Journal of Physiology (1992), **453**, *pp.* 547–574
With 9 *figures*
Printed in Great Britain

ESTIMATING THE STRENGTH OF COMMON INPUT TO HUMAN MOTONEURONS FROM THE CROSS-CORRELOGRAM

BY MICHAEL A. NORDSTROM*‡, ANDREW J. FUGLEVAND† AND ROGER M. ENOKA*†

*From the Departments of * Physiology and † Exercise and Sport Sciences,*
University of Arizona, Tucson, AZ 85724, USA

(*Received 27 June 1991*)

SUMMARY

1. The relationship between the motor unit discharge pattern (rate and variability) and synchronization of motor unit pairs was studied in the first dorsal interosseus muscle of human subjects. In separate trials of up to 4 min duration, subjects voluntarily controlled the mean discharge rate of an identified motor unit at one of several prescribed rates (range 7·5–17·5 Hz).

2. The effect of discharge rate on the synchronous peak in the cross-correlogram was examined in eighty motor unit pairs from six subjects. Five commonly used synchronization indices were used to quantify synchrony in the cross-correlograms constructed from different discharge-rate trials. For each synchronization index, the apparent magnitude of synchrony increased at lower motor unit discharge rates. The synchronization indices were not equally sensitive to discharge rate; increases in the different indices ranged from 72 to 494% between the highest and lowest discharge rates.

3. A model of the membrane potential trajectory underlying rhythmic motoneuron discharge was used to explain the observed increase in the magnitude of the synchronization indices at lower discharge rates. The essential feature of this model is that the probability of a common-input EPSP causing a synchronous discharge in two motoneurons is independent of discharge rate. This means that the number of synchronous action potentials *in excess of chance* in any trial depends on the properties of the common-input EPSPs and the duration of the trial, but is not related to motor unit discharge rates. The model also demonstrated that when the excess synchronous counts are normalized to motor unit discharge rate, or baseline counts in the histogram (as in the conventional synchronization indices), the magnitude of the index increases when the motor unit discharge rates are low.

4. The strength of common input to motoneurons could be misinterpreted if conventional synchronization indices are used because of discharge-rate effects. The model was used to derive an index of the strength of common input to motoneurons (CIS) that was independent of motor unit discharge rate. CIS is the frequency of synchronous action potentials in the motor unit pair in excess of those expected due

---

‡ Present address: Department of Physiology, University of Adelaide, Adelaide, Australia.

to chance (calculated during periods of tonic discharge in both units). The mean CIS in first dorsal interosseus motor unit pairs ranged from 0·052 to 1·005 extra synchronous action potentials per second across subjects.

5. Discharge variability was correlated with each of the synchronization indices and the CIS. Discharge variability differed across subjects (mean coefficient of variation of interspike intervals ranged from 12·1 to 26·4%) and subjects with more variable discharge had higher synchrony and CIS values. Differences in discharge variability accounted for 45% of the variation in CIS in different motor unit pairs. Large differences in CIS and discharge variability between subjects, and the correlation of synchrony and CIS with the variability of motoneuron discharge, suggest that either the properties of last-order presynaptic inputs to motoneurons, or intrinsic repetitive discharge properties of motoneurons, may differ substantially in the first dorsal interosseus motor pool among human subjects.

INTRODUCTION

Motoneurons that are activated in voluntary contractions have a weak tendency for synchronous discharge, which is revealed in the cross-correlogram (Moore, Perkel & Segundo, 1966) as an increased number of near-coincident action potentials above those expected due to chance. The synchronous peak in the cross-correlogram is often restricted to a narrow region near the discharge time of the reference unit. This 'short-term' synchronization (Sears & Stagg, 1976) is believed to arise from common-stem presynaptic inputs that increase the probability of simultaneous discharge in the target neurons sharing these inputs (Kirkwood & Sears, 1978; Datta & Stephens, 1990). The dependence of synchronization on the properties of shared last-order inputs has prompted studies of motoneuron synchronization in an attempt to uncover details of the neural circuitry underlying voluntary contractions. From this perspective, motoneuron synchronization has been recently studied in human spinal (Datta & Stephens, 1990) and trigeminal motor pools (Nordstrom, Miles & Türker, 1990), in different muscles of the same subject (Bremner, Baker & Stephens, 1991*a*, *b*), in the same muscle under different conditions of activation (Adams, Datta & Guz, 1989), in pathologic states (Datta, Farmer & Stephens, 1991), and even with pharmacological intervention (Logigian, Wierzbicka, Bruyninckx, Wiegner, Shahani & Young, 1988).

There is no current standard synchronization index derived from the cross-correlogram. Although it is recognized that the synchronous counts must be normalized to baseline discharge to allow for comparison with other cross-correlograms, a variety of normalization procedures have been proposed. At least four different indices have been used in recent studies of human motoneuron synchrony (Logigian *et al.* 1988; Datta & Stephens, 1990; Nordstrom *et al.* 1990; Bremner *et al.* 1991*b*). Despite this diversity, the magnitude of synchrony estimated from the cross-correlogram is thought to be positively correlated with the strength of common input (i.e. the number of shared branches of common-stem presynaptic fibres, and the frequency and amplitude of the postsynaptic potentials arising in both neurons from these sources).

The use of these indices to assess the strength of common input is complicated by the observation in the decerebrate, spinal cat of a decrease in synchrony of γ-

motoneurons when the neurons were discharging at higher rates (Ellaway & Murthy, 1985). This finding raises several concerns: In studies of motor unit synchrony in conscious humans, are the conventional indices affected by discharge rate? Is the effect similar for all indices? How does discharge rate affect the relationship between the synchronization index and the strength of common input to motoneurons? In human studies, the effect of discharge rate on cross-correlograms is generally thought to be weak. This assumption, however, had not been tested directly until Bremner *et al.* (1991*b*) examined the effects of discharge rate on a synchronization index for twenty-two motor unit pairs. Although an effect was seen for some pairs, no consistent pattern emerged.

The aim of the present study was to investigate the relationship between motoneuron discharge pattern (rate and variability) and synchronization of motor unit pairs in human subjects. This was accomplished by testing the same pairs of motor units over a range of mean discharge rates and quantifying the synchronization with five commonly used indices. Both the discharge rate and variability were found to be significantly correlated with each of the synchronization indices. A model of the trajectory of the membrane potential between action potentials was used to explain the observed dependence of the synchronization indices on motoneuron discharge rates. The model was also used to derive a new expression, which was independent of discharge rate, to quantify the strength of common input (CIS) to motoneurons. The CIS describes the strength of the common input in terms that can be directly related to the underlying physiological processes responsible for synchronous discharge, i.e. the total number, frequency and amplitude of common excitatory inputs. Preliminary results of these experiments have appeared previously in abstract form (Nordstrom, Fuglevand, Enoka & Türker, 1990).

METHODS

Seven healthy adults (three female, four male; ages 21–40 years) volunteered to participate in the study and gave informed consent to the procedures. The experiments were approved by the Human Subjects Ethics Committee of the University of Arizona. Details of the experimental arrangement were given in a previous report (Enoka, Robinson & Kossev, 1989). Briefly, subjects were seated with their left arm supported and their left hand comfortably secured in a manipulandum. Isometric abduction and flexion forces of the index finger were measured by strain gauges. Motor unit activity of first dorsal interosseus muscle was recorded simultaneously with two separate pairs of fine-wire electrodes. The distance between the two pairs of electrodes was generally 1–2 cm. The electrodes comprised insulated fine wires (50 μm core diameter) and were positioned either subcutaneously (Enoka, Robinson & Kossev, 1988), or in a conventional intramuscular location. The latter were less prone to detecting the same motor unit activity in both channels. Force and myoelectrical signals were amplified and recorded on FM tape for off-line analysis.

Protocol

Subjects were provided with visual and audio feedback of motor unit potentials during the motor unit isolation procedures and were asked to perform steady, low-force contractions. Electrode positions were optimized for reliable discrimination of motor units, and myoelectrical signals were checked to ensure that the two pairs of electrodes did not sample any common motor unit activity. Following these initial procedures, a motor unit was chosen by the experimenters for the subject to control at a prescribed discharge rate (termed the feedback unit). To help the subjects control the mean discharge rate of the feedback motor unit at the prescribed level they were instructed to watch an oscilloscope screen that displayed a horizontal line showing the mean discharge rate of the feedback unit and the target level. They were also provided with the audio signal of the motor unit potentials from the electrode channel containing the feedback unit. Discrimination of the feedback motor unit was performed on-line using a computer-based template-matching algorithm (SPS 8701; Signal Processing Systems, Prospect, South Australia, Australia). As many additional motor units (termed background units) as possible were recorded during the experiment at the two electrode sites while the feedback unit was voluntarily controlled at various mean discharge rates.

The subject was instructed to slowly increase the mean discharge rate of the feedback unit by performing an isometric abduction of the index finger. With this manoeuvre, the range of discharge rates consistent with accurate unit discrimination was established. Once this range had been determined, the subject was requested to slowly increase the abduction force until the mean discharge rate of the feedback unit matched a selected target rate. The subject was requested to keep the feedback unit discharging continuously at the target rate for 2–4 min with the aid of visual and audio feedback of frequency. Following a 2 min rest, the subject repeated the task at a different target discharge rate. The target rate typically varied about 2·5 Hz between trials. In some trials at high discharge rates, it was necessary to perform several trials of shorter duration (around 30 s) because discrimination accuracy was compromised by the appearance of additional large action potentials after the onset of the trial. For each trial following a rest, the subject increased force gradually to facilitate identification of units in the event of changes in action potential waveform due to minor shifts in muscle fibre–electrode geometry.

Analysis

Motor unit discrimination

All analyses were performed off-line from the taped records. The signal from each intramuscular electrode pair was led to a separate SPS 8701 and discrimination of the constituent motor unit potentials was carried out simultaneously by two experienced operators. Action potentials belonging to a particular motor unit were identified on the basis of waveform shape, and great care was taken to confirm the identity of units discriminated during different trials. Interspike intervals of identified motor units were measured (± 125 μs resolution) using an in-built function of the SPS 8701 and stored on computer.

Interspike interval records were scrutinized for every trial and each discriminated motor unit to assess discrimination accuracy. An example is shown in Fig. 1. A characteristic sign of discrimination error is the presence of abnormally short interspike intervals (< 20 ms), which are indicative of false-positive acceptances, and occur when action potentials with similar waveforms from different motor units have been accepted as belonging to the identified motor unit. In Fig. 1*A*, only two such intervals are seen, both occurring in the first 20 s of the trial, which indicates a highly reliable discrimination. Records accepted for analysis contained less than 5% of false-positive errors (i.e. 5% of interspike intervals < 20 ms). In multiunit recordings, some motor unit action potentials are not recognized due to chance superimposition with action potentials of other active units. This false-negative discrimination error is evident in Fig. 1*A* as a banding of intervals at multiples of the mean interspike interval (cf. Dorfman, Howard & McGill, 1989). This type of error is not serious for cross-correlation analysis as it simply means that fewer counts are available for the histogram. Because this study addressed the effects of discharge pattern on synchrony, however, it was necessary to obtain accurate estimates of the mean and standard deviation (S.D.) of the interspike intervals. Interval histograms were calculated for each record of motor unit discharge (Fig. 1*B*), and interspike intervals that were clearly outside the main distribution because of discrimination error were excluded from the calculation of the mean (±S.D.) interspike interval in each trial (i.e. only the intervals between the dashed lines in Fig. 1*B* were included). Discharge variability was expressed as the percentage coefficient of variation of the mean interspike interval

$$\left(\text{coefficient of variation} = \frac{\text{S.D.}}{\text{mean interspike interval}} \times 100\right).$$

In order to compare the relative excitation of pairs of motor units, the geometric mean discharge rate of both units of a pair was used (geometric mean rate = (mean discharge rate unit A × mean discharge rate unit B)$^{\frac{1}{2}}$). This measure has been used in previous studies on the effect of discharge rate on synchrony (Ellaway & Murthy, 1985; Davey & Ellaway, 1988) because the total number of counts in the cross-correlogram is related to the product of the discharge rate of both units of the pair. The effect of discharge rate on synchronization was examined by expressing discharge rate as the product of mean interspike intervals of the unit pair, because a linear relationship was predicted between the product of interspike intervals and the synchronization indices (see Discussion). The product of interspike intervals is equivalent to the inverse of the square of the geometric mean discharge rate.

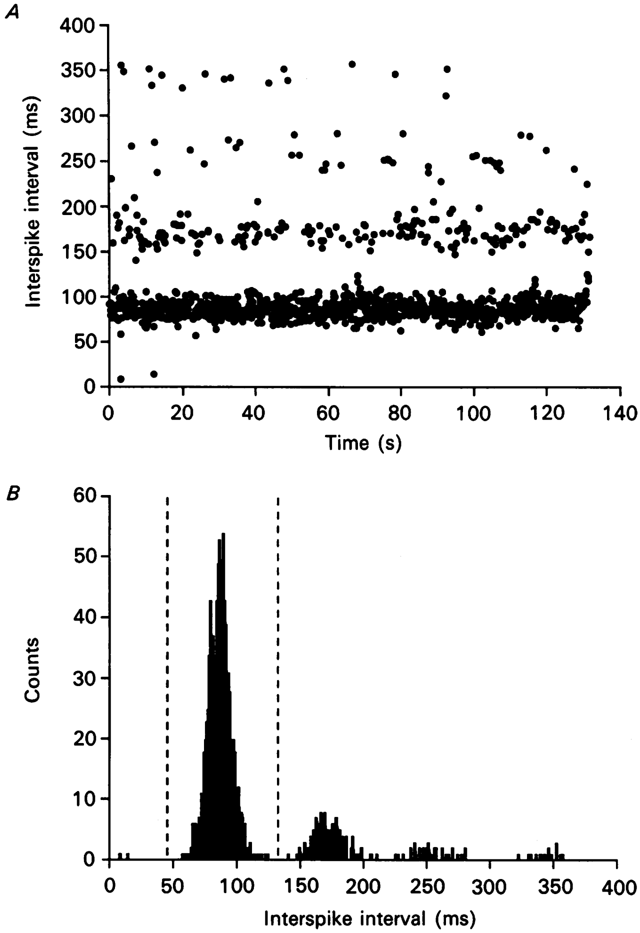

Fig. 1. Verification of discrimination accuracy, and quantification of discharge variability. *A*, interspike interval (ISI) time course for a motor unit discriminated from a multiunit record with few incorrect acceptances, but some missed discharge events. Two interspike intervals shorter than 20 ms in the early part of the record are indicative of false-positive discrimination error, but these were insignificant compared to the total number of acceptances. The banding of intervals at multiples greater than the main distribution around 87 ms is indicative of false-negative discrimination error arising predominantly from chance superimposition of the motor unit potential with potentials from other active units. *B*, interval histogram from the record shown in *A*. High and low cut-off limits were imposed on the interspike interval distribution (dashed vertical lines) following visual inspection to exclude intervals due to discrimination error from the calculation of mean ± S.D. of interspike intervals.

Cross-correlation histograms

Cross-correlograms of the discharge times of discriminated motor units were constructed for motor unit pairs detected with separate electrodes. Synchronized discharge is revealed as a peak in the histogram near the time of discharge of the reference unit. All histograms had 1 ms bin widths and spanned a period 100 ms before and after the discharge of the reference unit (201 bins). Cross-correlation analysis was restricted to periods in which both units of the pair were tonically active.

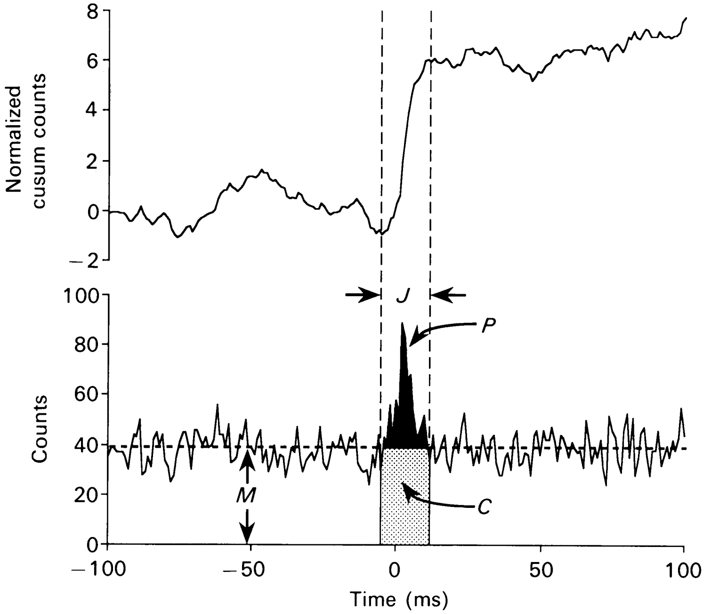

Fig. 2. Quantification of the synchronous peak in the cross-correlogram. The cross-correlogram from a pair of motor units in first dorsal interosseus is shown (lower trace) with its corresponding cusum (upper trace). The position and duration (J) of the synchronous peak was judged from the cusum. The mean bin count in the region of the histogram outside of the peak was denoted as M. The value of M served to distinguish those counts expected due to chance (region C) from those counts in excess of chance (region P) within the synchronous peak.

The cumulative sum procedure (cusum; Ellaway, 1978) was used to identify synchronous peaks in the cross-correlogram (Fig. 2, upper trace). The cusum was normalized by dividing the contents of each cusum bin by the mean bin count of the non-peak bins (M) calculated from the cross-correlogram. The position and duration of the peak was judged from the cusum. Mean peak duration was 11 ms (range 5–23 ms), and peaks could be offset ± 6 ms from the discharge time of the reference unit (time 0) due to differences in axonal and muscle fibre conduction velocities. The region of the cross-correlogram containing the peak comprises two sub-areas: an area representing the counts expected due to chance (Fig. 2, region C); and the region containing counts in excess of those expected due to chance (Fig. 2, region P). The number of counts within area C is given by the product of the number of bins in the peak (J) and the mean bin count in the non-peak region (M). The number of counts within area P is the difference between total counts in the peak region of the histogram and those in area C. Histograms with $M < 4$ were not analysed.

There is no current consensus on which synchronization index derived from the cross-correlogram peak is most appropriate for expressing the strength of the correlated activity in the two neurons. Normalization typically involves expressing the extra counts in the synchronous peak relative to some measure related to motor unit discharge rate, or the baseline counts in the histogram. In order to compare various normalization procedures, we measured five commonly used indices of synchronization from each cross-correlogram. These were:

(a) The index k', the ratio of the total counts in the peak region of the cross-correlogram ($P+C$) to the chance counts in that region (C) (Ellaway & Murthy, 1985; Nordstrom *et al.* 1990).

(b) The index $k'-1$, given by P/C.

(c) The index E, the total number of extra counts within the peak above that expected due to chance (P) relative to the total number of reference unit discharges (Datta & Stephens, 1990; Datta *et al.* 1991). To facilitate comparison with other studies, the unit with the lower discharge rate of the pair was used as the reference unit for each cross-correlogram.

(d) The index S, the total number of extra counts within the peak above that expected due to chance (P) relative to the total number of discharges for both units (Adams *et al.* 1989; Bremner *et al.* 1991*b*).

(e) The index SI, the total number of extra counts within the peak above that expected due to chance (P) relative to the total number of counts in the cross-correlogram (Logigian *et al.* 1988).

Linear regression was used to assess the relationships between synchronization and discharge pattern for each index, with significance reported for probability < 0.05.

RESULTS

One hundred and sixty-three cross-correlograms from eighty motor unit pairs were examined for the effect of discharge rate on estimates of synchrony. Each cross-correlogram represents a unique combination of motor unit pair and discharge rate. Thus, on average, each motor unit pair was tested at two different discharge rates. Thirty-nine additional motor unit pairs (53 cross-correlograms) were studied from a subject in whom synchrony was found to be virtually absent. These data were excluded from the study of the effect of discharge rate on synchrony, but were included for analysis of discharge variability.

It was difficult to test the same motor unit pair(s) over a wide range of discharge rates. Unambiguous identification of the units and reliable discrimination were complicated by changes in motor unit action potential waveform and the recruitment of additional units at higher force levels. Mean discharge rates of single motor units ranged from 6·1 to 17·4 Hz (median 11·8 Hz). The largest difference in mean discharge rates of two units of a pair within a single trial was 7·2 Hz (median difference was 1·5 Hz). The geometric mean discharge rate of both units of the pair ranged from 7·6 to 16·9 Hz. For the forty-five motor unit pairs examined at two or more discharge rates, the largest difference in geometric mean discharge rate of the pair for different trials was 5·4 Hz, with a median difference of 2·2 Hz.

In general, the synchronization indices were smaller when the motor units discharged at higher mean rates; that is, the ratio of extra counts in the synchronous peak normalized to baseline discharge became smaller at higher discharge rates. This effect is shown for one unit pair in Fig. 3, as the discharge rates changed from low (panel *A*: Unit 1, 7·7 Hz; Unit 2, 10·9 Hz) to higher values (panel *B*: Unit 1, 10·4 Hz; Unit 2, 12·9 Hz). The normalized cusum derived from the cross-correlogram is shown in the uppermost trace, with the cross-correlogram immediately below. The size of the synchronous peak relative to baseline discharge was larger in *A* (low discharge rate) than in *B*. This can be visualized by comparing the amplitude of the peak in the normalized cusum in *A* and *B* (the amplitude is proportional to $k'-1$). The two lowermost traces in *A* and *B* show the instantaneous interspike intervals (ISIs)

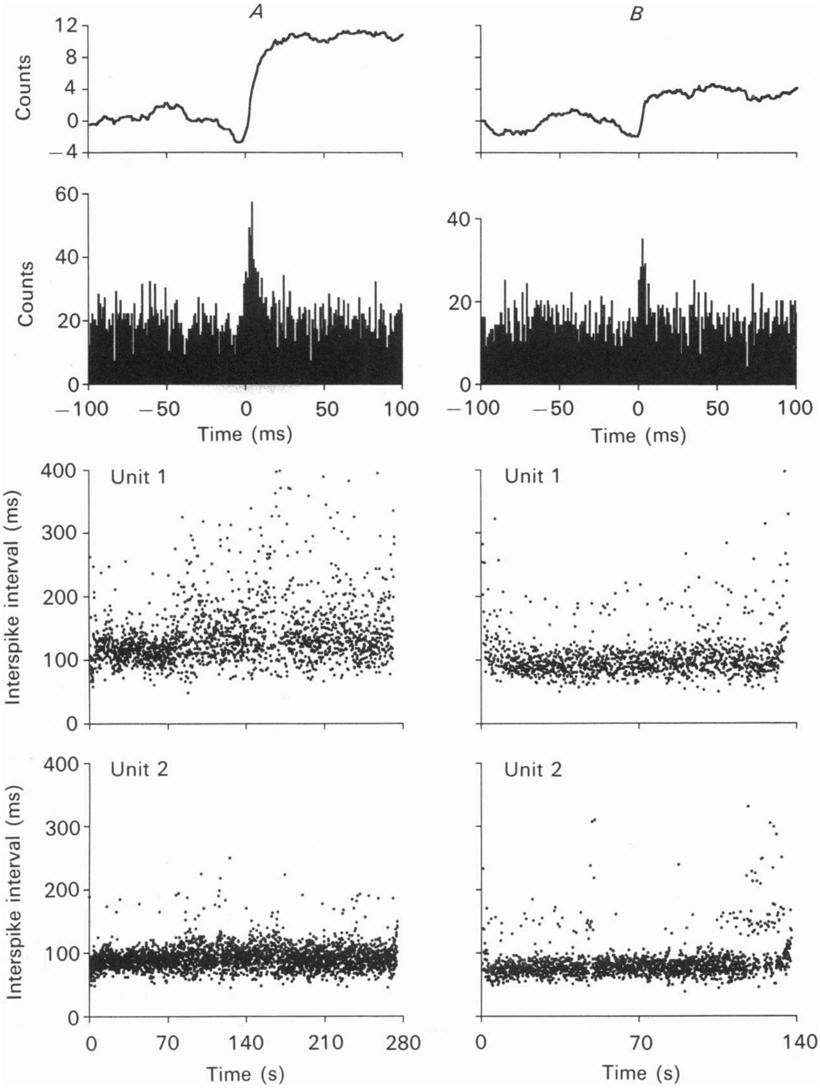

Fig. 3. Cross-correlation of a motor unit pair at two different discharge rates. *A*, low discharge rate. The four traces in each panel, beginning from the top, are the normalized cusum derived from the cross-correlogram, the cross-correlogram, and the time course of the interspike intervals for the two motor units. Unit 1, mean interspike interval 130·4 ms; Unit 2, mean interspike interval 91·4 ms. *B*, higher discharge rate. Unit 1, mean interspike interval 96·2 ms; Unit 2, mean interspike interval 77·5 ms. The duration of the trial was shorter than the low-rate trial, resulting in fewer total counts in the histogram. The size of the cross-correlogram peak relative to baseline discharge is smaller in the high-rate trial. This is best seen by comparing the amplitude of the normalized cusum peak (which is proportional to $k'-1$) in *B* and *A*.

plotted against time for the two motor units used for the cross-correlogram. Note the absence of abnormally short intervals (< 20 ms) in the interspike interval plots, and the stability of the mean interspike interval within each trial (Fig. 3).

For individual pairs, there were generally too few trials with insufficiently large differences in discharge frequency between them, for individual linear regression analyses (as were applied to the population data; see below) to be useful. The tendency for the synchronization indices for the same pair of units to be smaller when the units were discharging at higher rates were evaluated in the following manner. The analysis was restricted to motor unit pairs for which data was available for at least two different discharge-rate trials. The geometric mean discharge rate of both units of the pair contributing to the cross-correlogram was used as the index of discharge rate for comparison of synchrony among cross-correlograms constructed from the different discharge-rate trials for the same pair. For each unit pair, a comparison of the magnitude of the synchronization index k' in high-rate and low-rate trials were performed, for all combinations of the available trials for that unit pair. Trials in which the geometric mean discharge rate differed by > 1 Hz were considered appropriate for comparison. In 82·5 % of such comparisons (66 out of 80; 26 motor unit pairs) the synchronization index k' was smaller in the high-frequency trial. This distribution was significantly different from that expected due to chance

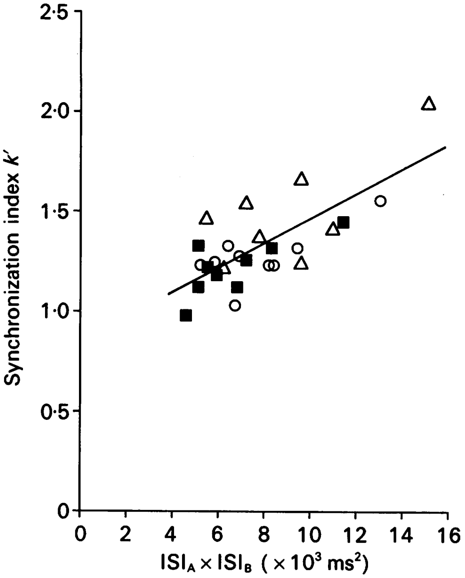

Fig. 4. The effect of discharge rate on the synchronization index k' for nine motor unit pairs recorded during the same experiment. Each symbol represents subgroups of k' values obtained from cross-correlograms using a particular unit in one electrode channel as the reference with each of three units in the other channel (e.g. ○ correspond to values from cross-correlograms in which unit A in one channel was cross-correlated with units D, E and F in the second electrode channel, at each of three different discharge rates). The index k' is plotted against the product of the mean interspike interval of each unit of the pair. There was a significant positive correlation between k' and the product of motor unit interspike intervals, both for the population as a whole (regression line shown; $y = (6{\cdot}2 \times 10^{-5})x + 0{\cdot}85$, $r^2 = 0{\cdot}53$) and for the grouped unit pairs (○, $r^2 = 0{\cdot}50$; ■, $r^2 = 0{\cdot}55$; △, $r^2 = 0{\cdot}50$).

($\chi^2 = 38{\cdot}8$, $P < 0{\cdot}001$). These data support the contention, for individual motor unit pairs, that the synchronization index k' tended to be smaller when the units were discharging at higher rates.

Occasionally it was possible to record from several identified motor unit pairs at different discharge rates in the same experiment. The data from a single experiment in which three different motor units were concurrently recorded by each of two electrodes are shown in Fig. 4. Since motor units were only cross-correlated between separate electrodes, this arrangement yielded nine possible unit-pair combinations for cross-correlation. Each of these units was identified during three different discharge-rate trials, thus yielding twenty-seven cross-correlograms for analysis. The data from one cross-correlogram with a mean bin count (M) of less than 4 were not used. The synchronization index k' for the remaining twenty-six cross-correlograms is plotted against the product of the motor unit interspike intervals in Fig. 4. There was a significant positive correlation between the synchronization index $k \times$ and the product of the motor unit interspike intervals, both for all units (A,B,C *vs.* D,E,F), and for subgroups of the population that shared a common reference motor unit (e.g. ○; A *vs.* D,E,F).

For each of the synchronization indices, the apparent strength of synchrony increased as the mean interspike intervals of the motor units increased (discharge rate decreased). These relationships are shown for k', E, S and SI indices in Fig. 5. The relationship for index $k'-1$ is not shown, as it is simply a vertical translation of that shown for k'. Despite substantial scatter for some indices, linear regression suggested significant correlations in each case, with the least variability for the k', $k'-1$ and SI indices ($r^2 = 0{\cdot}25$, $0{\cdot}25$ and $0{\cdot}27$, respectively). The sensitivity of each index to discharge rate was assessed by estimating from the regression line the percentage change in the index values at the extremes of the interspike interval range. For the data in Fig. 5, the largest value for the product of motor unit interspike intervals was 17300 ms^2, and the smallest was 3300 ms^2. These values correspond to a geometric mean discharge rate for the pair of 7·6 and 16·9 Hz, respectively. Over this range of discharge rates, the range of values for the synchronization indices were: k', 1·17–2·01 (72 % increase between high-rate and low-rate conditions); $k'-1$, 0·17–1·01 (494 % increase); E, 0·030–0·087 (190 % increase); S, 0·013–0·037 (185 % increase); SI, 0·009–0·053 (489 % increase). This analysis indicates that each index was sensitive to discharge rate. Although the same data set was used in each case, the indices $k'-1$ and SI were more sensitive (greater slope) than indices k', E and S. Index k' was the least sensitive to the discharge rate of the motor units because it is only the component of the index in excess of 1 that actually corresponds to extra synchronous discharge, and this was relatively small compared to the component due to chance (which remained unchanged at different rates).

The effect of the difference in discharge rate of the motor unit pair contributing to the cross-correlogram was also examined, as this may affect the strength of synchrony (Logigian *et al.* 1988). No significant correlations were found for the absolute difference in discharge rate of the motor unit pair and any of the synchronization indices (r^2 values all less than 0·02). There were weak correlations for the indices k' ($r^2 = 0{\cdot}07$), $k'-1$ ($r^2 = 0{\cdot}07$), E ($r^2 = 0{\cdot}04$) and SI ($r^2 = 0{\cdot}08$) with the absolute difference in interspike interval of the motor unit pair. These latter results were attributed to the strong covariation of the difference in interspike interval of the unit pair and the product of the interspike intervals ($r^2 = 0{\cdot}39$), and the correlation of the latter with synchronization.

In addition to the effect of discharge rate (product of interspike intervals) on synchrony, the synchronization indices were also related to the variability of motor

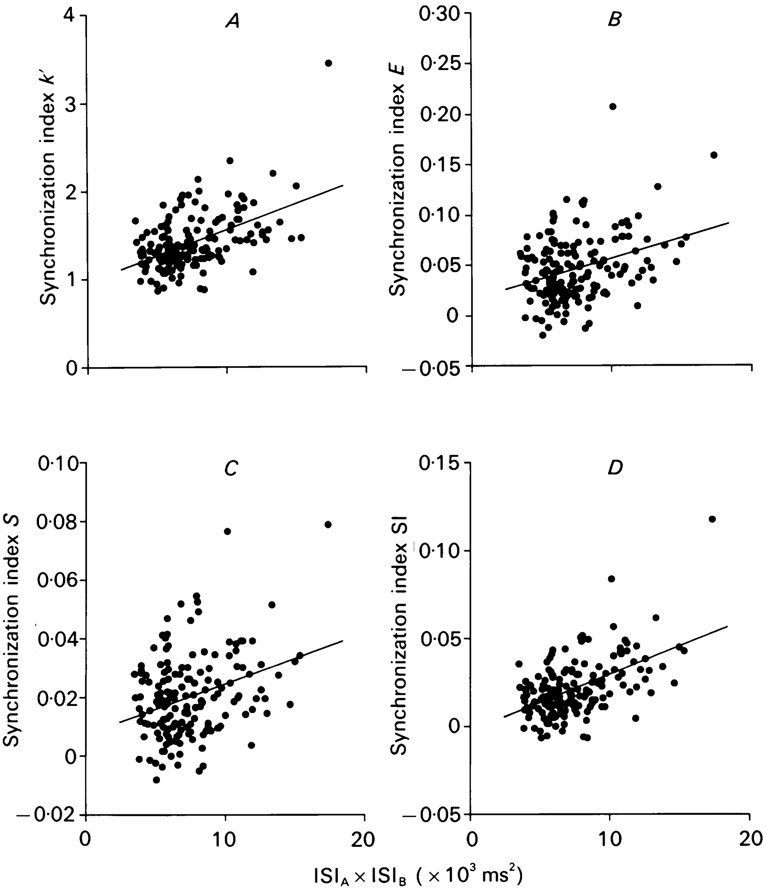

Fig. 5. Significant correlations for synchronization indices with motor unit interspike interval. The data comprise 163 cross-correlograms from 80 motor unit pairs discharging at various rates in six subjects. The data include cross-correlograms from the same motor unit pair tested at different mean discharge rates. Each panel (*A–D*) shows a different synchronization index as a function of the product of the mean interspike intervals ($\mathrm{ISI_A} \times \mathrm{ISI_B}$) of the motor units in the cross-correlogram. Significant linear regression lines are shown for each case. *A*, index k' (linear regression $y = (6{\cdot}00 \times 10^{-5})x + 0{\cdot}97$, $r^2 = 0{\cdot}25$); *B*, index E ($y = (4{\cdot}13 \times 10^{-6})x + 0{\cdot}016$, $r^2 = 0{\cdot}11$); *C*, index S ($y = (1{\cdot}74 \times 10^{-6})x + 0{\cdot}007$, $r^2 = 0{\cdot}11$); *D*, index SI ($y = (3{\cdot}20 \times 10^{-6})x - 0{\cdot}002$, $r^2 = 0{\cdot}27$). Data for index $k' - 1$ ($r^2 = 0{\cdot}25$) are not shown, as the regression is equivalent to a simple vertical translation of that shown for index k'. For all indices, a significant amount (11–27 %) of the variability could be attributed to discharge rate.

unit discharge. The relationships between the synchronization indices and mean coefficient of variation of the unit pair comprising the cross-correlogram are shown in Fig. 6. The consistent tendency was for the synchronization index to be higher when motor units with more variable discharge patterns were cross-correlated. The

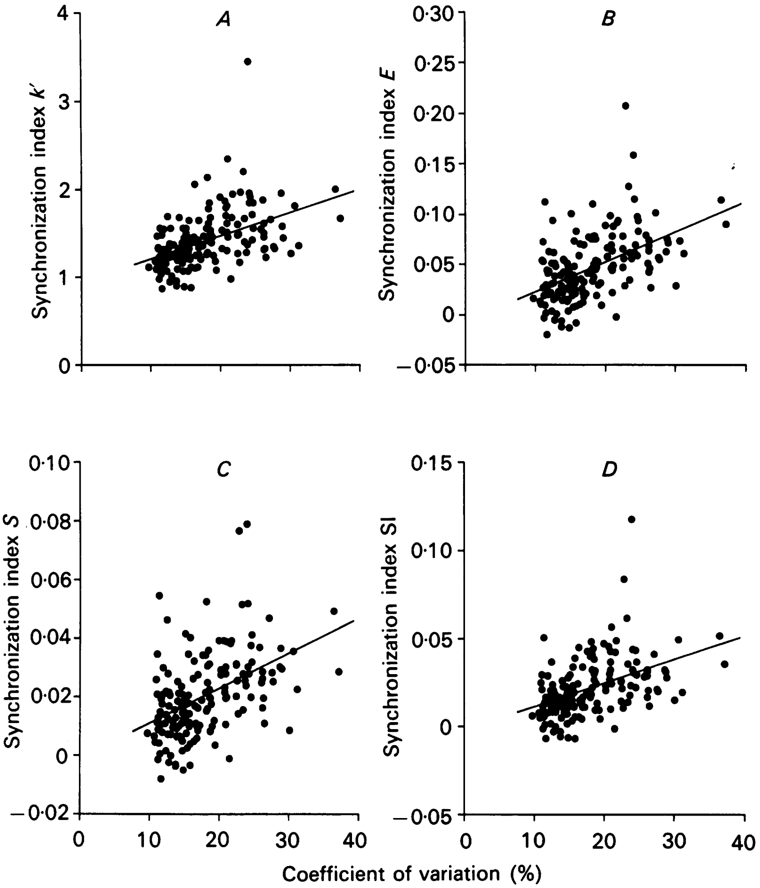

Fig. 6. Synchronization indices were positively correlated with discharge variability of the motor units. Four synchronization indices are shown as functions of the geometric mean coefficient of variation of both units of the pair used in the cross-correlation. Significant linear regression lines are shown. *A*, index k' (linear regression $y = 0·027x+0·933$, $r^2 = 0·22$); *B*, index E ($y = 0·003x-0·007$, $r^2 = 0·25$); *C*, index S ($y = 0·0019x-0·001$, $r^2 = 0·21$); *D*, index SI ($y = 0·0013x-0·002$, $r^2 = 0·21$). The data for index $k'-1$ ($r^2 = 0·22$) are not shown. For all indices, a significant amount (21–25%) of the variability could be attributed to the regularity of motor unit discharge.

sensitivity of each index to discharge variability was assessed by estimating from the regression line the percentage change in the index values at the extremes of discharge variability. For motor unit pairs with a mean coefficient of variation in the range 9·8–37·2% (a 280% increase), the range of values for the synchronization indices were: k', 1·20–1·94 (62% increase as variability increased); $k'-1$, 0·20–0·94 (373%

increase); E, 0·022–0·105 (366 % increase); S, 0·011–0·043 (395 % increase); SI, 0·011–0·048 (341 % increase). Apart from the index k', the synchronization indices showed an essentially proportionate increase with mean coefficient of variation.

The significant correlations of synchrony with coefficient of variation were not simply the result of a covariation of discharge variability with interspike interval. Although significant, the relationship between motor unit interspike interval and coefficient of variation was weak ($r^2 = 0{\cdot}02$, $n = 326$). The effect of both discharge rate and variability on synchronization was examined by combining the product of the mean interspike interval of the unit pair and their mean coefficient of variation into a single measure of discharge pattern ($\mathrm{ISI_A} \times \mathrm{ISI_B} \times$ mean coefficient of variation). In each case the correlations with discharge pattern were better than those found with either the product of interspike intervals, or coefficient of variation, alone (k', $r^2 = 0{\cdot}48$; $k'-1$, $r^2 = 0{\cdot}48$; E, $r^2 = 0{\cdot}32$; S, $r^2 = 0{\cdot}28$; SI, $r^2 = 0{\cdot}49$). A substantial proportion (28–49 %) of the variation in synchronization for the different indices could be accounted for by combined effects of the mean interspike interval and coefficient of variation of the units contributing to the cross-correlograms.

DISCUSSION

The main finding of the present study was that motor unit discharge patterns influence estimates of synchrony obtained from the cross-correlogram. Both the discharge rate and variability were significantly correlated with synchronization when it was quantified by each of the conventional indices. It is generally considered that these synchronization indices provide a measure of the strength of common inputs in motoneurons. The fundamental question that arises from these findings is whether these correlations reflect differences in the strength of common input to motoneurons with different discharge patterns. One problem with these indices is that the magnitude of the discharge-rate effects varied substantially across the five indices for the same data set. This means that inferences about the strength of common synaptic input under different discharge-rate conditions appear to be strongly affected by the particular index chosen to quantify synchrony from the cross-correlogram. We suggest that the discharge-rate effects on the synchronization indices can be attributed to the mathematical procedures involved in their calculation from the cross-correlogram. In contrast, the relationship between discharge variability and synchrony appears to have some physiological relevance.

Effect of motor unit discharge rate on synchronization indices

The usual, but not invariant, result when the same motor unit pair was tested under different discharge-rate conditions was for the synchronization index to be higher in cross-correlograms constructed from the low discharge-rate trials (e.g. Fig. 4). For the data from different motor units and subjects, significant correlations were evident between the amount of synchrony and the product of motor unit mean interspike intervals for each of the indices (Fig. 5). In previous studies on humans, discharge rate has generally been dismissed as having an insignificant effect on synchrony, although this assumption had not been tested directly in the same pairs at different discharge rates until the recent study of Bremner *et al.* (1991*b*). They found no consistent effect in twenty-two pairs of motor units; for some unit pairs synchronization (index S) increased with discharge rate, in others it decreased, and in the remainder there was no consistent effect. For the index S, which was used by Bremner *et al.* (1991*b*), we found the weakest correlation between discharge rate and the magnitude of synchrony (Fig. 5*C*). Another possible explanation for the absence of a clear discharge-rate effect in the Bremner *et al.* study may be the more restricted range of discharge rates over which units were tested since in that study the mean discharge rate of one unit of the pair was controlled at 10 Hz by the subject.

For motor unit pairs with mean discharge rates in the range 6·1–17·4 Hz, we found that a significant proportion (11–27 %) of the variation in synchronization could be accounted for by differences in discharge rate. Each of the conventional synchronization indices tend to have higher values when motor units discharge at low rates, but the various indices were not equally sensitive to discharge rate. Depending on the index used, linear regression revealed a 72–494 % increase between the highest (17·4 Hz) and lowest (6·1 Hz) discharge rates encountered in the present study (Fig. 5). These rates are representative of the range of motor unit discharge rates likely to be encountered in experiments using conventional intramuscular electrodes in this muscle, and the results suggest that discharge rate is an important source of variation in synchronization as assessed by the conventional indices.

The difference in discharge rate of the cross-correlated units has been reported to influence synchrony in human extensor carpi radialis (Logigian *et al.* 1988). In that study, units discharging at frequencies within 1 Hz of each other had larger and broader (20–40 ms) peaks in the cross-correlogram than unit pairs with a greater difference in discharge rate. We found no such relationship between the difference in discharge rate of the units and the strength of synchrony in first dorsal interosseus. Broad peaks in the range 20–40 ms were infrequent and the width of the synchronous peak was unaffected by differences in discharge rate. Logigian *et al.* interpreted their findings as indicating that either the strength of common input was greater in motoneurons of similar recruitment threshold, or that units discharging at similar rates were more easily synchronized by a given common input. Another possible explanation for broader peaks in the cross-correlogram is synchronization of the common inputs themselves (Kirkwood, Sears, Tuck & Westgaard, 1982). Whatever the explanation for the findings in extensor carpi radialis, the difference in rate does not appear to be important for synchrony among motor units in first dorsal interosseus.

Explanation of the discharge-rate effects in the cross-correlogram

The critical question arising from the observed correlation between interspike interval and synchronization for the different indices (Fig. 5) is whether this finding reflects a real reduction in the strength of common input to the motoneurons when they are discharging at higher rates. To the contrary, the following theoretical arguments suggest that the observed relationship between interspike interval and synchronization is a consequence of the mathematical procedures involved in estimating synchrony from the cross-correlogram peak.

A proportion of the inputs to motoneurons arise from branched axons of common presynaptic neurons. The joint arrival of EPSPs from these common sources causes a slight increase in the probability that the two neurons will discharge within a few milliseconds of each other, and this is the physiological basis for the synchronous peak in the cross-correlogram (Kirkwood & Sears, 1978; Datta & Stephens, 1990). In order to examine the effect of motoneuron discharge rate on the cross-correlogram, we first consider the probability that an EPSP will produce an action potential in a single tonically active motoneuron and then we consider the probability that a common-input EPSP will produce synchronous action potentials in two tonically active motoneurons.

The probability of an EPSP producing an action potential in an active motoneuron

The ability of an EPSP to influence discharge probability in a single tonically active motoneuron has been studied experimentally in both the cat (Fetz & Gustafsson, 1983; Gustafsson & McCrea, 1984; Cope, Fetz & Matsumura, 1987) and the human (Ashby & Zilm, 1982*b*; Miles, Türker & Le, 1989) by cross-correlation of motoneuron discharge with Ia afferent EPSPs. The cat studies suggest that both EPSP amplitude and rise time influence discharge probability, with amplitude likely to assume the predominant role under more natural conditions of small unitary EPSPs in the presence of synaptic noise (Cope *et al.* 1987). The human studies have shown that the probability of evoking an action potential in a tonically active motoneuron with a constant H-reflex stimulus is independent of discharge rate.

The effects of EPSP amplitude and motoneuron discharge rate on the probability of an EPSP producing an action potential can be schematized as in Fig. 7. This simple model, first suggested by Ashby & Zilm (1982*a*, *b*) as an appropriate model for repetitive discharge of motoneurons in conscious human subjects, show the trajectory of the membrane potential between action potentials in a tonically active motoneuron discharging at different rates in *A* and *B*. The trajectory of the membrane potential is simplified as a linear ramp to a constant discharge threshold that begins from a constant peak after-hyperpolarization potential (AHP) following each action potential. With this model, a change in interspike interval is accomplished by an alteration of the slope of the linear trajectory (angle α in *A*, angle β in *B*), with an unchanged AHP.

The experimental evidence on which the model is based has been derived from the study of the responses of tonically active human motor units involved in the H-reflex (Ashby & Zilm, 1982*a*, *b*; Miles *et al.* 1989). The timing of the stimulus, and hence the arrival of the stimulus-evoked compound EPSP with respect to the preceding motoneuron action potential, is varied to probe the difference between the membrane potential and discharge threshold at times within the interspike interval. If the EPSP arrives at the motoneuron when the membrane potential is too far from threshold, no action potential is elicited by the stimulus. If the EPSP arrives when the membrane potential is sufficiently close to threshold, an action potential is produced. In both tibialis anterior (Ashby & Zilm, 1982*b*) and soleus (Miles *et al.* 1989), when the same motor unit was tested at different mean discharge rates with a constant-intensity stimulus, the probability of the stimulus evoking an action potential in the motoneuron was *independent of discharge rate*. The proportion of the interspike interval in which the EPSP elicited an action potential remained constant at different mean rates (Miles *et al.* 1989). On the basis of the H-reflex results, both groups have suggested that a model of membrane potential trajectories like that shown in Fig. 7 is appropriate for repetitive discharge of human motoneurons during voluntary activity.

In a reduced-animal preparation, it is possible to measure directly the membrane potential during repetitive discharge in motoneurons using an intracellular microelectrode. Usually, the animals are deeply anaesthetized and repetitive discharge is induced by passing a depolarizing current into the cell via the microelectrode. With this technique the most common description of the membrane potential trajectory between action potentials for cells discharging within the primary range of the current–frequency relationship is a rapid repolarization following the action potential followed by a predominantly linear rise to threshold for the next action potential (Calvin, 1975). For injected currents just above threshold for rhythmic discharge, the membrane potential trajectories at different discharge rates are consistent with the model shown in Fig. 7 (cf. Fig. 2*B*1 in Schwindt & Calvin, 1972). Over the remainder of the primary range of the current–frequency relationship (generally higher discharge rates than the present study), an increase in frequency is accompanied by a reduction in the magnitude of the AHP with an unchanged rate of rise to threshold (Schwindt & Calvin, 1972, 1973; Calvin, 1975). If the membrane potential trajectory had these characteristics (reduced AHP, constant rate of rise to threshold) at higher rates during the previously described H-reflex paradigm, one would expect a rate-dependent increase in the probability of a stimulus-evoked motoneuron action potential. This would occur because the membrane potential would be closer to threshold for a greater proportion of the interspike interval due to the reduced magnitude of the AHP. This was not seen in the human data (Ashby & Zilm, 1982*a*, *b*; Miles *et al.* 1989).

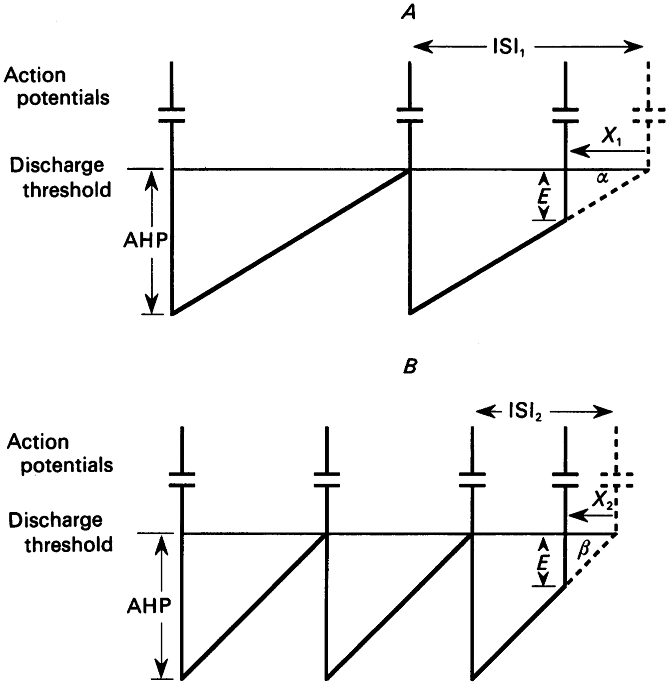

Fig. 7. Model of the trajectory of the membrane potential between action potentials in a tonically active motoneuron discharging at a different mean interspike interval in *A* and *B*. The trajectory is represented as a linear approach to discharge threshold commencing from a constant after-hyperpolarization potential (AHP) following each action potential. A change in interspike interval (ISI) is accomplished by altering the slope of the linear trajectory (angle α in *A*, angle β in *B*), with an unchanged AHP. The effect of an EPSP of amplitude *E* is to advance the time of discharge of the next action potential when the membrane potential is sufficiently close to discharge threshold (dashed line). The shortening (*X*) of the interspike interval is a function of the amplitude *E* with respect to AHP, but is independent of the interspike interval.

Another feature of the model in Fig. 7 is the assumption of a constant threshold for action potential generation. With current injection, threshold shifts to a more depolarized potential at higher discharge rates (Schwindt & Crill, 1982); however, it does seem to be stable over the frequency range encountered in our study (6–18 Hz) (cf. Figs 1 *C* and 3 *A* in Schwindt & Crill, 1982). It has also been reported that the threshold for action potential generation may vary within the interspike interval (Calvin, 1974), but this was not considered to be important for the present purposes as the threshold-crossing events of interest arise from small, presumably unitary, EPSPs which can only elicit an action potential when they arrive in the terminal portion of the interspike interval.

While the features of the model are not inconsistent with available intracellular data, this evidence is not conclusive. Furthermore, in the unanaesthetized, decerebrate cat marked differences have been observed in the membrane potential trajectory between action potentials *in the same motoneuron* when activated by current injection or synaptic input via stimulation of the midbrain locomotor region (Brownstone, Jordan, Kriellaars, Noga & Shefchyk, 1992). Until studies of repetitive discharge have been extended in animals under more 'natural' conditions in which extrinsic influences may play an important role, we should be cautious about inferring details of repetitive discharge in conscious humans from current-injection studies. In determining the features of the model, therefore, we have chosen to give the most weight to the experimental findings in humans (the H-reflex studies of Ashby & Zilm, 1982*a*, *b* and Miles *et al.* 1989), which show that the probability of a compound EPSP eliciting an action potential in a tonically active motoneuron is independent of discharge rate. This is the essential feature for our analysis of the effects of common inputs on the cross-correlogram. The model shown in Fig. 7 provides a simple conceptual basis for this effect.

The effect of a particular EPSP (amplitude E) on action potential timing is shown schematically for different mean interspike intervals in Fig. 7*A* and *B*. For simplicity, E is represented as an impulse, and its relative magnitude has been magnified for illustrative purposes. In the absence of an input E, the trajectory would ramp to threshold over the duration of the mean interspike interval (ISI). Synaptic noise, which causes variability in individual interspike intervals, is not considered in this representation of the mean trajectory. The ability of an input E to raise the membrane potential to discharge threshold is dependent on the membrane potential at the time of occurrence of E. If E occurs when the membrane potential is well below threshold (the portion of the ramp represented by a continuous line), no action potential is generated as a result of E and the trajectory continues upward to reach threshold at the projected interspike interval. If, however, E occurs when the membrane potential is sufficiently close to threshold (the portion of the ramp represented by a dashed line), an action potential is generated that is advanced in time from the expected time of occurrence. The portion of the interspike interval in which presentation of E can produce a threshold crossing is given by X. The probability that E produces an action potential is therefore X/ISI (or E/AHP using simple geometry). Since the AHP is unchanged in this model when the motoneuron discharges at different mean rates, E/AHP is assumed to be constant at different rates. That is:

$$\frac{E}{\mathrm{AHP}} = \frac{X_1}{\mathrm{ISI}_1} = \frac{X_2}{\mathrm{ISI}_2} = P. \tag{1}$$

With this model of repetitive discharge, the probability (P) of an EPSP of amplitude E producing an action potential in a tonically active motoneuron is independent of the interspike interval. This has been confirmed experimentally for Ia-afferent inputs to human soleus motor units by testing a motor unit at different mean discharge rates with a constant test (compound) EPSP evoked under H-reflex conditions (Ashby & Zilm, 1982*b*; Miles *et al.* 1989).

The probability of a common EPSP producing synchronous action potentials in a pair of motoneurons

Consider now the case of an EPSP arising from a branched presynaptic axon that is common to a pair of motoneurons, A and B. For a common EPSP to induce a synchronous action potential in these two tonically active motoneurons, it is necessary for the membrane potential of *each* motoneuron to be sufficiently close to threshold at the time of occurrence of the EPSP. The probability, therefore, of the common EPSP producing an action potential in both motoneurons (P_{AB}) is given by:

$$P_{AB} = P_A \times P_B, \tag{2}$$

where P_A and P_B are the probabilities of the EPSP producing an action potential in motoneurons A and B, respectively. Equation (2) can be rewritten by substituting from eqn (1) as:

$$P_{AB} = \frac{E_A}{\mathrm{AHP}_A} \times \frac{E_B}{\mathrm{AHP}_B}, \tag{3}$$

where E_A and E_B are the amplitudes of the EPSPs in motoneurons A and B, respectively, and AHP_A and AHP_B are the amplitudes of the after-hyperpolarization following an action potential in motoneurons A and B, respectively. This relationship indicates that the probability of a common EPSP producing synchronous action potentials is a function of its amplitude in proportion to the maximum excursion of the AHP following each action potential but is independent of the mean interspike interval of the two motoneurons.

The effects of common EPSPs on the cross-correlogram

In order to understand how the conventional synchronization indices are dependent on motoneuron discharge rates, it is necessary to relate the predictions of the model to the construction of the cross-correlogram. Consider two motoneurons A and B, which are tonically active with mean rates F_A and F_B, respectively. Construction of a cross-correlogram for neuron A with respect to B is shown in Fig. 8. For each B action potential, the number of counts (Q) added to the cross-correlogram will be equal to the number of A action potentials occurring within the duration (d) of the histogram. On average, Q is given by $F_A \times d$. The total number of action potentials generated by the reference neuron B (N_B) for a trial of duration D is $F_B \times D$. The duration of the trial (D) is defined as the period of time in which both motor units were tonically active, and contributing counts to the cross-correlogram. Therefore, the total number of counts in the histogram (N_H) is given by:

$$N_H = N_B \times Q = F_B \times D \times F_A \times d. \tag{4}$$

The duration of the histogram (d) is equal to the product of the number of bins in the histogram (N_b) and the duration of each bin (d_b):

$$d = N_b \times d_b. \tag{5}$$

By substitution, the total number of counts in the histogram becomes:

$$N_H = F_A \times F_B \times D \times N_b \times d_b. \tag{6}$$

The expected number of counts per bin ($N_{\mathrm{H/b}}$) in the histogram due to chance is:

$$\begin{aligned} N_{\mathrm{H/b}} &= N_{\mathrm{H}}/N_{\mathrm{b}} \\ &= F_{\mathrm{A}} \times F_{\mathrm{B}} \times D \times d_{\mathrm{b}}. \end{aligned} \tag{7}$$

For independent events in neurons A and B this is the expected count in any bin of the cross-correlogram. $N_{\mathrm{H/b}}$ can be estimated from the cross-correlogram by calculating the mean bin count in the region that does not include the peak (M in Fig. 2).

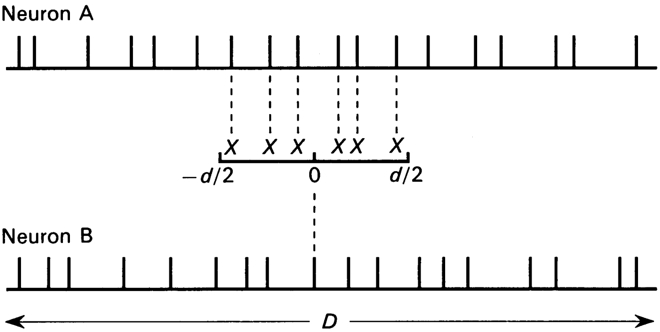

Fig. 8. Scheme showing construction of the cross-correlogram. Action potentials of two motoneurons (A and B) are represented as vertical lines. In the cross-correlation histogram of neuron A *vs.* neuron B, each action potential in neuron B is used as the reference for the histogram (time 0). For each reference event in neuron B, a count (x) is added to the histogram for all forward and backward occurrence times of neuron A action potentials within the duration (d) of the histogram (i.e. within $\pm d/2$ s). The cross-correlogram is completed by repeating this process sequentially for all reference neuron B action potentials occurring during the duration (D) of the trial used for the cross-correlogram.

Consider now that these neurons have at least one common excitatory input. The amplitude of the common EPSP in neurons A and B is denoted E_{A} and E_{B}, respectively. The common EPSP leads to an increase in the number of counts in the region of the histogram near time 0 (these are counts shifted from their expected time of occurrence in other bins near time 0). The number of additional synchronous action potential counts in the histogram (N_{CI}) due to a common-input EPSP (E) is equivalent to the total number of E events multiplied by the probability that E produces an action potential in both A and B:

$$N_{\mathrm{CI}} = (F_E \times D) \times \left(\frac{E_{\mathrm{A}}}{\mathrm{AHP_A}} \times \frac{E_{\mathrm{B}}}{\mathrm{AHP_B}}\right), \tag{8}$$

where F_E is the discharge rate of the common input.

The extra action potential counts in the peak due to common input from *all* sources will be

$$N_{\mathrm{CI}} = \sum_{i=1}^{n} \left(F_{E_i} \times D \times \frac{E_{\mathrm{A}_i}}{\mathrm{AHP}_{\mathrm{A}_i}} \times \frac{E_{\mathrm{B}_i}}{\mathrm{AHP}_{\mathrm{B}_i}}\right), \tag{9}$$

where n is the total number of shared axons from common presynaptic neurons. Equation (9) represents the theoretical number of synchronous events in the histogram due to common inputs, with the reasonable assumption that the effects of common inputs in parallel summate linearly. The synchronous peak in the histogram commonly has a duration (J) of several 1 ms bins due to differences in conduction time and EPSP rise time among the common inputs, and noisy membrane potential trajectories. N_{CI} can be considered equivalent to the number of counts in area P of the histogram (see Fig. 2) and may be estimated as the difference between the total counts (T) in the peak region and those expected due to chance (Fig. 2, area C). Thus we can write:

$$N_{\mathrm{CI}} \approx P = T - C. \tag{10}$$

As an example of the effects of common EPSPs on the cross-correlogram and the influence of motoneuron discharge rates on the commonly used synchronization indices, consider the synchronization index k':

$$k' = \frac{\text{total counts in the histogram peak } (T)}{\text{counts expected due to chance } (C)}.$$

From eqn (10), we can express k' as:

$$k' = \frac{C+P}{C} = 1 + \frac{P}{C}. \tag{11}$$

The number of expected counts due to chance in the peak region of the histogram (C) is equivalent to the expected counts per bin due to chance ($N_{\mathrm{H/b}}$) multiplied by the number of bins in the peak (J). The number of extra counts due to common input (P) is equivalent to N_{CI} (eqn (10)). Thus, we can write:

$$k' = 1 + \frac{N_{\mathrm{CI}}}{N_{\mathrm{H/b}} \times J}. \tag{12}$$

Expanding terms gives:

$$k' = 1 + \left(\frac{\sum_{i=1}^{n} \left(F_{E_i} \times D \times \frac{E_{\mathrm{A}_i}}{\mathrm{AHP}_{\mathrm{A}_i}} \times \frac{E_{\mathrm{B}_i}}{\mathrm{AHP}_{\mathrm{B}_i}} \right)}{F_{\mathrm{A}} \times F_{\mathrm{B}} \times D \times d_{\mathrm{b}} \times J} \right). \tag{13}$$

And now, expressing motoneuron discharge rates (F_{A} and F_{B}) in terms of interspike intervals ($\mathrm{ISI_A}$ and $\mathrm{ISI_B}$), and eliminating D, we obtain:

$$k' = 1 + \left(\frac{\sum_{i=1}^{n} \left(F_{E_i} \times \frac{E_{\mathrm{A}_i}}{\mathrm{AHP}_{\mathrm{A}_i}} \times \frac{E_{\mathrm{B}_i}}{\mathrm{AHP}_{\mathrm{B}_i}} \right)}{d_{\mathrm{b}} \times J} \times \mathrm{ISI_A} \times \mathrm{ISI_B} \right). \tag{14}$$

This theoretically derived expression for k' predicts that as interspike intervals become small, the value of k' approaches 1. Furthermore, this expression predicts that k' will increase linearly as the product of interspike intervals increases (decreased discharge rate). The best-fit regression line for the experimental data (Figs

4 and 5*A*) has a form similar to that predicted by eqn (14). This relationship is equivalent to the hyperbolic relationship between k' and discharge frequency of γ-motoneurons in the decerebrate cat reported by Ellaway & Murthy (1985).

A new expression for the strength of common input from the cross-correlogram

Synchronous action potentials in excess of those expected due to chance arise because a proportion of inputs to motoneurons are from shared axons of common presynaptic neurons. The preceding arguments (i.e. eqn (9)) lead to the conclusion that the *number* of extra synchronous action potentials above chance in motoneurons A and B is proportional to the total number of axons shared by the motoneuron pair, the mean rate and amplitude of the EPSPs produced in each neuron by these common inputs, and the duration of the trial. However, the number of extra synchronous counts is not influenced by the discharge rate of the motoneurons (provided they are tonically active) (eqn (9)).

Given this association, it is possible to examine some properties of this common input from the cross-correlogram. To do this, we can define the strength of common input (CIS) in two neurons as:

$$\mathrm{CIS} = \sum_{i=1}^{n} F_{E_i} \times \frac{E_{\mathrm{A}_i}}{\mathrm{AHP}_{\mathrm{A}_i}} \times \frac{E_{\mathrm{B}_i}}{\mathrm{AHP}_{\mathrm{B}_i}}, \tag{15}$$

where CIS describes the strength of common input in terms of the total number of common excitatory inputs to the motoneurons, and their average rate and amplitude. Using this definition of CIS, we can rewrite eqn (9) as:

$$N_{\mathrm{CI}} = \mathrm{CIS} \times D. \tag{16}$$

Then, from eqn (10), we can estimate CIS from the cross-correlogram as:

$$\mathrm{CIS} = \frac{N_{\mathrm{CI}}}{D} \approx \frac{P}{D}. \tag{17}$$

The common-input strength, therefore, is estimated from the cross-correlogram as the number of extra counts in the synchronous peak above that expected due to chance (i.e. area *P* in Fig. 2) normalized to the duration (*D*) of the trial. It is thus given by the *frequency* of synchronous action potentials of the motoneurons in excess of those expected due to chance. CIS provides a simpler, and more intuitive expression of the tendency for synchronous discharge than conventional indices. It can be directly related to common synaptic events affecting action potential timing in each neuron (eqn (15)), it is mathematically independent of motoneuron discharge rate, and it is thus a more robust estimate of common-input strength than conventional synchronization indices.

The strength of common input

Based on this rationale, common-input strength (CIS) in motoneuron pairs can be calculated from the cross-correlogram (eqns (15) and (17)). It is of physiological interest to ascertain whether the CIS itself varies with discharge rate, since one might expect that the total common-input strength would increase with increasing excitatory drive to the motoneuron pool. The dependence of CIS on discharge rate for individual unit pairs was evaluated in the manner previously described for the index k'. The analysis was restricted to motor unit pairs for which data was available for at least two different discharge rate trials. The geometric mean discharge rate of both units of the pair contributing to the cross-correlogram was used as the index of discharge rate. For each unit pair, a comparison of the magnitude of CIS values in high-rate and low-rate trials was performed, for all combinations of the available trials for that unit pair. Trials in which the geometric mean discharge rate differed by > 1 Hz were considered appropriate for comparison. In 60·5% of such comparisons (49 of 81; 26 motor unit pairs) the CIS value was smaller in the high-frequency trial. This distribution was not significantly different from that expected due to chance ($\chi^2 = 3{\cdot}57$, $P > 0{\cdot}05$), suggesting that for individual unit pairs, CIS was not dependent on discharge frequency. For the population of motor units there was no significant correlation between CIS and the product of the mean interspike intervals of units used for the cross-correlogram (Fig. 9*A*). CIS was also not influenced by the difference in discharge rate of the motor units used for the cross-correlogram (data not shown; CIS *vs.* absolute interspike interval difference, $r^2 = 0{\cdot}002$; CIS *vs.* absolute discharge rate difference, $r^2 = 0{\cdot}002$). The absence of a correlation between common-input strength and our indices of excitatory drive to motoneurons (either geometric mean firing frequency or $\mathrm{ISI_A} \times \mathrm{ISI_B}$) probably occurs because the presumed increase is small in proportion to the total excitatory activity necessary for tonic discharge, and is not evident over the range of discharge rates examined here.

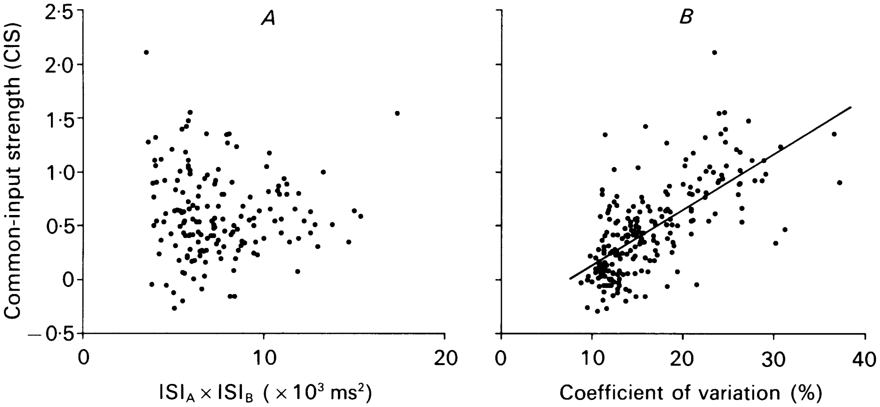

Fig. 9. Relationships between the index of common-input strength (CIS) and motor unit discharge pattern. *A*, CIS as a function of the product of mean interspike intervals ($\mathrm{ISI_A} \times \mathrm{ISI_B}$) of units contributing to the cross-correlogram. No significant correlation was found ($r^2 = 0{\cdot}001$, $n = 163$). *B*, CIS as a function of geometric mean coefficient of variation of units used in the cross-correlogram. Data from subject B.C. are included. Significant linear regression line shown ($y = 0{\cdot}052x - 0{\cdot}39$, $r^2 = 0{\cdot}45$, $n = 216$). CIS is independent of motor unit discharge rates, but tends to be larger when units with more variable discharge are cross-correlated.

Table 1 summarizes the synchrony data and ranks the subjects in order of decreasing CIS. The mean discharge rate of the pair of units used in each cross-correlogram differed considerably among the subjects, ranging from 9·7 Hz for subject R.C. to 14·3 Hz for subject A.T. This difference in discharge rates among the subjects could influence the conclusions made regarding the strength of common input depending on the synchronization index used. For example, the largest mean values for each of the indices k', E, S and SI were generally found in subjects A.F. and R.E., suggesting that the strength of common input was greatest for motor units in these subjects. This result can be explained, however, by the relatively low mean discharge rate of motor units used for the cross-correlograms in these subjects. When the cross-correlogram peak was expressed in terms of the CIS, one concludes that subjects A.T. and E.N. exhibited the strongest degree of common input; this result was masked in the conventional synchronization indices because of the substantially higher discharge rates of motor units used for the cross-correlograms in these subjects.

The synchronization indices tended to be larger when motor units with more variable discharge were cross-correlated (Fig. 6). Similarly, the index of common-input strength (CIS) was correlated with discharge variability. This is clearly seen in Fig. 9*B*, which shows CIS as a function of mean coefficient of variation of the motor unit pair used for the cross-correlogram. The data from subject B.C. has been included, as it was evident that the virtual absence of synchrony in this subject (which prompted his exclusion from the study of the effects of discharge rate on synchronization indices) was related to the extremely regular discharge pattern of his first dorsal interosseus motor units (see Table 1). There was a significant positive correlation between CIS and the mean coefficient of variation for the population of motor units studied ($r^2 = 0{\cdot}45$, $n = 216$). Exclusion of subject B.C. from this analysis did not alter this result ($r^2 = 0{\cdot}34$, $n = 163$). The linear regression line in Fig. 9*B* shows that an increase in mean coefficient of variation from 9·8 to 37·2% (a 280% increase), was accompanied by an increase in CIS from 0·12 to 1·54 (a 1190% increase). The coefficient of determination (r^2) of the population data indicates that 45% of the variation in *common-input strength* could be explained by differences in discharge variability of the motor units. This finding in human motor units responding to voluntary drive extends the previous findings of Davey & Ellaway (1988), who observed a similar relationship between synchronization index k' and coefficient of variation for γ-motoneurons in the decerebrate cat with spinal lesions.

The data in Table 1 indicate that the mean synchronization indices and CIS varied over almost a 20-fold range across the seven subjects. This range of values is comparable to that found for first dorsal interosseus motor units in previous studies (Datta & Stephens, 1990; Bremner *et al.* 1991*b*). Motor unit discharge variability also varied across subjects (CV in Table 1), ranging from a mean of 12% in subject B.C. to 26% in subject A.T. The individual differences in motor unit discharge variability were an important source of the subject differences in CIS. There was a tendency for subjects with less-regular discharge to have higher values for CIS. For example, subject B.C. had the most regular discharge and the lowest CIS, while subjects E.N. and A.T. had the least regular discharge and the highest values for CIS.

TABLE 1. Summary of synchrony data by subject

Subject	No. of MU pairs	No. of correlo-grams*	MU mean ISI (ms)	MU mean CV (%)	Pair mean rate (Hz)**	Synchrony index *k′*	Synchrony index *E*	Synchrony index *S*	Synchrony index SI	CIS index
A.T.	10	21	71·7 (57·3–104·1)	26·4 (19·1–45·2)	14·3 (10·6–16·9)	1·46 (0·98–2·00)	0·063 (−0·003 to 0·116)	0·026 (−0·001 to 0·052)	0·025 (−0·001 to 0·052)	1·005 (−0·049 to 2·109)
E.N.	6	22	76·8 (62·7–101·0)	23·6 (14·3–30·2)	13·2 (11·2–15·8)	1·58 (1·11–2·13)	0·062 (0·012–0·110)	0·027 (0·006–0·053)	0·026 (0·005–0·048)	0·909 (0·179–1·558)
A.F.	18	26	104·1 (68·2–142·1)	20·6 (13·0–53·7)	9·9 (7·6–13·1)	1·74 (1·25–3·44)	0·078 (0·029–0·208)	0·033 (0·010–0·079)	0·041 (0·013–0·118)	0·760 (0·309–1·544)
R.E.	3	8	105·0 (84·8–164·9)	15·5 (10·0–34·7)	9·8 (8·1–11·6)	1·51 (1·32–1·69)	0·073 (0·053–0·112)	0·033 (0·018–0·055)	0·034 (0·024–0·051)	0·703 (0·703–1·351)
C.Z.	42	84	83·6 (64·0–125·7)	14·0 (9·1–21·4)	12·2 (8·2–15·5)	1·25 (0·88–2·06)	0·027 (−0·020 to 0·083)	0·012 (−0·008 to 0·040)	0·013 (−0·007 to 0·045)	0·376 (−0·273 to 1·423)
R.C.	1	2	104·1 (94·0–126·1)	18·4 (16·0–22·5)	9·7 (9·2–10·2)	1·14 (1·08–1·20)	0·015 (0·009–0·021)	0·007 (0·004–0·010)	0·008 (0·004–0·011)	0·154 (0·076–0·231)
B.C.***	39	53	92·1 (65·3–135·1)	12·1 (8·3–18·7)	11·1 (7·7–14·3)	1·04 (0·79–1·32)	0·004 (−0·024 to 0·026)	0·002 (−0·011 to 0·017)	0·002 (−0·011 to 0·017)	0·052 (−0·293 to 0·461)

Values for measured parameters are means with range in parentheses. MU, motor unit. * Each cross-correlogram corresponds to a separate discharge-rate trial for the motor unit pairs. ** Geometric mean of discharge rates of both motor units of the pair used for cross-correlograms. *** This subject was excluded from the analyses of the effects of motor unit discharge rate on synchrony. In cases in which no clear peak was evident in the cusum, analysis was performed using an 11 ms peak duration that was centred around time 0.

The relationship between motoneuron discharge variability and synchrony

It is likely that the correlation between discharge variability and synchrony has a physiological basis, although at present we can only speculate on its nature. The degree of fluctuation in the membrane potential about the mean trajectory is an important factor contributing to the variability of motoneuron interspike intervals. The asynchronous arrival of postsynaptic potentials from many sources gives rise to these fluctuations in membrane potential (Calvin & Stevens, 1968; Stålberg & Thiele, 1973). As unitary EPSPs become larger, the fluctuations in membrane potential will increase. Based on the model in Fig. 7 (see also eqn (3)), larger common-input EPSP amplitudes will also increase the probability of synchronous discharge. Thus, the correlations between motor unit discharge variability and synchrony could be related to unitary EPSP amplitudes. Differences in EPSP amplitude could arise from extrinsic (source and location of inputs) or intrinsic (motoneuron input resistance) factors. Extrinsic factors seem to be important for synchrony (Adams *et al.* 1989), but appear to be relatively unimportant for discharge variability, which is insensitive to moderate alterations in afferent input (Stålberg & Thiele, 1973). It seems unlikely that extrinsic factors could be responsible for large differences in discharge variability and synchrony among motoneurons in a single motor pool. An alternative suggestion is that these differences are associated with intrinsic motoneuron properties related to repetitive discharge of action potentials. For example, if the repolarization following each action potential (AHP in Fig. 7) were smaller, discharge would be more irregular, because noise on the membrane potential trajectory would be effective in producing a threshold-crossing over a greater proportion of the interspike interval. The model in Fig. 7 suggests that a smaller AHP would also increase the probability of a common-input EPSP inducing synchronous discharge. Thus, intrinsic repetitive discharge properties of motoneurons could provide a link between discharge variability and synchrony. If discharge variability is related to intrinsic properties of the motoneuron, however, one would expect differences with motor unit type. At present the issue is not resolved, with evidence both for and against this notion (Nordstrom & Miles, 1991).

In comparing different motor pools, one should not assume that highly irregular discharge is indicative of strong common input. Masseter motor units have extremely irregular discharge (mean coefficient of variation of five subjects = 34·0%; Nordstrom & Miles, 1991) compared to first dorsal interosseus units (mean coefficient of variation of seven subjects = 18·6%; present study). In contrast, motor unit synchrony is much weaker in masseter (mean k' for 90 pairs = 1·13; Nordstrom *et al.* 1990) than first dorsal interosseus (mean k' for 119 pairs = 1·32; present study).

Quantifying the strength of common input to first dorsal interosseus motoneurons

The mean values for CIS in first dorsal interosseus motor units ranged from 0·052 to 1·005 extra synchronous action potentials per second in different subjects. It is instructive to examine what this large range may mean in terms of the underlying synaptic events responsible for synchrony. A general estimate can be made by assigning a value of 100 μV for average unitary EPSP amplitude (Sears & Stagg, 1976; Tuck, 1977), and 10 mV for AHP amplitude (Schwindt & Calvin, 1972; Calvin, 1974). With these values, the total frequency of common inputs can be calculated using eqn (17). For the subject (A.T.) with the highest CIS, this calculation yields a value of 10050 common EPSPs s^{-1}, while for the subject (B.C.) with the lowest CIS the value was 520 s^{-1}. The total frequency of asynchronous input EPSPs (amplitude 100 μV) required to bring a motoneuron to discharge threshold has been estimated to be 12 kHz (Sears & Stagg, 1976; Tuck, 1977). Although the cross-correlogram underestimates synaptic connectivity (Kirkwood, 1979), the estimated common-input strength in the present study ranges from 4 to 84% of active inputs to motoneurons in these subjects (with 100 μV EPSPs). Alternatively, if both subjects had the same rate of common EPSPs, then a difference in average EPSP amplitude between subjects may be the source of differences in CIS. Using a common-input EPSP rate of 10050 s^{-1}, the mean amplitude of the common EPSPs in subject B.C. would be 23 μV, compared to 100 μV in subject A.T.

In summary, the conventional synchronization indices are all sensitive to motor unit discharge rate, a finding which compromises their usefulness in assessing the strength of common input to motoneurons under conditions in which discharge rates vary. As an alternative, we have suggested a new index of common-input strength (CIS) that is independent of discharge rate and is more directly related to the physiological processes responsible for synchronous discharge of motoneurons. Large differences in CIS and discharge variability between subjects, and the correlation of synchrony and CIS with motoneuron discharge variability, suggest that either the properties of last-order presynaptic inputs to motoneurons, or the intrinsic repetitive discharge properties of motoneurons, may differ substantially in the first dorsal interosseus motor pool among human subjects.

The authors thank Peter Worden and Laurie Hubbard for their technical contributions to the study, and Drs Timothy Miles and Kemal Türker for their helpful advice. This research was supported by USPHS Grants NS 07309, NS 20544 and AG 09000. Michael Nordstrom was a C.J. Martin Fellow of the National Health and Medical Research Council of Australia. Andrew Fuglevand was an NRSA Postdoctoral Fellow of the NIH (NS 08634).

REFERENCES

Adams, L., Datta, A. K. & Guz, A. (1989). Synchronization of motor unit firing during different respiratory and postural tasks in human sternocleidomastoid muscle. *Journal of Physiology* **413**, 213–231.

Ashby, P. & Zilm, D. (1982*a*). Relationship between EPSP shape and cross-correlation profile explored by computer simulation for studies on human motoneurons. *Experimental Brain Research* **47**, 41–48.

Ashby, P. & Zilm, D. (1982*b*). Characteristics of postsynaptic potentials produced in single human motoneurons by homonymous Group 1 volleys. *Experimental Brain Research* **47**, 41–48.

Bremner, F. D., Baker, J. R. & Stephens, J. A. (1991*a*). Correlation between the discharges of motor units recorded from the same and from different finger muscles in man. *Journal of Physiology* **432**, 355–380.

Bremner, F. D., Baker, J. R. & Stephens, J. A. (1991*b*). Variation in the degree of synchronization exhibited by motor units lying in different finger muscles in man. *Journal of Physiology* **432**, 381–399.

Brownstone, R. M., Jordan, L. M., Kriellaars, D. J., Noga, B. R. & Shefchyk, S. J. (1992). On the regulation of repetitive firing in lumbar motoneurones during fictive locomotion in the cat. *Experimental Brain Research* (in the Press).

Calvin, W. H. (1974). Three modes of repetitive firing and the role of threshold time course between spikes. *Brain Research* **69**, 341–346.

Calvin, W. H. (1975). Generation of spike trains in CNS neurons. *Brain Research* **84**, 1–22.

Calvin, W. H. & Stevens, C. F. (1968). Synaptic noise and other sources of randomness in motoneuron interspike intervals. *Journal of Neurophysiology* **31**, 574–587.

Cope, T. C., Fetz, E. E. & Matsumura, M. (1987). Cross-correlation assessment of synaptic strength of single Ia fibre connections with triceps surae motoneurones in cats. *Journal of Physiology* **390**, 161–188.

Datta, A. K., Farmer, S. F. & Stephens, J. A. (1991). Central nervous pathways underlying synchronization of human motor unit firing studied during voluntary contractions. *Journal of Physiology* **432**, 401–425.

Datta, A. K. & Stephens, J. A. (1990). Synchronization of motor unit activity during voluntary contraction in man. *Journal of Physiology* **422**, 397–419.

Davey, N. J. & Ellaway, P. H. (1988). Control from the brainstem of synchrony of discharge between gamma motoneurones in the cat. *Experimental Brain Research* **72**, 249–263.

Dorfman, L. J., Howard, J. E. & McGill, K. C. (1989). Motor unit firing rates and firing rate variability in the detection of neuromuscular disorders. *Electroencephalography and Clinical Neurophysiology* **73**, 215–224.

Ellaway, P. H. (1978). Cumulative sum technique and its application to the analysis of peristimulus time histograms. *Electroencephalography and Clinical Neurophysiology* **45**, 302–304.

Ellaway, P. H. & Murthy, K. S. K. (1985). The origins and characteristics of cross-correlated activity between γ-motoneurones in the cat. *Quarterly Journal of Experimental Physiology* **70**, 219–232.

Enoka, R. M., Robinson, G. A. & Kossev, A. R. (1988). A stable, selective electrode for recording single motor-unit potentials in humans. *Experimental Neurology* **99**, 761–764.

Enoka, R. M., Robinson, G. A. & Kossev, A. R. (1989). Task and fatigue effects on low threshold motor units in a human hand muscle. *Journal of Neurophysiology* **62**, 1344–1359.

Fetz, E. E. & Gustafsson, B. (1983). Relation between shapes of post-synaptic potentials and changes in firing probability of cat motoneurones. *Journal of Physiology* **341**, 387–410.

Gustafsson, B. & McCrea, D. (1984). Influence of stretch-evoked synaptic potentials on firing probability of cat spinal motoneurones. *Journal of Physiology* **347**, 431–451.

Kirkwood, P. A. (1979). On the use and interpretation of cross-correlation measurements in the mammalian central nervous system. *Journal of Neuroscience Methods* **1**, 107–132.

Kirkwood, P. A. & Sears, T. A. (1978). The synaptic connexions to intercostal motoneurones as revealed by their average common excitation potential. *Journal of Physiology* **275**, 103–134.

Kirkwood, P. A., Sears, T. A., Tuck, D. L. & Westgaard, R. H. (1982). Variations in the time course of the synchronization of intercostal motoneurones in the cat. *Journal of Physiology* **327**, 105–135.

Logigian, E. L., Wierzbicka, M. M., Bruyninckx, F., Wiegner, A. W., Shahani, B. T. & Young, R. R. (1988). Motor unit synchronization in physiologic, enhanced physiologic, and voluntary tremor in man. *Annals of Neurology* **23**, 242–250.

Miles, T. S., Türker, K. S. & Le, H. T. (1989). Ia reflexes and EPSPs in human soleus motor neurones. *Experimental Brain Research* **77**, 628–636.

Moore, G. P., Perkel, D. H. & Segundo, J. P. (1966). Statistical analysis and functional interpretation of neuronal spike data. *Annual Review of Physiology* **28**, 493–522.

Nordstrom, M. A., Fuglevand, A. J., Enoka, R. M. & Türker, K. S. (1990). Estimating the strength of common inputs to motor neurons from the cross-correlogram. *Society for Neuroscience Abstracts* **16**, 1320.

Nordstrom, M. A. & Miles, T. S. (1991). Discharge variability and physiological properties of human masseter motor units. *Brain Research* **541**, 50–56.

Nordstrom, M. A., Miles, T. S. & Türker, K. S. (1990). Synchronization of motor units in human masseter during a prolonged isometric contraction. *Journal of Physiology* **426**, 409–421.

Schwindt, P. C. & Calvin, W. H. (1972). Membrane potential trajectories between spikes underlying motoneurone firing rates. *Journal of Neurophysiology* **35**, 311–325.

Schwindt, P. C. & Calvin, W. H. (1973). Equivalence of synaptic and injected current in determining the membrane potential trajectory during motoneuron rhythmic firing. *Brain Research* **59**, 389–394.

SCHWINDT, P. C. & CRILL, W. E. (1982). Factors influencing motoneuron rhythmic firing: results from a voltage-clamp study. *Journal of Neurophysiology* **48**, 875–890.

SEARS, T. A. & STAGG, D. (1976). Short-term synchronization of intercostal motoneurone activity. *Journal of Physiology* **263**, 357–381.

STÅLBERG, E. & THIELE, B. (1973). Discharge pattern of motoneurones in humans. In *New Developments in Electromyography and Clinical Neurophysiology*, ed. DESMEDT J. E., vol. 3, pp. 234–241. Karger, Basel.

TUCK, D. L. (1977). Investigation of intercostal neuronal intracellular processes and connectivity by signal analysis and computer simulation. Ph.D. Thesis, University of London.