}$$

For the case of large e.p.s.p.s and/or no noise (eqn. (9)), the k value is related to the maximum rate of rise of the e.p.s.p.:

$$k = 1 + \frac{1}{\dot{v}}\left[\frac{de}{dt}\right]_{\max}. \tag{15}$$

To illustrate the effects of e.p.s.p. amplitude and rise time on these results, the general model (eqn. (8)) can be applied to simplified e.p.s.p.s which rise linearly to a peak height h in rise time T. Under these conditions, the correlogram measures depend on the e.p.s.p. parameters as follows:

$$\text{peak area} = h\left[\frac{aT}{2} + b\right], \tag{16}$$

$$\text{m.p.i.} = \frac{100}{f_0}\frac{h}{T_p}\left[\frac{aT}{2}+b\right], \tag{17}$$

$$k = 1+\frac{h}{T_p f_0}\left[aT+b\right]. \tag{18}$$

These expressions are further simplified when the derivative term is dominant, i.e. when $b \gg aT$.

The applicability of the above results to a population of e.p.s.p.s depends on the appropriateness of transform (eqn. (8) or (9)). In previous studies (Kirkwood & Sears, 1978; Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984) as well as the present data, the correlogram peak shape could be accounted for, in largest part, by a function proportional to the e.p.s.p. derivative. The presence of appreciable synaptic noise, however, made the correlogram peaks wider than the derivative, because noise introduced additional intercepts during the falling phase of the e.p.s.p. The noise can be thought to add another term to the derivative function, resulting in a wider peak. The best linear estimate of this additional term may be a function proportional to the e.p.s.p. itself, as suggested by Kirkwood & Sears (1982). In our data, subtracting the e.p.s.p. derivative from the correlogram sometimes yielded a negligible difference term, suggesting that the correlogram peak was essentially proportional to the derivative; in other cases the difference term was partially matched by the e.p.s.p., but was typically more brief (Fig. 9*B*). The brief duration of this remainder term may be qualitatively explained by the threshold crossings introduced by the noise (Fetz & Gustafsson, 1983). The synaptic noise is more likely to increase the number of threshold crossings near the peak of the e.p.s.p., as opposed to the tail, for two reasons: (a) the probability of additional stochastic intercepts is inversely related to the difference between membrane potential and threshold, and (b) threshold crossing is contingent on the absence of preceding interactions with the same e.p.s.p.; both factors reduce the probability of late *vs.* early intercepts near the peak.

To evaluate the best fits of our data with eqn. (8), we calculated the coefficients a and b that optimized the match with the correlogram. The magnitudes of the resulting derivative terms $(b\,\mathrm{d}e/\mathrm{d}t)_{max}$ were considerably larger than the e.p.s.p. terms ($a\,e$). Our values for the ratios of these coefficients (mean (b/a) = 8·2 ms; (mean b/mean a) = 2·3 ms) are compatible with previous estimates of this ratio (Kirkwood & Sears, 1978; Gustafsson & McCrea, 1984). Although the addition of the e.p.s.p. term widened the correlogram peak slightly, its size was ultimately limited by the mismatch with the correlogram trough. Consequently, even with the best estimates of a and b the linear sum did not match the correlogram peak much better than the derivative.

Another measure of this transform is the degree to which the predicted relations between gross parameters held. According to the linear transform, the correlogram peak measures would be roughly related to the e.p.s.p. parameters by eqns. (16)–(18). With the optimal values of a and b, our empirical data approximated the predicted relations with the following regression coefficients: for peak area (eqn. (16)), r = 0·85; for m.p.i. (eqn. (17)), r = 0·51; for k (eqn. (18)), r = 0·42. These expressions all contain

e.p.s.p. amplitude (h) multiplied by terms predicted by the linear model. Yet, even using the optimal coefficients for each case, these expressions did not correlate as strongly with m.p.i. and k as did e.p.s.p. amplitude alone ($r = 0{\cdot}76$ for m.p.i. and $0{\cdot}57$ for k). However, the peak area showed a somewhat stronger correlation with the predictions of the linear model than with e.p.s.p. amplitude alone ($r = 0{\cdot}80$).

Ramp e.p.s.p.s

The above comparisons were based on records of e.p.s.p.s obtained when the motoneurones were at rest. An underlying assumption is that the rest e.p.s.p.s are representative of the e.p.s.p.s which actually affect the change in firing probability, namely those occurring near threshold during motoneurone discharge. We tested this assumption because group Ia e.p.s.p.s may change with steady levels of depolarization (Edwards *et al.* 1976; Engberg & Marshall, 1979); however, e.p.s.p.s have never been characterized in repetitively firing motoneurones. To make a direct comparison, we documented single-fibre e.p.s.p.s occurring on the depolarizing ramp of motoneurone membrane potential near spike threshold during repetitive firing.

The e.p.s.p.s averaged during repetitive firing generally had amplitudes similar to e.p.s.p.s produced by the same Ia fibre in the motoneurone at rest. Although ramp-e.p.s.p. amplitude was usually somewhat diminished (by about 8%), it was strongly correlated ($r = 0{\cdot}98$) with rest-e.p.s.p. amplitude. Moreover, the slope of this relationship was nearly 1. Thus, the rest-e.p.s.p. amplitude is representative of the amplitude of e.p.s.p.s that produce the increase in motoneurone firing. Accordingly, the correlations of rest- and ramp-e.p.s.p. amplitude with m.p.i. in firing probability were similar.

In contrast, the change in e.p.s.p. rise time during repetitive firing was more striking: rise times sometimes differed by more than 2-fold for rest *vs.* ramp e.p.s.p.s, and changed in both directions. As a result, the rise time of ramp e.p.s.p.s was more poorly represented by the corresponding rest e.p.s.p. ($r = 0{\cdot}60$) than was amplitude. Whatever the underlying mechanism, it must explain the disproportionate change in rise time relative to amplitude, and the finding that the rising phase of e.p.s.p.s produced by two different Ia fibres in the same motoneurone often changed independently during repetitive discharge. More relevant to the present goal, the ramp-e.p.s.p. rate of rise was better correlated with the correlogram peak height than was rest-e.p.s.p. rate of rise.

Unfortunately, we could not match the time course of correlograms with the linear transform (eqn. (8)) for ramp e.p.s.p.s, since the e.p.s.p.s' falling phase was interrupted by action potentials.

Concluding comments

In conclusion, the direct relation we found between the amplitude of single-fibre e.p.s.p.s and the size of their cross-correlogram peaks is similar to that for compound e.p.s.p.s (Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984). Our data extend evaluation of this and other relations to the small-amplitude, fast-rising e.p.s.p.s generated by single Ia fibres. We demonstrate that single fibre e.p.s.p.s larger than 86 μV with rise times as brief as 0·2 ms could generate significant correlogram peaks sometimes with relatively few events (Table 1 and Fig. 4*D*).

Our results have two implications for interpreting correlogram features in terms of synaptic interactions. The measures of the correlogram peak which related most strongly to the underlying e.p.s.p. were those which involved the entire correlogram peak not just its maximum, namely, the peak area and m.p.i. in firing probability. These measures represent, therefore, the net effect of synaptic excitation, even when the peak is dispersed by synaptic noise. Secondly, because of its lower sensitivity compared to spike-triggered averages of membrane potential, cross-correlation may well underestimate synaptic connectivity.

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