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ANALYSIS OF PAIRS OF INDIVIDUAL Ia-E.P.S.P.S IN SINGLE MOTONEURONES

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SUMMARY

1. Recordings of individual e.p.s.p.s evoked by the action of single medial gastrocnemius Ia fibres have been made from medial gastrocnemius motoneurones. In many motoneurones the action of two Ia fibres has been observed and the properties of the e.p.s.p.s compared.

2. For sixty-three pairs of averaged e.p.s.p.s, each from the same motoneurone, the ratio of half-widths was plotted against the ratio of rise times. These results were compared with theoretical values derived from the Rall compartmental model. It was found that variations in synaptic current time courses and differences in the termination of localized synaptic terminals were not sufficient to account for all the data.

3. Amplitude and rise time were inversely related but the correlation coefficient was very low. For pairs of e.p.s.p.s in the same motoneurone the e.p.s.p. with the fast rise time was larger than that with the slow rise time in forty-eight of sixty-three cases.

4. In a given motoneurone individual e.p.s.p.s evoked by the action of different Ia fibres did not vary greatly in amplitude. The ratio of peak amplitudes was less than 3 for 86% of the pairs of e.p.s.p.s examined, and the maximum was 4·8.

5. Amplitude histograms were constructed for individual e.p.s.p.s at thirty-three synapses. Twenty-two of them could be shown to satisfy the Poisson law. The others satisfied the binomial law or neither.

6. Within a given motoneurone the amplitude of an e.p.s.p. is closely related to the mean number of quanta released but not to the amplitude of the unit e.p.s.p. produced by the action of a single quantum of transmitter.

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INTRODUCTION

The synaptic region of the spinal α-motoneurone is highly complex, consisting of a cell soma and an extensive network of dendrites. Synaptic input from Ia afferent fibres is not restricted to any one region, but there is some histological evidence that the proximal dendrites receive the greatest number of Ia terminals (Conradi, 1970). Preliminary anatomical evidence has been presented that the boutons of at least some single Ia fibres are localized to a restricted region of the motoneurone membrane (Iles, 1973), but the generality of this finding is not yet known.

In recent years a number of studies have been made of the individual e.p.s.p.s evoked in α-motoneurones by activation of single Ia fibres (Kuno, 1964; Burke & Nelson, 1966; Letbetter, Willis & Thomson, 1968; Mendell & Henneman, 1968; Jack, Miller, Porter & Redman, 1971; Iansek & Redman, 1973*b*). These investigations have revealed a wide range of individual e.p.s.p. shapes (rise time and half-width) which have been in general agreement with predictions derived from theoretical models of the motoneurone (Rall, 1967; Jack & Redman, 1971; Redman, 1973). The e.p.s.p. shape is determined by a number of parameters including time course of the synaptic current, location of the Ia terminals on the soma-dendritic tree, and properties of the motoneurone (e.g. time constant τ, electrotonic length L, dendritic/somatic conductance ρ). It is difficult at present to assign variations in e.p.s.p. shapes to any or all of these parameters.

Experimental work has also demonstrated a wide range of individual e.p.s.p. amplitudes. Most Ia-evoked individual e.p.s.p.s (i.e. activated by a single Ia fibre) have average peak amplitudes between 20 and 600 μV (Kuno & Miyahara, 1969*a*; Mendell & Henneman, 1971) but values up to 1·5 mV have been reported (Burke & Nelson, 1966; Burke, 1967). Factors which might account for this wide range of e.p.s.p. amplitudes include variations in the average number of quanta released from the presynaptic terminals per impulse (Kuno, 1964; Kuno & Miyahara, 1969*a*), reactivity of the post-synaptic receptor complex (Iansek & Redman, 1973*b*) and the attenuation introduced by the cable properties of the motoneurone dendrites (Rall, 1967; Jack & Redman, 1971; Redman, 1973; Barrett & Crill, 1974; Rinzel & Rall, 1974).

In order to assess the importance of these factors in determining e.p.s.p. amplitudes, we have tried as much as possible to compare the properties of individual e.p.s.p.s within the same motoneurone rather than making comparisons of e.p.s.p.s evoked in different motoneurones. E.p.s.p. amplitude or shape may be affected by variations in cell properties such as input impedance (Burke, 1968) and membrane potential following

electrode penetration both of which depend additionally on temperature (Klee, Pierau & Faber, 1974). The level of anaesthesia also will affect e.p.s.p. amplitude (Weakly, 1969; Mendell & Henneman, 1971). These potential sources of error can be minimized by comparison of e.p.s.p.s generated in the same motoneurone by activity in two independently activated Ia fibres. Furthermore the effect of tonic synaptic activity on e.p.s.p. characteristics (Barrett & Crill, 1974) is more nearly identical for pairs of e.p.s.p.s in the same motoneurone than for two e.p.s.p.s in separate motoneurones. Thus, whenever possible, pairs of individual e.p.s.p.s from the same motoneurone have been analysed.

Notation

unit e.p.s.p.: the e.p.s.p. measured at the soma resulting from the release of a single quantum of neurotransmitter

individual e.p.s.p.: the e.p.s.p. evoked in a neurone by a single activation of the presynaptic terminals of one afferent fibre, and again measured at the soma

V the peak amplitude of an individual e.p.s.p. (μV)

$\bar{V}$ the peak amplitude of an averaged individual e.p.s.p. (μV)

m the mean number of quanta of neurotransmitter released by a single presynaptic impulse (for a given afferent fibre and a given post-synaptic neurone)

v_1 the average peak amplitude of the unit e.p.s.p. (μV)

RT rise time of an averaged individual e.p.s.p. measured as the interval from 10 to 90% of peak amplitude (msec)

HW half-width of an averaged individual e.p.s.p. measured as the duration that the wave form is above one half peak amplitude (msec)

R_Z the ratio of values of the parameter Z (e.g. $\bar{V}$, m, v_1) of two e.p.s.p.s both recorded from the soma of the same motoneurone, and each evoked by a different afferent fibre. The value for Z for the e.p.s.p. with the fastest rise time is always in the numerator.

METHODS

On-line analysis of amplitude and shape

The monosynaptic connexions between medial gastrocnemius group Ia fibres and homonymous motoneurones in the cat were studied. Each animal was deeply anaesthetized with parenteral sodium pentobarbitone. A lower lumbar laminectomy was done to expose the spinal cord from L3 to S1 on the animal's left side. An extensive denervation of the tail and left hind limb was then carried out, leaving only the medial gastrocnemius nerve intact. One or two dorsal root filaments were located, each containing at least one medial gastrocnemius group Ia fibre. Afferent

impulses were recorded from these (Fig. 1*A*) simultaneously with the synaptic activity in a medial gastrocnemius motoneurone (Fig. 1*B*) impaled by a microelectrode filled with 3 M-KCl (impedance 2–5 MΩ). As shown in Fig. 1*A*, the signal from a medial gastrocnemius Ia afferent fibre during a period of uniform stretch of the medial gastrocnemius muscle consists of regularly occurring impulses. If the dorsal

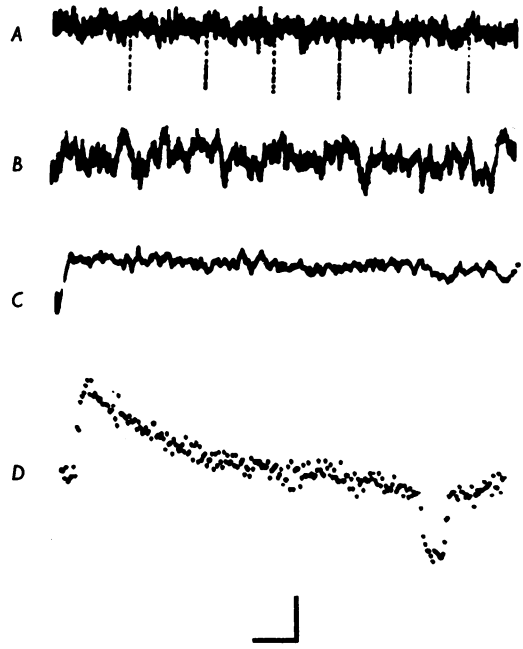

Fig. 1. *A*, impulses recorded from a single Ia fibre in response to steady stretch of the medial gastrocnemius muscle. Recording is made with bipolar platinum hook electrodes from an intact dorsal root filament. Time calibration is 20 msec. *B*, the intracellular potential from a medial gastrocnemius motoneurone over the same time period as *A*. Calibration is 1 mV and 20 msec. *C*, output from pulse height analyser triggers an oscilloscope which displays that impulse (same as in *A*) on a fast sweep. Pulse height analyser was required to select impulses of different amplitudes if present. Time calibration is 1 msec. *D*, the e.p.s.p. averaged from the intracellular potential (*B*) when the impulse in *C* triggers the signal averager. Square-wave calibration at the end of trace is 100 μV, 1 msec.

root filament contains more than one active fibre, the impulse trains from different axons are independent of one another and can be separated on the basis of amplitude, wave form and frequency. They represent 'time markers' for the occurrence of e.p.s.p. evoked by the action of individual Ia fibres. This is a crucial piece of information, because the e.p.s.p.s studied here (Fig. 1*D*) average only 100 μV in peak amplitude (Mendell & Henneman, 1971). They lie in a base line of electrical noise and background synaptic activity (Fig. 1*B*) which is so large that the only way to study e.p.s.p.s evoked by a specific single afferent fibre is to know when they occur.

The analysis of the data was done on-line using techniques described by Mendell & Henneman (1971). Impulses from group Ia fibres were distinguished from group

Ib or group II fibres by conventional criteria (Matthews, 1933). The muscle was stretched to a constant length, and impulses produced by single group Ia fibres were selected using a pulse height analyser. The output of the pulse height analyser was used to trigger an oscilloscope which displayed that impulse on a fast sweep (Fig. 1*C*). This was monitored continuously to verify that the same fibre was being selected by the pulse height analyser throughout the analysis. This beam was often slowed down to check the regularity of the afferent fibre discharge as a further control on the pulse height analysis. With the micro-electrode in a motoneurone, 256 or 512 consecutive impulses from each group Ia fibre available were used to trigger the averaging of the intracellular potential by a Fabritek no. 1010 averaging computer. The resulting averages (Fig. 1*D*) were estimates of the mean wave form of the individual e.p.s.p. associated with each afferent fibre, and these were stored on film.

The statistical analysis was done using the Statistical Program for the Social Sciences on the TUCC computer system at Duke University.

Off-line computer analysis of quantal variables

Data collection. Impulses from single medial gastrocnemius Ia fibres and simultaneous intracellular recordings from medial gastrocnemius motoneurones were obtained as above and were recorded on a Precision Instrument no. 6108 FM magnetic tape recorder. Using a pulse height analyser the discharges from individual group Ia fibres were extracted and these signals were used to trigger an EAI 580/PDP 15–35 hybrid computer system which carried out digital sampling of the intracellular potential and stored the resultant wave form (Weiner, 1973). The average individual e.p.s.p. wave form was calculated and plotted on external devices for each synaptic contact. The computer was used not only to store and average e.p.s.p.s for each afferent fibre studied, but also to further process the data, as described below. Computer programs were written specifically for the work being presented here in FOCAL (a proprietary language of the Digital Equipment Corporation).

The rejection of poor data by time interval criteria. For the Poisson analysis (see below) it was important to determine the probability that a Ia impulse evokes no e.p.s.p. (method of failures). Triggering the system from the inappropriate afferent fibre impulse could result in a poor estimate of this probability as well as errors in the average peak amplitude of the e.p.s.p. Impulses in a given group Ia fibre occur at fairly regular intervals in response to stretch of the muscle to a fixed length, and they also occur independently of impulses in other afferent fibres. A time interval histogram, i.e. a histogram of the time intervals between successive impulses, was used to monitor how well the spike potentials from afferent fibres had been separated from one another. A histogram with a narrow fundamental peak indicates a perfect separation. Otherwise, the separation of spike potentials from different afferent fibres was not exact, and the computer was able to reject the stored data corresponding to the extraneous spike potentials. Such spike potentials occurred at random intervals after a correct spike potential, and therefore the location of the corresponding stored intracellular potential data could easily be determined.

In the majority of connexions between group Ia fibres and spinal motoneurones studied (twenty out of thirty-three), the fundamental peak of the interspike time interval histogram, along with its harmonic elements, were clearly enough defined such that no data needed to be rejected on interspike time interval criteria. In thirteen out of thirty-three of the synaptic connexions studied, some data were rejected on the basis of the interspike interval criteria described above. For data in which such criteria were used, the number of impulses generated by the group Ia fibres ranged from 333 to 500. The fraction of data rejected on interval criteria ranged

from 0·03 to 0·46 with a mean of 0·18. If these 'rejected' impulses were having no synaptic action on the motoneurone, their inclusion would lead to an underestimate of $\bar{V}$ (and an over-estimate of the number of failures, see below). Eliminating their contribution should increase $\bar{V}$ by the inverse of the fraction of impulses that was eliminated. In five of thirteen cases studied this was found to be true within 5 %. In six of the remaining cases the increase in $\bar{V}$ was less than the decrease in N by amounts ranging from 10 to 40 % (four values below 18 %). Some rejected impulses must have generated e.p.s.p.s; either they were other group Ia or group II fibres (Kirkwood & Sears, 1974), or perhaps some impulses from the group Ia fibre under study were rejected. In two cases $\bar{V}$ decreased slightly after the rejection procedure, again suggesting the elimination of impulses causing e.p.s.p.s. These small errors in estimates of $\bar{V}$ were judged to be acceptable in order to obtain the best possible estimate of the number (and probability) of failures.

Quantal analysis. The peak amplitudes of individual e.p.s.p.s, V, recorded at the soma of mammalian neurones have in many cases been shown to be quantal in nature (Kuno, 1971). According to the quantum hypothesis (del Castillo & Katz, 1954), each V can be considered to be the sum of an integral number of 'quantal' components, with both the integer and the amplitude of each quantal component being random variables. For a given synaptic contact, quantal size is distributed normally about a mean, v_1, and the mean number of quanta contributing to the individual e.p.s.p. is m which implies $\bar{V} = mv_1$. Kuno (1964) and Kuno & Miyahara (1969*a*, *b*) have provided evidence that the number of quantal components liberated by a single group Ia presynaptic impulse is distributed according to Poisson statistics.

The probability for the release of i quanta at a given synaptic contact according to Poisson statistics, i.e. that $V = iv_1$, is

$$P_i = \frac{m^i e^{-m}}{i!}.$$

Given a Poisson distribution, m and v_1 can be determined by knowing the number of 'failures', i.e. the number of times that $i = 0$ (Hubbard, Llinas & Quastel, 1969). From the preceding equation, the fraction of failures, $P_0 = e^{-m}$. Transposing, $m = \ln (1/P_0)$. Since $\bar{V} = mv_1$, we can find $v_1 = \bar{V}/m$. As will be shown, the fraction of failures can be estimated from analysis of the peak amplitudes of the individual e.p.s.p.s stored in the computer.

The e.p.s.p. peak amplitude histogram. In order to study the statistical nature of synaptic transmission, the computer was programmed to calculate and to plot the histogram of the peak amplitudes of a series of individual e.p.s.p.s evoked by a single Ia fibre in a given motoneurone (Weiner, 1973): the 'peak amplitude histogram'. An analogous histogram for the intracellular noise (Weiner, 1973) was also calculated and plotted for each synaptic contact by calculating the peak amplitude of the intracellular potential 20 msec after the trigger signal, i.e. when the e.p.s.p. was no longer present. This 'noise amplitude histogram' is a measure of how much the noise contributes to the variance of the peak amplitude histogram (Hubbard *et al.* 1969). These have been found to be Gaussian in appearance with zero mean.

It was apparent from the beginning that the motoneurone intracellular noise very often led to large inaccuracies in the measurement of the individual e.p.s.p. peak amplitudes. To compensate for this, the computer was programmed to reject those e.p.s.p.s whose wave forms were most contaminated by noise. This was accomplished (Weiner, 1973) by assuming the shape of all e.p.s.p.s evoked by a given group Ia fibre in a given motoneurone to be constant. Those e.p.s.p.s whose shape differed by the largest amount from that of the average e.p.s.p. wave form for the synaptic contact being studied were assumed to be the most contaminated by noise and were

rejected. Since the noise was found to be normally distributed with zero mean, the rejection of the noisiest individual e.p.s.p.s should not drastically alter the resulting e.p.s.p. peak amplitude histogram, and the mean e.p.s.p. peak amplitude after rejection on e.p.s.p. shape criteria should be the same as the mean before rejection. It turned out, however, that these two mean e.p.s.p. peak amplitudes differed. This difference appeared to be ascribable to an increased deviance of the shape of the larger individual e.p.s.p.s from the shape of the average e.p.s.p.s. The reason for this is unclear, but may be related at least in part to possible asynchrony in the release of multiple quanta of neurotransmitter (Burke, 1968; Jack *et al.* 1971) or perhaps non-linear summation of unit e.p.s.p.s (Kuno & Miyahara, 1969*b*). This amplitude related e.p.s.p. shape dependence goes against our assumption that the shapes of all individual e.p.s.p.s evoked by a particular group Ia fibre in a particular motoneurone are identical. This finding necessitated an attempt to remove this bias, i.e. to make sure that larger individual e.p.s.p.s were not being selectively rejected. This was accomplished by using an empirically determined factor such that resulting difference between the average e.p.s.p. peak amplitude after rejection on e.p.s.p. shape fit criteria was the same as the average e.p.s.p. peak amplitude before. A sample e.p.s.p. peak amplitude histogram for e.p.s.p.s evoked by a single group Ia fibre in one motoneurone is shown in Fig. 2.

The estimation of e.p.s.p. 'failures'. The e.p.s.p. peak amplitude histogram is an estimate of the distribution of individual e.p.s.p. peak amplitudes, V, for a given synaptic contact. To estimate the fraction of 'failures', or instances of release of zero quanta of transmitter, we must first consider the differences between an ideal Poisson process and the somewhat less ideal biological system of group Ia e.p.s.p.s in

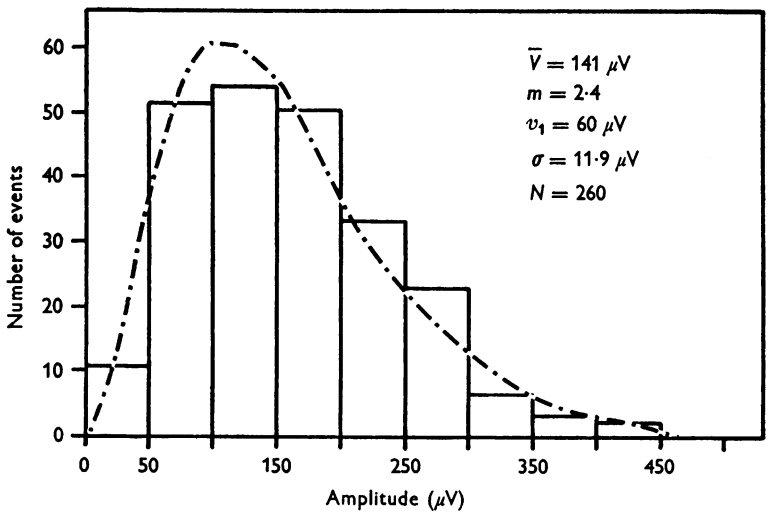

Fig. 2. Amplitude histogram of the individual e.p.s.p.s at a Ia-motoneurone synaptic contact. Only potentials greater than cut-off level (0 μV) are shown. The number of failures was estimated as those events which had an amplitude less than 0 μV. This was measured as twenty-four events giving a value of m = 2·4. The continuous curve is the best fit to the histogram (P > 0·6) using the Poisson relation with the value of m determined as above. Complete details and definition of symbols in text.

homonymous motoneurones. A Poisson process is discrete, and assuming the size of each quantum of transmitter to be uniform, we would expect a discrete distribution for individual e.p.s.p. peak amplitudes with the location of each peak corresponding to an integral multiple of the unit e.p.s.p. (v_1) and the height proportional to the probability that such an event is observed, i.e. P_i. Because the noise level is comparable to the amplitude of the unit potential, it is likely that failures will appear as non-failures, and vice versa, which makes it impossible to accurately determine the actual fraction of failures (Martin & Pilar, 1964).

Three values of m were calculated for each amplitude histogram by estimating the probability of failures as the fraction of total number events with amplitudes below 0, 50, and 100 μV. In other words three cutoff levels were investigated for each histogram in order to determine the most accurate estimate of m. From each value of m and $\bar{V}$, a continuous curve was constructed on a digital computer using the trial and error method described by Boyd & Martin (1956) and Kuno (1964). This curve is the sum of n normal curves centred at v_1, $2v_1$, ..., nv_1 having variance σ^2, $2\sigma^2$, ..., $n\sigma^2$, each curve being multiplied by the appropriate Poisson probability P_i. If f_{ab} = the expected number of individual e.p.s.p.s with amplitudes between a and b μV, conditional on the release of 1–8 quanta, and on the amplitude ranging between the cut-off (X_C) and the highest value (X_H) in the amplitude histogram, then

$$f_{ab} = N_p \sum_{i=1}^{8} \frac{P_i}{\sum_{j=1}^{8} P_j} \int_a^b \left[\frac{\frac{1}{i\sigma\sqrt{2\pi}} \exp\{-(x-iv_1)^2/2i\sigma^2\}}{\int_{X_C}^{X_H} \frac{1}{i\sigma\sqrt{2\pi}} \exp\{-(w-iv_1)^2/2i\sigma^2\}\, dw} \right] dx,$$

where $i, j = 1, 2, \ldots, 8$ denotes integral multiples of v_1. A maximum of 8 was considered because P_i converged to 0 sufficiently rapidly. $P_i = (m^i e^{-m})/i!$, the Poisson probabilities; σ^2 is the variance of the normal curve centred at v_1; N_p is the number of events out of N total events for which $X_C \leqslant V \leqslant X_H$.

An initial value of σ was chosen such that the height of the normal curve centred at v_1 was equal to the height of the observed histogram at v_1 and from this the expected distribution was constructed. This was compared to the observed distribution by computing the function

$$Z^2 = \sum_{a=X_C}^{X_H-50} \frac{(f_{ab} - y_{ab})^2}{f_{ab}},$$

where f_{ab} is the expected number of events between a and b, y_{ab} is the observed number of events between a and b, K is the number of bins in the histogram, $a = X_C, X_C+50, \ldots, X_H-50$, $b = a+50$. At the upper end of some distributions, 2 or 3 adjacent 50 μV bins were lumped together to keep the number of observed events above three simplifying the statistical analysis, i.e. $b = a+100$ or $b = a+150$. Different values of σ were then chosen using an iterative procedure until by trial and error the value of Z^2 was minimized (Fig. 2). σ was allowed to vary from 5·0 to 400·0 because of the mechanics of the search procedure but the final value of σ gave a coefficient variation (σ/v_1) averaging 0·7. The minimum value of Z^2 is distributed as χ^2 (Rao, 1965) with degrees of freedom = number of bins − 2. The deviation between observed and expected was judged to be significant (i.e. Poisson hypothesis rejected) when the null hypothesis was rejected (i.e. $P < 0{\cdot}05$). This entire computation was repeated for each cut-off (0, 50, 100 μV) for each of the thirty-three distributions. The result for each distribution was an assessment of whether a cut-off (i.e. a value of m) could be found for which the calculated Poisson distribution could be judged not to differ significantly from the observed distribution. This was possible

for twenty-two of the thirty-three distributions tested. A similar procedure was carried out using the binomial (B_i) rather than the Poisson law (P_i) where

$$B_i = \frac{n!}{i!(n-i)!}[p^i(1-p)^{n-i}].$$

We chose values of $p(0 \leqslant p \leqslant 1)$ between 0·2 and 0·8 where $m = np$.

In order to obtain an independent estimate of m which was free of the necessity to obtain the proportion of failures, the variance of the individual e.p.s.p. peak amplitudes and the corresponding background noise peak amplitudes were each calculated by the computer, for each synaptic contact, using equivalent algorithms (Weiner, 1973). These variances were used to get a noise independent measure of the Poisson quantal statistics of e.p.s.p. peak amplitude as determined by the method of the coefficient of variation (Hubbard *et al.* 1969). According to this method

$$m_{cv} = \frac{(\bar{V})^2}{\sigma^2(\text{e.p.s.p.}) - \sigma^2(N)},$$

where σ^2(e.p.s.p.) is the variance of the individual e.p.s.p. peak amplitudes, and $\sigma^2(N)$ is the variance of the background noise peak amplitude. For technical reasons related to estimating the variance of the noise (Weiner, 1973), m_{cv} could only be determined for about one third of the e.p.s.p.s analysed by the methods of failures. Therefore, the coefficient of variation data were only used to verify that the conclusions derived from the method of failures data were not a function of the noise. The values of m estimated by the coefficient of variation method were larger than those estimated by the failures method (see Results). This difference did not appear to be dependent on the size of the e.p.s.p.s, as the relationships between e.p.s.p. peak amplitude and both measures of m were quite similar in form.

RESULTS

The relationship between amplitude and rise time

A total of 121 averaged individual e.p.s.p.s were obtained from fifty-eight motoneurones. The data from a cell were not considered unless its action potential was above 40 mV. Care was taken to avoid e.p.s.p.s with clearly compound decays such as illustrated by Mendell & Henneman (1971, Fig. 7). Some very small e.p.s.p.s could not be included because of the difficulty in measuring rise time and particularly half-width. In some cases the e.p.s.p. did not come down to the base line presumably because of large stretch evoked intracellular potentials which were not averaged out. This made the half-width impossible to measure and so these e.p.s.p.s were eliminated from the data to be analysed. In general the rise time could be obtained much more easily. In all, sixty-three pairs of averaged individual e.p.s.p.s evoked in single motoneurones by the action of different Ia fibres were selected as being suitable for the analysis outlined in the following sections.

Shape–index curve

Using a ten compartment model of the motoneurone, Rall (1967) has produced a theoretical shape–index curve relating the normalized half-width and rise time of individual e.p.s.p.s which are assumed to be generated at different localized electrotonic distances from the soma by synaptic currents of constant time course and uniform intensity. According to this curve, half-width is a monotonic increasing function of rise time although for the largest rise times, half-width increases very slowly. The synapses producing these e.p.s.p.s with long rise times are located on the most distal dendritic processes; recent evidence indicates that most Ia synapses are located on the proximal portion of the dendritic tree (Conradi, 1970; Jack *et al.* 1971), where increases in rise time are almost matched by increases in half-width. Most experimental data do not fall on this line (Rall, Burke, Smith, Nelson & Frank, 1967) even when the data are normalized by dividing by τ (Jack *et al.* 1971). This could be due, at least in part, to comparisons of e.p.s.p.s from different motoneurones; variations in synaptic current time courses, difficulties in measuring τ, and differences in the experimental conditions could introduce errors. These might be avoided by comparing pairs of e.p.s.p.s from the same motoneurone. By taking the ratios of half-widths and rise times, τ is eliminated and does not need to be measured even if it is not identical at both synapses (Fatt, 1957; Iansek & Redman, 1973*a*).

In Fig. 3 the ratio of half-widths for each pair of averaged individual e.p.s.p.s (HW_2/HW_1) has been plotted against the ratio of the rise times (RT_2/RT_1) which has been defined as greater than unity (i.e. $RT_2 > RT_1$ always). The wide scatter of experimental values indicates a wide range of e.p.s.p. shapes in the same motoneurone. The dashed line encloses a region in which the ratios of rise times and half-widths of e.p.s.p.s evoked by inputs to single compartments would lie. These have been obtained from Table 2 of Rall (1967) which presents the calculated rise times and half-widths of theoretical e.p.s.p.s generated in compartments 1, 2, 3, 4, 6, 8, 10 of a ten compartment model by slow and by fast synaptic current transients. The ratios have been computed for each possible pair of compartments (distal–proximal) keeping $RT_2/RT_1 > 1$ and using each of the four possible current transient combinations, i.e. fast–fast, slow–slow, slow–fast and fast-slow. Of sixty–three pairs of experimental individual e.p.s.p.s, thirty-four pairs lie inside the region enclosed by the dashed line and twenty-nine pairs lie outside. It is possible that the number of points falling outside this region has been underestimated or over-estimated by the choice of the allowable range of synaptic current time course. It seems unlikely that this departure from the theoretically expected values

can be ascribed to measurement error since e.p.s.p.s with ambiguous rise time and/or half-width were excluded. The extent of this region of expected values (between dashed lines) takes into account variations in both synaptic current time course and location of Ia terminals in various single compartments. Therefore, it seems likely that experimental values falling

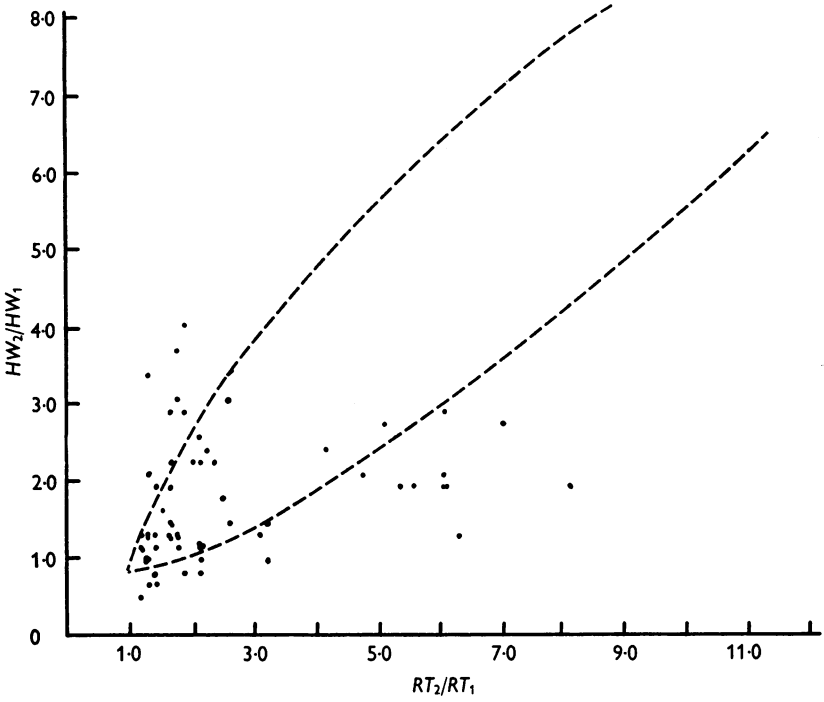

Fig. 3. Relationship between HW_2/HW_1 and RT_2/RT_1, for pairs of averaged individual e.p.s.p.s in the same motoneurone. $RT_2/RT_1 > 1$ by definition. Dashed line is obtained from theoretical data of Rall (1967) (see text).

outside this region are the result of synaptic input not being localized to a single compartment which agrees with the conclusion of Rall *et al.* (1967). Such e.p.s.p.s might have short rise times contributed by more proximal boutons and long half-widths produced by distal boutons. When compared with e.p.s.p.s having short rise times and short half-widths or long rise times and long half-widths one would expect to find points for which $R_{HW} \gg R_{RT}$ and $R_{RT} \gg R_{HW}$, i.e. points which are outside the expected region.

Amplitudes of averaged individual e.p.s.p.s

The distribution of average peak amplitudes for all e.p.s.p.s is shown in Fig. 4*A*. The mean of this distribution is 137 μV which is somewhat larger

than the value of 102 μV found by Mendell & Henneman (1971). This may be partly accounted for by the elimination of e.p.s.p.s mostly with small peak amplitudes because of the difficulty in measuring half-widths. In each of these neurones it has been possible to obtain at least two e.p.s.p.s which permits calculation of the ratios of the average peak e.p.s.p. amplitudes in the same motoneurone. The distribution of these ratios (here defined as > 1) is shown in Fig. 4*B*, and it can be seen that most (92%) of these values are less than 3 and none is greater than 4·8. This distribution of ratios is consistent with the small number of e.p.s.p. amplitudes below 50 μV(11%) and above 150 μV(34%) as shown in Fig. 4*A*.

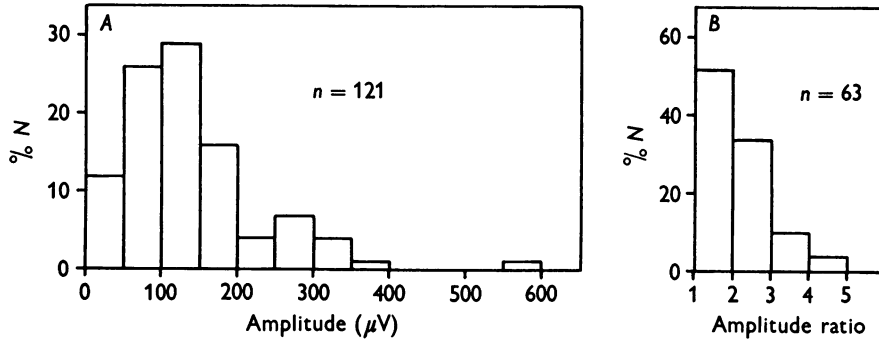

Fig. 4. *A*, histogram of peak amplitudes of averaged individual e.p.s.p.s. *B*, histogram of ratios of peak amplitudes of averaged individual e.p.s.p.s evoked by pairs of Ia fibres in the same motoneurone. In each case the peak amplitude of the larger e.p.s.p. is in the numerator, i.e. ratios are always > 1.

E.p.s.p. peak amplitude as a function of rise time

At some synapses rise time of composite e.p.s.p.s has been found to be directly related to the distance of the terminals from the soma (microelectrode) (Fadiga & Brookhart, 1960; Tsukahara & Kosaka, 1966). Accordingly the relationship between peak amplitude and rise time for our data is shown in Fig. 5 along with the straight line fit to these points by the method of least squares. It is similar to the relationship reported by Mendell & Henneman (1971) for Ia-motoneurone synapses. While small e.p.s.p.s are observed for all values of rise time, a tendency for large e.p.s.p.s to have short rise times suggests that there is an apparent decrease in both the mean and the variability of $\bar{V}$ as rise time increases. The negative correlation between the variables is small ($r = -0{\cdot}21$) but significant ($P < 0{\cdot}02$). Other factors (e.g. quantal content) must play a larger role than e.p.s.p. rise time in the determination of e.p.s.p. peak amplitude.

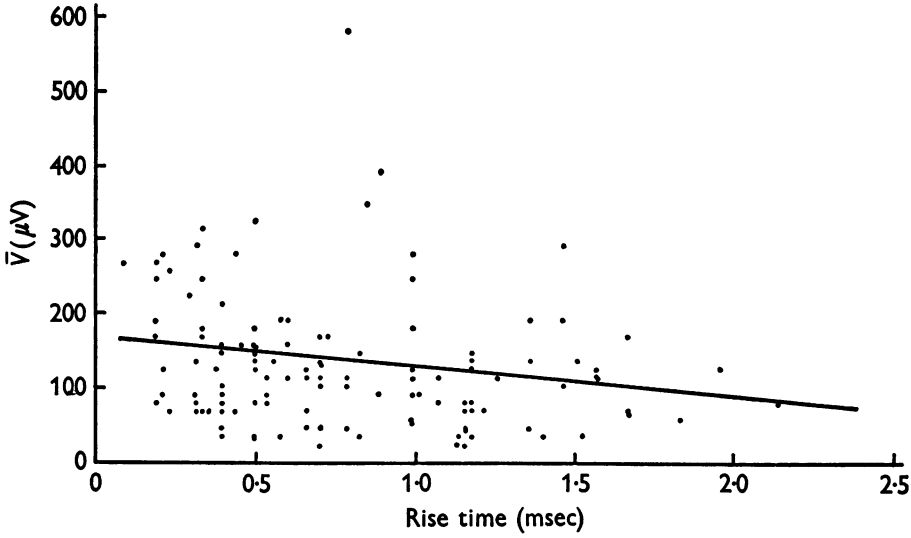

Fig. 5. Plot of peak amplitude of averaged individual e.p.s.p.s ($\bar{V}$) versus rise time. The straight line is fitted by method of least mean squares.

Relationship between peak amplitudes and rise times of e.p.s.p.s generated in the same motoneurone by pairs of group Ia fibres

In order to analyse the relationship between amplitude and rise time more accurately, pairs of averaged individual e.p.s.p.s occurring within the same motoneurone were examined in sixty-three cases. For each such e.p.s.p. pair the ratio of the peak amplitudes ($R_{\bar{V}} = \bar{V}_1/\bar{V}_2$) and the ratio of the rise times $R_{RT} = RT_2/RT_1$ were calculated (where the subscript 1 denotes the parameter value for the e.p.s.p. with the faster rise time). By this definition $R_{RT} > 1$ always and $R_{\bar{V}} \lesssim 1$; in fact $R_{\bar{V}} > 1$ for 75% of these e.p.s.p. pairs. Thus there is a significantly greater than even chance ($P < 0{\cdot}001$) that the e.p.s.p. with the fast rise time is larger than the e.p.s.p. with the slow rise time. A plot of $R_{\bar{V}}$ *vs.* R_{RT} is shown in Fig. 6 with the regression line fitted by least squares. Again the correlation between these variables is very low ($r = +0{\cdot}24$) and barely lacks significance ($P < 0{\cdot}06$) suggesting that factors other than R_{RT} account for variations in $R_{\bar{V}}$. This relationship indicates that, on the average, even for pairs of e.p.s.p.s with large rise time ratios (e.g. $R_{RT} = 5$), $R_{\bar{V}}$ is not very large ($R_{\bar{V}} = 1{\cdot}9$). Use of rise time differences ($\triangle RT$) rather than rise time ratios (R_{RT}) for comparison of each e.p.s.p. pair also yields a low correlation coefficient ($r = +0{\cdot}25$, $P < 0{\cdot}06$). Both of these relations indicate that for pairs of e.p.s.p.s in the same motoneurone, greater disparities in amplitude tend to be associated with larger ratios or differences in rise

time but that this association is very weak. Analysis of pairs of individual e.p.s.p.s in the same motoneurone yields a similar relationship between peak amplitude and rise time as consideration of e.p.s.p.s in different motoneurones.

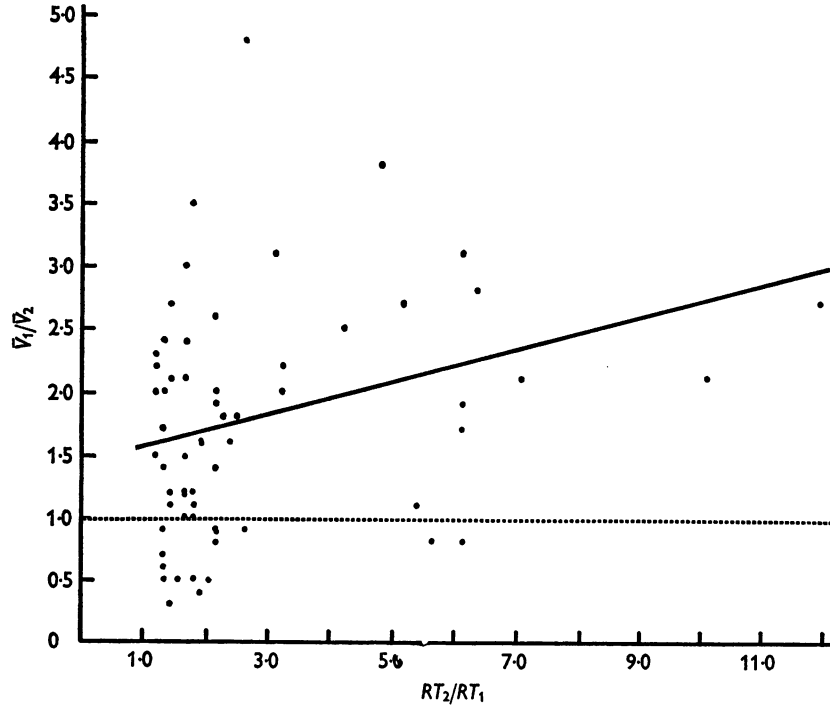

Fig. 6. Plot of ratio of peak amplitudes of pairs of averaged individual e.p.s.p.s in the same motoneurone ($\bar{V}_1/\bar{V}_2$) versus the ratio of their rise times (RT_2/RT_1). $RT_2/RT_1 > 1$ by definition and $\bar{V}_1/\bar{V}_2$ can be less than or greater than 1. Dotted line corresponds to $\bar{V}_1/\bar{V}_2 = 1$; only 15 of 63 points are below this line indicating that if $RT_2 > RT_1$ then $\bar{V}_2$ is usually less than $\bar{V}_1$. Continuous line is fitted to these points by method of least mean squares.

Relationship between amplitude and half-width

From the inverse relation between amplitude and rise time (Fig. 5) and the direct relation between half-width and rise time (Rall, 1967), it was expected that $\bar{V}$ and HW should be inversely related. A consideration of the $\bar{V}$–HW relation revealed a weak positive correlation ($r = +0{\cdot}09$) between $\bar{V}$ and HW which was not significant ($P > 0{\cdot}28$).

The positive correlation between $\bar{V}$ and HW was examined in more detail by calculating the partial correlation between $\bar{V}$ and HW, i.e. keeping RT constant. This permitted a truer evaluation of the relationship between $\bar{V}$ and HW since variations resulting from the functional dependence of $\bar{V}$ and HW on RT were

eliminated. Under these conditions a weak positive correlation of +0·20 was found which was statistically significant ($P < 0·03$). The larger e.p.s.p. may decay more slowly (longer half-width) because the synaptic current is prolonged. This could occur either because of asynchrony in transmitter release (Burke, 1967; Jack *et al.* 1971) or because of repeated binding of neuro-transmitter to post-synaptic receptors (Katz & Miledi, 1973; Magleby & Terrar, 1975). This confirms that rise time and half-width of Ia-e.p.s.p.s are not the tightly coupled variables (Fig. 3) which might have been predicted from the theoretical Rall shape–index curve. Partial correlation coefficients were calculated for the other relations ($\bar{V}$ *vs.* RT; $R_{\bar{V}}$ *vs.* R_{RT}); these were only slightly different than the zero order coefficients reported above with similar statistical significance.

Quantal analysis

In twenty-two of the thirty-three cases analysed by the method of failures, it was possible using the Poisson relation to construct an expected continuous curve which did not deviate significantly from the observed amplitude histogram. The optimal cut-off point, below which the events were considered failures, was not the same in each case. In nine cases 0 μV was optimal, in seven cases 50 μV was optimal, and in four cases 100 μV was optimal. In the other two cases it was impossible to judge between the cut-offs and so 50 μV was chosen. The four largest e.p.s.p.s (> 200 μV) had an optimal cut-off at 0 μV but otherwise there was no obvious correlation between e.p.s.p. amplitude and the optimal cut-off.

For each amplitude distribution following the Poisson law, an estimate of m was calculated using the variance method ($= m_{cv}$, see Methods). This was successful in only about one-third of the cases attempted because the high variance of the noise, often equalling that of the signal (e.p.s.p. + noise), led to a very high (and probably inaccurate) value for m_{cv}. In those cases where the variance of the noise was less than one-half that of the e.p.s.p. signal, m_{cv} was calculated and was found to be uniformly larger than m estimated by failures (m_f) suggesting a binomial distribution with $p > 0·5$ (Johnson & Wernig, 1971). It should be recalled that the Poisson distribution is the limiting case of the binomial; in both cases $m = np$ where m is the mean number of quanta released, n is the number of quanta available and p is the probability of release. For large n and small p the binomial law can be approximated by the Poisson law. An attempt was made to fit the binomial distribution to these twenty-two histograms with mixed results. In most cases the binomial clearly did not fit as well particularly at high p ($> 0·5$); in other cases there was little difference between the binomial with $p > 0·5$ and the Poisson. The technique of fitting a theoretical distribution to observed amplitude histograms allows possible models to be tested but the solution is not necessarily unique, e.g. the binomial distribution may equally well apply (Kuno, 1964). The failure of the variance method to adequately support the Poisson hypothesis, i.e. $m_{cv} > m_f$ may be the result of interaction between signal and noise. For example, if synaptic noise shunted the e.p.s.p.s. (signal) (Barrett & Crill, 1974), the variance of the 'e.p.s.p. + noise' would be artificially low which would lead to an overestimate of m_{cv}.

In six of the eleven synapses not following the Poisson law, the binomial distribution was found to apply with p ranging from 0·20 to 0·69 with a mean of 0·39. Those synapses for which the binomial law applied were the ones which came closest to being fit by the Poisson relation. This may be because the Poisson distribution is

the limiting case of the binomial where n is large and p is small. This also may have been the natural outcome of the inherently variable conditions under which these e.p.s.p.s have been evoked since McLachlan (1975) has reported that the laws governing transmitter release (i.e. Poisson and binomial) depend on experimental conditions. We assume that our inability to fit either a Poisson or a binomial distribution to the remaining five synapses arose from deviations introduced by synaptic noise, shunting of e.p.s.p.s resulting from synaptic activity, non-linear summation of unit potentials (Kuno & Miyahara, 1969*b*), or external stochastic processes related perhaps to membrane potential of afferent terminals (Rudomin, Burke, Nuñez, Madrid & Dutton, 1975). Some of these factors may also have prevented the six synapses described only by the binomial relation from following the Poisson law.

Relationship between $\bar{V}$, m and v_1

Histograms of m (mean of 1·7) and v_1 (mean of 92 μV) for the twenty-two synapses following the Poisson law are shown in Fig. 7. Both means are similar to those of Kuno (1964) and Kuno & Miyahara (1969*a*). Values

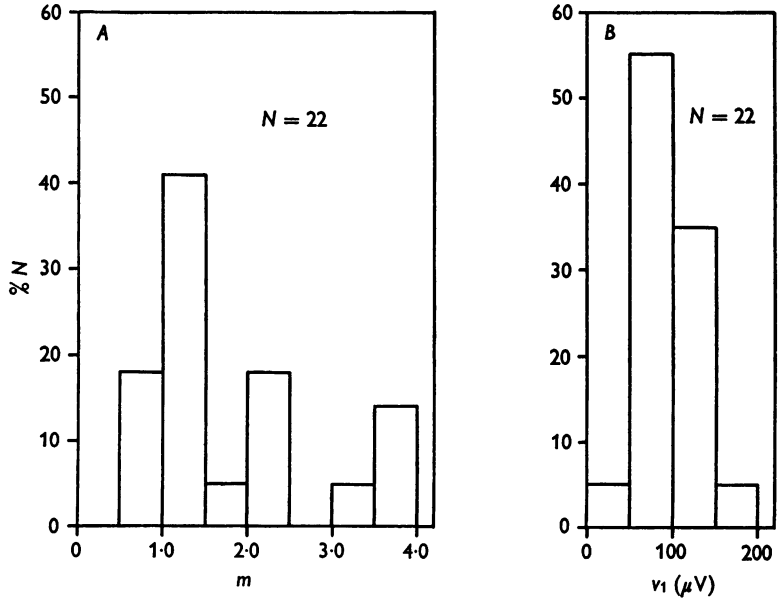

Fig. 7. *A*, histogram of m determined by method of failures. *B*, histogram of $v_1 (= \bar{V}/m)$.

of m for three large e.p.s.p.s with no failures (average e.p.s.p. peak amplitude, $\bar{V}$, between 435 and 457 μV) ranged from 21 to 25, as calculated by the coefficient of variation method (Hubbard *et al.* 1969) again assuming Poisson statistics. This also agrees with the results of Kuno & Miyahara (1969*a*). Since the method of calculation was different, the data from these three e.p.s.p.s are not included below.

As shown in Fig. 8*A*, $\bar{V}$, is linearly proportional to m, as previously reported by Kuno (1964). The correlation coefficient is +0·79 which is significant ($P < 0{\cdot}001$). From this linear relationship one would predict that $\bar{V}$ should be independent of v_1 ($= \bar{V}/m$). The correlation coefficient for this relationship (Fig. 8*B*) is +0·03 which is not significant ($P > 0{\cdot}8$).

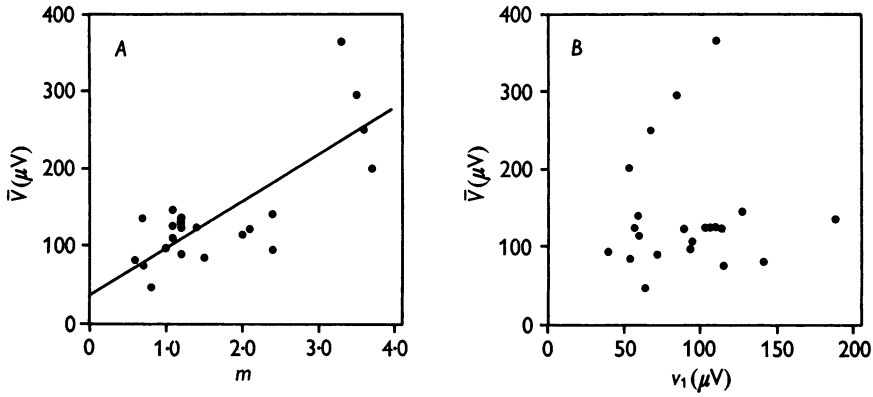

Fig. 8. *A*, relationship between peak amplitude of an averaged individual e.p.s.p. ($\bar{V}$) and its quantal content (m) determined by method of failures. Continuous line is regression line fit by method of least mean squares. *B*, relationship between $\bar{V}$ and v_1.

Quantal analysis of pairs of e.p.s.p.s in the same motoneurone

The above findings suggest that the size of a group Ia e.p.s.p. is more closely correlated with the mean number of quanta of neurotransmitter released from the presynaptic terminal (m), than with the size of the post-synaptic potential (measured at the soma) evoked by a single quantum of neurotransmitter (v_1). Some of the variability in the relationship between $\bar{V}$ and m and v_1 may have been introduced by variability in anaesthetic levels (Weakly, 1969) and in other motoneurone properties which might cause systematic inaccuracies in the estimation of $\bar{V}$ and v_1. An attempt was made to eliminate these causes of variability of comparing values of $\bar{V}$, m and v_1 in the same motoneurone. This could be investigated only in a preliminary way because of the small amount of data available (six pairs of e.p.s.p.s).

As shown in Fig. 9*A*, ratios of $\bar{V}$ for pairs of e.p.s.p.s within the same motoneurone are highly correlated with ratios of m (the correlation coefficient is 0·96 which is significant, $P < 0{\cdot}007$). The equation of the line fitted by the method of least squares is $R_{\bar{V}} = 0{\cdot}95\, R_m + 0{\cdot}08$ which indicates that $R_{\bar{V}} \simeq R_m$. This finding confirms the close dependence of $\bar{V}$ on m found for e.p.s.p.s from different motoneurones.

From the relationship $\bar{V} = mv_1$ and the result $R_{\bar{V}} \simeq R_m$, one would expect that $R_{v_1} = 1$ independent of values of $R_{\bar{V}}$. As shown in Fig. 9*B*, ratios of $\bar{V}$ within the same motoneurone are uncorrelated with v_1 (correlation coefficient $r = +0{\cdot}71$, $P > 0{\cdot}7$). This finding validates the similar relationship found for e.p.s.p.s from different motoneurones. Furthermore R_{v_1} averages about unity.

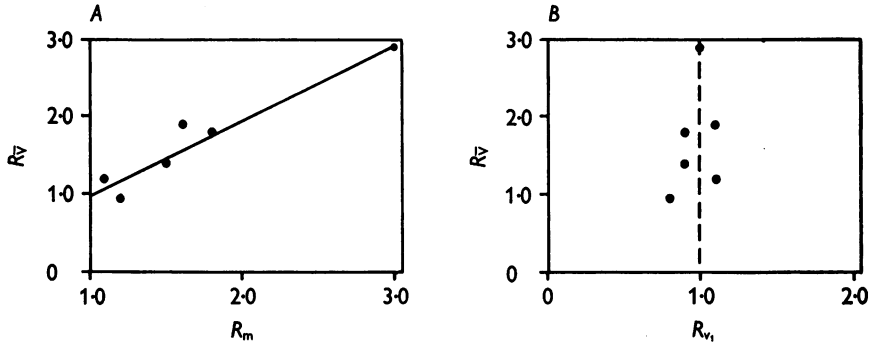

Fig. 9. *A*, relationship between ratio of peak amplitude ($R_{\bar{V}}$) and ratio of average quantal content (R_m) for six pairs of averaged individual e.p.s.p.s each in the same motoneurone. Continuous line fit by method of least mean squares. *B*, relationship between ratio of $R_{\bar{V}}$ and R_{v_1} (the ratio of unit e.p.s.p.s) for e.p.s.p.s in the same motoneurone. Dashed line is $R_{v_1} = 1$.

Since for any average e.p.s.p., $\bar{V} = mv_1$, the preceding findings suggest that differences in sizes of e.p.s.p.s evoked by different group Ia fibres in the same motoneurone are determined by differences in m, while v_1 remains the same for all homonymous group Ia synapses on the same motoneurone. This invariance of v_1 has been demonstrated by Blankenship & Kuno (1968), who investigated spontaneously occurring e.p.s.p.s from large numbers of synapses in spinal motoneurones, only a small fraction of which were presumed to be evoked by group Ia fibres.

DISCUSSION

The dependence of peak amplitude ($\bar{V}$) on quantal content (m) is similar to that obtained by Kuno (1964) in spinal motoneurones although the e.p.s.p.s in this study are true individual e.p.s.p.s (i.e. from a single fibre) whereas in the earlier investigation m may have been a function of the number of fibres stimulated. The role of quantal content in the determination of peak amplitude of an individual e.p.s.p. has received further support from the comparison of e.p.s.p.s in cells of Clarke's column and motoneurones (Kuno, 1971; Kuno & Miyahara, 1968). The close depend-

ence of $\overline{V}$ on m, in sharp contrast to the small negative correlation of $\overline{V}$ and e.p.s.p. rise time and the reported lack of correlation between $\overline{V}$ and distance between the synapse and the soma (Iansek & Redman, 1973*b*) indicates that the amplitude of an individual e.p.s.p. is more closely related to presynaptic factors than to post-synaptic spatial ones. The physical difference between synapses with high m and low m is not known, but the inference that at least some Ia fibres contact a motoneurone at more than one location (Fig. 3) suggests that synapses with high m may be ones which provide larger numbers of boutons to a post-synaptic cell (Kuno, Muñoz-Martinez & Randic, 1973). If transmitter release from each bouton is described by the Poisson law, the amplitude histogram of individual e.p.s.p.s for the total synaptic complex will also follow Poisson statistics provided that the release from each bouton is independent of the others.

The difficulty in estimating the true number of failures may influence the relationship between $\overline{V}$ and m. As $\overline{V}$ decreases, the number of events having an amplitude smaller than any arbitrary cut-off increases. It is possible that some of these potentials are not failures as was assumed in calculating m, but are synaptic potentials which would result in an over-estimate in the number of failures. Thus m would be underestimated by an increasing amount as $\overline{V}$ decreased, and this could account for the relationship between $\overline{V}$ and m. Although we cannot directly rule out this possibility, it seems unlikely because we find that the number of failures, estimated in this way, predicts a continuous amplitude distribution which agrees well with the experimental histogram for amplitudes larger than the cut-off level (Fig. 2). If the number of failures were estimated very inaccurately, this should not be possible. Also, $\overline{V}$ is a monotonic increasing function of m_{cv} which does not depend on the estimate of failures. Finally, the optimal cut-off does not increase with $\overline{V}$; in fact the synapses which generated the four largest average e.p.s.p.s had optimal cut-offs at 0 μV.

The range in v_1 observed here is similar to that observed by Kuno & Miyahara (1969*a*). In view of the direct proportionality between $\overline{V}$ and motoneurone input resistance (Burke, 1968) and between v_1 and motoneurone input resistance (Kuno & Miyahara, 1969*a*), one might expect that motoneurones with large v_1 should have a large $\overline{V}$. Our data have revealed no such tendency. This probably reflects the variability in m which represents the slope of the $\overline{V}$ *vs.* v_1 relation. For reasons similar to those mentioned in connexion with the estimate of m, we do not feel that v_1 has been over-estimated for small $\overline{V}$.

Rall (1967) has shown theoretically that inputs localized to particular compartments and with a uniform time course evoke e.p.s.p.s with unique rise times and half-widths which increase as the distance from synapse to

soma increases. Inferring synpatic location from measurements of rise time (the inverse procedure) is ambiguous because synaptic current time course may not be identical at all Ia-motoneurone synapses (Iansek & Redman, 1973*b*) perhaps reflecting asynchronous release of transmitter (Burke, 1967; Jack *et al.* 1971). Also, the possibility that the terminals of some single Ia fibres may be scattered about the motoneurone (cf. Rall *et al.* 1967) makes it difficult in some cases to assign a distance between synapse and soma. These ambiguities in the association between rise time and synaptic location confound any conclusion drawn from the negative correlation between peak amplitude and rise time. Nevertheless, the finding of this weak negative correlation despite these uncertainties suggests that activation of proximal Ia synapses evokes larger e.p.s.p.s on the average than activation of distal Ia synapses. The weakness of the correlation indicates that other factors (e.g. mean quantal content) must play a more important role in the determination of e.p.s.p. amplitude. Burke's (1967) finding of no relationship between stretch evoked individual e.p.s.p. peak amplitude and rise time may be the result of activation of a different portion of the Ia fibre spectrum, or perhaps even another class of inputs since the e.p.s.p. amplitudes were greater than 500 μV.

Burke (1968) has demonstrated with electrical stimulation of the medial gastrocnemius nerve that the largest aggregate monosynaptic e.p.s.p.s have the longest rise times on the average. Since these large e.p.s.p.s occur in the smallest motoneurones, this unexpected direct relationship between amplitude and rise time may be explained by the finding of Jack, Miller, Porter & Redman (1970) that small α-motoneurones possess a larger proportion of dendritic Ia synapses than large α-motoneurones. These distal synapses would evoke slower rising e.p.s.p.s assuming no systematic effect of location on time course of the synaptic current (Iansek & Redman, 1973*a*). The large amplitudes are probably accounted for by the high input impedance of these small α-motoneurones (Burke, 1968).

Recent theoretical (Rinzel & Rall, 1974; Barrett & Crill, 1974) and experimental work (Jack *et al.* 1971; Iansek & Redman, 1973*b*) has shown that a unit conductance change at the distal dendrites generates an e.p.s.p. recorded at the soma which is about 20% as large as one generated at the soma. This attenuation is the result of electrotonic decay in the dendritic cable. Our data from pairs of individual e.p.s.p.s in the same motoneurone are consistent with this notion. We have never seen a pair of e.p.s.p.s with a ratio of peak amplitudes greater than 5 even with large differences in rise time, and most ratios are less than 3. However, this must be interpreted cautiously since it is possible that some events scored as no e.p.s.p. (Mendell & Henneman, 1971) are in fact very small e.p.s.p.s which cannot be resolved by our averaging technique (peak amplitude less than 15–25

μV depending on rise time). This would tend to exclude large amplitude ratios. Furthermore, our estimate of relative distance of each synapse from the soma for those e.p.s.p. pairs we have analysed may not be reliable. Thus it may very well be that none of the e.p.s.p.s in our sample was generated at the distal dendrites particularly since few Ia synapses are located there (Conradi, 1970; McLaughlin, 1972).

Since individual e.p.s.p.s are evoked by the release of varying numbers of quanta of transmitter (characteristic average number (m) at each synapse), the unit e.p.s.p. produced by a single quantum may better represent the effect of a unit conductance increase. We have observed no correlation between v_1 and rise time which ranged from 0·3 to 1·5 msec for the twenty-two synapses in our sample. In six cases we have found that Rv_1 for two synapses on the same motoneurone varied from 0·8 to 1·1 (mean of 1·0) with no correlation with the ratio of rise times which ranged up to 1·7. We have never seen ratios as high as 5 which might have been expected on the basis of theoretical results. It is of course quite possible, again, that none of our e.p.s.p. pairs included synapses located at significantly different electrotonic distances from the soma. Nevertheless, these data are consistent with the conclusions of Iansek & Redman (1973*b*) who have suggested from independent evidence that dendritic synapses might have larger v_1 than somatic ones because of larger quanta, an increased post-synaptic receptor reactivity or a higher specific membrane resistivity of the dendrites compared to the soma (Fatt, 1957; Katz & Miledi, 1963; Iansek & Redman, 1973*a*). This would account in a qualitative way for dendritic synaptic currents being larger than somatic ones (Iansek & Redman, 1973*b*). Bolstering the effect of dendritic synapses will balance the attenuation introduced by the cable properties of the dendrites and might account for the inability of electrotonic decay to account for variations in peak amplitude of spontaneous individual e.p.s.p.s (cf. Kuno, 1971). The implication that $\overline{V}$, but not v_1, is larger for more proximally, generated e.p.s.p.s indicates that m may be larger for somatic than for dendritic synapses but this will require direct verification.

Despite the uncertainty introduced by our use of rise time to estimate synaptic distance, our conclusions do not differ greatly from those of Iansek & Redman (1973*b*) who employed a much more quantitative technique to calculate synaptic distance. They concluded on the basis of calculations of synaptic charge transfer that the reactivity of dendritic synapses must be larger than that for somatic synapses. Our conclusion based on measurement of quantal variables is consistent with this. They also reported that the average peak amplitude of individual e.p.s.p.s ($\overline{V}$) is not a function of the electrotonic distance between the synapse and the soma. We have found only a very weak negative correlation between

e.p.s.p. amplitude and distance as measured by rise time. There is not much difference in these results particularly in view of the fact that the eight largest e.p.s.p.s in Iansek & Redman's sample were generated at comparatively short distances (within one space constant) of the soma. The method used by Iansek & Redman to compute synaptic distance rests on assumptions of localized synaptic terminals as well as uniform τ which we have seen may not be accurate. It seems that further studies along these lines will require correlative electrophysiological and anatomical observations in order to verify the estimates of synaptic distance.

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