J. Physiol. (1985), **365**, *pp.* 77–102
With 11 *text-figures*
Printed in Great Britain

SYNCHRONOUS AFFERENT DISCHARGE FROM A PASSIVE MUSCLE OF THE CAT: SIGNIFICANCE FOR INTERPRETING SPIKE-TRIGGERED AVERAGES

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(*Received 19 June 1984*)

SUMMARY

1. Evidence is presented for the existence of synchrony between the spike trains of muscle afferents of the passive cat medial gastrocnemius muscle held at fixed length.

2. Synchrony between the spike trains of a population of muscle afferents was quantified by means of a synchronization index (I_s), derived from spike-triggered averages of the muscle–nerve neurogram and the rectified neurogram. A previously used test based solely upon the neurogram average (Watt, Stauffer, Taylor, Reinking & Stuart, 1976) is shown to be invalid.

3. The differences between experimentally derived I_s values and theoretical I_s values derived for the condition of asynchrony were compared to estimated confidence limits for those differences. This comparison revealed that twenty-two of fifty-three muscle-afferent spike trains whose rectified averages satisfied certain conditions for interpreting the I_s were synchronized with the discharge of other afferents. The form of the rectified averages of another eight afferents suggested that these afferents might also have been synchronized.

4. Synchrony in the discharge of muscle afferents was found in three experiments in which the neurogram was recorded from a single nerve branch to medial gastrocnemius, as well as in the data of experiments in which the whole muscle nerve was used.

5. The degree of synchrony was similar for Ia, spindle group II and Ib afferents.

6. The magnitude of the synchrony found in these experiments was judged by comparison to the results of analog simulations and the increase in I_s values resulting from the application of small, quick stretches to the medial gastrocnemius muscle. The degree of synchrony found on average was approximately equivalent to that of a single spike occurring once for every four discharges of the reference spike train.

7. Simulations were performed to determine the distortion of monosynaptic excitatory post-synaptic potentials (e.p.s.p.s) obtained by spike-triggered averaging which would be produced by synchrony between the spike trains of Ia and spindle group II afferents of the magnitude found in this study. These simulations indicate

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that the apparent amplitude would be increased by approximately 4 μV on average. Both the 10–90 % rise time and the half-width would increase, the effects being greater for smaller e.p.s.p.s. Consequently, the synchrony found in this study is of most concern in the study of small post-synaptic potentials, such as those due to spindle group II afferents.

INTRODUCTION

The technique of spike-triggered averaging (for review: Fetz, Henneman, Mendell, Stein & Stuart, 1979; Kirkwood & Sears, 1980) has been of considerable value in the study of the properties and organization of neural pathways. Its application to neuronal systems in the spinal cord of the cat, for example, has provided information on the properties of single-fibre excitatory post-synaptic potentials (e.p.s.p.s; e.g. Mendell & Henneman, 1971; Munson & Sypert, 1979) and on the patterns of connexions of single type-identified muscle afferents (e.g. Kirkwood & Sears, 1974; Scott & Mendell, 1976; Watt, Stauffer, Taylor, Reinking & Stuart, 1976; Stauffer, Watt, Taylor, Reinking & Stuart, 1976; Lüscher, Ruenzel & Henneman, 1980; Fleshman, Munson & Sypert, 1981; Munson, Sypert, Zengel, Lofton & Fleshman, 1982; Lucas, Cope & Binder, 1984). The fundamental assumption of spike-triggered averaging is the absence of synchrony between the spike train serving as the trigger (reference) in the averaging process and any other spike trains. The presence of synchrony in a spike-triggered averaging experiment would yield post-synaptic potentials (p.s.p.s) contaminated by contributions from the synchronized neurones.

The importance of testing this assumption in spike-triggered averaging experiments is stressed by findings of correlations between spindle discharge and arterial pulse in decerebrate rabbits (Ellaway & Furness, 1977) and in human subjects (McKeon & Burke, 1981) and by synchrony in the discharge of spindles from intercostal muscles of anaesthetized cats (Kirkwood & Sears, 1982). Despite its importance, this assumption has been tested infrequently as a part of spike-triggered averaging experiments, in part because of the difficulty of testing for synchrony between the discharge of the reference afferent and the discharge of a representative sample of other afferents. Watt *et al.* (1976) suggested that synchrony would be revealed by the presence of more than one extracellular wave form in a spike-triggered average of the neurogram from the muscle nerve containing the reference afferent (see also Lüscher, Ruenzel, Fetz & Henneman, 1979). However, as will be shown in this report, this test is inadequate.

Milner-Brown, Stein & Yemm (1973) successfully employed a test for synchrony in the discharge of motor units based on a comparison of the spike-triggered averages of rectified and non-rectified surface electromyograms. We have adapted their method to averages of neurograms and of rectified neurograms, providing a quantitative synchronization index (I_s) to detect synchrony in the discharge of muscle afferents as well as in that of motor units (Roscoe, Botterman, Cameron, Reinking & Stuart, 1979, 1981). Application of this technique to afferents from the medial gastrocnemius muscles of deeply anaesthetized cats, a preparation frequently used in spike-triggered averaging studies of spinal synaptic connectivity, failed to reveal the presence of synchrony in the muscle afferent population (Reinking, Roscoe, Hamm, Botterman, Cameron & Stuart, 1981). The I_s has since been revised to increase its sensitivity (Hamm, Roscoe, Reinking & Stuart, 1985; Roscoe, Hamm, Reinking &

Stuart, 1985). Application of this revised test in the present report reveals a subtle degree of synchrony in the discharge of muscle afferents from the medial gastrocnemius muscle of anaesthetized cats. This degree of synchrony has been evaluated, showing that its effects on p.s.p.s obtained by spike-triggered averaging would be of most concern for potentials of small amplitude (e.g. $< 25\ \mu V$).

A preliminary communication has been presented (Hamm, Roscoe, Reinking & Stuart, 1983).

METHODS

Analysis was made of afferent signals in seven cats from a passive (i.e. electrically silent) medial gastrocnemius muscle held at a fixed length under moderate (1 N) tension. The cats were deeply anaesthetized with a mixture of halothane, nitrous oxide and oxygen. In two cats, the muscle was also subjected to quick stretches. The surgical, muscle stretching, bioelectric recording, and data processing techniques are described in detail in previous reports from this laboratory (Stuart, Mosher, Gerlach & Reinking, 1970, 1972; Reinking & Stuart, 1974; Binder, Kroin, Moore, Stauffer & Stuart, 1976; Cameron, Binder, Botterman, Reinking & Stuart, 1981; Reinking & Roscoe, 1982; Hamm *et al.* 1985; Roscoe *et al.* 1985).

The animal was fixed to a rigid Göteborg-type frame by clamps supporting the spine at T3, L4 and S2. The knee was fixed at an angle of approximately 130 deg, with clamps at the ankle and at just below or above the knee. The tendon of medial gastrocnemius was separated from the calcaneal tendon and attached to a strain gauge with a short length of low-compliance Dacron line. The measurements reported in this study, except those involving quick stretch, were made under isometric conditions with the muscle attached to a strain gauge affixed to a rigid, massive support. For the application of quick stretch and, in some cases, for afferent identification, the muscle was attached to a servo-controlled electromagnetic puller. This puller system has a peak-to-peak noise level of less than 5 μm. Although this noise level would be sufficient to induce synchrony in the case of some afferents, interpretation of the quick-stretch results depended upon the increase of the I_s with the application of brief triangular stretches above the value obtained in the absence of such stretches. Consequently, this background level of noise would not have been of consequence for the phase of this study in which the sensitivity of I_s was evaluated (see below).

Rectal and hind-limb pool temperatures were controlled at 37 ± 1 °C and 30–35 °C, respectively. These experiments were from a series involving the use of intramuscular nerve branches (Cameron *et al.* 1981), in which a wide exposure of the hind limb was required to provide sufficient space for handling the nerve branches. Heat loss from the pool was increased by this wide exposure, making temperature regulation more difficult, and resulting in the range of temperatures given above, which are lower than those reported previously by this laboratory. Consequently, the axonal conduction velocity used to separate Ia from spindle group II (spII) afferents was lowered from the conventional value of 72 m/s. Based upon a Q_{10} of 1·6 between 27 and 37 °C (Paintal, 1965) and a mean bath temperaure of 32·5 °C, spindle afferents with conduction velocities of 59 m/s or greater were classified as Ia while those with conduction velocities of 58 m/s or less were classified as spII.

The recording arrangement involved functional isolation of single medial gastrocnemius afferent spike trains from among L7–S1 dorsal root filaments mounted on a monopolar stainless-steel Canberra-type electrode. The indifferent electrode was a stainless-steel needle inserted into adjacent denervated back musculature. One to four spike trains were recorded simultaneously on analog tape together with a multi-unit neurogram recording made with a coiled silver spring monopolar electrode holding either the whole muscle nerve or an intramuscular nerve branch. This electrode was also used to stimulate the intact medial gastrocnemius muscle (all other muscles were denervated) as part of standard afferent identification procedures (reviewed in Matthews, 1972).

The detection of synchrony

The synchronization index (I_s). The I_s is the ratio of areas within two neurogram averages: (1) an average of the raw neurogram (A) triggered by the spike train of a functionally isolated muscle afferent; and (2) an average of the rectified neurogram (A_r) triggered from the same spike train (Roscoe *et al.* 1981; Hamm *et al.* 1985). Examples are shown in Fig. 1 of averages triggered by the spike train of a Ia afferent. Each average represents a period of 10 ms about the reference spike.

The areas used in computing the I_s are the absolute area (S_a) under the wave form in the A average and the area (S_r) under the wave form in the A_r average with respect to the base line of background activity in the rectified neurogram ($\bar{A}_{r_0}$; determined for these purposes from the first and last quarters of the average). These areas are given by

$$S_a = \sum_{i=t_2}^{t_3} |A_i|, \tag{1}$$

$$S_r = \sum_{i=t_1}^{t_4} (A_{r_i} - \bar{A}_{r_0}), \tag{2}$$

where

$$\bar{A}_{r_0} = \frac{\sum_{i=t_0}^{t_1-1} A_{r_i} + \sum_{i=t_4+1}^{t_5} A_{r_i}}{(t_1-t_0)+(t_5-t_4)}. \tag{3}$$

These summations are taken over the points in the digitized averages with the limits (t_0, ..., t_5) as shown in Fig. 1.

The S_a area is determined solely by the wave form of the extracellular action potential of the reference afferent, since the positive and negative phases of any synchronized potentials will cancel due to jitter in their time of occurrence (see below). The S_r area, on the other hand, depends on the profile of the A wave form and on the presence of any synchronized potentials, since the rectification process eliminates cancellation of the positive and negative components. Consequently, the I_s, which is given by

$$I_s = S_r/S_a, \tag{4}$$

increases with the degree of synchrony, provided that this synchrony is centred around the time of the reference spike. The occurrence of synchronized discharge predominantly in either of the two base-line areas (t_0-t_1 and t_4-t_5 intervals), however, would make the relation between the I_s and synchrony indirect. Each record was examined for this condition by comparing the mean values of the first and last quarters of the rectified average. All records which had a difference between these means which was greater than could be ascribed to chance are so indicated in the Results section. The I_s values obtained from these records were excluded from calculation of mean values.

Estimated I_s values for asynchrony ($\hat{I}_s$). To determine if an I_s value represents synchrony, it is necessary to determine the I_s value which would be obtained in the absence of synchrony ($\hat{I}_s$). Milner-Brown *et al.* (1973) derived an equation for determining the profile of the rectified wave form from the non-rectified wave form, assuming that the background noise has a Gaussian amplitude distribution (their eqn. (3)). We have observed that the amplitude distribution of neurograms often deviates to some extent from normality (Hamm *et al.* 1985). We have therefore used a more general equation for this determination:

$$\begin{aligned}\hat{A}_r(y) &= \int_{-\infty}^{\infty} |x+y|\, p(x)\, dx \\ &= \int_{-y}^{\infty} (x+y)\, p(x)\, dx - \int_{-\infty}^{-y} (x+y)\, p(x)\, dx,\end{aligned} \tag{5}$$

where $\hat{A}_r(y)$ is the value in the rectified average in the condition of asynchrony corresponding to a value of y in the non-rectified average, and $p(x)$ is the probability density function of the neurogram signal, which can be estimated from an amplitude histogram. From eqn. (5) the expected asynchronous values of S_r ($\hat{S}_r$) and I_s ($\hat{I}_s$) can be determined:

$$\hat{S}_r = \sum_{i=t_1}^{t_4} [\hat{A}_r(A_i) - \hat{A}_r(0)], \tag{6}$$

$$\hat{I}_s = \hat{S}_r/S_a. \tag{7}$$

Use of eqn. (5) requires that the amplitude of the signal in the neurogram average be expressed in relation to the amplitude of the background noise in the neurogram, which determines the amplitude distribution. Signal amplitudes have therefore been expressed as the signal-to-noise ratios (S/N) in the original neurograms (before averaging). The signal-to-noise ratio has been determined by taking the ratio of the peak-to-peak amplitude in the A average to the standard

deviation of the variations in the base line of the average and dividing by the square root of the number of samples in the average.

Determination of I_s and $\hat{I}_s$. I_s and $\hat{I}_s$ were computed from the A and A_r averages, which were collected in a signal averager following passage of the neurogram signal through an analog delay, an active filter (100–10 kHz), and, in the case of A_r, a precision full-wave rectifier. I_s was calculated from A and A_r according to eqns. (1)–(4), while $\hat{I}_s$ was calculated from these averages according

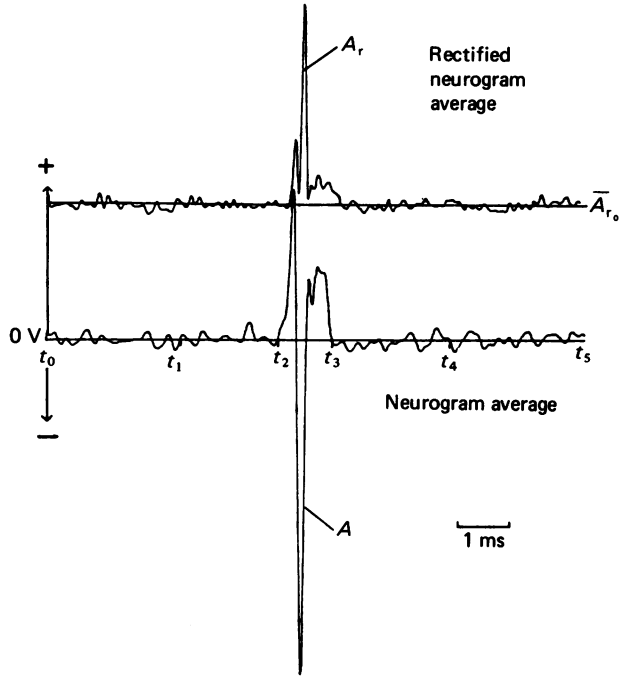

Fig. 1. Averages used for calculating the synchronization index (I_s). The A average, derived from a muscle–nerve (medial gastronemius) neurogram, is the extracellularly recorded action potential of a I a afferent. The A_r average is an average of the neurogram following rectification. Note the non-zero base-line level which is due to asynchronous noise in the neurogram. $\bar{A}_{r_0}$ shows the average value of the first and fourth quarters of this rectified average. t_0, t_1, t_2, t_3, t_4, and t_5 are the limits of summation used in calculating the I_s (see text).

to eqns. (5)–(7). The test for synchrony involves a comparison of I_s and $\hat{I}_s$, the test relying on the assumption that no difference would exist between I_s and $\hat{I}_s$ in the case of asynchrony. This assumption rests, in turn, on the equivalency of the procedures used to collect the A_r average and calculate S_r from that average to those procedures used to collect the A average and calculate $\hat{S}_r$. (The effect of S_a can be neglected, since this term is common to both I_s and $\hat{I}_s$.) In other words, the process of rectifying and averaging the neurogram must be equivalent to averaging the neurogram and calculating the expected rectified average from the neurogram average, using the estimated probability density function (eqns. (5) and (6)).

The first important factor in satisfying these requirements is the rectifier, the characteristics of which should approach those of an absolute value function. A precision rectifier was constructed using a circuit based on the design of Graeme (1973). The rectifier was built with amplifiers having a high slew rate (24 μV/s) to avoid distorting the higher frequencies present in rectified signals. Furthermore, components were specially selected to minimize drift and errors at zero crossing. Before each analysis session, the over-all gain, the relative gains for positive and negative signals, and the offset voltage of the rectifier were carefully adjusted. In collecting averages of rectified

signals, the high-frequency filtering at the input of the averager was removed, to avoid distorting the high-frequency components of the rectified signal. The sampling rate used (25 kHz) was sufficient to avoid aliasing errors, given the limited band width of neurogram signals (e.g. Andreassen, Stein & Oguztöreli, 1979), even considering the effects of rectification. These procedures and precautions ensured the attainment of an accurate rectified average.

In the calculation of $\hat{S}_r$, histograms representing the amplitude distributions of the neurogram signals were compiled using a small laboratory computer to provide estimates of the probability density functions of the neurogram for use in eqn. (5). These histograms were compiled from 16000 to 40000 points, spanning the period of record from which the averages were taken. Eqn. (5) was evaluated numerically using points in the A average as the argument to yield estimates of the expected rectified values of the neurogram ($\hat{A}_r(A_i)$); these estimates were then used in eqn. (6). These procedures were checked by comparing the expected rectified values obtained in this manner, using a histogram computed from a Gaussian pseudorandom noise source, with the expected rectified values calculated under the assumption of an ideal Gaussian distribution. With a variation in the signal-to-noise ratio (amplitude of y in eqn. (5)) from -4 to 4 (covering the range of interest in these experiments), the error between the two sets of estimates was no greater than 0·03%.

An additional check on the accuracy and comparability of our measurements of I_s and $\hat{I}_s$ is provided by the analog simulations which were performed (see below). Comparison of I_s and $\hat{I}_s$ values obtained in these simulations in the absence of any simulated synchrony yields good agreement between the two measures (see Fig. 8; also Fig. 3 in Roscoe *et al.* 1985). Differences found between I_s and $\hat{I}_s$ were less than the estimated 95% confidence limits (see below).

One additional factor to be considered is the effect of variability of the trigger pulse used in collecting the averages. A window discriminator was used to generate trigger pulses from the reference spike train. Any noise in the spike-train record would result in a variability in the relative timing between the reference spikes and the trigger pulses, and consequently between the trigger pulses and the extracellularly recorded action potential in the neurogram. The effect of this variability would be to reduce the peak-to-peak amplitude of the action-potential wave form in the A average while slightly increasing its duration. The total (absolute value) of the area in the averaged wave form would be reduced due to partial cancellation of the positive and negative components of the wave form; however, part of this difference in area would appear in the rectified average (cf. Fig. 2). The effect would be to reduce the value of $\hat{I}_s$ and, to a lesser extent, that of I_s. Consequently, variability in the trigger could introduce a false indication of synchrony, if such variability was great enough. Inspection of the reference spike trains revealed only a small degree of variability in their timing with respect to the trigger pulses derived from them; typical values for the standard deviation of this relative timing varied from less than 5 to 15 μs. Simulations were performed to estimate the effect of such variability on $\hat{I}_s$ values. With a standard deviation of 15 μs, the decrease of $\hat{I}_s$ relative to its value with no variability ranges from 0·015 to 0·018 depending on the signal-to-noise ratio of the wave form. The actual difference between I_s and $\hat{I}_s$ which would occur due to this effect would be less than these values, for the actual value of I_s would be smaller than the value of I_s which would have been obtained with no variability, due to the non-linear relation between the amplitude of synchronized signals and their contribution to the rectified neurogram and the I_s (see Fig. 8; Roscoe *et al.* 1985). Consequently, variability of the reference trigger is not a significant factor in the results of this study.

Confidence limits of $I_s - \hat{I}_s$. Due to the presence of background noise, both I_s and $\hat{I}_s$ have variances. The detection of synchrony therefore depends on whether or not $I_s - \hat{I}_s$ is significantly greater than 0. Two methods have been used to determine this significance. The first requires a population of I_s values. In this case, synchrony exists in the population if the mean of the $I_s - \hat{I}_s$ values is significantly greater than 0 as judged by use of t statistics. The second method of determination has been used for individual I_s values. In this case the condition of asynchrony is rejected if

$$I_s - \hat{I}_s = \frac{S_r - \hat{S}_r}{S_a} > \frac{2\sigma_{\Delta S_r}}{S_a}, \tag{8}$$

where $\sigma_{\Delta S_r}$ is the standard deviation of $S_r - \hat{S}_r$, which is given by:

$$\sigma_{\Delta S_r} = [\sigma^2_{S_r} + \sigma^2_{\hat{S}_r} - 2\,\mathrm{cov}\,(S_r, \hat{S}_r)]^{\frac{1}{2}}, \tag{9}$$

where $\sigma^2_{S_r}$ is the variance of S_r, $\sigma^2_{\hat{S}_r}$ is the variance of $\hat{S}_r$ and cov $(S_r, \hat{S}_r)$ is the covariance of S_r

and $\hat{S}_r$. These quantities may be determined approximately by the following equations (Hamm *et al.* 1985). $\sigma^2_{S_r}$ is given by:

$$\sigma^2_{S_r} \cong \left(\sum_{i,j-t_1}^{t_4} \rho_{yy}(i-j)\, \sigma^2_{y_i} + \sum_{i,j-t_0}^{t_1} \rho_{yy}(i-j)\, \sigma^2_{y_i} + \sum_{i,j-t_4}^{t_5} \rho_{yy}(i-j)\, \sigma^2_{y_i} - 2\sum_{i-t_0}^{t_1} \sum_{j-t_1}^{t_4} \rho_{yy}(i-j)\, \sigma^2_{y_i} - 2\sum_{i-t_1}^{t_4} \sum_{j-t_4}^{t_5} \rho_{yy}(i-j)\, \sigma^2_{y_i} \right) \Big/ n, \quad (10)$$

where $\rho_{yy}(i-j)$, the normalized autocovariance of the rectified neurogram signal and $\sigma^2_{y_i}$, the variance of the average of the neurogram signal, are given for a Gaussian distribution by:

$$\rho_{yy}(i-j) = \frac{1}{\pi/2-1} \left[\rho_{xx}(i-j) \sin^{-1} \rho_{xx}(i-j) + (1-\rho^2_{xx}(i-j))^{\frac{1}{2}} - 1\right] \quad (11)$$

and

$$\sigma^2_{y_i} = \sigma^2 + \mu^2_i - \mu^2_{r_i}. \quad (12)$$

In eqn. (11) $\rho_{xx}(i-j)$ is the correlation coefficient between points separated by an interval of $i-j$ in the neurogram signal, while in eqn. (12), σ^2 is the variance of the neurogram signal, μ_i and μ_{r_i} are the mean values of the ith point in the neurogram and rectified neurogram, respectively, and n (eqn. (10)) is the number of samples in the average. The variance of $\hat{S}_r$ is given by:

$$\sigma^2_{\hat{S}_r} \cong \sum_{i,j-t_1}^{t_4} K_i\, K_j\, \sigma^2_{xx}(i-j)/n, \quad (13)$$

where $\sigma^2_{xx}(i-j)$ is the autocovariance of the neurogram signal and K_i is given by

$$K_i = \left. \frac{d\hat{A}_r(x)}{dx} \right|_{x=A_i}. \quad (14)$$

The covariance of S_r and $\hat{S}_r$ is given by:

$$\text{cov}\,(S_r, \hat{S}_r) \cong \sum_{i-t_2}^{t_3} \sum_{j-t_1}^{t_4} K_i\, \text{cov}\,(A_i, A_{r_j}), \quad (15)$$

where cov (A_i, A_{r_j}) is the covariance of points i and j in the A and A_r averages, respectively. For a Gaussian distribution, this covariance is determined by

$$\text{cov}\,(A_i, A_{r_j}) = \frac{\sigma \rho_{xx}(i-j)}{n(2\pi)^{\frac{1}{2}}} \int_{-\mu_j}^{\mu_j} \exp\left(\frac{-\xi^2}{2\sigma^2}\right) d\xi, \quad (16)$$

where μ_j is the amplitude of the point in the neurogram signal corresponding to the jth point in the rectified average, A_r.

The autocovariances and correlation coefficients required in these calculations were computed in a small laboratory computer using a representative sample of neurogram recordings from each experiment.

Several of these equations (11, 12 and 16) were derived for Gaussian distributions. While the non-normality of the amplitude distributions of some neurogram records produces some deviation from the values given by these equations, use of these equations provides approximate confidence limits with which the $I_s - \hat{I}_s$ values can be compared, as judged by the general agreement between the lower limits of the distribution of $I_s - \hat{I}_s$ values and the lower confidence limits (Figs. 4–6).

Sensitivity of the I_s

The sensitivity of the I_s to small amounts of synchrony was judged from I_s values obtained in analog simulations and during the application of quick stretches to the medial gastrocnemius muscle. The circuit used in the analog simulations has been described elsewhere (Reinking & Roscoe, 1982). Briefly, this circuit provided a 'neurogram' signal consisting of a reference spike train, a synchronized non-reference spike train and pseudorandom background noise. Amplitude of the triphasic spikes and the degree of synchrony (amount of jitter) between the non-reference spikes and the reference spikes were adjustable. For the simulations presented here, the non-reference spikes were locked one-to-one to the reference spikes within a 1 ms time window. The simulated neurogram signal and its rectified version were averaged and I_s values calculated for known degrees of synchrony.

Quick (2 ms rise time, 5 ms duration) triangular stretches of varying amplitude (1–100 μm) were applied to the medial gastrocnemius muscle in two experiments. At each amplitude, 1024 consecutive stretches were given at random intervals (mean interval of 32 ms, range of 27–37 ms).

Simulations of spike-triggered averages

The effects of the synchrony found in the present study on the amplitude and time course of e.p.s.p.s obtained by spike-triggered averaging were investigated by performing numerical simulations. The simplifying assumption was made that the p.s.p.s produced by the synchronized afferents could be treated as a single potential having the mean characteristics of those potentials (i.e. amplitude, rise time, etc.). This assumption was made on the basis of the linearity of summation of p.s.p.s in α-motoneurones (Burke, 1967; Koehler, Hamm, Enoka, Stuart & Windhorst, 1984). To select the time course and amplitude of this composite synchronized p.s.p., the assumption was made that synchrony existed uniformly throughout the different types of afferents, in accordance with our results (see below). Consequently, the composite synchronized p.s.p. was represented as the sum of representative single-fibre Ia and spII e.p.s.p.s, each being weighted according to the numbers of muscle afferents supplying medial gastrocnemius and the connectivity of each afferent type with α-motoneurones (Ib p.s.p.s were assumed to be small enough to be neglected; Watt *et al.* 1976). Given approximately sixty Ia, sixty spII and forty Ib afferents supplying medial gastrocnemius (Boyd & Davey, 1968) and connectivities of 90% for Ia afferents (Mendell & Henneman, 1971; Scott & Mendell, 1976; Watt *et al.* 1976; Fleshman *et al.* 1981) and 50% for spII afferents (Stauffer *et al.* 1976; Munson *et al.* 1982), weighting factors of 0·34 (0·9 × 60/160) and 0·19 (0·5 × 60/160) were selected for the representative Ia and spII e.p.s.p.s, respectively. The amplitudes for the representative Ia and sp II e.p.s.p.s were chosen as 100 and 25 μV, respectively; both were given 10–90% rise times of 0·6 ms and half-widths of 3·9 ms, in accordance with established values (Mendell & Henneman, 1971; Scott & Mendell, 1976; Stauffer *et al.* 1976; Watt *et al.* 1976; Munson, Fleshman & Sypert, 1980; Fleshman *et al.* 1981; Munson *et al.* 1982). With these amplitudes and weighting factors, the composite synchronized p.s.p. was given an amplitude of 40 μV and represented by the following equation (Kirkwood & Sears, 1978):

$$f(t) = \frac{V\alpha^2}{(\beta-\alpha)^2}\{[(\beta-\alpha)t-1]\exp(-\alpha t)+\exp(-\beta t)\} \quad (\text{for } t \geqslant 0). \tag{17}$$

Values of the scale factor V and the time constants α and β were chosen to yield the desired amplitude and time course. The time at which this synchronized p.s.p. occurred with respect to the p.s.p. produced by the reference afferent was represented by a Gaussian distribution, the standard deviation and total probability of which were varied to represent different degrees of synchrony. The contribution to the averaged p.s.p. due to synchrony was calculated by convolving this 'synchronized-discharge' distribution with the wave form of the synchronized p.s.p. The resulting wave form was then added to an e.p.s.p. of amplitude appropriate to represent a Ia or spII e.p.s.p.

The probability of the synchronized discharge was set to 1 to represent the greatest degree of synchrony found in the present results: the equivalent of one afferent locked one-to-one with the reference spike (see Results). The width of the probability distribution of the synchronized spike, specified by its standard deviation, was chosen in accord with the constraints of the I_s. Since, for a given level of synchrony, the I_s value would decrease as the width of the distribution increased beyond the t_1–t_4 interval (see Fig. 1), the standard deviation was set at 1·25 ms for this case. The possibility of a broader distribution was treated by increasing the total probability of the distribution by such an amount that the I_s would correspond to that obtained for the narrower distribution. For small degrees of synchrony, the I_s decreases in proportion to the probability that the synchronized spikes occur outside the t_1–t_4 interval as compared to within it (T. M. Hamm, unpublished observations; cf. Roscoe *et al.* 1985). A distribution with a standard deviation of 2·5 ms would therefore yield an I_s of approximately 40% (the 68% probability that the synchronized spikes occur within the t_1–t_4 interval minus the 27% probability that they occur in the t_0–t_1 or t_4–t_5 intervals) that of a distribution which was within the t_1–t_4 interval. For a distribution with a standard deviation of 2·5 ms, then, the total probability was set to 2·5 (representing the equivalent of 2·5 synchronized afferents) to represent the greatest degree of synchrony found in these experiments. Lesser degrees of synchrony were specified by smaller values of total probability.

RESULTS

Inadequacy of averaging non-rectified signals for detecting synchrony

Fig. 2*A* shows an average of the wave form of a Ia afferent from medial gastrocnemius extracted from a multi-unit neurogram. The averaging interval in *A* is extended to include the epoch in which the afferent spike (Fig. 2*B*) should have

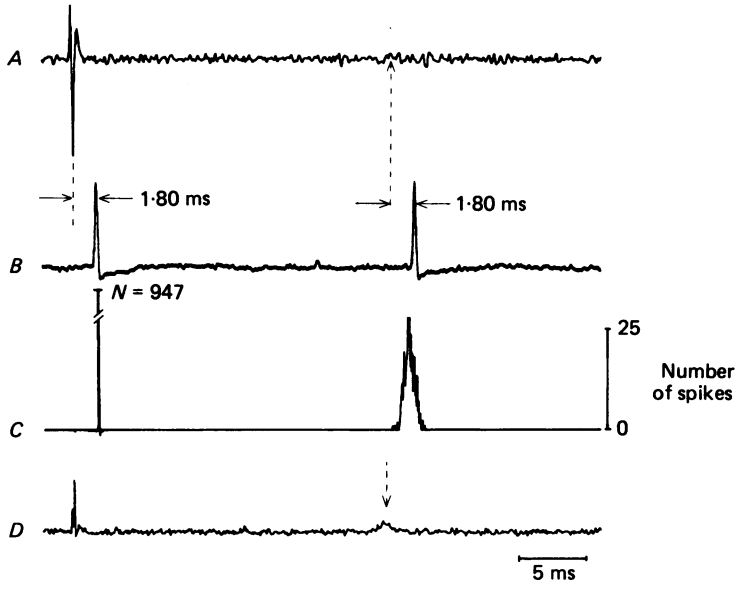

Fig. 2. Limitations of averaging non-rectified neurograms. *A* shows an average of a neurogram (512 sweeps) triggered by a single Ia afferent spike train recorded from a cut dorsal root filament. A sample of the triggering Ia spike train is shown in *B*. Conduction time from neurogram recording level to dorsal root filament electrode was 1·80 ms. The neurogram was aligned by passage through a variable analog delay line. *C* shows an interspike-interval histogram (40 μs bin width) triggered by 947 reference afferent spikes. The duration of the peak of the histogram indicates the variability of the afferent's interspike interval. Dashed vertical arrow indicates when spike following trigger spike would appear in the neurogram average (*A*), *if it was detectable*. *D* shows the same averaging epoch as in *A* and the same trigger signal (arrow) but with the average based on a rectified version of the neurogram. Note appearance of spike following trigger spike in this rectified average, albeit in an attenuated and broadened form.

'reappeared' at the neurogram recording level (i.e. the next spike following the reference trigger spike). However, the average of the neurogram shows no evidence of this second spike. Fig. 2*C* shows an autocorrelogram of this afferent's spike train. The interspike-interval variance (Matthews & Stein, 1969*b*) is evident in the width of the second peak in the autocorrelogram. This variance is responsible for the non-appearance in Fig. 2*A* of the next spike following the reference spike. In contrast, Fig. 2*D* shows that this next spike does indeed appear, albeit in attenuated and broadened form, in an average based on a rectified version of the neurogram.

The repetitive firing of the same single reference spike train is, of course, analogous to synchronous discharge. If the averaging of an unrectified neurogram cannot detect

such simple 'synchrony', then a second spike, with its own interspike-interval variance, must surely escape detection even if it is synchronous with the reference spike. Thus, the synchrony test introduced for spike-triggered averaging by Watt *et al.* (1976) is invalid.

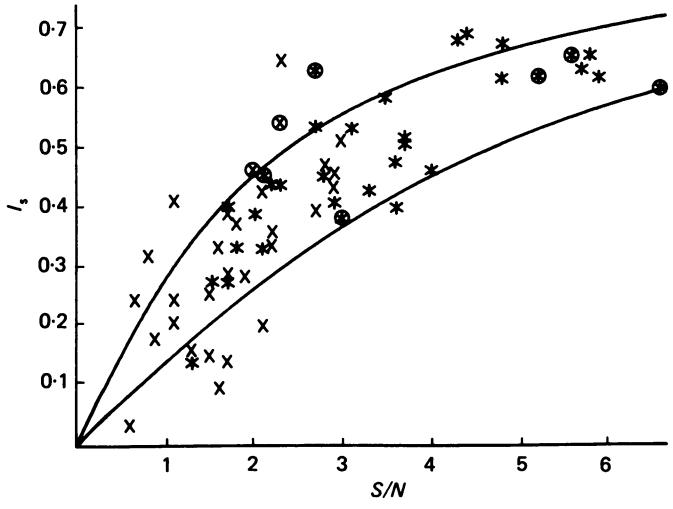

Fig. 3. Distribution of I_s values. This Figure shows the I_s values found for sixty-one muscle afferents, plotted as a function of the signal-to-noise ratio (S/N) of the neurogram wave form of each afferent. The two curves give the I_s values expected for asynchrony ($\hat{I}_s$). The upper curve gives the $\hat{I}_s$ values calculated using the amplitude distribution from one nerve-branch experiment and represents the upper limit of $\hat{I}_s$ values; the lower curve, calculated according to a Gaussian distribution, is more representative of $\hat{I}_s$ values in this data base. Asterisks indicate data drawn from experiments in which the neurogram recordings were made from a nerve branch. Crosses indicate data from experiments in which the whole muscle nerve was used. The circled symbols indicate that the rectified averages used in computing these I_s values had uneven base lines (see Methods).

Synchrony in the discharge of muscle afferents

The spike trains of sixty-one muscle afferents were used as references in collecting neurogram and rectified-neurogram averages. The I_s values calculated using these pairs of averages are shown in Fig. 3. Also shown in Fig. 3 are two curves representing the range of expected values of I_s in the condition of asynchrony ($\hat{I}_s$). As noted in Methods, $\hat{I}_s$ is dependent on the amplitude distribution of background noise in the neurogram. The lower curve is based on a Gaussian distribution, the other curve on a markedly non-Gaussian distribution from a nerve-branch experiment. The curve representing this nerve-branch experiment is an extreme; all other experiments gave $\hat{I}_s$ values which were closer to those given by the Gaussian distribution. Both curves were calculated using an idealized triphasic wave form to represent values of the neurogram average required for eqn. (6) (see Fig. 2, Hamm *et al.* 1985). As shown in Fig. 3, the I_s is dependent on the signal-to-noise ratio (S/N) of the reference spike. Comparison of the I_s values to the more representative Gaussian curve suggests the presence of synchrony although variations in $\hat{I}_s$ with variations in amplitude

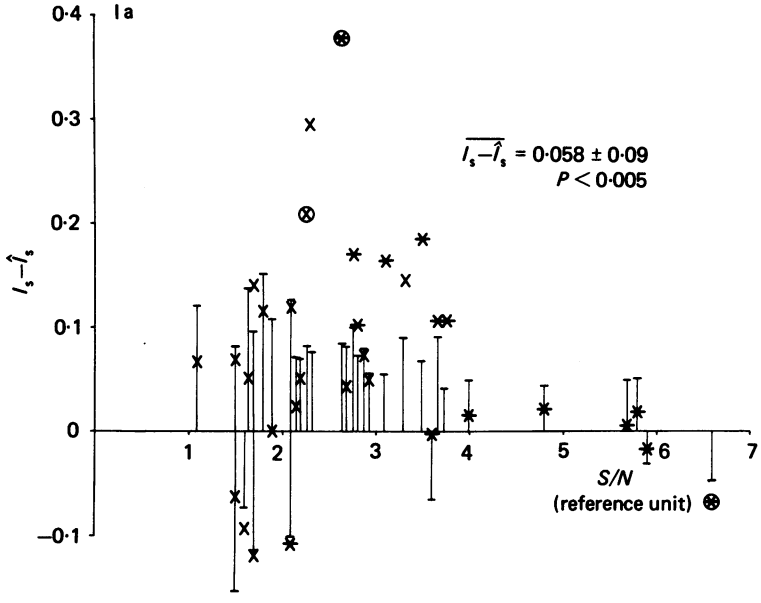

Fig. 4. Synchrony in the discharge of Ia afferents. This graph shows the differences between the I_s and $\hat{I}_s$ values for a population of thirty-three Ia afferents. The symbols representing the $I_s - \hat{I}_s$ differences have the same meaning as in Fig. 3. The vertical lines indicate the estimated 95 % confidence limits for these differences. $I_s - \hat{I}_s$ values which were calculated from records having rectified averages with uneven base lines (circled symbols) were excluded in the calculation of the mean and standard deviation shown in this and the following two Figures.

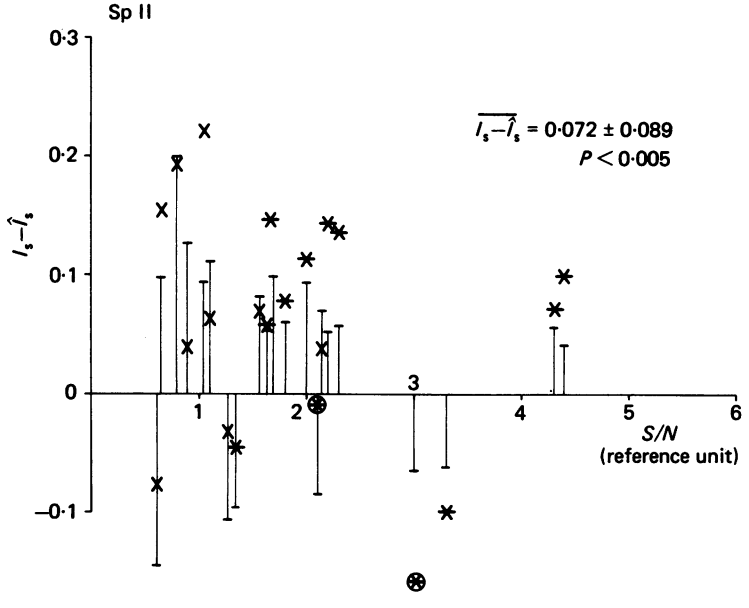

Fig. 5. Synchrony in the discharge of sp II afferents. Data derived from the discharge of twenty-one sp II afferents are presented in this graph. The format is the same as in Fig. 4.

distribution and neurogram wave form prevent a conclusive analysis using this Figure.

Figs. 4, 5 and 6 provide a more accurate indication of the presence of synchrony. These Figures are the findings for I a, sp II and I b afferents, respectively. Each plotted point is the difference between the measured I_s and the value calculated for $\hat{I}_s$. The

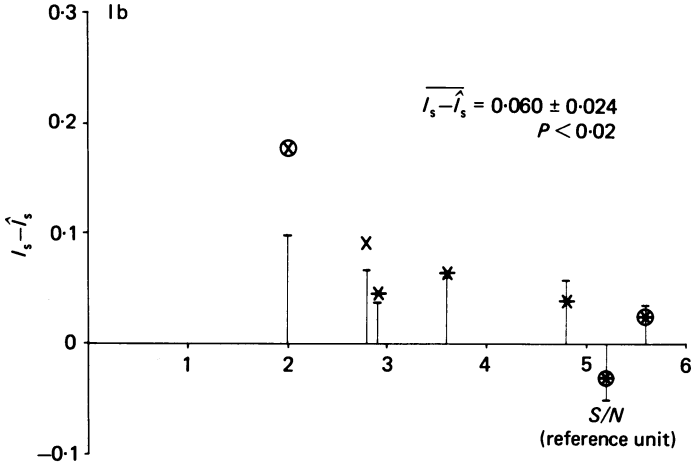

Fig. 6. Synchrony in the discharge of I b afferents. Data derived from the discharge of seven I b afferents are presented in this graph. The format is the same as in Fig. 4.

averaged neurogram wave forms (A) and the noise distribution measured from the section of recording containing each reference spike train were used in calculating these $\hat{I}_s$ values. Also shown are 95% confidence limits for each $I_s - \hat{I}_s$ difference, calculated as discussed in Methods. For the moment attention will be confined to the values obtained for those afferents whose rectified averages had uniform base lines (see Methods). Of the fifty-three afferents in this population, twenty-two display synchrony as judged by the difference between I_s and $\hat{I}_s$ being greater than the associated confidence limits. In addition, the mean value of $I_s - \hat{I}_s$ for this group of afferents is 0·063, a value significantly greater than 0 ($P < 0·001$).

Examination of each type (I a, sp II, I b) of muscle afferent reveals that synchrony was present in the discharge of each type. The mean of each type is significantly greater than 0, and a number of afferents are evident for each type the $I_s - \hat{I}_s$ values of which are greater than their confidence limits. The means of $I_s - \hat{I}_s$ for the I a (0·058), sp II (0·072) and I b (0·060) populations are not significantly different, as shown by an analysis of variance ($F = 0·16$); furthermore, a χ^2 analysis failed to reveal any differences in the incidence of synchrony in these groups of afferents (nine of thirty for I a; ten of nineteen for sp II; and three of four for I b; $\chi^2 = 1·82$; $0·25 < P < 0·5$). Consequently, for the present sample of afferents and within the limitations of the I_s, the degree of synchrony was similar for the discharge of I a, sp II and I b afferents.

The uncertainties of separating I a from sp II afferents on the basis of conduction velocity (cf. Matthews, 1972) raise the possibility that this even distribution of synchrony in the discharge of I a and sp II afferents could be due to improper

classification. However, examination of spindle afferents with conduction velocities of less than 51·8 m/s or greater than 65·5 m/s (corresponding to 0·6 times the expected maximum conduction velocity of 120 m/s after adjustment for the extreme muscle bath temperatures, 30 and 35 °C, respectively) yields mean values for $I_s - \hat{I}_s$ of $0{\cdot}054 \pm 0{\cdot}095$ and $0{\cdot}056 \pm 0{\cdot}095$, respectively. The similarity of these values to those of the Ia and spII averages in Figs. 4 and 5 supports the validity of the findings of a similar degree of synchrony for each type of muscle afferent.

In three experiments recordings were made from nerve branches of the medial gastrocnemius nerve. The $I_s - \hat{I}_s$ values representing data collected from these experiments are indicated in Figs. 4–6. Synchrony is evident in these values obtained for averages from nerve branches as well as those obtained for averages from the whole medial gastrocnemius nerve. The mean value of $I_s - \hat{I}_s$ for the nerve-branch data is $0{\cdot}067 \pm 0{\cdot}071$, while that for the whole-nerve data is $0{\cdot}060 \pm 0{\cdot}103$. Previous work has shown that the nerve branches of medial gastrocnemius innervate muscle compartments which are largely separate (Letbetter, 1974) and that the spindle afferents in each nerve branch supply spindles within the corresponding muscular compartment (Farina & Letbetter, 1977). These results, which show that sampling the discharge of afferents from a localized muscle area yields I_s increases which are as great or greater than those obtained by sampling afferent discharge from the entire muscle, suggest that the synchrony found in this study may be due predominantly to the discharge of receptors contained within the subvolume of muscle surrounding the receptor of the reference afferent.

The rectified averages of eight afferents had uneven base lines. The I_s values corresponding to these afferents have not been mentioned to this point since, with violation of one of the assumptions underlying the I_s, interpretation is uncertain. However, this inequality of the first and last quarters of the rectified average indicates an increase (or decrease) of activity in these segments, suggesting that these afferents may have been synchronized to others. This set of $I_s - \hat{I}_s$ values included three which clearly exceeded the upper bound of their confidence limits. The mean value of $I_s - \hat{I}_s$ ($0{\cdot}066 \pm 0{\cdot}176$) was not significantly different from 0, however, due in part to the presence of two values which fell below the lower bounds of their confidence limits.

We were concerned that the synchrony found in this study might have been produced by a mechanical source driven by line frequency, such as a fan motor in a nearby piece of equipment (cf. Kirkwood & Sears, 1982). Cross-correlation histograms were computed between a trigger pulse occurring at 60 Hz and afferent spike trains recorded in nerve-branch experiments, in which synchrony was most easily recognized in individual cases. (To ensure that the 60 Hz trigger was phase locked to any potential power-line-induced effects in the recorded data, 60 Hz contamination on one of the channels of the tape recorder was examined on an oscilloscope, using the 60 cycle pulse to trigger the sweep of the oscilloscope. The contamination was tightly locked to the trigger.) These histograms, based on a limited number of spikes (< 1000), showed peaks in some cases. However, it is difficult to state that such peaks indicate that these afferent spike trains were dependent on 60 Hz vibration, given the difficulty of determining whether two periodic spike trains are dependent based on the presence of a peak in a cross-correlation histogram (cf. Perkel, Gerstein & Moore, 1967). This issue was examined

further by calculating the $I_s - \hat{I}_s$ value which would be due to synchronization induced by 60 Hz vibration. These calculations were based on the following line of reasoning. If 60 Hz vibration was the sole synchronizing influence, then the afferent pool would have discharged preferentially at a particular phase of each 16·7 ms period. This increased discharge would be evident as a peak in an average of the rectified neurogram, triggered by a 60 Hz signal. The I_s calculated for an afferent spike train would show synchrony to the extent that the afferent discharged most frequently at

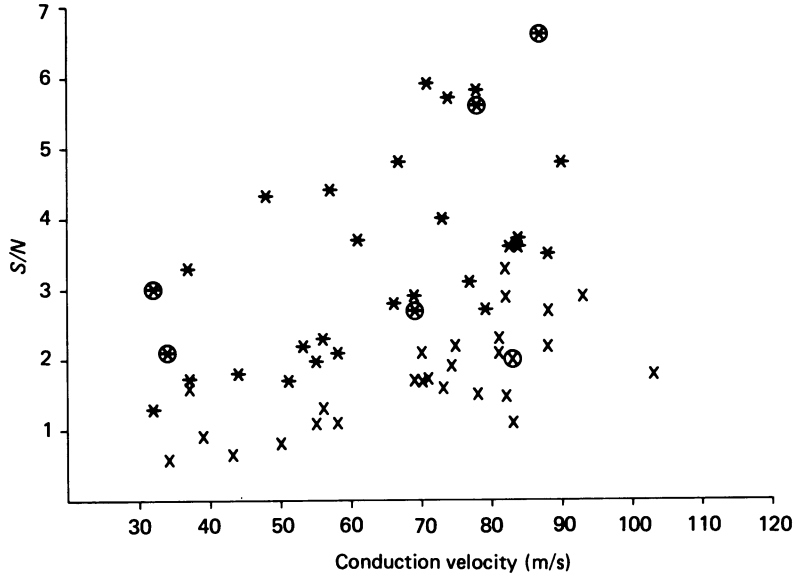

Fig. 7. Relation between afferent axonal conduction velocity and action potential signal-to-noise ratio. The signal-to-noise ratio of the action potential of sixty-one afferents (seven experiments) is shown plotted against the corresponding afferent's axonal conduction velocity. Symbols are same as in Fig. 3.

the time of this peak. Consequently, raw and rectified neurogram averages and cross-correlation histograms of afferent discharge, triggered by a 60 Hz signal, were collected simultaneously in a signal averager. (The raw neurogram average was used to calculate the noise level, to which the rectified average was scaled for comparison with the results obtained using averages triggered by afferent spike trains.) The histograms, composed originally of 16 μs bins, were shifted by an amount equal to the conduction delay between the neurogram and dorsal root filament electrodes and regrouped into sixteen equal bins that spanned the 16·7 ms interval. S_r values were calculated for the rectified average at points corresponding to the centre of each histogram bin (each S_r value was the sum of points in a 5 ms interval minus the sum of the two adjacent 2·5 ms intervals; cf. eqn. (2)). These S_r values were summed, each value being weighted by the fraction of spikes in its corresponding histogram bin relative to the total number of spikes in the 16·7 ms period. This summed S_r value was then divided by S_a, obtained from the original neurogram average collected with an afferent spike train as trigger, to yield the difference between I_s and I_s contributed

by any synchrony induced by a source of vibration at line frequency. In no individual case did these values indicate the presence of synchrony, and the mean value of the examined averages (representing the spike trains of thirty-three afferents) was not significantly different from 0 ($0{\cdot}001 \pm 0{\cdot}004$). We concluded from this analysis that vibration at line frequency was not a significant source of synchrony.

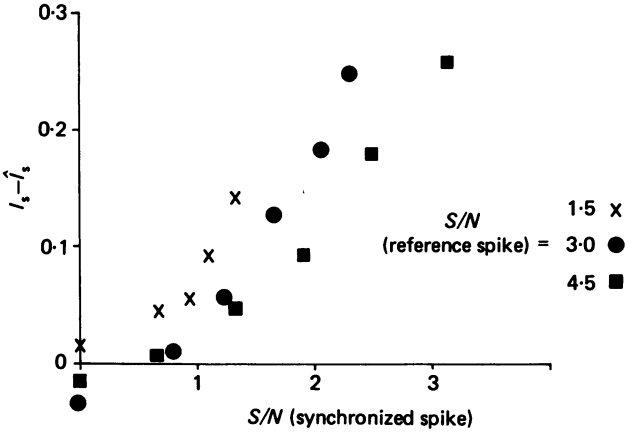

Fig. 8. Sensitivity of the I_s to simulated synchrony. This Figure shows the increase in the I_s as the signal-to-noise ratio of a single spike, locked one-to-one with the discharge of a reference spike in an analog simulation, is varied. Values are shown for three different signal-to-noise ratios of the reference spike. The increase of the I_s for a synchronized spike with a signal-to-noise ratio of 3 (approximately the mid-range of the signal-to-noise ratios found in this study) is comparable to that of the largest $I_s - \hat{I}_s$ differences found in the afferent population of this study.

The range of afferent fibre conduction velocities sampled in the present study is indicated in Fig. 7, which shows the relation between signal-to-noise ratio and conduction velocity. We have shown elsewhere (Roscoe *et al.* 1985) the difficulty of detecting the synchronized discharge of an afferent whose signal-to-noise ratio is much less than 1. The data in Fig. 7 indicate that a large proportion of the muscle afferent population, including all of the Ia and Ib and part of the spII afferents, would have been sampled in these experiments. The sampling was more complete in the nerve-branch experiments, since the signal-to-noise ratios are larger for this set of data for a given conduction velocity.

Sensitivity of the I_s

The degree of synchrony indicated by an increase of I_s above $\hat{I}_s$ was judged by two methods. In the first method analog simulations were performed, I_s values being computed for the case of two simulated spike trains synchronized with one-to-one coupling. The results of one such set of simulations are shown in Fig. 8. A more complete analysis is presented elsewhere (Roscoe *et al.* 1985).

Fig. 8 shows that the difference between I_s and $\hat{I}_s$ increases progressively as the signal-to-noise ratio of the synchronized spike increases. At a signal-to-noise ratio of the synchronized spike of 3 (roughly the mid-range of the signal-to-noise ratios in this study), $I_s - \hat{I}_s$ would have a value of approximately 0·25, a value which is in the

neighbourhood of the largest found in this study. This result is applicable to more general situations by virtue of the fact that the number of synchronized spikes and the degree of coupling of each synchronized spike contribute equally to the I_s (Hamm, 1982; Roscoe *et al.* 1985). For example, the I_s for the condition of one synchronized spike with one-to-one coupling should be the same as for the condition of four synchronized spikes with one-to-four coupling. Thus, the simulations represented in

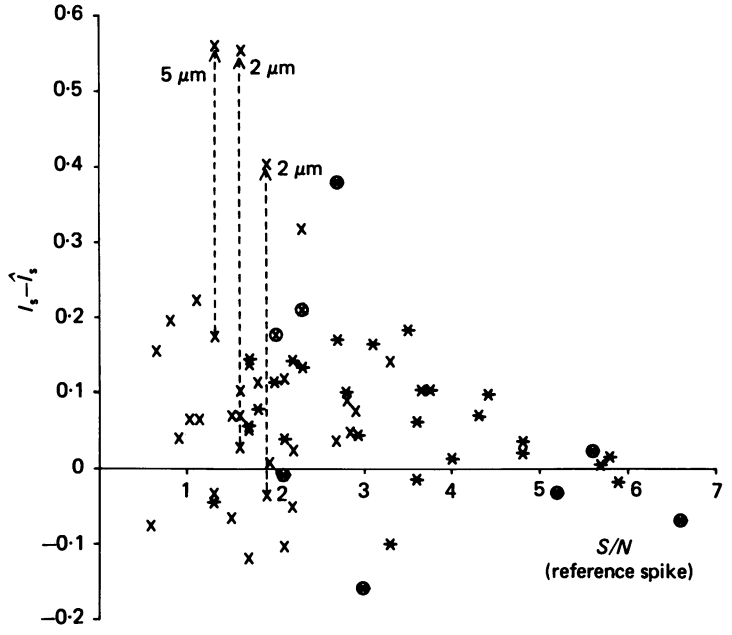

Fig. 9. Sensitivity of the I_s to the synchrony induced by quick stretches. The increase in the I_s for three afferents during the application of a quick (2 ms rise time, 5 ms duration) stretch is indicated by the dashed arrows. The amplitude of the stretches are indicated in the Figure. The increase produced by this subtle degree of synchrony may be compared to the $I_s - \hat{I}_s$ values for the afferents with the muscle at fixed length (data from Figs. 4–6; the confidence limits have been omitted for clarity). Symbols are the same as in Fig. 3.

Fig. 8 suggest that the greatest degree of synchrony in the present study is the *equivalent* of one spike locked one-to-one with the reference spike. The average degree of synchrony, as judged by mean $I_s - \hat{I}_s$ values of approximately 0·06, is the equivalent of one spike which discharges in synchrony with one of every four reference spikes.

The subtlety of the synchrony present in these experiments is confirmed in Fig. 9, which shows the effects of applying quick stretches to the medial gastrocnemius muscle. Lucas & Willis (1974) found that stretches of a similar time course applied to medial gastrocnemius held at a passive tension of about 1 N induced discharge of Ia afferents starting at amplitudes of stretch of about 3 μm. Application of 10 μm stretches activated only a few of the Ia afferents. Consequently, the stretches represented in Fig. 9 (of 2 and 5 μm) likely produced only modulation of discharge in a number of Ia afferents or the overt discharge of one or two Ia afferents. This small degree of induced synchrony produced increases in the I_s which are just slightly

larger than the largest increases of I_s above $\hat{I}_s$ found when the muscle was held at fixed length.

Contributions of synchrony to p.s.p.s measured by spike-triggered averaging

The contributions of afferent fibre synchrony to e.p.s.p.s obtained by spike-triggered averaging were evaluated by performing numerical simulations, using the previously presented results to estimate the level of synchrony which might be anticipated in a spike-triggered averaging experiment. Figs. 10 and 11 show results of these

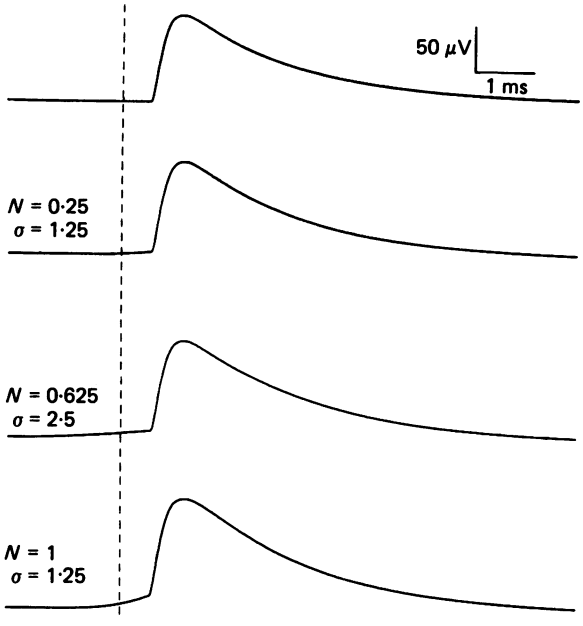

Fig. 10. Contributions of synchrony to Ia e.p.s.p.s obtained by spike-triggered averaging. The wave forms of simulated Ia e.p.s.p.s are shown as measured in the absence (top) and presence of varying degrees of synchrony. N indicates the total probability of the distribution describing the time of occurrence of the synchronized spikes with respect to that of the reference spike. (N can also be interpreted as the equivalent number of synchronized afferents.) σ gives the standard deviation of this distribution. The mean degree of synchrony found in the present experiments is represented by $N = 0{\cdot}25$, $\sigma = 1{\cdot}25$ ms and $N = 0{\cdot}625$, $\sigma = 0{\cdot}25$ ms. The maximum degree of synchrony is represented by $N = 1$, $\sigma = 1{\cdot}25$ ms.

simulations for e.p.s.p.s averaged when using a Ia and a spII afferent as references, respectively. Each e.p.s.p. can be seen to be superimposed on a sloping base line, which would be obscured to some extent in an actual average due to the presence of noise. The effect of the synchrony on the wave form is not great. However, the effect is significant for the spII e.p.s.p.s given their small amplitudes. Moreover, changes in time course are greater for these e.p.s.p.s.

These notions are made more precise in Table 1. The amplitudes, 10–90 % rise times and half-widths were measured for the contaminated e.p.s.p.s, using the point 0·5 ms before the foot of the e.p.s.p. as the reference level from which the potential was

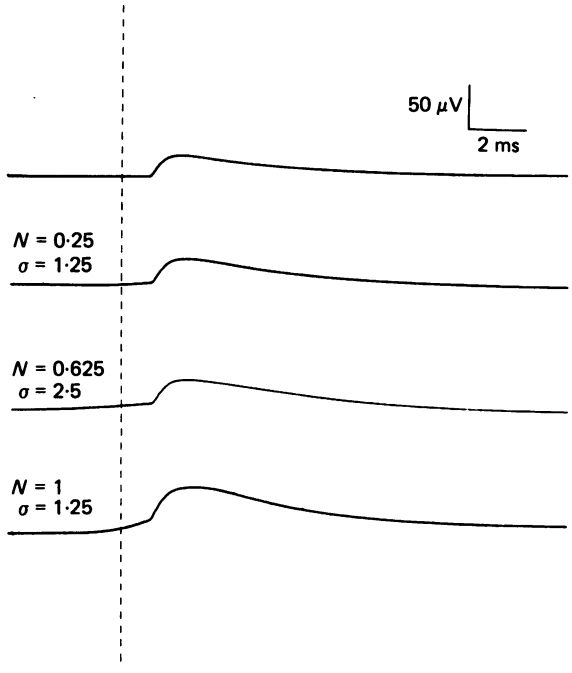

Fig. 11. Contributions of synchrony to sp II e.p.s.p.s obtained by spike-triggered averaging. The format of this Figure is the same as for Fig. 10.

TABLE 1. Parameters of simulated monosynaptic e.p.s.p.s obtained by spike-triggered averaging in the presence of synchrony

	Ia e.p.s.p.s			SpII e.p.s.p.s		
Condition	Amplitude (μV)	Rise time (ms)	Half-width (ms)	Amplitude (μV)	Rise time (ms)	Half-width (ms)
Asynchrony...	100	0·60	3·90	25	0·60	3·90
$N = 0{\cdot}25$ $\sigma = 1{\cdot}25$	104·1	0·62	3·98	29·2	0·66	4·10
$N = 0{\cdot}625$ $\sigma = 2{\cdot}50$	104·1	0·62	4·10	29·1	0·66	4·50
$N = 0{\cdot}50$ $\sigma = 1{\cdot}25$	108·2	0·64	4·02	33·5	0·74	4·20
$N = 1{\cdot}25$ $\sigma = 2{\cdot}50$	108·2	0·64	4·26	33·5	0·76	4·76
$N = 1{\cdot}0$ $\sigma = 1{\cdot}25$	116·6	0·67	4·10	42·6	0·94	4·26
$N = 2{\cdot}5$ $\sigma = 2{\cdot}50$	116·6	0·68	4·50	43·2	1·00	4·96

Values are given for the amplitude, 10–90 % rise time and half-width for simulated monosynaptic e.p.s.p.s measured in the presence of synchrony by spike-triggered averaging. The degree of synchrony is specified by the parameters N and σ. N is the total probability of the distribution describing the discharge of synchronized afferents with respect to a reference afferent (or the equivalent number of synchronized afferents). σ is the standard deviation of this distribution. The maximum degree of synchrony found in the present study is represented by $N = 1$, $\sigma = 1{\cdot}25$ ms and $N = 2{\cdot}5$, $\sigma = 2{\cdot}5$ ms. The mean degree of synchrony is represented by $N = 0{\cdot}25$, $\sigma = 1{\cdot}25$ ms and $N = 0{\cdot}625$, $\sigma = 2{\cdot}5$ ms.

measured. The increase in amplitude for the spII e.p.s.p.s produced by the synchronously discharging fibre amounts to a 17% increase, on average, although the increase can be as great as 73%. Similarly, rise time and half-width increase on average 10 and 5%, respectively, but as much as 67 and 27% in some cases. Although wider distributions have little effect on amplitude, both rise time and half-width increase as the synchrony occurs over a wider interval. The results of these simulations show that the degree of synchrony found in these experiments would have only a minor effect on e.p.s.p.s of large amplitude, but that the effect on e.p.s.p.s of small amplitude is of consequence.

DISCUSSION

Contrary to previous reports from this laboratory, using tests now considered either invalid (Watt *et al.* 1976) or less sensitive (Reinking, Roscoe, Hamm, Botterman, Cameron & Stuart, 1981), the present study has demonstrated a subtle degree of synchrony between the spike trains of some of the Ia, sp II and Ib afferent fibres and other unspecified afferent spike trains in a passive muscle held at fixed length. No claim is made of representative afferent sampling as is now clearly needed to help resolve the two key issues (see below) raised by the present findings, which we believe have provided a new opening in the study of neuronal synchrony and a cautionary note on the evaluation of p.s.p.s when measured by spike-triggered averaging.

Synchronization of muscle afferents

The possibility of synchrony between the discharge of afferents from a passive muscle held at fixed length was first raised directly by Watt *et al.* (1976) in a study which included recordings of very small ($< 20\ \mu V$) p.s.p.s. The critical nature of the assumption of asynchrony in spike-triggered averaging experiments in which such small p.s.p.s are measured has been reinforced by studies showing that the discharge of some muscle afferents is modulated by the arterial pulse (see below). Afferent synchrony and controls for synchrony have been studied subsequently by this laboratory (Roscoe *et al.* 1979; Roscoe, 1980; Roscoe *et al.* 1981; Reinking *et al.* 1981; Hamm *et al.* 1985; Roscoe *et al.* 1985) and by Kirkwood & Sears (1982). In their study of spindle afferents of the intercostal muscles, Kirkwood & Sears found evidence using cross-correlation analysis for synchrony induced in the discharge of afferents by the arterial pulse and slight mechanical perturbations originating in laboratory equipment. We did not attempt to identify sources of synchrony in the experiments reported here, except to exclude a significant contribution by vibration locked to line frequency. Given the sources of synchrony implicated by Kirkwood & Sears (1982), the great sensitivity of primary spindle afferents, at least to mechanical perturbations of small amplitude (Matthews, 1972), and the modulating effect of the arterial pulse on receptor discharge, we will confine our discussion to the effects of the arterial pulse on the discharge of muscle receptors and to the sensitivity of Ia, spII and Ib afferents to mechanical perturbations of small amplitude.

The great sensitivity of primary spindle endings to stretches of small amplitude has been well documented (Matthews & Stein, 1969*a*; Poppele & Bowman, 1970; Hasan & Houk, 1975). Consequently, reports of locking of the discharge of spindle afferents

to the arterial pulse, dating from the classic study of Matthews (1933), are not surprising. Ellaway & Furness (1977) reported that spindle discharge in anaesthetized rabbits was correlated with the electrocardiogram. In decerebrated cats (Ellaway, Furness & Murthy, 1979), the discharge of motoneurones innervating muscles of the neck and forelimbs was found to be correlated with the electrocardiogram, an effect presumably due to the correlated discharge of muscle spindles with the electrocardiogram (however, this effect was not observed in the discharge of motoneurones innervating hind-limb muscles). The most complete description to date on the influence of the arterial pulse on spindle discharge is that of McKeon & Burke (1981), a study in which the discharges of the spindles of human pretibial muscles were examined. Few spindles were found whose discharge was tightly locked to the electrocardiogram. A majority of the spindles examined, however, displayed a frequency of discharge which was modulated by the electrocardiogram. Thus, the modulation of spindle discharge by the arterial pulse is likely to be a common occurrence.

Since the discharges of only a few spindles were tightly locked to the arterial pulse in the data of McKeon & Burke, they concluded that the spike trains of muscle spindles are unlikely to be synchronized. However, even a modulation of receptor discharge, as McKeon & Burke found for the majority of the spindles in their study, would be equivalent to a change in the probability of receptor discharge at the time of the arterial pulse. The discharge of an afferent the frequency of which increases in response to the pulse would be correlated with that of other such afferents, the net effect being an increase in afferent activity at the time of the pulse. This form of synchrony, although not evident as pairs of spike trains locked to each other at the time of the arterial pulse, could presumably contribute to the I_s and to spike-triggered averages.

At first inspection, such an effect would not be expected for sp II afferents given their lower sensitivity (compared to the Ia afferents) to brief muscle stretches (Lundberg & Winsbury, 1960; Stuart *et al.* 1970; Lucas & Willis, 1974) and vibration or small-amplitude sinusoidal stretches (Brown, Engberg & Matthews, 1967; Trott, 1967; Matthews & Stein, 1969*a*; Poppele & Bowman, 1970; Hasan & Houk, 1975; Cussons, Hulliger & Matthews, 1977). However, comparison of the relative sensitivities of the Ia and sp II endings in these various studies reveals that the Ia–sp II difference is less in some studies than in others (e.g. Stuart *et al.* 1970; Trott, 1976), a finding which is no doubt due in part to slight differences in such factors as the character of the wave form being applied or the mode of application. Bianconi & Van der Meulen (1963) found, for example, that some secondary endings were capable of following vibration applied to the area of a muscle overlying a muscle spindle, but failed to follow the vibration when applied to the tendon. Consequently, without knowing the source of the synchrony in these experiments or the nature of the coupling of that source to individual receptors, estimation of the degree of synchrony in the afferents supplying different receptor types is difficult. While the synchronization of the discharge of sp II afferents seems less likely than that of Ia afferents, the synchronization of at least part of the sp II population cannot be ruled out. Indeed, afferents the discharge of which was modulated by the arterial pulse in the study of McKeon & Burke (1981) included three presumed secondary endings (cf. Matthews, 1933).

Similar considerations apply to the issue of synchrony in the discharge of Ib afferents. Tendon organs are quite sensitive to forces developed in series to them, having thresholds as low as 40 μN for the production of an impulse as shown by

indirect calculations (Binder, Kroin, Moore & Stuart, 1977), and by measurements on isolated tendon organs (Fukami, 1981). The Ib afferents studied in the present set of experiments were part of that select group which had a maintained discharge with the muscle held at a moderate degree of passive tension. With some force being applied passively to the receptor, modulation of the discharge of the receptor by slight perturbations is conceivable, provided that the disturbance is coupled appropriately to the receptor. Again, while first expectations are that synchrony in the discharge of Ib afferents would be less than that of Ia afferents, definite conclusions cannot be drawn without knowledge of the source of synchrony and its mode of coupling to the receptors. The examination of the discharge of statically firing tendon organs would be of interest to determine whether or not that discharge is modulated by the arterial pulse.

The present results show that the discharge of each of the three primary types of muscle afferents is synchronized to that of other afferents. While we think it likely, based on the sensitivity of muscle receptors to small-amplitude stretches and vibration, that the degree and incidence of synchrony of the different afferent types is likely to increase in the order Ib < spII < Ia, we are unable to detect such a difference in the present set of data. The resolution of this issue must await further studies in which the source of synchrony is firmly identified and a representative sampling of afferents is obtained.

An issue raised in the pioneering studies of electrophysiology was whether or not the currents associated with the propagation of the action potential in a nerve fibre could influence activity in adjacent axons, entraining action potentials and thereby producing synchrony (Blair & Erlanger, 1932). This question was answered in the affirmative by Katz & Schmitt (1940), who used a preparation consisting of a pair of non-myelinated axons from the limb nerve of a crab. Such an effect has not been observed in myelinated axons, except in cases of hyperexcitability or injury (Adrian, 1930; Blair & Erlanger, 1932). Even should such an effect be present in myelinated axons, however, it is unlikely to have contributed significantly to the present findings, for unless the action potentials in adjacent axons occurred within a short interval of one another (i.e. were already synchronized) they would not have influenced each other. The influence of such a phenomenon would be no more than to 'tighten' a pre-existing synchronization.

Contamination of p.s.p.s measured by spike-triggered averaging

The issue of contamination of p.s.p.s measured by spike-triggered averaging, the primary focus of this study, became a serious concern with the demonstration of monosynaptic e.p.s.p.s from spII afferents by Kirkwood & Sears (1974, 1975). This concern has subsequently become more acute with further studies of spII e.p.s.p.s (Stauffer *et al.* 1976; Munson *et al.* 1980, 1982) and other small-amplitude p.s.p.s (e.g. Watt *et al.* 1976).

The contamination of single-fibre Ia e.p.s.p.s due to synchronous discharge of other fibres does not appear to be a major issue as judged by the results of the present study, which indicate that the mean increase in apparent amplitude due to synchrony would be on the order of 4 μV. The largest increase as judged by the simulations of this study would be approximately 16 μV. However, this figure might vary considerably, depending on the uniformity of the distribution of synchrony in the afferent pool. The present results suggest that synchrony exists preferentially between the discharge of receptors occupying localized areas of the test muscle. In this case, the contribution

of synchronized afferent fibres to the apparent amplitude of single-fibre p.s.p.s would be more variable than in the case of a widely-distributed synchrony, since the variability of the mean amplitude of p.s.p.s produced by a smaller number of synchronized afferents would be greater. In any event, the contamination will not make a large contribution, on average, to e.p.s.p.s which have an amplitude on the order of 100 μV. Previous comparisons of Ia e.p.s.p.s measured by spike-triggered averaging with those produced by electrical stimulation of a muscle nerve with only a single in-continuity, dorsal root filament (Mendell & Hennemann, 1971) or by intra-axonal stimulation (Honig, Collins & Mendell, 1983) reveal quite similar profiles for e.p.s.p.s of such an amplitude. Any differences between e.p.s.p.s measured by spike-triggered averaging and more direct methods may be accounted for possibly by the effects of stimulation history on e.p.s.p. amplitude (Honig *et al.* 1983). Indeed, such frequency effects are comparable to the effects of synchrony found in the present study, at least for large Ia e.p.s.p.s.

The contamination of spII e.p.s.p.s measured by spike-triggered averaging was suggested by an apparent discrepancy noted by Lundberg, Malmgren & Schomburg (1977) between the composite monosynaptic group II e.p.s.p.s produced by electrical stimulation with that predicted from single-fibre spII e.p.s.p.s, as based on the data of Stauffer *et al.* (1976). Munson *et al.* (1980) argued that this discrepancy is probably not as great as thought by Lundberg *et al.* (1977), especially when considering such factors as temporal dispersion of the group II volley and non-linear summation of e.p.s.p.s. Nevertheless, the present results indicate that caution is in order when examining spII e.p.s.p.s measured by spike-triggered averaging. It is worth emphasizing that the existence of monosynaptic spII connexions to α-motoneurones is not in question, given its demonstration by Kirkwood & Sears (1982), using spike-triggered averaging in a preparation in which all relevant dorsal root filaments were cut, save those containing the reference afferents. Furthermore, we think it unlikely that the smoothed and attentuated profile of a synchronized e.p.s.p., produced by the dispersion of the discharge of the synchronized spikes with respect to the trigger spike, would be readily mistaken for a monosynaptic e.p.s.p., even considering the background of noise which would be prominent in an average in which the apparent e.p.s.p. amplitude is small. The critical issue is the increase in amplitude produced by even the modest degree of synchrony observed in this study, for a slight increase would be large in relation to the small amplitudes which have been observed for spII e.p.s.p.s measured by spike-triggered averaging. The simulations performed in the present study provide a first approximation to this increase, showing that it may be 17 % of the actual e.p.s.p. amplitude, on average. A more definitive answer must await studies which provide a representative sample of recordings in which spII e.p.s.p.s measured with spike-triggered averaging can be compared with those measured with alternative methods (e.g. Kirkwood & Sears, 1982; Honig *et al.* 1983).

The measurement of Ib p.s.p.s by spike-triggered averaging is particularly vulnerable to contamination by synchronization of muscle afferents, given the small amplitude of single-fibre p.s.p.s ($< 10\,\mu$V; Watt *et al.* 1976) and post-synaptic population potentials (Lüscher *et al.* 1980). Watt and colleagues reported a number of e.p.s.p.s in synergist and homonymous motoneurones as well as inhibitory post-synaptic potentials (i.p.s.p.s), and also a mixture of e.p.s.p.s and i.p.s.p.s in

antagonist motoneurones. A waxing and waning of p.s.p.s during the averaging period, suggestive of labile interneuronal pathways, was also reported. These observations are called into question by the present findings. However, the I b sample size of this study and those by Watt *et al.* (1976) and Lüscher *et al.* (1980) are too small to draw reliable conclusions.

In conclusion, our findings indicate that experiments in which small-amplitude p.s.p.s are measured by spike-triggered averaging must contain a control for synchrony, if the results are to be interpreted without ambiguity. Alternatively, a technique of measurement must be used which excludes the possibility of contamination due to synchrony.

We thank Drs M. D. Binder, E. E. Fetz, Z. Hasan and P. A. Kirkwood for their criticism of various drafts of this manuscript and Ms P. Pierce for help with the illustrations. We are indebted to Drs B. R. Botterman and W. E. Cameron for their assistance in these experiments. This work was supported by the Fan Kane Fund and by U.S.P.H.S. Grants NS 07888 (to Douglas G. Stuart), FR 05745 (to the College of Medicine) and NS 17887 (to Thomas M. Hamm). D. D. Roscoe was supported by U.S.P.H.S. training grant HL 07249 (to the Department of Physiology).

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