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RELATION BETWEEN STRUCTURAL AND RELEASE PARAMETERS AT THE FROG SENSORY–MOTOR SYNAPSE

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SUMMARY

1. The sensory–motor synaptic connexions in the frog lumbar cord have been used to examine the relationship between the statistical characteristics of the unitary excitatory post-synaptic potential (e.p.s.p.) and the number and organization of synaptic contacts determined when the primary afferent fibre used in evoking the e.p.s.p., and a motoneurone in which it was recorded, were both labelled with horseradish peroxidase (HRP).

2. A significant correlation is found between the number of contacting boutons and the amplitude of the chemical component of the unitary e.p.s.p.s generated at the same connexions.

3. The amplitude fluctuation patterns of the single-fibre e.p.s.p.s could be fitted by both Poisson and binomial distribution. The number of presumed Poisson release sites as estimated from the ratio V_{max}/v (where V_{max} is the maximal amplitude of the chemical component of e.p.s.p. and v is quantal size) is always less than or equal to the total number of boutons observed histologically. In three connexions there was a close correspondence between the number of binomial release units, n, and the number of contact regions formed by the tight clusters of contacting boutons.

4. The unit potential amplitude estimated from the Poisson distribution is found to be two to three times smaller than the quantal size calculated from binomial distribution. A similar numerical relationship was found between the number of contacting boutons and the number of contact regions. It is suggested that at a single bouton, transmission results in release of a single quantum of transmitter, whereas the binomial quantum probably reflects the multi-quantal release occurring simultaneously at boutons comprising a contact region.

5. A significant correlation is found between the mean quantum content estimated either from Poisson or binomial distribution and the number of contacting boutons and contact regions respectively, indicating the dependence of quantal release on the magnitude of synaptic surface.

6. No correlation is found between the motoneuronal soma diameter and the quantal size, although the former is significantly correlated with the number of contacting boutons.

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INTRODUCTION

At present there is no consensus of opinion about the mechanisms governing transmitter release at central excitatory synapses and the morphological correlates to the release parameters. Although Kuno (1964) has been able to demonstrate quantal release of neurotransmitter at Ia synapses in the cat, later studies on the same junction have suggested that transmission might be non-quantal (Edwards, Redman & Walmsley, 1976*a*, *b*) or could not be described using Poisson or binomial distribution (Jack, Redman & Wong, 1981). There is also evidence raising the possibility that the fluctuations of unitary synaptic actions may be ascribed to variations in impulse propagation at different presynaptic fibre branch points (Lüscher, Ruenzel & Henneman, 1979).

The amplitude fluctuations of excitatory post-synaptic potentials (e.p.s.p.s) evoked in frog motoneurones by weak stimulation of dorsal roots or muscle nerves could not be predicted by a Poisson distribution, although the spontaneous synaptic potentials recorded from these cells may be considered to result from quantal release (Katz & Miledi, 1963). The identified sensory–motor synapses in the frog spinal cord which are homologous to the Ia synapses in the cat (Shapovalov, 1980; Shiriaev, 1983) have allowed increasingly rigorous study of the quantal nature of evoked release with intracellular access to both pre- and post-synaptic elements (Shapovalov & Shiriaev, 1979, 1980). The dual mode of junctional transmission at frog sensory–motor synapses has made it possible to use the coupling potential as an indicator of the stability of presynaptic invasion and has provided the basis for demonstrating the quantal nature of amplitude fluctuations of a single-fibre e.p.s.p. However, the further clarification of quantal mechanisms at this synapse requires a correlation of e.p.s.p. statistics with the morphology of the presynaptic apparatus.

When a motoneurone and a sensory fibre which directly contact one another are both injected with horseradish peroxidase (HRP), the subsequent reconstruction of labelled elements allowed the number and distribution of synaptic contacts on the identified post-synaptic cell to be determined (Grantyn, Shapovalov & Shiriaev, 1982, 1984). In the present paper we compare the structural and quantal parameters determined at the same sensory–motor connexion.

METHODS

Isolated hemisected spinal cords of frogs *Rana ridibunda* were used in all experiments. The methods of preparing and mounting the spinal cord together with ventral and dorsal roots and simultaneous intracellular recording from motoneurones and single dorsal root fibres afferent to them were essentially the same as those described by Shapovalov & Shiriaev (1980). The techniques of HRP injection and subsequent reconstruction of labelled elements were described in a previous paper (Grantyn *et al.* 1984).

The micro-electrode signals were conventionally amplified, monitored on an oscilloscope and successive sweeps were photographed for later detailed analysis. For statistical analysis of amplitude fluctuations of the chemical component 180–430 individual e.p.s.p.s were used. All responses for each connexion were averaged with a DIDAC-4000. The mean amplitudes of the electrical and chemical components of the e.p.s.p. were measured from the averaged records.

The statistical analysis of single-fibre e.p.s.p.s was similar to that used by Shapovalov & Shiriaev

(1980). Initial estimation of the quantal size, v, for the Poisson distribution was calculated from

$$m = \frac{1}{cv^2}, \quad v = \frac{\overline{V}}{m};$$

and for the binomial distribution (Ballanter, 1977):

$$p = \frac{\overline{V}}{V_{max}}; \quad m = \frac{1-p}{cv^2}; \quad v = \frac{\overline{V}}{m}.$$

The number of binomial units, n, was derived from:

$$n = \frac{m}{p}$$

where m is mean quantum content; cv, coefficient of variation; p, binomial probability of release; $\overline{V}$, mean amplitude of chemical component of e.p.s.p.; V_{max}, maximal amplitude of chemical component of e.p.s.p.

There are some differences in the statistical analysis used by Shapovalov & Shiriaev (1980) and in the present paper. The optimum quantal size was determined by the method of maximal likelihood. After initial estimation of the quantal size from the equations given above, its amplitude was shifted by steps of 2 μV in the range ± 20 μV from the initial estimate. The theoretical histogram was plotted for each value of quantal size and its correspondence to the experimental histogram of e.p.s.p. fluctuations was estimated by the χ^2 test. In the present analysis, the theoretical distribution with the best goodness of fit to the experimental distribution was taken.

RESULTS

General. The results of the present study are based on observations made on six primary afferent fibre–motoneurone connexions for which the afferent fibre could be reliably traced. These connexions have been used for detailed morphological reconstruction (Grantyn *et al.* 1984), and for each of these connexions a sequence of e.p.s.p.s was recorded and used for statistical analysis of the fluctuation in the e.p.s.p. All six connexions revealed combined electrical–chemical transmission, although the degree of electrical coupling at each synapse was quite different.

While in some motoneurones the electrical component of the single-fibre e.p.s.p. was very prominent and could be even larger than the chemical component, the former was negligible in the others.

The small amplitude of the coupling potential suggests that the relevant synapse bears especially close similarity to the homologous Ia junctions in mammals, where transmission is entirely chemical (Tamarova, Shapovalov & Shiriaev, 1978; Shapovalov, Shiriaev & Tamarova, 1979; Flatman, Engberg & Lambert, 1982). The scatter diagram of Fig. 1 demonstrates the ratio between the amplitudes of chemical and electrical components of the e.p.s.p. observed in the present sample. It reveals that same tendency which was found previously for the much larger population of single-fibre e.p.s.p.s recorded at frog sensory–motor synapses (Shapovalov & Shiriaev, 1980). The ratio of the mean amplitude of the electrical and chemical components was used as an index for describing the connexion, and all six connexions were numbered according to this coefficient value (see also Grantyn *et al.* 1984).

Relation between the structural pattern of the sensory–motor connexions and the properties of the single-fibre e.p.s.p.s. Reconstructions of the dendritic arborizations and schematic representation of the synaptic contacts established on a given

motoneurone by the collaterals of the afferent fibre in three different sensory–motor connexions, together with the averaged records of the presynaptic spike and resulting e.p.s.p. generated at that connexion, are presented in Figs. 2–4. The records of potentials are included to illustrate two points, one which bears upon the considerable

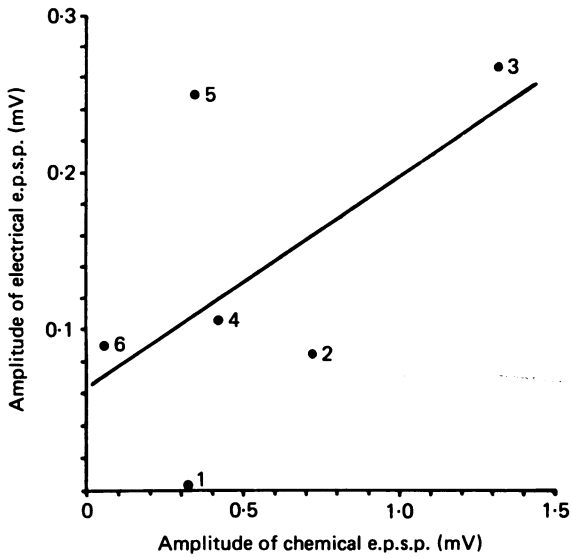

Fig. 1. Relation between the peak amplitudes of electrotonic e.p.s.p.s (ordinate) and the peak amplitudes of the chemical e.p.s.p.s (abscissa) measured in the same motoneurone. Numbers denote connexion number.

variation in average amplitudes of single-fibre e.p.s.p.s, and the other the differences in ratio between electrical and chemical components. In contrast to detailed reconstruction of the terminal arborizations of the same connexions presented in the preceding paper (Grantyn *et al.* 1984), the individual synaptic contacts as determined histologically are, for simplification, outlined schematically only with filled circles. Non-relevant dendritic systems are omitted. The individual synaptic contacts clustered in close vicinity to one another and defined as contact regions are numbered in each Figure.

The unitary synaptic response shown in Fig. 2*A* is relatively small (316 μV) and its electrical component is quite negligible (about 10 μV). The number of synaptic boutons is also moderate (twenty-six) and only eight contact regions can be found (Fig. 2*B*).

The single-fibre e.p.s.p. presented in Fig. 3*A* has a much larger mean amplitude, and although its peak is determined by the chemical e.p.s.p. the electrical component is very well pronounced, its amplitude reaching 270 μV. The number of individual synaptic contacts and the number of contact regions is much larger than in the preceding example (seventy-two and twenty-three respectively). In the case presented in Fig. 4 the amplitudes of the electrical and chemical components of the e.p.s.p. are nearly similar. In accordance with the relatively small size of the chemical e.p.s.p.

the number of individual synaptic boutons and the number of contact regions is smaller (forty-five and thirteen respectively) than in connexion 3.

In spite of variation in unitary e.p.s.p. amplitudes, different ratios between electrical and chemical component and variations in number and complexity of synaptic contacts seen in Figs. 2–4, several common features of investigated

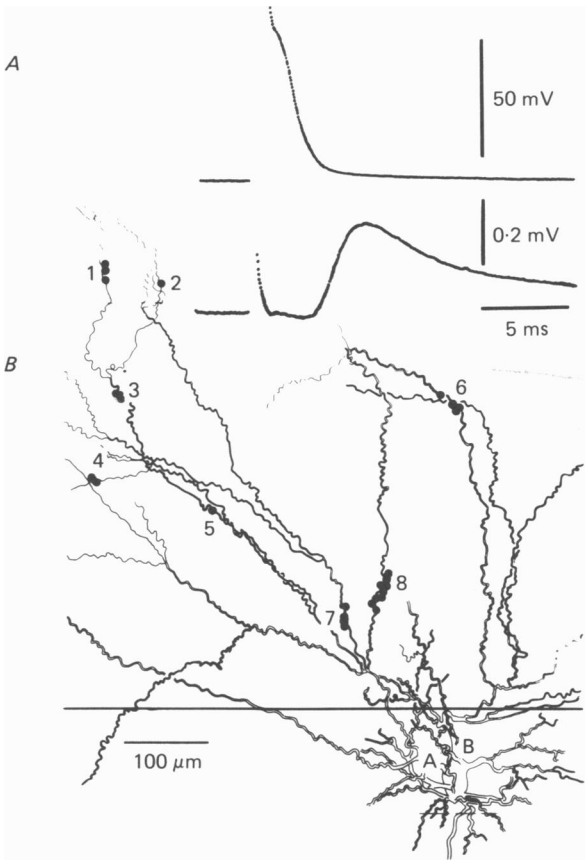

Fig. 2. *A*, average records of the presynaptic spike (above) and the single-fibre e.p.s.p. (below) and *B*, location of contacting boutons (filled circles on dendrites of a motoneurone belonging to the same connexion (connexion 1)). Numbers refer to contact regions formed by tight clusters of boutons. The horizontal line indicates the dorsal limit of the soma layer.

connexions emerge from the above examples. (1) Each unitary e.p.s.p. results from the summed activity of many synaptic contacts widely distributed along proximal and distal dendritic branches; (2) the amplitude of the chemical component of the unitary e.p.s.p. increases with an increase in the number of individual synaptic contacts and (3) the individual synaptic contacts reveal the tendency to make clusters at different locations from the soma.

When the mean and maximal amplitudes of the chemical component were plotted *versus* the number of contacting boutons (Fig. 5), significant correlation was found

between these values. An especially high level of significance ($r = 0{\cdot}92$, $P < 0{\cdot}01$, by t test, where r is the linear correlation coefficient) characterizes the relation between the mean amplitude of the chemical e.p.s.p. and the number of boutons. The same tendency appears to be present for the electrical component, although in the latter case the correlation is not statistically significant ($r = 0{\cdot}79$, $P > 0{\cdot}5$, by t test).

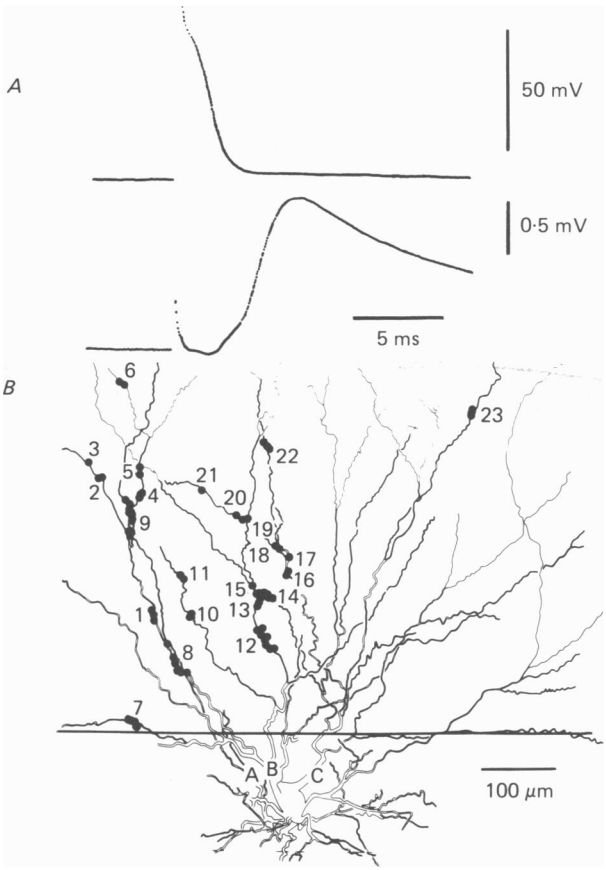

Fig. 3. Averaged records and location of contacting boutons at connexion 3. See legend to Fig. 2 for full explanation.

Quantal analysis of transmitter release at labelled synapses. Although the resistance of HRP-filled micro-electrodes was usually relatively high, the single-fibre e.p.s.p.s were well above the noise level to achieve an accurate measurement of the absolute values of the recorded potentials. The consecutive records taken from connexion 5 and illustrated in Fig. 6 show typical biphasic responses with a stable electrical component and a fluctuating chemical component.

Based on the evidence that the electrotonic e.p.s.p. remains stable from trial to trial, indicating a constant degree of invasion of presynaptic terminals by the propagating impulses, the fluctuation of the chemically mediated event could be accounted for by variations in the number of quanta of transmitter released per

presynaptic impulse (Shapovalov & Shiriaev, 1980). Fig. 7 gives examples of the statistical variability in successive chemical e.p.s.p.s. The histograms of the peak responses are shown together with the theoretical curves for a convolution of both binomial and Poisson distributions with a normal distribution of the background noise. The distribution of amplitudes of Fig. 7*A* and *B* (connexion 1 and 3

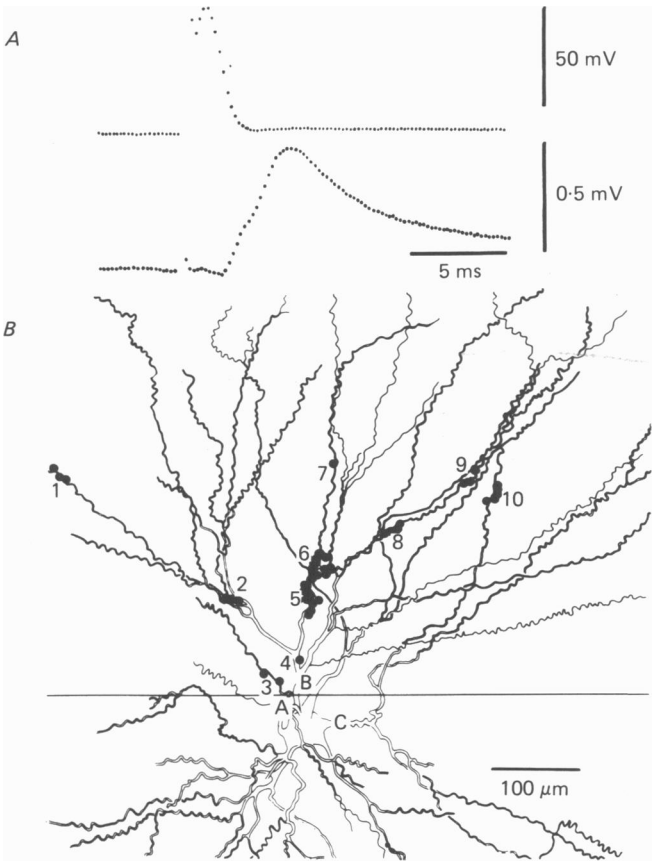

Fig. 4. Averaged records and location of contacting boutons at connexion 5. See legend to Fig. 2 for full explanation.

respectively) fits with predictions based both on the binomial and Poisson models. The distribution of amplitudes presented in Fig. 7*C* (connexion 6) fits with predictions based on a Poisson model ($P = 0{\cdot}3$, where P is goodness of fit by χ^2 test) and does not fit with predictions based on a binomial model ($P = 0{\cdot}01$).

The correspondence of experimental histograms to the Poisson and binomial models makes it possible to estimate the release parameters which characterize the labelled synapses and to relate these parameters to the morphological features of the identified contacts. Table 1 summarizes the results of a combined morphological and statistical analysis of all six primary afferent fibre–motoneurone connexions so far investigated.

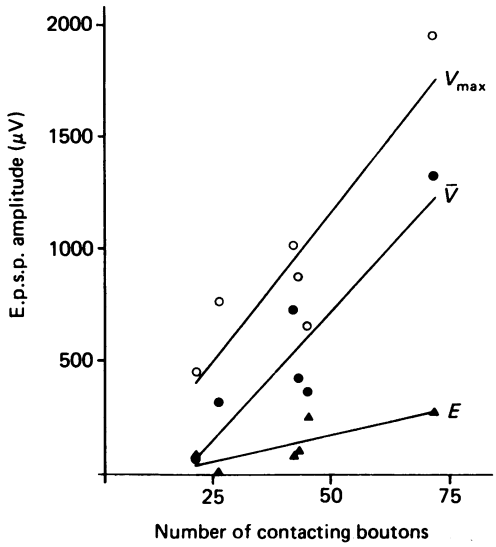

Fig. 5. Relation between single-fibre e.p.s.p. amplitudes and number of contacting boutons for six connexions. Maximal amplitudes of the chemical component: open circles (the linear correlation coefficient, $r = 0{\cdot}9$; $P < 0{\cdot}01$; by t test). Mean amplitudes of the chemical component: filled circles ($r = 0{\cdot}92$; $P < 0{\cdot}01$; by t test). Mean amplitudes of the electrical component: triangles ($r = 0{\cdot}79$; $P > 0{\cdot}05$; by t test).

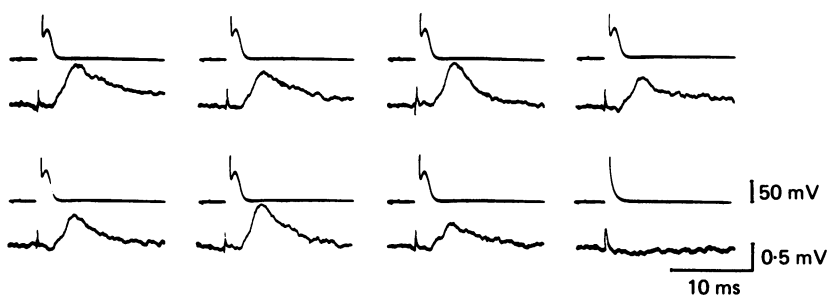

Fig. 6. Single traces of monosynaptic e.p.s.p.s (below) elicited in motoneurone by action potentials in a primary afferent fibre (above). Note fluctuation in size of the late, chemical component of the e.p.s.p. The last trace is shown to demonstrate the background noise when the stimulus strength is subthreshold for eliciting a spike.

Comparison of binomial and Poisson characterization of the same synaptic connexions. It is clear from Table 1 that almost all e.p.s.p.s analysed in the present work could be fitted by both Poisson and binomial models. In two connexions (2 and 5) the goodness of fit for binomial and Poisson models was practically equal, but it differed considerably in connexions 3 and 4. The Poisson and binomial curves were often rather similar for the same connexion, and sometimes hardly distinguishable (Fig. 7*B*).

Unexpectedly, the calculated size of the unit potential equivalent to a single quantum of the transmitter was found to be two to three times smaller when the

chemical e.p.s.p. fluctuation pattern followed Poisson distribution than when the same amplitude histogram was fitted by the binomial distribution (Table 1, quantal parameters). This unexpected feature may be due to an error in estimating the real size of the unit potential, since at frog sensory–motor connexions, as at other central

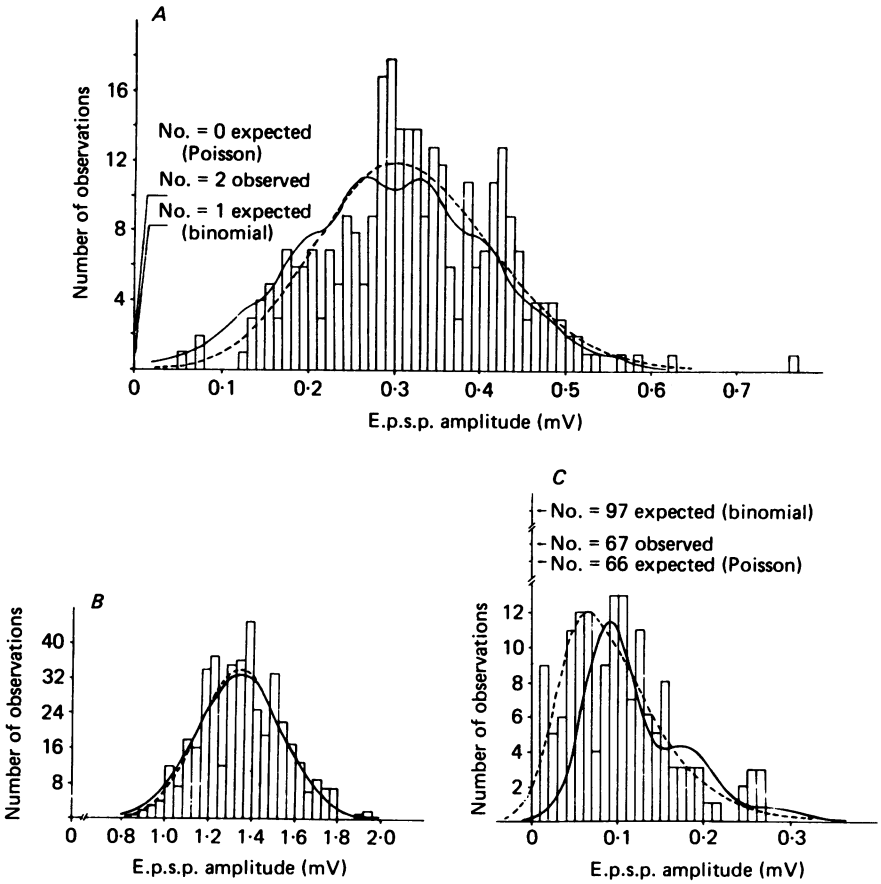

Fig. 7. Histograms of amplitude distributions of e.p.s.p.s at connexion 1 (*A*), connexion 3 (*B*) and connexion 5 (*C*). The dashed and continuous curves in each histogram are the Poisson and binomial predictions, respectively.

synapses, spontaneous miniature e.p.s.p.s cannot be used for determining the size of the quantal event. On the other hand, the estimated unit potentials (25–90 μV) fall into the same range of values as the smallest spontaneous miniature e.p.s.p.s recorded under low-noise conditions using KCl-filled low-resistance micro-electrodes (Shapovalov & Shiriaev, 1980). It appears reasonable, therefore, to suggest that the difference between unit potentials corresponding to Poisson and binomial models of transmitter release may reflect the difference between quantum potential produced by a single bouton and by a tight cluster of boutons forming a contact region.

Table 1 further demonstrates that the estimates of mean quantum content, m, derived with the Poisson equations were significantly greater than those obtained

TABLE 1. Comparison of release and structural parameters determined in six experiments. E, peak average amplitude of electrical component, μV; $\bar{V}$, peak average amplitude of chemical component, μV; V_{max}, maximal amplitude of chemical component, μV; v, amplitude of unit potential, μV; m, mean quantum content; N, number of Poisson release sites (V_{max}/v); n, number of binomial units; P, goodness of fit (by χ^2 test); p, binomial probability of release

Connexion number	E.p.s.p. parameters				Quantal parameters									Structural parameters			
					Poisson				Binomial								
	E	$\bar{V}$	V_{max}	$\bar{V}/E$	v	m	N	P	v	m	n	p	P	Number of contacting boutons	Number of contact regions	Mean number of boutons per contact region	Number of collaterals
1	10	316	760	31·6	30	10·6	25	0·11	69	4·6	9	0·50	0·20	26	8	3·3	2
2	85	725	1010	8·5	24	29·6	42	0·61	64	11·3	19	0·60	0·50	42	15	2·8	2
3	270	1314	1940	4·9	27	48·0	69	0·30	89	14·7	21	0·69	0·06	72	23	3·1	3
4	105	421	870	4·0	25	16·9	35	0·25	88	4·8	10	0·48	0·07	43	14	3·1	1
5	251	359	650	1·4	42	8·7	16	0·24	61	5·9	24	0·26	0·25	45	13	3·5	3
6	90	67·6	450	0·8	56	1·2	8	0·30	90	0·8	5	0·15	0·01	21	5	4·2	2

with the binomial model. Similar differences were mentioned by Korn, Mallet, Triller & Faber (1982).

Transmitter release and the number and organization of contacting boutons. When the amplitude fluctuation pattern could be fitted by a Poisson distribution, it is quite probable that the largest number of release sites is brought into play when the

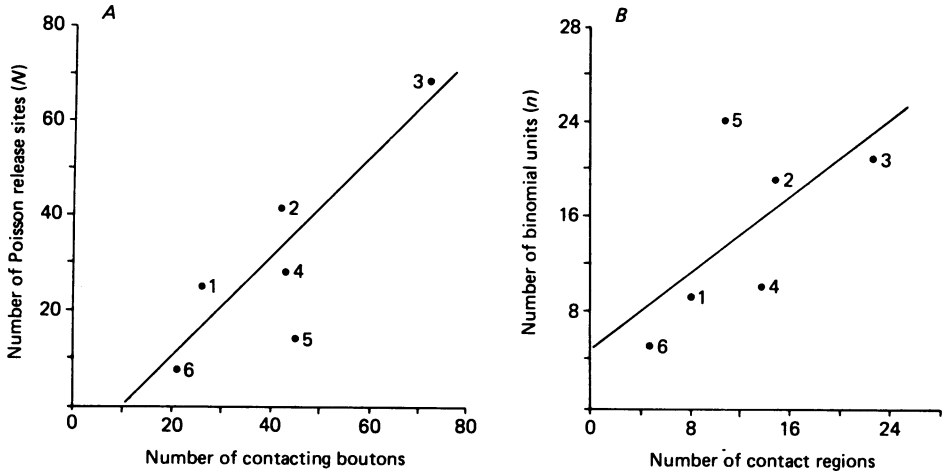

Fig. 8. *A*, relation between presumed Poisson release sites (ordinate) and the number of contacting boutons (abscissa) ($r = 0{\cdot}86$; $P < 0{\cdot}05$). *B*, relation between binomial n (ordinate) and the number of contact regions (abscissa) ($r = 0{\cdot}72$; $P > 0{\cdot}05$). Numbers denote connexion number.

maximal amplitude of the chemical e.p.s.p. is recorded. Then the total number of release sites, N, may be calculated from the ratio V_{max}/v, where v is the amplitude of a single quantum potential. Fig. 8*A* shows that when the number of contacting boutons is plotted *versus* the number of presumed Poisson release sites there is a positive correlation between these values. The linear correlation coefficient r computed for the total population of labelled sensory–motor connexions is 0·87. The correlation is statistically significant ($P < 0{\cdot}05$, by t test).

The correspondence between the number of boutons and presumed Poisson release sites is especially close for connexions 1–3, where there is almost absolute identity of both parameters. All these connexions are characterized by the dominance of the chemical component of the e.p.s.p. (Table 1). In contrast, the connexions 4–6 with the considerable deviation from the linear relationship reveal a relatively large coupling potential ($\overline{V}/E$ ratio 4·0–0·8, where E is peak average amplitude of electrical component). It may be suggested that at those junctions where electrical transmission occurs, the probability of transmitter release is especially low.

When the number of binomial release sites, n, was plotted *versus* the number of contact regions (Fig. 8*B*) there is also apparent tendency for correlation, but the latter is not statistically significant ($r = 0{\cdot}72$; $P > 0{\cdot}05$, by t test). However, for three pairs (connexions 1, 3 and 6) almost one-to-one correspondence between the binomial n and the number of contact regions may be seen. Thus, the lack of equivalence between release and histological parameters in some connexions may be ascribed to a wide

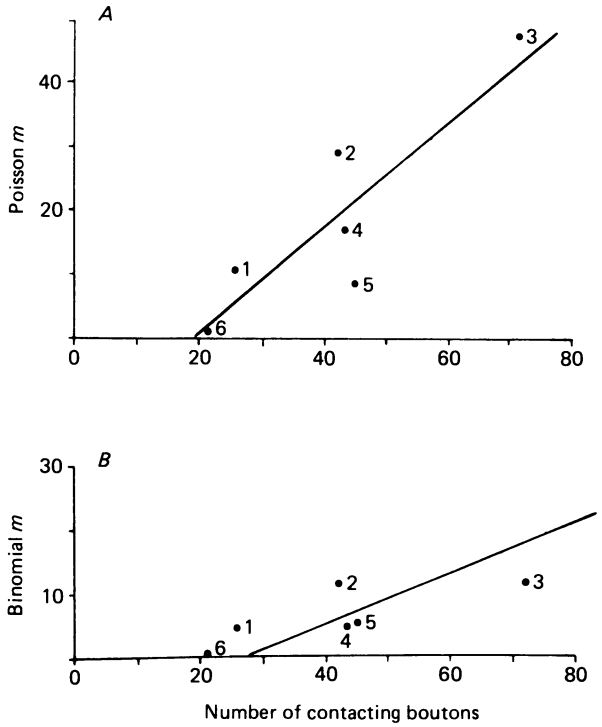

Fig. 9. Relationship between mean quantum content, m, calculated for Poisson (A) and binomial (B) distribution (ordinate) and the number of contacting boutons (abscissa) $r = 0{\cdot}88$; $P < 0{\cdot}05$ for A and $r = 0{\cdot}87$; $P < 0{\cdot}05$ for B. Numbers denote connexion number.

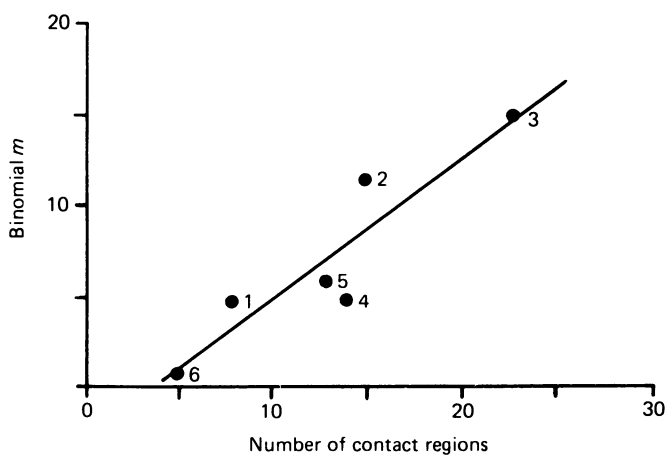

Fig. 10. Relation between binomial m (ordinate) and the number of contact regions (abscissa) $r = 0{\cdot}92$; $P < 0{\cdot}01$. Numbers denote connexion number.

variability in the number of boutons comprising each contact region (see Grantyn *et al.* 1984 and Figs. 2–4).

It may be suggested that a Poisson model based on the assumption that a single bouton may release only one quantum of the transmitter provides a better correlate of the synaptic structural pattern at the frog sensory–motor synapse.

Relation between mean quantum content and the number and organization of contacting boutons. The value of m seems to be related to the extent of neuromuscular junction where a wide range of size has been observed (Kuno, Turkanis & Weakly, 1971). Assuming that at sensory–motor synapse each bouton may produce only one quantum of the transmitter in all-or-nothing maner (Jack *et al.* 1981; Hirst, Redman & Wong, 1981 and evidence presented above) one may suggest that it is not the bouton size, but the total number of boutons which characterize the size of the synaptic apparatus.

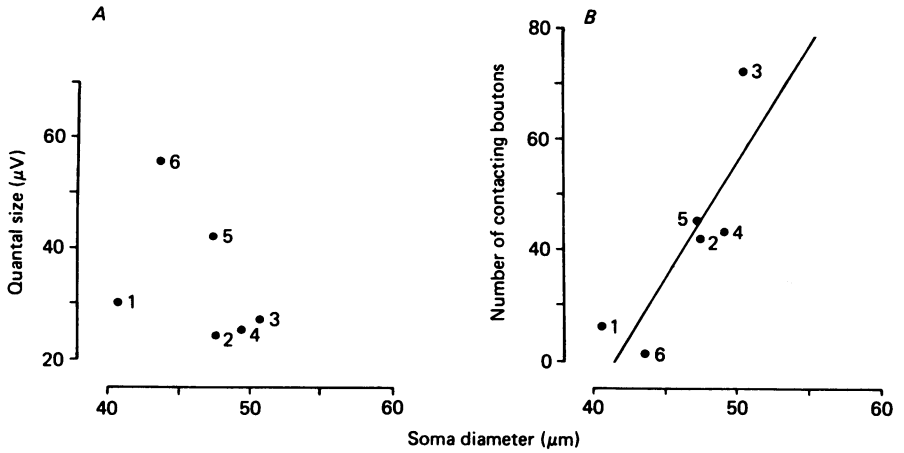

Fig. 11. Relation between soma diameter and the quantal size (A) and the number of contacting boutons (B). Numbers denote connexion number. $r = 0{\cdot}41$; $P > 0{\cdot}05$ for A and $r = 0{\cdot}85$; $P < 0{\cdot}05$ for B.

The association between m derived either with Poisson or binomial statistics and the number of contacting boutons is shown in Fig. 9. Both plots demonstrate that there is a significant correlation between parameter m and the number of boutons ($r = 0{\cdot}88$, $P < 0{\cdot}05$ for Poisson m; $r = 0{\cdot}087$, $P < 0{\cdot}05$ for binomial m).

If a binomial quantal unit represents a specific quantal multiple produced by simultaneous release at several boutons constituting the contact region, then a similar correlation should exist between binomial m and the number of contact regions. Fig. 10 demonstrates that in fact this correlation exists and is statistically significant ($r = 0{\cdot}92$, $P < 0{\cdot}01$).

Motoneurone size, number of boutons and quantal size. It is well established that the amplitude of the unit potential is directly related to the input resistance of the post-synaptic cell where it is generated (Katz & Thesleff, 1957; Kuno & Miyahara, 1969). As the input resistance in its turn is dependent on the motoneuronal size (Kernell, 1966) it would be reasonable to suggest that the estimated quantal size would be correlated with the diameter of the motoneurone in which it was recorded. The relation presented in Fig. 11*A* shows that it is not so. There is no significant correlation between these parameters ($r = 0{\cdot}41$; $P > 0{\cdot}05$).

On the other hand, there is an obvious positive correlation between the diameter of the motoneuronal soma and the number of contacting boutons. Plot of Fig. 11*B* gives the linear correlation coefficient $r = 0{\cdot}85$; $P < 0{\cdot}05$. As most boutons are located

on the dendritic branches the relation between motoneuronal soma size and the number of boutons probably coincides with the larger dendritic tree of big neurones.

The fact that all labelled presynaptic collaterals contact the target motoneurones at many locations on dendritic sites implies that the impedance of subsynaptic dendritic twigs may play an especially important role in determining the quantal size, as was suggested by Katz & Miledi (1963). (See also Gage & McBurney, 1973.)

DISCUSSION

The main result of the present work is the demonstration of a close correspondence between the number of statistical units building up the unitary e.p.s.p. and structural parameters of the sensory–motor connexion generating that e.p.s.p.

In five out of the six pairs that we have analysed, amplitude distributions of the unitary e.p.s.p.s could be fitted by both the Poisson and binomial models, and in one only by the Poisson distribution. As was pointed out by Del Castillo & Katz (1954) the Poisson process represents the limit of a binomial case when the probability of release, p, is small. It may be suggested further that p is not uniform at different boutons of the same connexion. In fact, Bennett & Lavidis (1979, 1982) have obtained direct evidence for spatial non-uniformity in the probability of release at the amphibian neuromuscular junction.

If a single bouton represents the structural correlate for a release site producing a Poisson unit, then a two to three times larger binomial quantum might represent a specific quantal multiple provided that boutons forming a contact region exhibit correlation behaviour. Such non-independent release could be produced by a concomitant increase in internal calcium concentration in boutons located in close vicinity to one another. In most cases (connexions 1–4 and 6) the estimated number of binomial release sites, n, and the number of contact regions fall into the same range of values. Moreover, the mean number of boutons comprising a contact region and the ratio between the binomial and Poisson quantal units are similar in connexions 1–4 (2·3–3·2; 2·6–2·8; 3·3–3·1 and 3·5–3·1, respectively). Thus it may be suggested that the number of contact regions represents a structural correlate for binomial n.

The best correlation between the number of presumed Poisson release sites and the number of boutons was found at synapses with largest $\overline{V}/E$ ratio. If at junctions where electrical transmission takes place the probability of transmitter release is very low, then such mixed boutons may obscure the simple relation between structural and quantal parameters. A junction providing joint electrical and chemical transmission is a morphologically mixed synapse, and such junctions have been observed also in the frog spinal cord (Sotelo & Grofova, 1976; Taugner, Sonnhof, Richter & Schiller, 1978). Recently such mixed synapses belonging to sensory–motor connexions were identified with HRP labelling (Adanina & Shapovalov, 1983).

Whether boutons establishing mixed synapses have the same release properties as those establishing purely chemical synapses remains to be clarified. It was pointed out, however, that the coexistence of chemical and electrical transmission at the same location may be functionally disadvantageous (Shapovalov, 1980), providing the possibility of limited participation of such boutons in transmitter release.

The applicability of quantal models of transmitter release to transmission process at the frog sensory–motor synapse suggests that there may be a certain uniformity among the potential changes produced by a quantum of transmitter from each release site when recorded in the soma of the motoneurone. Since it is quite obvious that numerous synaptic contacts are made at very distinct locations on the dendritic arborization (Figs. 2–4), it may be suggested that a special mechanism exists which compensates for the distance differences. It was shown recently by Jack *et al.* (1981) in cat motoneurones that there is a relative constancy of amplitude of the quantal units whatever their time course and hence deduced site of origin on the dendrites.

It is also instructive to compare the results of the present analysis with the morpho-functional correlations obtained by Korn *et al.* (1982) at goldfish inhibitory synapses. While their findings agree with our general conclusion about the relationship between termination pattern and release parameters, they stress that the binomial relation always gives a more adequate description of the quantal release process than does the Poisson model. Moreover, they describe a striking equivalence between the binomial n and the number of boutons. Apart from any differences between excitatory and inhibitory synapses, these differences may be due to the fact that the boutons *en passant* were never seen at inhibitory synapses labelled with HRP by Korn *et al.* (1982), whereas such boutons were the dominating feature of the frog sensory–motor connexion (see Grantyn *et al.* 1984). The average number of boutons per connexion in our material was also much higher. These features together with the tendency to form tight clusters of contacts may explain the differences in physical correlates for binomial n suggested for the sensory–motor synapse and for the inhibitory synapse on the Mauthner cell.

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REFERENCES

ADANINA, V. O. & SHAPOVALOV, A. I. (1983). Mixed synapses of primary afferent fibres on frog motoneurones identified by use of the neuroplasmic transport of horseradish peroxidase and electron microscopy *Dokl. Akad. Nauk SSSR* **269**, 231–232.

BALLANTER, B. I. (1977). *Probability Models in Physiology*. Moscow: Nauka.

BENNETT, M. R. & LAVIDIS, N. A. (1979). The effect of calcium ions on the secretion of quanta evoked by an impulse at nerve terminal release sites. *J. gen. Physiol.* **74**, 429–456.

BENNETT, M. R. & LAVIDIS, N. A. (1982). Variations in quantal secretion at different release sites along developing and mature motor terminal branches. *Dev. Brain Res.* **281**, 1–10.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol.* **124**, 560–573.

EDWARDS, E. R., REDMAN, S. J. & WALMSLEY, B. (1976*a*). Statistical fluctuations in charge transfer at Ia synapses on spinal motoneurones. *J. Physiol.* **259**, 665–688.

EDWARDS, E. R., REDMAN, S. J. & WALMSLEY, B. (1976*b*). Non-quantal fluctuations and transmission failures in charge transfer at Ia synapses on spinal motoneurones. *J. Physiol.* **259**, 689–704.

FLATMAN, J. A., ENGBERG, I. & LAMBERT, J. D. (1982). Reversibility of Ia EPSP investigated with intracellularly iontophorezed QX-222. *J. Neurophysiol.* **48**, 419–430.

GAGE, P. W. & MCBURNEY, R. N. (1973). An analysis of the relationship between the current and potential generated by a quantum of acetylcholine in muscle fibres without transverse tubules. *J. Membrane Biol.* **12**, 247–272.

GRANTYN, R., SHAPOVALOV, A. I. & SHIRIAEV, B. I. (1982). Combined morphological and electrophysiological description of connexions between single primary afferent fibres and individual motoneurones in the frog spinal cord. *Exp. Brain Res.* **48**, 459–462.

GRANTYN, R., SHAPOVALOV, A. I. & SHIRIAEV, B. I. (1984). Tracing of frog sensory–motor synapses by intracellular injection of horseradish peroxidase. *J. Physiol.* **349**, 441–458.

HIRST, G. D. S., REDMAN, S. J. & WONG, K. (1981). Post-tetanic potentiation and facilitation of synaptic potentials evoked in cat spinal motoneurones by impulses in group Ia fibres. *J. Physiol.* **321**, 97–109.

JACK, J. J. B., REDMAN, S. J. & WONG, K. (1981). The components of synaptic potentials evoked in cat spinal motoneurones by impulses in single group Ia afferents. *J. Physiol.* **321**, 65–96.

KATZ, B. & MILEDI, R. (1963). A study of spontaneous miniature potentials in spinal motoneurones. *J. Physiol.* **168**, 389–422.

KATZ, B. & THESLEFF, S. (1957). On the factors which determine the amplitude of the 'miniature end-plate potential'. *J. Physiol.* **137**, 267–278.

KERNELL, D. (1966). Input resistance, electrical excitability and size of ventral horn cells in cat spinal cord. *Science, N.Y.* **152**, 1637–1640.

KORN, H., MALLET, A., TRILLER, A. & FABER, D. S. (1982). Transmission at a central inhibitory synapse. II. Quantal description of release with a physical correlate for binomial *n*. *J. Neurophysiol.* **48**, 679–707.

KUNO, M. (1964). Quantal components of excitatory potentials in spinal motoneurones. *J. Physiol.* **175**, 81–99.

KUNO, M. & MIYAHARA, G. T. (1969). Analysis of synaptic efficacy in spinal motoneurones from 'quantum' aspects. *J. Physiol.* **201**, 479–493.

KUNO, M., TURKANIS, S. A. & WEAKLY, J. N. (1971). Correlation between nerve terminal size and transmitter release at the neuromuscular junction of the frog. *J. Physiol.* **213**, 545–556.

LÜSCHER, H. R., RUENZEL, P. & HENNEMAN, E. (1979). How the size of motoneurones determines their susceptibility to discharge. *Nature, Lond.* **282**, 859–861.

SHAPOVALOV, A. I. (1980). Interneuronal synapses with electrical, dual and chemical mode of transmission in vertebrates. *Neuroscience* **5**, 1113–1124.

SHAPOVALOV, A. I. & SHIRIAEV, B. I. (1979). Single-fibre EPSPs in amphibian motoneurones. *Brain Res.* **160**, 519–523.

SHAPOVALOV, A. I. & SHIRIAEV, B. I. (1980). Dual mode of junctional transmission at synapses between single primary afferent fibres and motoneurones in the amphibian. *J. Physiol.* **306**, 1–15.

SHAPOVALOV, A. I., SHIRIAEV, B. I. & TAMAROVA, Z. A. (1979). Synaptic activity in motoneurones of the immature cat spinal cord *in vitro*. Effects of manganese and tetrodotoxin. *Brain Res.* **160**, 524–528.

SHIRIAEV, B. I. (1983). Synaptic actions evoked in frog spinal motoneurones by adequate muscle spindle stimulation. *Sechenov Physiol. J. USSR* **69**, 1157–1163.

SOTELO, C. L. & GROFOVA, I. (1976). Ultrastructural features of the spinal cord. In *Frog Neurobiology*, ed. LLINAS, R. & PRECHT, W., pp. 707–727. Berlin: Springer.

TAMAROVA, Z. A., SHAPOVALOV, A. I. & SHIRIAEV, B. I. (1978). Action of magnesium and manganese ions and calcium deficiency on the synaptic transmission in the isolated perfused rat spinal cord. *Neirofiziologia* **10**, 530–533.

TAUGNER, R., SONNHOF, U., RICHTER, D. D. & SCHILLER, A. (1978). Mixed (chemical and electrical) synapses on frog spinal motoneurons. *Cell & Tissue Res.* **193**, 41–50.