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# QUANTAL COMPONENTS OF EXCITATORY SYNAPTIC POTENTIALS IN SPINAL MOTONEURONES

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Spinal motoneurones of the cat may be activated monosynaptically by impulses in the large afferent fibres from the muscle innervated by the motoneurone or the synergists (Lloyd, 1943*a*, *b*, 1946*a*, *b*). This effect is associated with the generation of a localized depolarization or excitatory post-synaptic potential (EPSP) in individual motoneurones following such afferent volleys (Brock, Coombs & Eccles, 1952). Since the amplitude of EPSPs so obtained is a function of the number of the afferent fibres stimulated, it is possible to adjust the strength of stimulation so that a number of steps in the size of EPSPs may be recognized (Brock *et al.* 1952). It was thus observed that the least afferent volley may generate an EPSP as small as or less than 1 mV (Eccles, Eccles & Lundberg, 1957*a*, *b*).

In the absence of added stimulation, the majority of motoneurones also show irregularly spaced small depolarizing potentials. Some of these 'spontaneous' small potentials are similar in size and time course to the least 'evoked' EPSP, ranging from 0·2 to 1·5 mV in amplitude (Brock *et al.* 1952). Their origin has been attributed to the synaptic bombardment of motoneurones by afferent impulses or by background discharge of spinal interneurones (Brock *et al.* 1952; Kolmodin & Skoglund, 1958; Eccles, 1961*b*). With spinal motoneurones of the frog, however, Katz & Miledi (1963) have recently shown that while synaptic responses to afferent volleys are virtually abolished by application of excess potassium or magnesium, the 'spontaneous' small potentials still persist without appreciable changes in their frequency. This suggests that the spontaneous subthreshold activity in spinal motoneurones may arise locally within individual synaptic terminals, the mechanism being similar to that for 'miniature end-plate potentials' (Fatt & Katz, 1952). Based on this similarity, Katz & Miledi (1963) have postulated that the spontaneous subthreshold potentials in motoneurones form the unit components of normal synaptic responses as in the neuromuscular junction (del Castillo &

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Katz, 1954). In contrast to the latter, however, the amplitude distribution of synaptic responses in motoneurones to successive constant stimuli did not always show satisfactory agreement with the statistical assumptions on which the Poisson analysis was based (Katz & Miledi, 1963).

These problems provide the basis of the present study in which the amplitudes of monosynaptic EPSPs evoked by the least afferent volleys are evaluated in greater detail. For this purpose, only one or a few fibres were used as afferent stimulation. It will be shown that monosynaptic EPSPs produced by impulses in a single afferent fibre are composed of discrete units evoked with a certain probability. A preliminary report of some of the results has already appeared (Kuno, 1963).

## METHODS

Adult cats were made decapitate by spinal section at the first cervical segment under ether or pentobarbital sodium (Nembutal, Abbott Laboratories) anaesthesia. The animal was thereafter maintained on artificial respiration and paralysed by intravenous injection of gallamine triethiodide (Flaxedil, American Cyanamid Company). In most cases, intravenous injection of pentobarbital sodium, 5–10 mg/kg, was repeated every 2–5 hr during the experiments. This helped to reduce the background biological noise and hence to stabilize the membrane potential of the motoneurone recorded (Frank & Fuortes, 1956). After lumbosacral laminectomy, the muscle nerve to the triceps surae (TS, medial and lateral gastrocnemius plus soleus) was dissected and cut distally. This nerve was then divided into several naturally occurring small bundles. Usually, the medial gastrocnemius (MG) nerve was found to have seven main bundles, and the lateral gastrocnemius and soleus (LGS) nerve could easily be divided into four lateral gastrocnemius (LG) and one soleus (S) bundles. All these bundles were placed on a glass plate and prepared for further dissection. Other branches of the sciatic nerve were severed. The exposed nervous tissues were covered with pools of mineral oil, and external heat aided in keeping the body temperature between 34·5 and 40·0° C.

In each experiment, antidromic sensory impulses were recorded from the TS nerve following stimulation of the lumbosacral dorsal roots. Without exception, impulses in the TS nerve were found only on stimulation of dorsal roots L-7 and/or S-1. The ipsilateral L-6 and S-2 dorsal roots were then cut, leaving all other ventral and dorsal roots intact except in a few experiments in which the L-7 and S-1 ventral roots were also cut. For internal recording from spinal motoneurones, glass micro-electrodes filled with 2·7 M-KCl solution were used. Recording from TS motoneurones was identified by antidromic spikes following stimulation of the muscle nerve, or by monosynaptic activation in cases with section of the ventral roots. Once the motoneurone was impaled with a stable resting potential, stimuli were successively applied to each of the previously dissected small bundles of the TS nerve. The appropriate bundle was then further dissected into fine filaments on the glass surface under a binocular microscope. After each division, the filaments obtained were stimulated, and the EPSP was observed in the motoneurone. The afferent fibres which produced the monosynaptic EPSP in the motoneurone might be accompanied by motor axons of other motoneurones or by some other afferent fibres such as Groups Ib, II and III fibres which are not responsible for monosynaptic activation (Lloyd, 1943*a*, *b*, 1946*a*, *b*; Hunt, 1952, 1954; Bradley & Eccles, 1953; Eccles *et al.* 1957*a*). The number of Group I fibres stimulated, however, could be estimated approximately by two methods with the electrode arrangement illustrated in Fig. 1*A*. In the first method, antidromic sensory impulses following stimulation of the dorsal roots ($S_1$) were recorded from the filament ($R_3$) after the experi-

ment. For example, in Fig. 1*B*, when the stimulus intensity was gradually increased to twice threshold strength, which was presumably strong enough to activate all Group Ia fibres in the dorsal roots, two different sizes of the response could be recognized. It was thus assumed that two Group I fibres had been stimulated. The response in Fig. 1*B* which appeared with a delay of about 2·6 msec was probably evoked reflexly through the intact ventral root, indicating that this filament included at least one motor axon. In later experiments, the dissected filament was stimulated ($S_3$), and the evoked impulses were recorded from the TS nerve ($R_2$). Figure 2*A* shows three steps in the size of such a response when the stimulus intensity was progressively increased. In Fig. 2*B* (lower record), the

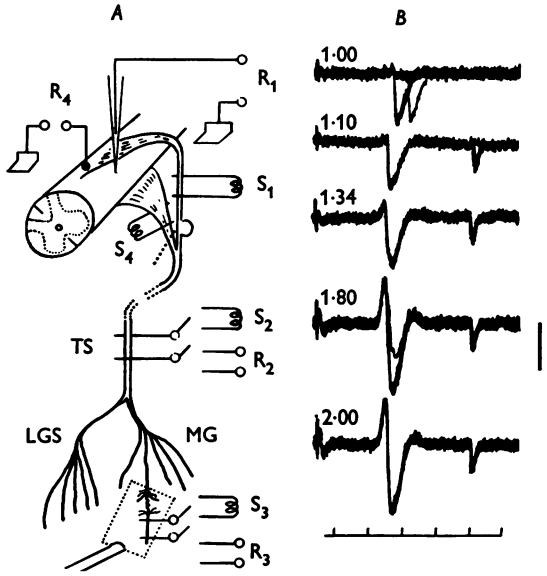


Fig. 1. *A*. Scheme of experimental arrangement. $R_1$, for intracellular recording from spinal motoneurones. $R_2$, for recording of afferent impulses from the triceps surae nerve. $R_3$, for recording of antidromic sensory impulses from dissected filaments. $R_4$, for recording of afferent volleys at the root-cord junction. $S_1$, for stimulation of the intact dorsal roots (L-7 and S-1). $S_2$, for stimulation of the triceps surae nerve. $S_3$, for stimulation of dissected filaments. $S_4$, for stimulation of the ventral roots (L-7 and S-1), which were distally cut in some experiments. TS, the triceps surae nerve. MG, medial gastrocnemius bundles. LGS, lateral gastrocnemius and soleus bundles. *B*. Responses recorded from dissected filaments ($R_3$ in *A*) following stimulation of the dorsal roots ($S_1$ in *A*). Stimulus intensities progressively increased from the top downward as indicated on each record relative to the threshold. Time, 1 msec. Voltage, 0·05 mV.

ventral roots were also stimulated ($S_4$ in Fig. 1*A*) to occlude the responses of motor fibres. One may thus estimate the number of afferent fibres from the sizes of responses remaining and/or lost by collision of the two impulses. However, both the methods could not exclude the presence of Group Ib fibres. Further, there was no evidence that all the Group Ia fibres stimulated contributed to the production of monosynaptic EPSPs in the motoneurone under observation. These uncertainties may have led the number of Group Ia fibres to be over-estimated (see Fig. 10).

When it was established that the single motoneurone was responding to impulses from

a small number of Group Ia afferent fibres, the nerve filament was stimulated at a rate of once every two sec. The accuracy in measurements of the size of such small EPSPs was limited by the high noise level (Fig. 3) although the latter could be reduced to some extent with the aid of a high-frequency filter (Fig. 4). Before and after a series of observations on the monosynaptic EPSPs, spike potentials of the motoneurone were produced by stimulation of the TS nerve ($S_2$ in Fig. 1*A*) or the ventral roots ($S_4$ in Fig. 1*A*). Those units with a spike less than 50 mV were discarded. The equipment employed for intracellular recording and the fixation of the spinal cord was essentially similar to that described previously (Hunt & Kuno, 1959).

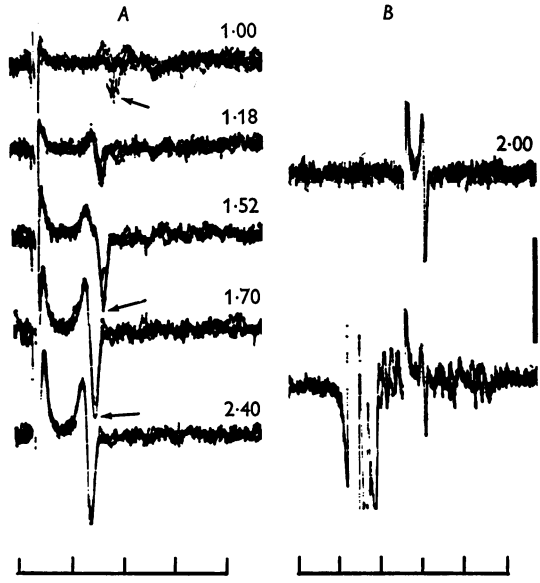


Fig. 2. *A*. Responses recorded from the triceps surae nerve ($R_2$ in Fig. 1) following stimulation of dissected filaments ($S_3$ in Fig. 1). Stimulus intensity gradually increased from the top downward as indicated on each record relative to the threshold. Arrows indicate three different sizes in the response. *B*. Upper record, same as in *A* but recorded at a slower sweep speed. Lower record shows a reduction in size of the above response owing to collision with motor impulses evoked by stimulation of the ventral roots ($S_4$ in Fig. 1). Note, filaments illustrated in Fig. 1*B* and Fig. 2 were obtained from different experiments. Time, 0·5 msec for *A* and 1 msec for *B*. Voltage, 0·05 mV.

## RESULTS

### *The least monosynaptic EPSPs*

Impulses in large afferent fibres from the TS nerve invariably produced EPSPs in TS motoneurones with a short latency which is attributable to monsynaptic delay (Brock *et al.* 1952). However, the maximum size of the monosynaptic EPSPs generated by stimulation of small natural bundles of the TS nerve varied considerably from bundle to bundle. This was obviously dependent upon the number of Group Ia fibres involved

in each bundle and upon the amount of monosynaptic connexion of the Group Ia fibres to the motoneurone under test. For example, stimulation of one small bundle evoked EPSPs of less than 1·5 mV as the maximal response (Fig. 3). When the stimulus intensity was gradually decreased from twice to just-threshold strength, the EPSPs showed parallel decreases in size, and the least response obtained was 0·2 mV or less (Fig. 3). In several experiments, the least monosynaptic EPSP thus obtained was

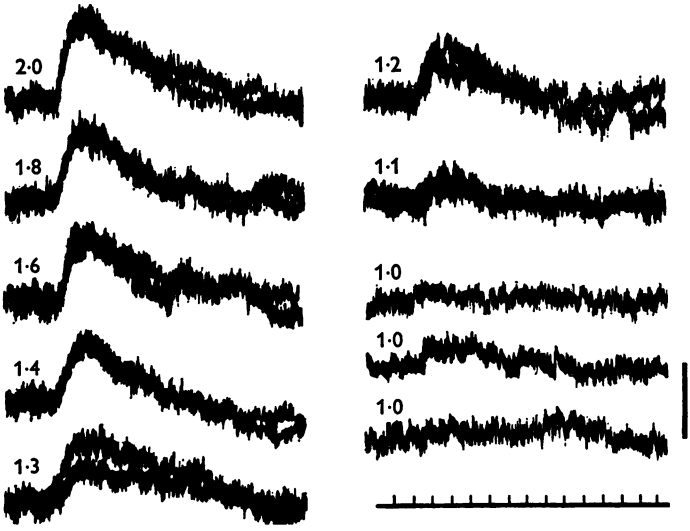


Fig. 3. Monosynaptic EPSPs in a LG motoneurone produced by afferent impulses in a small natural bundle of the LG nerve. Strengths of stimulation are indicated on each record relative to the threshold for the afferent impulse. Time, 1 msec. Voltage, 1 mV.

fairly constant in size, the range being 0·10–0·25 mV, except one motoneurone in which the least EPSP was about 0·45 mV. During the course of this series of observations, it was also noticed that the size of EPSPs showed a considerable variation at any fixed level of the stimulus intensity. Such fluctuations in the size of EPSPs were more evident with a weaker stimulus strength than with a stronger stimulation. Failures of the monosynaptic responses were occasionally observed even with the intensity level of 1·3 times the threshold strength. In order to exclude accidental changes in the number of afferent fibres stimulated, the small natural bundle was further dissected into a fine filament and stimulated with an intensity of twice threshold strength, which was considered to be supramaximal for all the Group Ia fibres in the muscle nerve (Bradley & Eccles, 1953). Since the preliminary experiments had shown that the least EPSP is about 0·2 mV, dissection of the bundle was repeated until it gave a fine

filament, stimulation of which produced monosynaptic EPSPs with a mode of approximately 0·2 mV. Figure 4*A* illustrates a series of monosynaptic EPSPs evoked by stimulation of such a filament every 2 sec. Obviously, the constant stimulation produced EPSPs of various sizes, and some of the

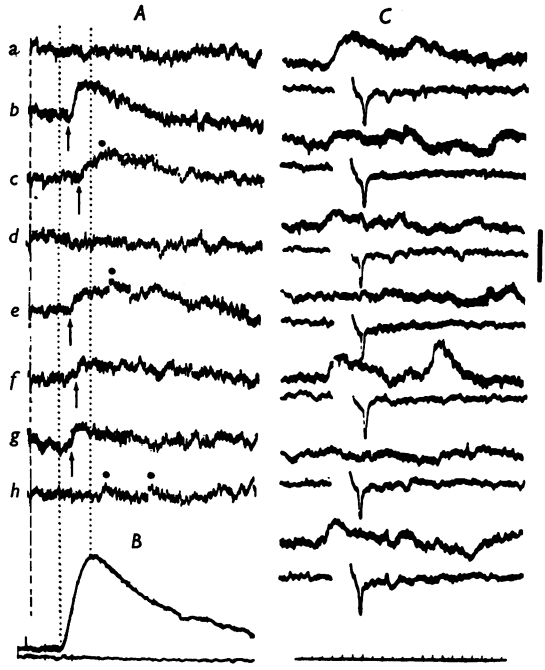


Fig. 4. *A*. Monosynaptic EPSPs in a MG motoneurone produced by afferent impulses in a dissected fine filament of the MG nerve at a rate of 0·5/sec. The number of afferent fibres measured in this filament was more than five. The filament was stimulated at intensity of twice the threshold, but the input impulses were not continuously monitored. Arrows indicate the onset of the EPSP responses. Two interrupted lines show the limits of latency fluctuations of the responses which are defined by the onset and peak of the large monosynaptic EPSP evoked by stimulation of the TS nerve (*B*). For large dots on some records, see text. *C*. Upper beams, same as in *A* but in another experiment. Lower beams, simultaneous recording of afferent impulses from the TS nerve ($R_2$ in Fig. 1), indicating that only one fibre contributes to the action potential at stimulus intensity of twice the threshold. Note: the upper and lower traces are recorded simultaneously but at different sweep speeds. Time, 1 msec for intracellular potentials and 0·1 msec for afferent impulses. Voltage, 1 mV for EPSPs in *A* and *C* and 4 mV for *B*.

volleys failed to evoke any detectable monosynaptic responses (Fig. 4*A*, *a*, *d*, *h*). Figure 4*C* shows similar records obtained from another experiment. In the latter, the afferent impulses evoked were simultaneously recorded from the central part of the TS nerve (see Fig. 1*A*) in the lower

beams. Since the size of the input was constant throughout the observations, fluctuations in the amplitude of EPSPs were not due to changes in the number of afferent fibres stimulated. In fact, in the experiment illustrated in Fig. 4*C*, only one afferent fibre was stimulated. Thus, although the least afferent volley usually evoked EPSPs of about 0·2 mV, the same volley could also give larger EPSPs or fail to produce any responses (see also Katz & Miledi, 1963). However, it was still possible that some of the failures observed could be due to the afferent impulses failing to reach the cord or the motoneurone.

Because the 'evoked' EPSPs and irregularly spaced 'spontaneous' potentials were similar in size, it was difficult to distinguish between them. Consequently, the distinction of the 'evoked' EPSPs from 'spontaneous' potentials was based entirely on the latency of the response. As shown in Fig. 4, however, such small monosynaptic responses often showed a considerable fluctuation in their latency. To assign certain limits for the latency fluctuations, a large monosynaptic EPSP was obtained by stimulation of the TS nerve (Fig. 4*B*), and any potentials which arose outside the interval between the onset and the summit of the large EPSP (indicated by two dotted lines in Fig. 4*A*, *B*) were not accepted as 'evoked EPSPs'. The maximal latency fluctuation thus allowed was occasionally more than 2 msec. Since this value exceeds the range of additional synaptic delay (0·5–1·0 msec), it could be argued that some of the 'evoked' EPSPs are 'polysynaptic' rather than 'monosynaptic'. However, no excitatory action on TS motoneurones by impulses from the TS nerve has been demonstrated other than monosynaptic activation by Group Ia afferent volleys (Laporte & Lloyd, 1952). Further, all the synaptic potentials produced by impulses in the muscle nerve other than Group Ia fibres have longer latencies so that they do not commence before the summit of the monosynaptic EPSP (Eccles *et al.* 1957*a*, *b*).

Although subthreshold potentials occurred spontaneously in all spinal motoneurones observed, their occurrence was often more evident for about 20 msec after each afferent stimulation than in the 'resting' state. Accordingly, some small depolarizing potentials indicated by dots in Fig. 4*A*, could be 'recruitment' of small EPSPs but not 'spontaneous' potentials. Observations of increases in the occurrence of small EPSPs after afferent stimulation were possible only in those units in which the background subthreshold activity was relatively quiescent. As will be described below, this phenomenon provided an independent measure of the unit size of monosynaptic EPSPs (Fig. 6*B*). Further information about this behaviour will be given elsewhere.

### *Statistical analysis of evoked EPSPs*

At the neuromuscular junction depressed by excess magnesium and/or reduction in calcium, the amplitude of the end-plate potential fluctuates in a manner described by Poisson's law, suggesting the presence of a large

number of contributing units at the junction, each with a small probability of responding to a nerve impulse (del Castillo & Katz, 1954; Boyd & Martin, 1956; Liley, 1956). This is also the case in 'normal' transmission at the neuromuscular junction of the crayfish (Dudel & Kuffler, 1961). The striking fluctuations in the size of monosynaptic EPSPs shown in Fig. 4 suggest that the mechanism involved in the spinal monosynaptic transmission may be similar to that at the neuromuscular junction. On the basis of this surmise, the observed monosynaptic EPSPs may be considered to be built up of units whose chance of occurrence to an afferent impulse may be described by Poisson's law. The mean number of units responding to one afferent impulse, $m$, may then be derived from two independent relations (del Castillo & Katz, 1954):

$$m = \frac{\text{mean amplitudes of EPSP responses } (\bar{v})}{\text{mean amplitudes of spontaneous potentials } (v_1)}, \tag{1}$$

$$m = \log_e \frac{\text{number of afferent impulses } (N)}{\text{number of failures of EPSP responses } (n_0)}. \tag{2}$$

However, because of the several origins of the synaptic contacts on a motoneurone, it was impossible to distinguish which 'spontaneous miniature potential', $v_1$, arose from the Group Ia fibre under observation (however, see below). Consequently, equation (1) could not be used to obtain a value for $m$. The validity of the above assumptions was tested by plotting the relation between the mean amplitude of EPSPs ($\bar{v}$) in the equation (1) and $m$ obtained from equation (2). As would be expected, fewer failures of the monosynaptic responses and therefore larger values of $m$, were observed when a larger number of Group Ia afferent fibres were stimulated (Fig. 10). However, this calculation of $m$ was subject to a limitation, since no failures of responses were recognized even in 200 trials if more than a certain number of Group Ia fibres were stimulated. The lower value of $m$ was limited by using single Group Ia fibres. The range of $m$ thus obtained was 0·63–5·4. Figure 5 illustrates the results of twenty-three experiments in which the applicability of the above assumption was tested. Although the points for various experiments are distributed along a straight line with a considerable scatter, the general tendency supports the relations expected from equations (1) and (2). Theoretically, however, the relation between these two factors should pass through the origin, since if the mean number of units responding to afferent impulses, $m$, is zero, then the mean amplitude of EPSPs should also be zero. The deviation in Fig. 5 might have arisen from two possible sources. As described above, there was no way to discriminate between 'spontaneous' and 'evoked' EPSPs other than the latency of EPSP responses. Under this condition, it was possible that some 'spontaneous' potentials might have been taken

as 'evoked' EPSPs. Secondly, the tests in Fig. 5 were extended to larger monosynaptic EPSPs while, as will be seen below, the Poisson analysis failed to apply to those EPSPs with $m$ in excess of two or three. The average value of the unit size of monosynaptic EPSPs ($v_1$) with $m$ less than three was calculated as 0·17 ± 0·03 (S.D.) mV, the range being 0·12–0·24 mV, which agreed with the size of the least 'evoked' EPSPs observed in the previous section.

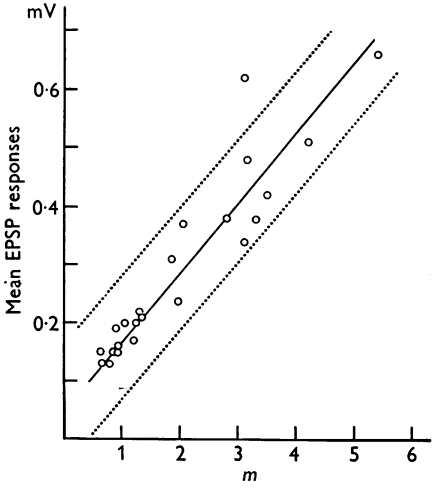


Fig. 5

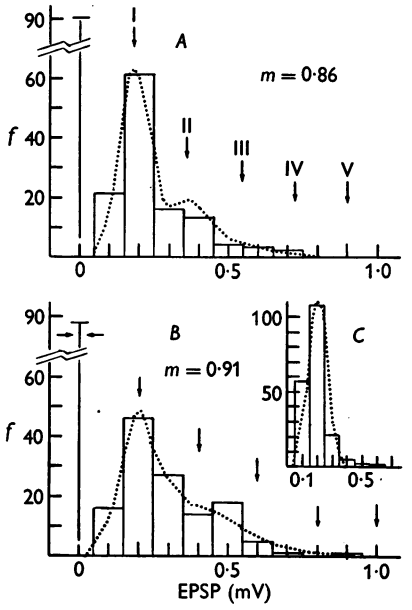


Fig. 6

Fig. 5. Relation between $m$ calculated from equation (2) and mean amplitudes of monosynaptic EPSPs ($\bar{v}$) in twenty-three experiments. Dotted lines show 2 S.E. of the correlation.

Fig. 6. *A*. Histogram showing distribution of amplitudes of monosynaptic EPSPs in the experiment illustrated in Fig. 4*C*. Failures of responses shown by a vertical line at 0. The theoretical distribution is drawn by the interrupted curve. *B*. Same as in *A*, but in another experiment illustrated in Fig. 4*A*. Horizontal arrows at 0 indicate the predicted number of failures of the responses. *C*. Histogram showing distribution of amplitudes of miniature EPSPs recruited after afferent stimulation in the experiment shown in *B*. $f$ = number of observations.

The applicability of Poisson's law can also be tested by a statistical analysis of the entire distribution of sizes of observed monosynaptic EPSPs. Figure 6*A* illustrates an example in which 212 stimuli ($N$) were applied to one Group Ia afferent fibre every two sec. The distribution shows a mean monosynaptic EPSP of 0·15 mV, including 90 failures of responses,

and the maximum EPSP of about 0·70 mV. The mean size of unit potentials, $v_1$, can again be calculated from the equations (1) and (2), since other values, $\bar{v}$, $N$ and $n_0$, are known from the experiment. Multiples of the unit size are then shown as the mean sizes of EPSPs in consecutive groups, $v_{II}$, $v_{III}$, $v_{IV}$,.... The expected numbers of occurrence of EPSPs in each group were calculated from the Poisson equation, $N(m^x/x!)e^{-m}$, using $m$ derived from the equation (2). In order to draw a Gaussian curve for the first group, the variance, $\sigma^2$, was obtained by trial and error, finding the best fitting curve (Dudel & Kuffler, 1961). The Gaussian curves were then drawn for the second and third groups about the means of $v_{II}$ and $v_{III}$ with the variances of $2\sigma^2$ and $3\sigma^2$ (Boyd & Martin, 1956). Since the number

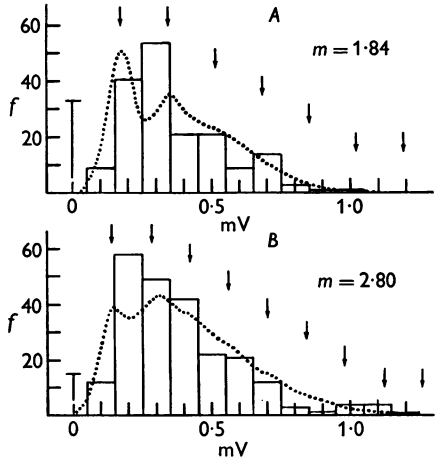


Fig. 7

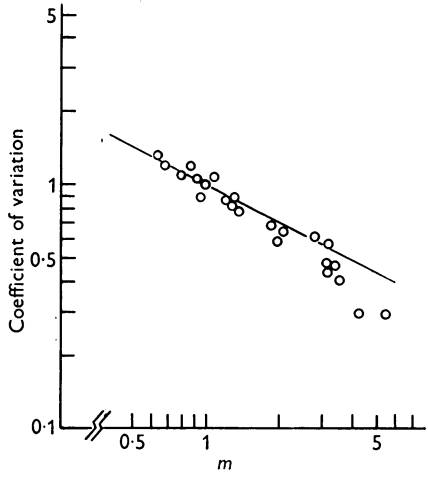


Fig. 8

Fig. 7. As in Fig. 6*A*, but for two other experiments with high level of $m$.

Fig. 8. Relation between coefficient of variation and $m$ calculated from the equation (2) in twenty-three experiments. Full line shows theoretical relation for Poisson distributions.

of occurrences in the remaining groups was virtually negligible, the curves were obtained only for the first three groups in this experiment. But this procedure was extended to the first five groups in Fig. 7*A* and to seven groups for Fig. 7*B*, respectively. Adding the three Gaussian curves together, the theoretical distribution was constructed. The theoretical curve so obtained was in good agreement with the observed distribution (Fig. 6*A*). In the analysis of another experiment shown in Fig. 6*B*, a different approach was made. As mentioned above (p. 87), there was occasionally 'recruitment of miniature EPSPs' following afferent stimulation. These miniature EPSPs may be considered to arise from the Group Ia

fibre under test, and therefore their mean size may give an independent measure of unit amplitude of the monosynaptic EPSPs. $m$ can then be obtained from relation (1). The theoretical amplitude distribution of the EPSP responses can be calculated from the mean and variance of the miniature potential distribution (Fig. 6*C*). This curve again agreed with the experimental distribution (Fig. 6*B*). Further, as indicated by horizontal arrows in Fig. 6*B*, the number of failures of the responses predicted from relation (2) was very close to the observed value. Figure 7 shows a further extension of this test to larger EPSPs. Although the range of fluctuations in the EPSP responses still agreed with the theoretical curve, the number of occurrences of EPSPs in the first and/or second groups was more than predicted from the assumptions (Fig. 7*A*, *B*). Such a deviation from the Poisson law may not be striking, but this tendency was consistent in all experiments with $m$ larger than approximately two.

The final test of the present assumptions is provided by the relation between the coefficient of variation and $m$. The expected coefficient of variation for a Poisson distribution should equal $m^{-\frac{1}{2}}$. In each experiment, about 200 monosynaptic EPSPs were obtained by stimulation of dissected small filaments every two sec. The results of twenty-three experiments are illustrated in Fig. 8, in which the coefficient of variation of each series of EPSP responses is plotted against the $m$ calculated from the number of response failures. Although those points with large values of $m$ ($> 3$) again showed a consistent discrepancy, for small values of $m$ there was good agreement with the prediction.

Although a similar deviation from the expected coefficient at high values of $m$ has also been noted in the neuromuscular junction (del Castillo & Katz, 1954), the discrepancy was removed when allowance was made for non-linear summation of the unit potentials making up the end-plate potential (Martin, 1955). However, this correction is obviously not sufficient to account for the discrepancy in the spinal monosynaptic transmission, since the mean size of the EPSP responses with $m = 3$ is still less than 1 % of the driving potential of the EPSP, 70 mV (Coombs, Eccles & Fatt, 1955). If the number of units at the afferent terminals is relatively small, it is possible that the amplitude fluctuation of EPSP responses may be described by a binomial distribution rather than Poisson's law. In this case, the mean number of units responding to one afferent impulse, $m$, may again be given by $np$, where $n$ is the number of units at afferent terminals activated by one impulse and $p$ is the average probability of each unit responding to the impulse. In a binomial law, the size distribution of observed EPSPs should be described by $(q + p)^n$, where $q$ is the chance of failures and equals $(1 - p)$. The first term of the binomial series, $(1 - p)^n$, will then be given by

$$\frac{\text{number of failures of EPSP responses } (n_0)}{\text{number of afferent impulses } (N)}.$$

Since the standard deviation in a binomial distribution is $(nqp)^{\frac{1}{2}}$, the coefficient of variation of EPSP responses, $v$, should equal $(npq)/\frac{1}{2}m$ or $(1 - p)^{\frac{1}{2}}/(np)^{\frac{1}{2}}$. From these relations the following equations are obtained

$$\log n_0/N = n \log (1 - p), \tag{3}$$

$$v^2 = (1 - p)/(np). \tag{4}$$

Although both $n$ and $p$ are unknown, the left terms of these equations can be obtained from the experimental results. Accordingly, the applicability of a binomial distribution may be tested by plotting the relation

$$1/v^2 = k \log n_0/N \tag{5}$$

where $$k = \frac{p}{(1-p)\log(1-p)}.$$

In the present preparation, an increase of $m$ is simply due to addition of other afferent fibres and is therefore due to an increase in $n$. If $p$ is constant for all units in any afferent fibre, the relation between $1/v^2$ and $\log n_0/N$ should be linear. Figure 9 shows that this is the case only when $n_0/N$ is large, $p$ being approximately 0·25, and a discrepancy still exists at high values of $m$. Such a deviation may be corrected if it is assumed that $p$ does not remain constant by, for example, some interaction among the afferent terminals when a large number of afferent fibres are used. But the same assumption can also be applied to the Poisson analysis, and it does not seem worth while to elaborate further along the lines of a binomial distribution although the possibility cannot be excluded at present.

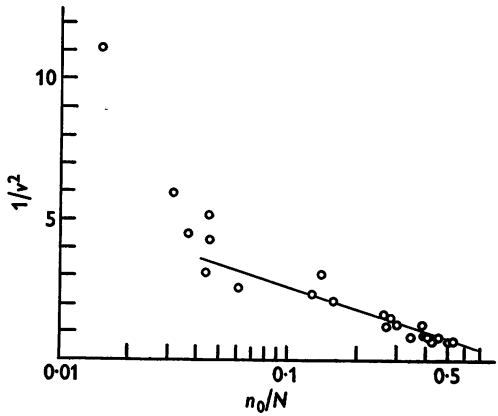


Fig. 9. A test for a binomial distribution. Relation between a reciprocal of (coefficient of variation)$^2$ and the number of failures relative to the number of applied stimuli. Full line shows a linear relation only when $n_0/N$ is large. One experiment is not shown, since the $n_0/N$ is too small.

### *Relation between m and the number of afferent fibres*

The size of monosynaptic EPSPs is apparently a function of the number of Group Ia afferent fibres stimulated (Fig. 3) and the value of $m$ shows a parallel increase with an increase of the mean size of monosynaptic EPSPs (Fig. 5). If the mean number of units responsing to one afferent impulse is approximately uniform for all Group Ia fibres, it would be expected that a correlation exists between the number of Group Ia fibres stimulated and $m$ for observed monosynaptic EPSPs. Figure 10$A$ shows this relation. It was, however, impossible to measure only the number of those Group Ia fibres which connect monosynaptically to the motoneurone under test. The number of afferent fibres measured might have included Group Ib

fibres and Group Ia fibres which have no monosynaptic connexion to the motoneurone under observation (see Methods). Consequently, the number in the abscissa of Fig. 10*A* gives only the maximum limit of the Group Ia fibres stimulated. The typical behaviour is seen in three experiments in which three afferent fibres were stimulated (Fig. 10*A*). In one of these experiments (the uppermost point at 3), the afferent fibres measured are probably all Group Ia fibres which have monosynaptic connexion to the motoneurone tested. In the other two experiments (lower two points at 3), the measurement might have included one or two unrelated afferent

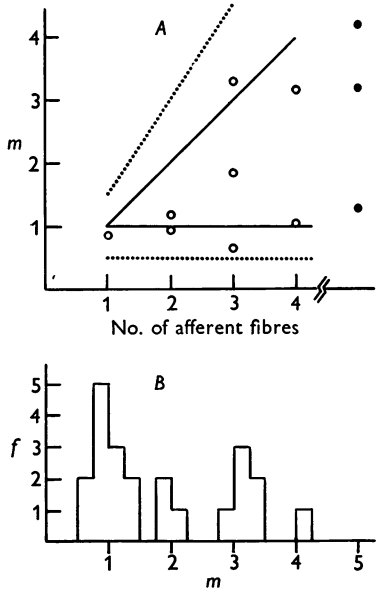


Fig. 10. *A*. Relation between $m$ calculated from the equation (2) and the number of Group I afferent fibres stimulated. Further explanation in text. *B*. Histogram showing distribution of $m$ calculated from the equation (2) in twenty-two experiments. One experiment with $m = 5{\cdot}4$ is not shown.

fibres. Accordingly, the actual number of Group Ia afferent fibres which produced monosynaptic EPSPs in all these experiments in Fig. 10*A* should be between the minimum, one, and the maximum, the number given by the measurement. When this is taken into consideration, the scatter of points in Fig. 10*A* is in good agreement with the assumption that $m$ is 1·0 for monosynaptic EPSPs produced by one Group Ia fibre (solid lines) or more satisfactorily, that $m$ is in a range of 0·5–1·5 (dotted lines). In three experiments shown by filled circles on the right, the number of afferent fibres measured was more than five, but the number could not be decided accurately.

Figure 10*B* shows a frequency distribution of $m$ obtained from twenty-two experiments. The distribution was apparently not continuous but multimodal, the first distribution being grouped at approximately $m = 1$ and others at the multiples. It might thus be assumed that in the experiments belonging to the first group, only one Group Ia afferent fibre was stimulated. The average value of $m$ in twelve experiments of the first group was 0·99 ± 0·23 (S.D.), the range being 0·63–1·34. It is concluded that one impulse in a single Group Ia afferent fibre may evoke, on the average, only one unit of the monosynaptic EPSP.

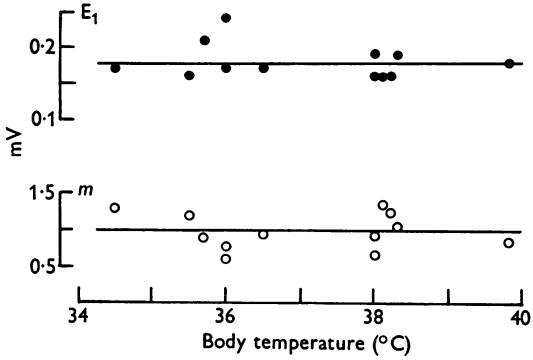


Fig. 11. Relation between body temperature and the unit size of EPSPs ($E_1$, filled circles) and $m$ (open circles). Twelve experiments with $m$ less than 1·5 are selected.

*Effects of temperature*

It is well known that monosynaptic transmission in the spinal cord is markedly influenced by changes in temperature (Brooks, Koizumi & Malcolm, 1955; Hunt, 1955; Suda, Koizumi & Brooks, 1957). One may surmise whether this influence is due to alteration in $m$ or in the unit size of EPSPs. While no observation on the temperature effect upon the same cell was made in the present study, a wide range of the body temperature in different experiments made it possible to test this problem. In Fig. 11, this relation is shown on twelve experiments in which presumably only one Group Ia fibre is used as afferent stimulation, since their values of $m$ are all less than 1·5. Obviously, a difference in the body temperature within this range (34–40° C) did not affect significantly either the unit size of EPSPs or $m$. Although more detailed studies are required, these results support a recent suggestion that the hyper-responsiveness in hypothermia may be due to changes in the post-synaptic properties (Koizumi, Ushiyama & Brooks, 1960; cf. however, Brooks *et al.* 1955).

## DISCUSSION

The present study has shown that when a strictly limited number of Group Ia afferent fibres are stimulated at a rate of 0·5/sec, the monosynaptic EPSPs in spinal motoneurones reveal a considerable fluctuation in size, including some failures of responses. Since the amplitude of the afferent impulses was constant throughout the observations, fluctuations in the EPSP size were not due to changes in the number of afferent fibres stimulated. The variations in amplitudes of successive EPSPs were analysed statistically on the same assumptions as used in the neuromuscular junction. However, the present preparation was subject to a limitation because of inability to discriminate only the 'spontaneous EPSPs' arising from the terminals of Group Ia fibres under observation. This did not allow an independent measure of the unit size of monosynaptic EPSPs, although in some experiments 'miniature EPSPs' recruited after afferent stimulation could be used for this purpose. Consequently, the statistical analysis was less accurate than that in the vertebrate neuromuscular junction (del Castillo & Katz, 1954; Boyd & Martin, 1956; Liley, 1956). Nevertheless, the mean number of units responding to one afferent impulse, $m$, calculated from the failures of responses, on the basis of the Poisson series, showed a linear relation with the mean amplitude of observed EPSPs. The assumptions were further supported by the fact that the entire distribution of the sizes of EPSP responses is in good agreement with the Poisson law, although there was a slight but consistent discrepancy when the test was extended to larger EPSP responses. Such a deviation was again noted in another test on the coefficient of variation of EPSP amplitudes, but below a certain limit ($m < 3$), the results agreed with the coefficients expected from the Poisson distribution. Thus, the mechanism of monosynaptic transmission in spinal motoneurones is similar to that in the neuromuscular junction. It may be concluded that the monosynaptic EPSPs are built up of units at the synapse, each with a small probability of responding to afferent impulses.

The unit EPSP size calculated from monosynaptic responses with $m$ less than three was in the range of 0·12–0·24 mV. This relatively constant value was rather surprising, since the size of unit EPSPs recorded would be influenced by the synaptic site of afferent terminals on the motoneurone surface (Fadiga & Brookhart, 1960; Katz & Miledi, 1963; Eccles, 1964). However, the theoretical calculation by Rall (1962*a*, *b*) has revealed that the maximum depolarization in the soma produced by a transient conductance change at the central part of dendrites is only twice as great as the depolarization produced by the same conductance change at the dendritic periphery. The range of the present values may, therefore, cover the

variations in the unit EPSP size due to different synaptic sites. The histological study has further shown that Group Ia fibres usually terminate near the origin of dendrites on the motoneurone soma (Szentágothai, 1958).

Although the afferent terminal knobs arising from Group Ia fibres may belong to the group with the largest size on a motoneurone (Szentágothai, 1958), their volume and synaptic area are still small compared with those of the motor nerve terminal. Accordingly, on the assumption that a terminal knob has the same density of quantal liberation as at the neuromuscular junction, excitation of one terminal knob may be considered to release no more than one quantum (Eccles, 1961*a*) or no more than two or three quanta of the transmitter (Eccles, 1961*b*, 1964). In approximate agreement with this prediction, the present study has shown that one impulse in a single Group Ia fibre may release, on the average, only one quantum when stimulated at a rate of 0·5/sec. One Group Ia fibre may usually have three knobs on a motoneurone (cf. Szentágothai, 1958). This implies that the probability of the units in each knob responding to one afferent impulse is approximately 0·3.

From the unit size of EPSPs, $v_1$ (0·12–0·24 mV, see above), the driving potential of EPSPs, $V_0$ (70 mV, Coombs *et al.* 1955), and the critical firing level of the motoneurone, $v_f$ (10 mV, Brock *et al.* 1952), it is possible to calculate how many Group Ia afferent fibres ($N$) are required to evoke a reflex discharge. Taking the non-linear summation of EPSPs into consideration, the figure of approximately 50–100 fibres will be obtained from the following equation given by Martin (1955):

$$N = \frac{v_f}{v_1}\left(1 - \frac{v_f}{V_0}\right)^{-1}. \tag{6}$$

This has been based on the theoretical consideration that any EPSP ($\bar{v}$) is made up of a certain number ($m$) of units ($v_1$), and the action of each unit produces a small increment of conductance ($g$) in the motoneurone membrane. The EPSP ($\bar{v}$) across the membrane resistance ($1/G$) will then be given by:

$$\frac{\bar{v}}{V_0 - \bar{v}} = \frac{mg}{G}. \tag{7}$$

In the case of a unit EPSP, $m = 1$ and $\bar{v} = v_1$. Since the average membrane resistance of spinal motoneurones ($1/G$) is 1·2 MΩ (Coombs, Curtis & Eccles, 1959; see also Frank & Fuortes, 1956), the shunting resistance by a unit EPSP ($1/g$) may be calculated as 350–700 MΩ. This value is much greater than the shunt resistance of 6·8 MΩ by a unit end-plate potential (Takeuchi & Takeuchi, 1960), but is comparable with those by unit synaptic responses in the avian ciliary ganglion (400–800 MΩ, Martin & Pilar, 1964) and in the frog sympathetic ganglion (600 MΩ, C. C. Hunt &

P. G. Nelson, personal communication; but cf. 55–200 MΩ, Blackman, Ginsborg & Ray, 1963).

The above estimate, however, conflicts with the input–output relation existing for the monosynaptic reflex discharge. When monosynaptic responses are recorded from the ventral root following afferent volleys, an input volley in about 20% of the total Group Ia fibres of the muscle nerve can initiate a reflex discharge (Hunt, 1955; Rall, 1955). This implies that convergence of impulses from 10 to 20 afferent fibres, in contrast to 50–100 of the present estimate, produces monosynaptic excitation in some motoneurones, since the total number of Group Ia fibres (or muscle spindles) in the medial gastrocnemius nerve is in the range of 50–100 (Hagbarth & Wohlfart, 1952; Hunt, 1954; Swett & Eldred, 1960; Chin, Cope & Pang, 1962). A variation in the critical firing level in individual motoneurones (6–14 mV, Brock *et al.* 1952) may partially account for the discrepancy, but this is not sufficient. Two factors are considered as possible explanations for this discrepancy. Some motoneurones may have a high membrane resistance, and therefore, the size of unit EPSPs in these motoneurones could be relatively large (cf. Katz & Thesleff, 1957). Secondly, some Group Ia afferent fibres or the Group Ia fibres connected to some motoneurones may have a great number of terminal branchings, and therefore, the value of $m$ in these systems could be relatively large. It is possible that these particular motoneurones may belong to the 'tonic' type of motoneurones, since evidence exists that tonic motoneurones have a lower threshold for the stretch reflex (Denny-Brown, 1929) and have denser excitatory synaptic contacts (Eccles *et al.* 1957*b*; Kuno, 1959) than do 'phasic' motoneurones. The number of gastrocnemius motoneurones fired reflexly by such small afferent volleys was presumably less than 5% of the total gastrocnemius motoneurones (Hunt, 1955; Rall, 1955). Therefore, it is not surprising that these may not have been encountered in the present experiments.

## SUMMARY

1. Monosynaptic excitatory post-synaptic potentials (EPSPs) in triceps surae motoneurones of the cat were recorded with intracellular electrodes following stimulation of a strictly limited number of Group Ia afferent fibres in the muscle nerve.

2. With successive afferent stimulation at a frequency of 0·5/sec, the EPSP in a motoneurone showed a fluctuation in size, including some failures of responses. Since the amplitude of the afferent impulses was constant throughout the observations, fluctuations in the EPSP size were not due to changes in the number of afferent fibres stimulated.

3. The statistical analysis showed that the distribution of sizes of the

observed EPSPs could be described by Poisson's law, although there was a discrepancy when the analysis was extended to larger EPSPs.

4. The average size of the smallest or 'unit' EPSP was in the range of 0·12–0·24 mV. The mean number of units responding to one impulse in a single Group Ia afferent fibre was approximately one.

5. There was probably no marked effect of temperature either on the unit EPSP size or on the probability of the units responding to afferent impulses.

6. It was concluded that the monosynaptic transmission in spinal motoneurones occurs in quantal steps, and that one impulse in single afferent fibres may release, on the average, only one quantum.

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