Journal of Neurocytology **16**, 221–237 (1987)

Changes in the dimensions of release sites along terminal branches at amphibian neuromuscular synapses

MAX R. BENNETT, NICKOLAS A. LAVIDIS and FRANCES M. ARMSON

The Neurobiology Research Centre, University of Sydney, NSW 2006, Australia

Received 23 June 1986; revised 19 September 1986; accepted 6 October 1986

Summary

The probability of transmitter secretion from release sites declines along the length of most long terminal branches (> 78 μm) at toad (*Bufo marinus*) neuromuscular junctions; in contrast, few short terminal branches (< 78 μm) show such a decline. The present study was carried out to see if any of the dimensions of release sites change along the length of terminal branches in a way that can be correlated with the decrease in secretion probability. The size of presynaptic release site structures was determined by examining serial transverse sections through entire terminal branches with the transmission electron microscope; the size of postsynaptic release site structures was determined by examining terminal gutters with the scanning electron microscope after the removal of terminal branches. Long terminal branches showed a significant decrease in the length of their synaptic contact and cross-sectional area (terminal size) with distance from the origin of the branch. In contrast, there was no significant difference in the length of close apposition (< 0.2 μm) between the nerve terminal and postsynaptic muscle membrane; furthermore, neither the length of postsynaptic folds nor the frequency of the folds along the length of the terminal gutter changed. Short terminal branches showed no significant differences in the dimensions of either presynaptic or postsynaptic release site structures. The decline in the length of synaptic contacts whilst the length of close apposition remains relatively constant is due to the progressive encroachment of Schwann cell processes between presynaptic and postsynaptic membranes along the length of long terminal branches.

Introduction

Motor nerve terminals on amphibian fast-twitch fibres are composed of hundreds of release sites or active zones from which quanta of transmitter can be secreted on arrival of the nerve impulse (Couteaux & Pecot-Dechavassine, 1970; McMahan *et al.*, 1972; Dreyer *et al.*, 1973; Miller & Heuser, 1984). The quantal content of the endplate potential in low calcium concentrations does not fluctuate much during low frequency trains of impulses, so that it behaves as a binomial rather than a Poisson random variable (Bennett & Florin, 1974; Wernig, 1975; Bennett & Fisher, 1977). This occurs because some release sites have a relatively high probability for secretion and many have a very low probability (Bennett & Lavidis, 1979; see also Fig. 10 in De Cino, 1981). Furthermore, the spatial distribution of high and low probability release sites is not random along the length of terminal branches (Bennett & Lavidis, 1982). It is now possible to record quantal release with an extracellular electrode from living nerve terminals visualized using the fluorescent dye, 3-3 diethyloxardicarbocyanine iodide ($DiOC_2(5)$; Yoshikami & Okun, 1984); this technique shows that, in general, the probability of secretion is highest for release sites near the origins of long terminal branches and declines exponentially with a length constant of 54 μm for release sites further away (Bennett *et al.*, 1986a, b). Similar results have been obtained for the decline in the frequency of spontaneous transmitter secretion along terminal branches (Tremblay *et al.*, 1984) as well as for the decline of the probability of evoked secretion along the length of very long terminal branches (D'Alonzo & Grinnell, 1985).

The question arises as to whether there are any structural differences between nerve terminal release sites which may be correlated with their differences in secretion probability. Release sites may be identified in transverse sections of nerve terminals with the transmission electron microscope as regions of accumulation of synaptic vesicles in close apposition to an electron-dense presynaptic membrane overlying a muscle postsynaptic fold (Dreyer *et al.*, 1973). If the length of the synaptic contact at these release

0300-4864/87 $03.00 + .12

sites is taken as the length of close (< 0.2 μm) apposition, uninterrupted by Schwann cell, between presynaptic and postsynaptic membranes at the site, then contradictory claims have been made as to changes in the length of the synaptic contact at different positions along single terminal branches. Davey & Bennett (1982) found that the synaptic contact in the toad iliofibularis muscle tended to shorten for release sites along terminal branches more distal from the point of nerve entry; in contrast, Werle *et al.* (1984) failed to find any changes in synaptic contact along the length of terminal branches in the frog sartorius muscle. The difference may be due to the fact that Davey & Bennett (1982) first bathed their nerve–muscle preparation in horseradish peroxidase (HRP) for 1 h in Krebs solution and then stimulated the nerve at 1 Hz for 1 h in order to label the synaptic vesicles with HRP; this could change the dimensions of release sites.

Comparisons have been made between the average length of synaptic contacts at terminals which are known to secrete small numbers of quanta with those at terminals which secrete large numbers of quanta: terminals with a low average quantal secretion have synaptic contacts which are on average smaller than those at terminals with a high average quantal secretion (Fukunaga *et al.*, 1982; Herrera *et al.*, 1985a). Such observations lead to the expectation that the exponential decline in the probability of secretion along terminal branches should be correlated with a decrease in the length of synaptic contacts. This has been reinvestigated in the present work, which avoids possible changes to release site parameters by their prior stimulation, and makes quantitative comparisons between the changes in synaptic contact along the length of terminal branches and the changes in the probability of secretion observed by Bennett *et al.* (1986b).

Methods

PREPARATIONS

Simple *'en bushel'* terminals from the iliofibularis muscle of the toad (*Bufo marinus*) were studied. Mature toads weighing 70–100 g were anaesthetized with tricaine methanesulphonate (Rural Chem. Industries, Australia) and then pithed. Both muscles were dissected free from surrounding tissue and tendinous insertions. The muscle was pinned in a Perspex bath with a capacity of 3 ml and oriented with what had been the lateral surface upwards. The structural analysis was restricted to a small region of muscle distal to the point of nerve entry and away from the slow tonic muscle fibres (see Fig. 2 in Davey & Bennett, 1982). The bath was perfused at room temperature (18 ± 2° C) with a modified Ringer's solution of the following composition (mM): Na, 117; K, 3.0; Mg, 2.0; Cl, 103.9; $H_2PO_4^-$, 0.64; HPO_4^{2-}, 9.70; Ca, 0.2; glucose, 7.8. The solution was gassed continuously with 95% O_2 and 5% CO; this maintained the pH between 7.2 and 7.4.

LIGHT MICROSCOPY

The isolated muscle preparations were bathed for 30 s in 1.0 μM of the fluorescent dye $DiOC_2(5)$ (Yoshikami & Okun, 1984) and were then thoroughly washed with Ringer's solution. Endplates were chosen by viewing the fluorescent terminals on a video monitor attached via an image intensifier camera (Panasonic National) to an Olympus (BH2) fluorescent microscope.

Once a terminal or a set of terminals was chosen, the $DiOC_2(5)$-stained fluorescent terminals were photographed through a camera attached to the Olympus (BH2) microscope (see Fig. 1A, C, E). The pinned-out muscle was then fixed with 2.5% electron microscopic grade glutaraldehyde in Ringer's solution with the glucose removed, and stained for cholinesterase (see Fig. 1B, D, F) according to Karnovsky (1964). Both $DiOC_2(5)$ and cholinesterase staining was necessary to ensure that the terminals to be studied were associated with cholinesterase and to avoid cholinesterase deposits unaccompanied by nerve terminals (see Bennett *et al.*, 1986b). $DiOC_2(5)$, at a concentration 10 times that used in the present study, had no effect on the quantal content of the endplate potential after exposing the terminal to fluorescence for long periods (Bennett *et al.*, 1986b).

PREPARATION FOR TRANSMISSION ELECTRON MICROSCOPY

Small blocks containing the terminals of interest were teased and the muscle fibres cut transversely on one side about 100 μm from the cholinesterase-stained terminals. The block was then further fixed for 2 h in 2.5% glutaraldehyde in Ringer's solution free of glucose, and rinsed with several changes of the Ringer's solution. The block was then postfixed using osmium tetroxide (20 $g\,l^{-1}$ Ringer's solution, 2 h) stained with uranyl acetate (40 $g\,l^{-1}$, pH 5.0, 45 min) and dehydrated to 100% acetone. The tendon end of the muscle block was then fixed in a stick to enable transverse sectioning of the end of the block containing the end-plate region. The muscle fixed to the end of the stick was then left in Spurr's and acetone overnight and finally embedded in Spurr's (1969) resin.

SERIAL SECTIONING

Muscle blocks were sectioned using an LKBIV ultramicrotome equipped with an orientating specimen head. The holder was adjusted to give precisely transverse sections. Repeated cycles of 40 thick (0.5 μm) and 20 thin (90–160 nm) sections were obtained using glass and diamond knives, respectively. The two thick sections immediately prior to the thin sections were mounted on glass slides and stained for light microscopy with 1% toluidine blue in 1% borax. Thin sections were mounted on copper grids and stained with uranyl acetate (4 $g\,l^{-1}$, 7 min) and lead citrate (Reynolds, 1963) for electron microscopy. The total thickness of each cycle was recorded for analytical purposes.

TRANSMISSION ELECTRON MICROSCOPY

Camera lucida drawings of the thick sections were obtained for the purpose of muscle fibre and terminal identification using an Olympus microscope and a × 20 objective. Each

fibre was then given a number for the purpose of reconstruction. Thin sections were viewed using a Philips 300 transmission electron microscope. All terminals observed were photographed at an instrument magnification of × 15000. Photographic prints of these micrographs were produced by a further × 2 enlargement.

PREPARATION FOR SCANNING ELECTRON MICROSCOPY

Following staining of the muscle for cholinesterase the terminals were photographed and placed in 3% glutaraldehyde in Ringer's solution for 10 h. The muscle was then rinsed with several changes of the Ringer's solution and postfixed using osmium tetroxide ($20\,g\,l^{-1}$ in Ringer's solution) for 6 h. The muscle was then rinsed with several changes of distilled water and placed in warm (60° C) 8 M HCl for 25–70 min. During this time the preparation was gently shaken at intervals of 5–10 min, and was then rinsed with several changes of distilled water and dehydrated with 100% alcohol. Single muscle fibres were teased, after critical point drying, and placed on double-sided adhesive tape which was mounted on an aluminium stub; they were then spatter coated with a thin layer of platinum.

SCANNING ELECTRON MICROSCOPY

Muscle fibres mounted on platinum-coated aluminium stubs were viewed using a Jeol 35C scanning microscope. All terminal branches of interest were photographed at an instrument magnification of 4400. Synaptic gutters were photographed as montages.

THREE-DIMENSIONAL RECONSTRUCTION AND MORPHOMETRY

Micrographs from the transmission electron microscope

Reconstruction of endplate structure required the use of the thick-section camera lucida drawings and thin-section micrographs (× 1650). Once full reconstruction of interesting terminal branches was completed, the photomicrographs of terminals (microscope magnification of 15000 and final computer monitor magnification of 78000) were analysed. Analysis of endplate structure and endplate measurements such as terminal size, synaptic contact length, terminal apposition length, terminal height and terminal width were carried out with the assistance of an Apple IIe computer and an Apple mouse with a morphometry program (Magellan) written by Paul Halasz and Paul Martin.

Release sites have been identifed as regions of AChE accumulation with postsynaptic folds and accumulation of synapse vesicles at the presynaptic membrane. The following definition of release site parameters have been used, as indicated in Fig. 2F: synaptic contact length is the length of close apposition (less than 0.2 μm) between presynaptic and postsynaptic membranes which is not interposed by Schwann cell processes; the terminal apposition length is the length of close apposition between presynaptic and postsynaptic membranes whether there is interposing Schwann cell membrane or not; the terminal size is the cross-sectional area of the terminal at a release site; definitions of the remaining release site parameters are clearly defined in Fig. 2F.

The beginning of a terminal branch or its most proximal point is defined as that point which is closest to where the myelinated axon first contacts the muscle fibre; this is the case independent of whether the branch originates at this contact site or from another branch (for several examples see Bennett *et al.*, 1986b). Because of the way in which serial sections were collected (see above), the point of origin of a branch can be underestimated by up to 26 μm. It should be noted that this definition of the origin of a terminal branch implies that the distance along a short terminal branch near where the myelinated axon first contacts a muscle fibre is equivalent to that of a similar size branch located far from this contact point. However, only one of these latter kind of branches was included in the morphological study (see Fig. 1D) and none in a previous electrophysiological study (Bennett *et al.*, 1986b). It is therefore not possible to comment on whether the morphological properties of these branches or their transmitter release parameters change along their length.

Micrographs from the scanning electron microscope

Photomontages of micrographs of interesting synaptic gutters were constructed. Enlarged tracings of these montages were made from the negatives using a photographic enlarger (× 31680). Analysis of gutter width, fold length and fold frequency was carried out from these tracings with the assistance of the Apple IIe computer.

Results

Transmission electron microscopy

Motor nerve terminals in the toad iliofibularis muscle were chosen for ultrastructural study by staining with the fluorescent dye, $DiOC_2(5)$ (Fig. 1A, C, E). Once a group of neuromuscular junctions were seen by this method to contain suitably long terminal branches, they were stained for cholinesterase so as to enable their easy identification with the electron microscope (Fig. 1B, D, F). Release sites were identified in transverse serial sections through terminal branches as they possessed postsynaptic folds and a characteristic accumulation of synaptic vesicles in close apposition to the presynaptic membrane release-site density. Physiological studies have shown that the probability of quantal secretion varies in different ways along terminal branches, according to the length of the branch (Bennett *et al.*, 1986b). For short branches (less than 80 μm long) the probability of secretion remains constant for over 60% of the branches; for longer branches the probability declined approximately exponentially in 80% of cases, with a length constant of between 40 and 60 μm. In the present work, terminal branches have been divided into three categories of sizes, namely very short (less than 52 μm), short (less than 78 μm but greater than 52 μm) and long (greater than 78 μm).

Serial sections through a 1.2 to 2.8 μm sample of terminal were taken every 26 μm. From each of such samples the section with the greatest synaptic contact length was chosen for analysis.

Very short and short terminal branches

Eight short terminal branches were chosen for this analysis. These branches showed no significant differences in release site dimensions from the point of branch origin to the end (Fig. 2D, E). Table 1 shows the average size of each of the parameters measured, together with values that have been normalized to the size of the particular release site parameter at the beginning of a branch. Student's *t*-tests showed there was no significant change in the normalized values of synaptic contact length or terminal apposition length along the terminal branches, although the size of the terminal did decline near the ends of the branches (Table 1).

Long terminal branches

Seven long terminal branches were chosen for this analysis. The synaptic contact length declined continually along the branch from its origin in a highly significant way for these branches (Table 2). The branches could, however, be divided into two groups: all the branches in one of these groups possessed synaptic contact lengths which declined progressively along the branch from their point of origin (71% of branches belonged in this group, Table 2B; Fig. 2A–C); in the other group, which was less frequently observed (29% of branches, Table 2A), the synaptic contact length remained approximately constant until the very end of the terminal branch was reached. Fig. 3 shows that if the synaptic contact lengths along the terminal branch are normalized to their size at the origins of the terminal, then some synaptic contacts do not decline until about 60 μm beyond the terminal origin (Fig. 3B), whereas others decline within 25 μm of the terminal origin (Fig. 3A). As the probability of secretion decreases with distance along the length of a majority of long terminal branches, and this decline can be well fitted by an exponential with a length constant between 40 and 60 μm (Bennett *et al.*, 1986b), it was of interest to see if similar exponentials could be used to describe the synaptic contact length along the five terminal branches which showed a decline in this parameter (Table 2B). An exponential curve fitted to these results gave a length constant of 63 μm (Fig. 3A).

The terminal apposition length did not decline significantly for branches until the very end of the branch was reached (Table 2). It follows then that the ratio of synaptic contact length to terminal apposition length declined for that category of terminal branches that showed a decline in their synaptic contact length (Fig. 3A, C). For these branches the terminal size declined significantly from the point of branch origin (Table 2; Fig. 3D), as has been shown previously (Davey & Bennett, 1982).

Scanning electron microscopy

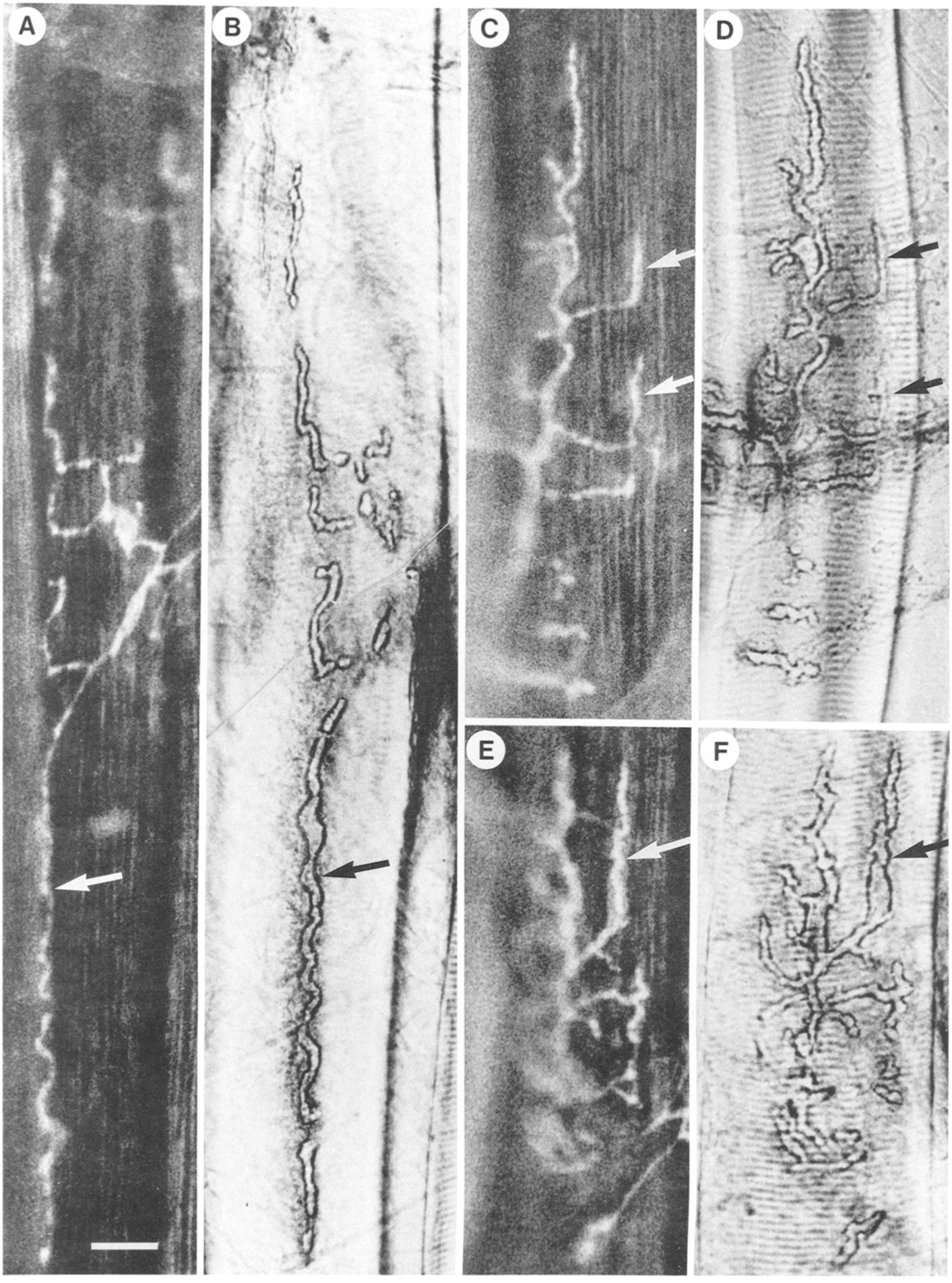
The dimensions of the postsynaptic gutter and folds along the length of terminal branches were determined, after removal of the branches, with the scanning electron microscope. These were obtained for both short (four terminals less than 78 μm) and long (two terminals greater than 78 μm) terminal branches (Fig. 4). There was no significant change in the width of the terminal gutter or the length of the postsynaptic folds until the very end (10–20 μm) of the gutter was reached (Table 3). Both short and long gutters, associated with short and long branches, showed a relative constancy of postsynaptic fold length until the last 10–20 μm of the gutters was reached (Fig. 5). The frequency of postsynaptic folds also remained constant at about one per micrometre along the length of the terminal gutters, as did the length of postsynaptic fold per 10 μm length of gutter (Table 3).

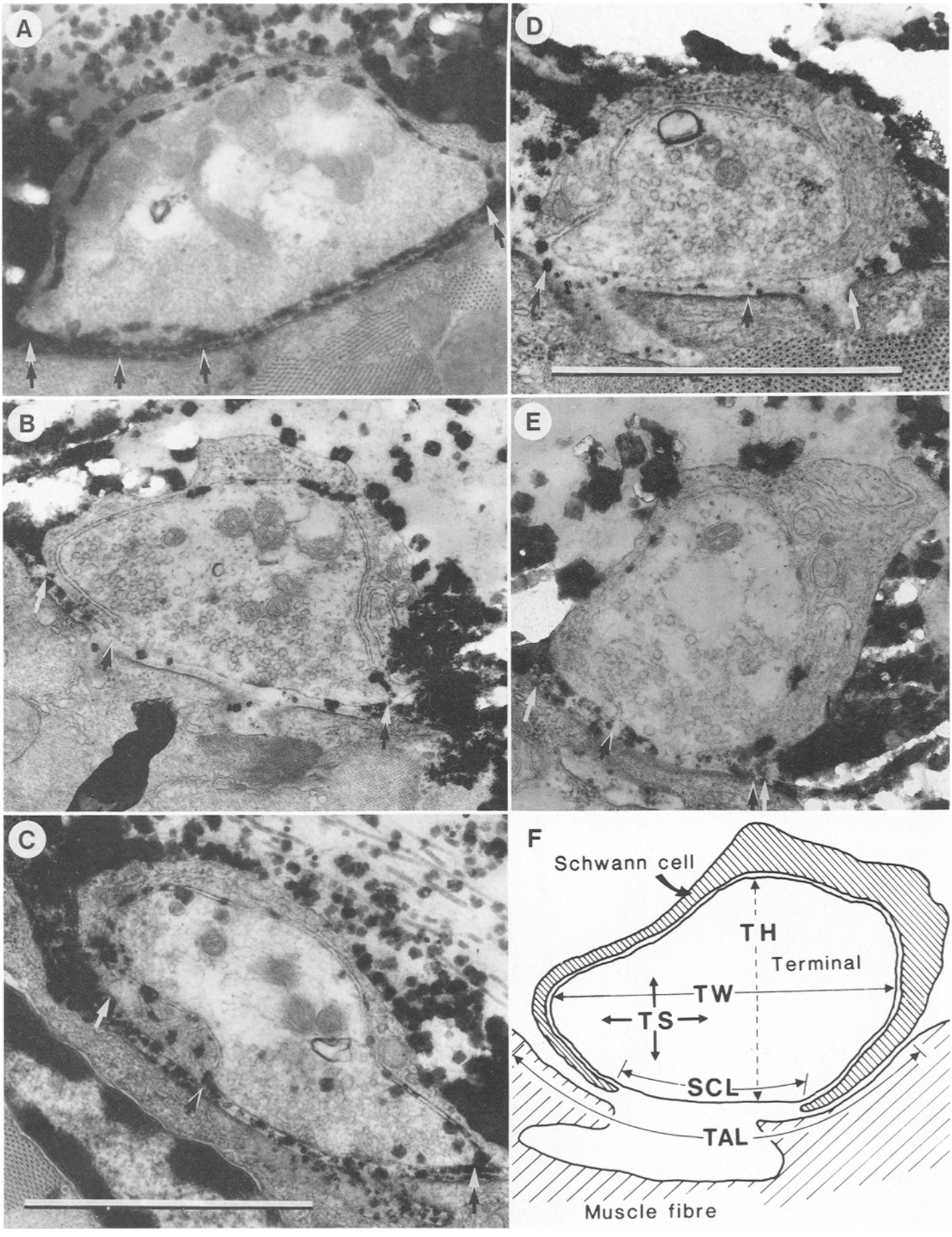
Discussion

Changes in active zones along the length of terminal branches

Active zones on twitch fibres consist of two double rows of 10 nm intramembranous particles at release sites; these are flanked by vesicles which may secrete their transmitter on arrival of the nerve impulse (Heuser *et al.*, 1974; Miller & Heuser, 1984). It is speculated that these intramembranous particles are calcium channels (Llinas, 1982). During development and reinnervation of amphibian fast-twitch fibres only short segments of active zone occur opposite the postsynaptic fold and scattered over the presynaptic membrane; these segments eventually double in length, join and form a single active zone opposite the postsynaptic fold (Ko, 1984, 1985) Quantal release can occur from vesicles flanking these short segments

Fig. 1. (A, C, E) $DiOC_2(5)$-stained fluorescent nerve terminals. (B, D, F) The same nerve terminals stained for cholinesterase (Karnovsky, 1964). Arrows in (A) and (B) show a terminal with a long terminal branch; in (C) and (D) show a terminal which has two very short terminal branches; in (E) and (F) show a medium length terminal branch. Endplates were identified in this way before being prepared for electron microscopy. Scale bar: 20 μm.


A
B
C
D
E
F


A
D
B
E
C
F
Schwann cell
TH
Terminal
TW
TS
SCL
TAL
Muscle fibre

of active zone in developing and reinnervated fast-twitch fibres as well as in the terminals of twitch and tonic fibres in mature lizard muscles which only possess scattered short segments of active zone (Walrond & Reese, 1985). In frog twitch fibres there is an apparent correlation between the quantal release per unit length of terminal branching in a muscle and the total length of active zone per 100 μm of terminal branching (Propst & Ko, 1985). Terminals in the cutaneous pectoris muscle, which on average release more quanta per unit length of terminal than do those in the cutaneous dorsi, also possess active zones which are on average longer than those in the cutaneous dorsi (Ko *et al.*, 1985; Herrera *et al.*, 1985b). Patients with Lambert–Eaton Myasthenic Syndrome, in which there is reduced quantal secretion, possess neuromuscular junctions with a marked decrease in active zones and in intramembranous particles of active zones (Fukunaga *et al.*, 1982). Taken together, these observations suggest that the probability of secretion of a quanta is in part determined by the length of active zones, which may vary substantially under different experimental conditions.

The question arises, then, as to whether active zones are continuous at release sites along the length of terminal branches on amphibian twitch fibres? Pumplin (1983) has shown that 87% of active zones along the length of terminal branches in amphibian twitch fibres have the usual appearance of two double rows of intramembranous particles; these only become dispersed in the last few active zones along the length of terminal branches. It therefore seems unlikely that progressive disruption of the active zone occurs along the length of terminal branches so as to provide a basis for the decrease in secretion probability.

The possibility exists that the length of active zones or their frequency changes along terminal branches. Unfortunately there has been no direct study of this problem, which requires freeze-fractures to be taken at regular intervals along individual terminal branches. It is not possible to use estimates of release site length, terminal apposition length or postsynaptic fold length along terminal branches as a measure of active zone length, as it has not been shown that there is any fixed relationship between these parameters.

Changes in synaptic contact length along terminal branches

In a previous study (Bennett *et al.*, 1986b), 60% of short terminal branches showed no decline in transmitter release along their length, while 40% showed an exponential decline. In contrast, 20% of long terminal branches showed no decline in transmitter release along the length of the branches, whereas 80% showed an exponential decline. In the present study 75% of short terminal branches showed no decline in synaptic contact length (Table 1), whereas 70% of the long terminal branches showed an exponential decline in synaptic contact length (Table 2). There is then reasonable agreement between the morphological and physiological observations, suggesting that the former give rise to the latter.

Similar results to those in the present study were obtained by Davey & Bennett (1982) (Fig. 6A), who showed that a significant decline in synaptic contact length occurs along terminal branches greater than 80 μm long in both mature and immature iliofibularis muscles of the toad (*Bufo marinus*) (see their Table 1). In contrast, Werle *et al.* (1984) failed to find any change in synaptic contact length along terminal branches in the frog (*Rana pipiens*) sartorius. It is not clear whether the methodological procedures used may provide an explanation for these differences. In the present work, 20 sections (sample size 1.2 to 2.8 μm) were taken every 26 μm along 20 terminal branches and, of these, 5 had either very small side branches or were associated with irregular cholinesterase rings which made them unsuitable for further analysis. When sections passed through a release site, the maximum length of the synaptic contact observed in any section was determined, not the mean of contact lengths observed in all the sections; this was carried out because the largest contact length observed is likely to indicate most faithfully the full extent of the active zone, rather than just giving an oblique view.

More recently Herrera *et al.* (1985a) have shown that the average length of the synaptic contact in the cutaneous pectoris muscle is 33% higher than that in the sartorius muscle, reflecting the higher quantal release per unit length of terminal in the cutaneous pectoris muscle. This laboratory has also shown that the average synaptic contact length increases in limb

Fig. 2. Serial sections through a long terminal branch (> 78 μm; A–C) and a short terminal branch (< 52 μm; D, E) viewed with the transmission electron microscope at × 16 500. Each section is labelled with white arrows which indicate terminal apposition length, and black arrows or black arrows with white highlight for synaptic contact length. (F) Diagram of a transverse section through a terminal branch, showing the definition of terms used to characterize the release site dimensions. The following are indicated: SCL, synaptic contact length; TAL, terminal apposition length; TS, terminal size; TH, terminal height; TW, terminal width. In both branches the synaptic contact length decreases continually from the point of the first close apposition with the muscle. (A–C) Branch 44T1 in Table 2, indicated by open triangles in Fig. 3A; (D, E) branch 583 in Table 1. Scale bars: (A–C), shown in C, 3 μm; (D, E) shown in D, 3 μm.

Table 1. Release site dimensions of very short terminal branches (A, less than 52 μm long but greater than 26 μm) and short terminal branches (B, less than 78 μm long but greater than 52 μm). Each parameter is given for three distances along the terminal branch: 0, 26 and 52 μm (see footnote).

		Synaptic contact length (μm)			*Terminal apposition length (μm)*			*Synaptic contact length/apposition length ratio*			*Terminal size (μm^2)*			*Terminal height (μm)*			*Terminal width (μm)*			*Terminal height/ terminal width ratio*		
		0	26	52	0	26	52	0	26	52	0	26	52	0	26	52	0	26	52	0	26	52
A	583	2.5	1.8		2.5	2.5		1.0	0.72		2.8	2.1		1.6	1.8		1.9	1.5		0.84	1.2	
		1.0	0.72		1.0	1.0		1.0	0.72		1.0	0.75		1.0	1.12		1.0	0.79		1.0	1.4	
	586	1.9	1.8		2.5	2.1		0.76	0.86		3.0	1.3		1.9	1.9		2.5	1.6		0.76	1.2	
		1.0	0.95		1.0	0.84		1.0	1.13		1.0	0.43		1.0	1.0		1.0	0.64		1.0	1.6	
	562	2.6	1.9		2.5	2.3		1.04	0.83		2.9	2.3		1.6	1.8		2.2	1.7		0.72	1.1	
		1.0	0.73		1.0			1.0	0.92		1.0	0.80		1.0	1.12		1.0	0.77		1.0	1.5	
B	525	2.1	2.7	1.2	2.4	2.7	2.6	0.88	1.0	0.46	3.1	2.6	2.3	1.9	1.5	1.2	1.9	2.1	2.1	1.0	0.71	0.57
		1.0	1.3	0.57	1.0	1.12	1.10	1.0	1.14	0.52	1.0	0.84	0.74	1.0	0.79	0.63	1.0	1.1	1.1	1.0	0.71	0.57
	526	2.4	2.6	3.4	3.2	3.2	3.4	0.75	0.81	1.0	3.9	5.0	3.0	1.9	1.8	1.4	2.4	2.8	2.8	0.79	0.64	0.50
		1.0	1.1	1.4	1.0	1.0	1.06	1.0	1.08	1.37	1.0	1.3	0.77	1.0	0.95	0.74	1.0	1.17	1.17	1.0	0.81	0.63
	585M	1.5	1.9	0.8	2.4	2.4	1.2	0.63	0.79	0.69	2.5	2.8	0.4	1.7	1.6	0.61	1.8	2.2	0.9	0.94	0.73	0.68
		1.0	1.27	0.53	1.0	1.0	0.50	1.0	0.25	1.06	1.0	1.1	0.16	1.0	0.94	0.36	1.0	1.22	0.50	1.0	0.78	0.72
	372	1.0	1.5	0.5	2.6	1.7	1.0	0.39	0.88	0.50	2.5	1.1	1.5	1.2	1.2	1.6	2.3	1.8	1.1	0.52	0.67	1.5
		1.0	1.5	0.5	1.0	0.65	0.38	1.0	2.26	1.28	1.0	0.64	0.62	1.0	1.0	1.3	1.0	0.78	0.48	1.0	1.3	2.9
	56T4	2.5	1.2	0.2	2.5	1.3	1.5	1.0	0.93	0.13	3.5	0.8	1.4	2.5	0.8	1.1	2.3	1.2	1.5	1.1	0.7	0.7
		1.0	0.48	0.08	1.0	0.52	0.60	1.0	0.93	0.13	1.0	0.23	0.40	1.0	0.32	0.44	1.0	0.52	0.65	1.0	0.64	0.64
Mean (±S.E.M.) of absolute values for series B		1.9 ±0.28	1.98 ±0.30	1.22 ±0.57	2.62 ±0.15	2.26 ±0.34	1.94 ±0.46	0.73 ±0.11	0.88 ±0.04	0.55 ±0.14	3.1 ±0.28	2.5 ±0.75	1.7 ±0.44	1.8 ±0.21	1.4 ±0.17	1.18 ±0.17	2.1 ±0.12	2.0 ±0.26	1.7 ±0.35	0.87 ±0.10	0.70 ±0.02	0.79 ±0.18
Mean (± S.E.M.) of normalized values for series B		1.0	1.13 ±0.17	0.62 ±0.22	1.0	0.86 ±0.11	0.73 ±0.15	1.0	1.33 ±0.24	0.86 ±0.23	1.0	0.82 ±0.19	0.54 ±0.12	1.0	0.80 ±0.13	0.69 ±0.17	1.0	0.96 ±0.13	0.78 ±0.15	1.0	0.85 ±0.12	1.10 ±0.45
P for normalized values of series B (different from 1.0)			>0.25	>0.10		>0.25	>0.05		>0.10	>0.25		>0.25	<0.01		>0.10	>0.10		>0.25	>0.10		>0.10	>0.25
P' for regression coefficient of normalized values of series B (different from 0)			>0.10			>0.10			>0.25			<0.02			>0.10			>0.10			>0.25	

The size of each parameter is given together with values normalized to the parameter size found at the beginning of the terminal branches in close apposition to the muscle membrane (at 0 μm). In row *P* are given the results of Student's *t*-test for significant differences between the normalized values at 0 μm and those at other distances for the B subset of branches; only the terminal size at 52 μm declined significantly along the terminal branch according to this test of significance. In row *P'* are given the results of Student's *t*-test for significant differences between the regression coefficients of linear regressions fitted to the results for each branch and zero; according to this criteria only the regression coefficients for terminal size are significantly smaller than zero.

Table 2. Release site dimensions of long terminal branches (less than 104 μm long but greater than 78 μm). Each parameter is given for four distances along the terminal branch: 0, 26, 52 and 78 μm (see footnote).

		Synaptic contact length (μm)				*Terminal apposition length (μm)*				*Synaptic contact length/ apposition length ratio*			
		0	26	52	78	0	26	52	78	0	26	52	78
A	58T5	2.2	2.6	2.2	0.8	3.2	3.2	2.5	0.9	0.69	0.88	0.88	0.88
		1.0	1.20	1.0	0.36	1.0	1.0	0.78	0.28	1.0	1.27	1.27	1.27
	58T1	1.4	1.5	1.4	1.2	1.5	2.2	1.9	1.2	0.93	0.68	0.74	0.63
		1.0	1.07	1.0	0.86	1.0	1.47	1.27	0.8	1.0	0.73	0.80	0.68
B	39T1	3.4	1.5		1.5	3.5	1.5	1.9	2.1	0.97	0.98		0.71
		1.0	0.44		0.44	1.0	0.43	0.54	0.60	1.0	1.01		0.73
	39T4	2.8	2.2	1.1	0.1	2.9	3.1	3.1	1.2	0.97	0.71	0.35	0.09
		1.0	0.79	0.39	0.04	1.0	1.07	1.07	0.41	1.0	0.73	0.36	0.09
	56T5	2.7	2.0	1.9	1.2	2.7	2.2	2.7	2.5	1.0	0.91	0.71	0.48
		1.0	0.74	0.71	0.44	1.0	0.81	1.0	0.93	1.0	0.91	0.71	0.48
	56T1	1.4	1.3	1.0	0.5	1.7	1.8	1.7	0.8	0.82	0.72	0.59	0.63
		1.0	0.92	0.71	0.35	1.0	1.1	1.0	0.47	1.0	0.88	0.72	0.77
	44T1	4.2	2.4	2.6	2.3	3.8	2.5	3.1	3.8	1.0	0.96	0.84	0.55
		1.0	0.57	0.62	0.55	1.0	0.66	0.81	1.0	1.0	0.96	0.84	0.55
Mean (±S.E.M.) of absolute values		2.59 ±0.39	1.93 ±0.19	1.70 ±0.26	1.09 ±0.27	2.76 ±0.33	2.36 ±0.24	2.41 ±0.22	1.79 ±0.41	0.91 ±0.04	0.83 ±0.05	0.69 ±0.08	0.57 ±0.09
Mean (±S.E.M.) of normalized values		1.0	0.82 ±0.10	0.74 ±0.10	0.43 ±0.09	1.0	0.93 ±0.13	0.92 ±0.09	0.64 ±0.10	1.0	0.93 ±0.07	0.78 ±0.12	0.65 ±0.13
P for normalized values (different from 1.0)			>0.05	<0.05	<0.001		>0.25	>0.25	<0.01		>0.25	>0.05	<0.05
P' for regression coefficient of normalized values (different from 0)			<0.002				<0.05				<0.05		

		Terminal size (μm²)				*Terminal height (μm)*				*Terminal width (μm)*				*Terminal height/terminal width ratio*			
		0	26	52	78	0	26	52	78	0	26	52	78	0	26	52	78
A	58T5	6.5	6.3	1.9	0.4	2.9	2.9	1.2	1.3	2.7	2.6	2.1	2.4	1.07	1.11	0.6	0.5
		1.0	0.97	0.29	0.05	1.0	1.0	0.41	0.45	1.0	0.96	0.78	0.89	1.0	1.04	0.53	0.50
	58T1	1.8	2.0	2.8	0.9	1.7	1.7	2.2	1.2	1.2	1.3	1.2	1.1	1.4	1.3	1.8	1.1
		1.0	1.1	1.56	0.50	1.0	1.0	1.3	1.3	1.0	1.08	1.0	0.92	1.0	0.93	1.30	0.78
B	39T1	3.1	2.1	1.8	1.5	1.4	1.1	1.0	1.1	2.9	1.5	2.1	1.8	0.5	0.7	0.5	0.6
		1.0	0.68	0.58	0.48	1.0	0.79	0.71	0.79	1.0	0.52	0.72	0.62	1.0	1.5	1.0	1.3
	39T4	3.8	2.9	2.1	0.96	1.9	1.5	1.6	0.8	2.5	2.2	1.7	1.2	0.8	0.7	0.9	0.7
		1.0	0.76	0.55	0.25	1.0	0.79	0.84	0.42	1.0	0.88	0.68	0.48	1.0	0.89	1.23	0.88
	56T5	3.1	1.9	2.5	2.5	1.3	1.2	1.5	1.8	2.2	1.9	2.0	1.7	0.6	0.6	0.8	1.1
		1.0	0.61	0.81	0.81	1.0	0.92	1.15	1.38	1.0	0.86	0.91	0.77	1.0	1.07	1.27	1.78
	56T1	2.0	1.8	2.2	1.25	1.4	1.3	1.3	1.9	1.6	1.9	2.3	1.9	0.9	0.7	1.0	1.0
		1.0	0.90	1.1	0.63	1.0	0.93	1.57	1.36	1.0	1.19	1.44	1.19	1.0	0.77	1.10	1.14
	44T1	5.8	3.8	3.5	3.1	1.9	2.2	1.8	1.3	3.2	1.9	2.5	2.7	0.6	1.2	0.7	0.5
		1.0	0.66	0.60	0.53	1.0	1.16	0.95	0.68	1.0	0.59	0.78	0.84	1.0	1.97	1.22	0.81
Mean (±S.E.M.) of absolute values		3.73 ±0.68	2.97 ±0.62	2.40 ±0.22	1.52 ±0.36	1.79 ±0.21	1.70 ±0.25	1.51 ±0.15	1.34 ±0.15	2.33 ±0.27	1.90 ±0.16	1.99 ±0.16	1.82 ±0.22	0.84 ±0.12	0.90 ±0.11	0.90 ±0.16	0.79 ±0.10
Mean (±S.E.M.) of normalized values		1.0	0.81 ±0.07	0.78 ±0.16	0.46 ±0.09	1.0	0.94 ±0.05	0.99 ±0.15	0.91 ±0.16	1.0	0.87 ±0.09	0.90 ±0.10	0.82 ±0.09	1.0	1.17 ±0.16	1.09 ±0.10	1.03 ±0.16
P for normalized values (different from 1.0)			<0.02	>0.10	<0.001		>0.25	>0.25	>0.25		>0.10	>0.25	>0.05		>0.25	>0.25	>0.25
P' for regression coefficient of normalized values (different from 0)			<0.01				>0.25				>0.10				>0.25		

The size of each parameter is given together with values normalized to the parameter size found at the beginning of the terminal branches in close apposition to the muscle membrane (at 0 μm). In row *P* are given the results of Student's *t*-test for significant differences between the normalized values at 0 μm and those at other distances; both the synaptic contact length and terminal size decline significantly according to this criteria. In row *P'* are given the results of Student's *t*-test for significant differences between the regression coefficients of linear regressions fitted to the results for each branch and zero; the regression coefficients for synaptic contact length and terminal size are very significantly different from zero according to this test of significance. Note that the seven terminal branches have been subdivided into two subsets: (A) in which synaptic contact length did not decline along the branch until the very end; (B) in which the length declined continually.

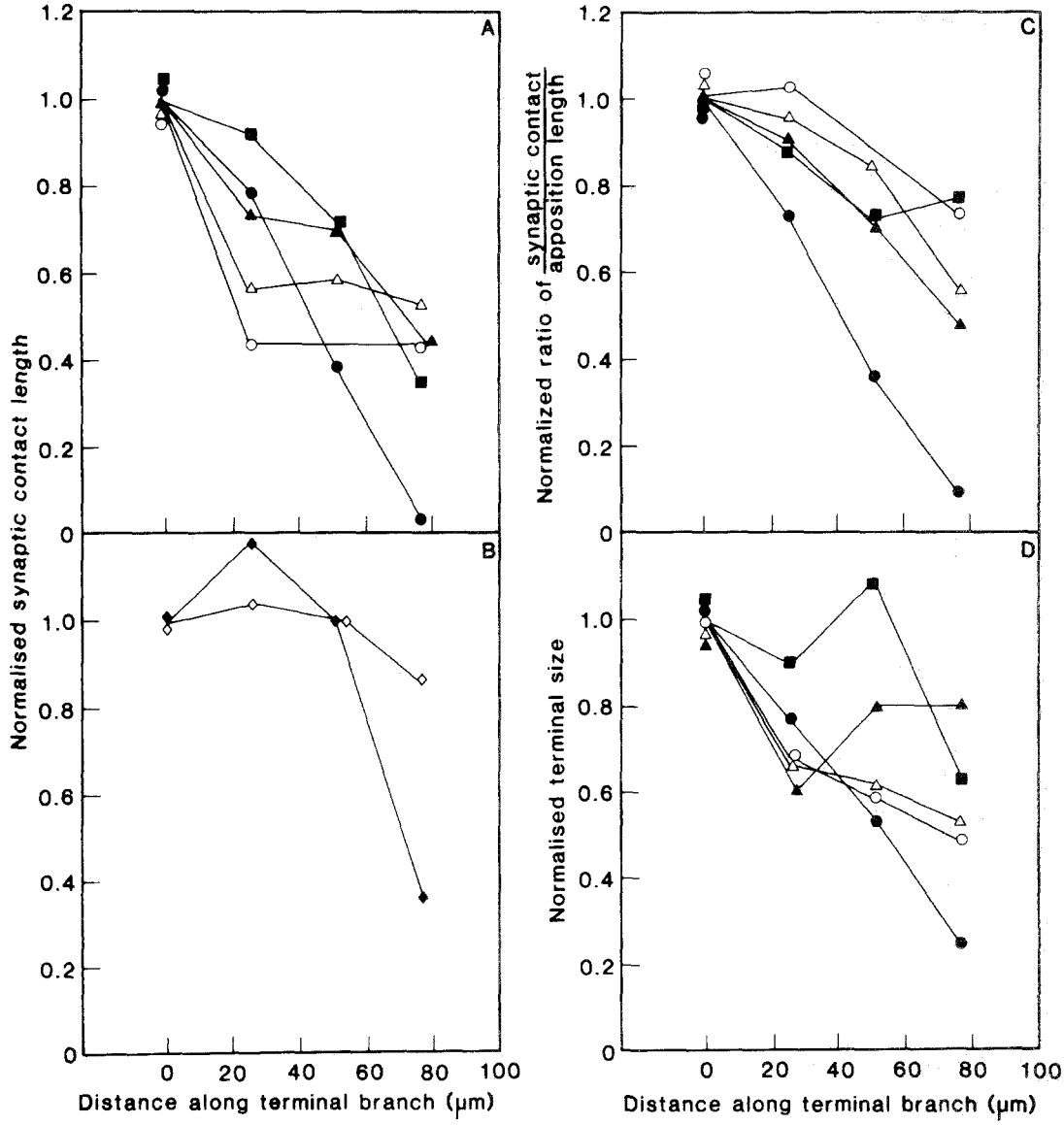


Fig. 3. Release site parameters for long (> 78 μm) terminal branches. (A, B) Changes in the normalized contact length with distance along terminal branches; the release site lengths have been normalized to the value at the beginning of the terminal branch. Of the seven terminal branches analysed in detail, five showed a continual decline in synaptic contact length with distance (A), and the remaining two showed no decline until the end of the branch (B); an exponential curve fitted to the results in (A) had a length constant of 62 μm and a correlation coefficient (r^2) of 0.44. (C) Decline in the normalized ratio of synaptic contact length to terminal apposition length with distance along terminal branches; the ratio of synaptic contact length to apposition length has been normalized to that at the beginning of the terminal branch. Only terminal branches which show a continual decline in synaptic contact length have been included (see A and Table 2, subset B). (D) Decline in the terminal size (cross-sectional area) of terminals at release sites with distance along terminal branches; this area has been normalized to that found at the beginning of the terminal branch.

Fig. 4. Terminal branch gutters viewed with the scanning electron microscope after removal of the nerve terminal. (A–C) and (D–F) represent high-power insets at intervals along the terminal branch gutter in (G) and (H), respectively. (G) Long terminal gutter (> 78 μm) showing a relatively uniform distribution of postsynaptic folds along the length. (H) Short terminal gutter (< 78 μm), with a similar distribution of postsynaptic folds. White arrows in (G) and (H) indicate where samples (A–F) are taken from. Scale bars: (A–F), shown in F, 5 μm; (G, H), shown in H, 5 μm.


A
B
C
D
E
F
G
a
b
c
H
d
e
f

Table 3. Dimensions of the gutter and postsynaptic folds of both short terminal branches (subset A, less than 78 μm but greater than 52 μm) and long terminal branches (subset B, less than 104 μm but greater than 78 μm). Each parameter is given for eight distances along the terminal branch: 10, 20, 30, 40, 50, 60, 70 and 80 μm (see footnote).

		Average width of terminal gutter (μm)								*Average length of postsynaptic fold (μm)*							
		10	20	30	40	50	60	70	80	10	20	30	40	50	60	70	80
A	2434	4.2	4.2	3.25	3.25	3.50	3.3			2.89	2.89	2.97	2.92	2.70	2.22		
		1.0	1.0	0.77	0.77	0.83	0.79			1.0	1.0	1.03	1.01	0.93	0.77		
	2718	2.7	2.5	1.95	2.05	2.35	1.9			1.51	1.39	1.41	1.39	1.00	0.40		
		1.0	0.93	0.72	0.76	0.87	0.70			1.0	0.92	0.93	0.92	0.66	0.26		
	2712A	2.45	2.65	2.10	2.30	2.50	1.40			1.88	2.03	2.27	1.78	1.89	1.06		
		1.0	1.08	0.86	0.94	1.02	0.57			1.0	1.08	1.21	0.95	1.01	0.56		
	1147	2.3	2.75	2.8	3.0	2.65	2.10			1.28	1.85	1.62	1.73	1.37	1.11		
		1.0	1.17	1.21	1.30	1.15	0.91			1.0	1.45	1.27	1.35	1.07	0.87		
B	2712	2.4	3.05	3.10	2.9	2.9	2.8	1.7	1.0	2.16	2.60	2.63	1.99	2.10	1.97	1.32	0.41
		1.0	1.27	1.29	1.21	1.21	1.17	0.71	0.42	1.0	1.20	1.22	0.92	0.97	0.91	0.61	0.19
	2569	4.7	4.25	5.25	4.45	4.70	4.15	4.85	3.0	2.44	2.42	3.25	3.30	2.13	2.68	2.36	2.18
		1.0	0.90	1.12	0.95	1.0	0.88	1.03	0.64	1.0	0.99	1.33	1.35	0.87	1.10	0.97	0.89
Mean (±S.E.M.) of absolute values of series A		2.91 ±0.44	3.03 ±0.40	2.53 ±0.30	2.65 ±0.28	2.75 ±0.26	2.18 ±0.40			1.89 ±0.36	2.04 ±0.31	2.07 ±0.35	1.96 ±0.33	1.74 ±0.37	1.20 ±0.38		
Mean (±S.E.M.) of normalized values of series A		1.0	1.05 ±0.05	0.89 ±0.11	0.94 ±0.12	0.97 ±0.07	0.74 ±0.07			1.0	1.11 ±0.12	1.11 ±0.08	1.06 ±0.10	0.92 ±0.09	0.62 ±0.13		
P for normalized values of series A (different from 1.0)			>0.25	>0.25	>0.25	>0.25	<0.02				>0.25	>0.10	>0.25	>0.25	<0.05		
P' for regression coefficient of normalized values of series A (different from 0)					<0.05								<0.05				

		Frequency of postsynaptic folds per 10 μm								*Length of postsynaptic folds per 10 μm*							
		10	20	30	40	50	60	70	80	10	20	30	40	50	60	70	80
A	2434	10	5	13	17	15	12			29	15	40	50	41	27		
		1.0	0.5	1.3	1.7	1.5	1.2			1.0	0.52	1.38	1.72	1.41	0.93		
	2718	20	17	16	16	20	8			30	24	22	22	20	3		
		1.0	0.85	0.80	0.80	1.0	0.40			1.0	0.80	0.73	0.73	0.67	0.10		
	2712A	12	12	11	13	11	5			23	24	25	23	21	5		
		1.0	1.0	0.92	1.1	0.92	0.42			1.0	1.0	1.1	1.0	0.91	0.22		
	1147	10	18	10	15	16	13			13	34	16	26	22	15		
		1.0	1.8	1.0	1.5	1.6	1.3			1.0	2.6	1.2	2.0	1.7	1.15		
B	2712	12	15	10	11	11	12	13	11	26	39	26	22	23	24	17	5
		1.0	1.25	0.83	0.92	0.92	1.0	1.08	1.08	1.0	1.5	1.0	0.85	0.88	0.92	0.65	0.02
	2569	11	10	8	8	7	8	6	4	27	24	26	26	15	21	14	9
		1.0	0.91	0.73	0.73	0.64	0.73	0.55	0.36	1.0	0.89	0.96	0.96	0.56	0.78	0.52	0.33
Mean (S.E.M.) of absolute values of series A		13 ±2.4	13 ±3.0	12.5 ±1.3	15.3 ±0.85	15.5 ±1.8	9.5 ±1.8			23.7 ±3.9	24.3 ±3.9	25.8 ±5.1	30.3 ±6.6	26.0 ±5.0	25.0 ±1.1		
Mean (±S.E.M.) of normalized values of series A		1.0	1.04 ±0.28	1.00 ±0.11	1.28 ±0.20	1.2 ±0.	0.83 ±0.24				1.23 ±0.47	1.10 ±0.14	1.36 ±0.29	1.17 ±0.23	0.60 ±0.26		
P for normalized values of series A (different from 1.0)			>0.25	>0.25		>0	>0.25				>0.25	>0.25	>0.25	>0.25	>0.10		
P' for regression coefficient of normalized values of series A (different from 0)					>0.25								>0.25				

The size of each parameter is given together with values normalized to the parameter size found at the beginning of the terminal gutter (at 10 μm). In row *P* are given the results of Student's *t*-test for significant differences between the normalized values at 10 μm and those at other distances for the A subset of terminal gutters; only the length of the postsynaptic fold and the width of the gutter decline significantly at the very end of the gutter according to these criteria. In row *P'* are given the results of Student's *t*-test for significant differences between the regression coefficients of linear regressions fitted to the results for each branch and zero; according to these criteria only the regression coefficients for the length of the postsynaptic fold and width of the gutter are significantly smaller than zero.

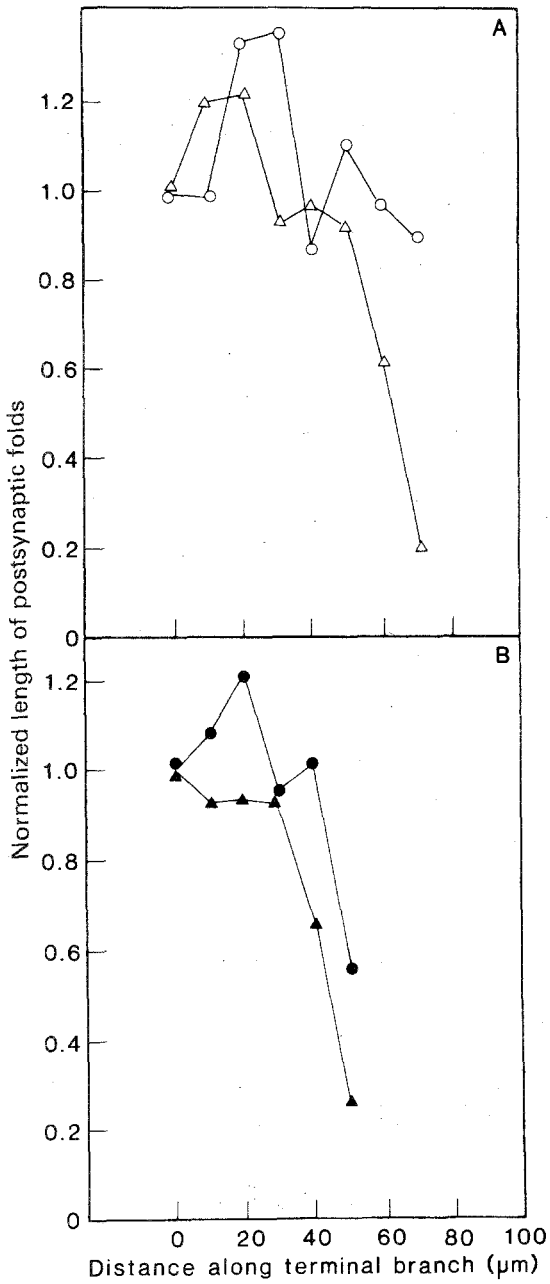


Fig. 5. Changes in the average length of postsynaptic folds, in successive 10-μm samples, for long terminal branches (A, greater than 78 μm but less than 104 μm) and short terminal branches (B, greater than 52 μm but less than 78 μm) along the length of terminal gutters. The length of the postsynaptic folds has been normalized to that found in the first 10-μm sample (beginning of the terminal branch). Note that the fold length does not decrease significantly until the end of the gutter has been reached.

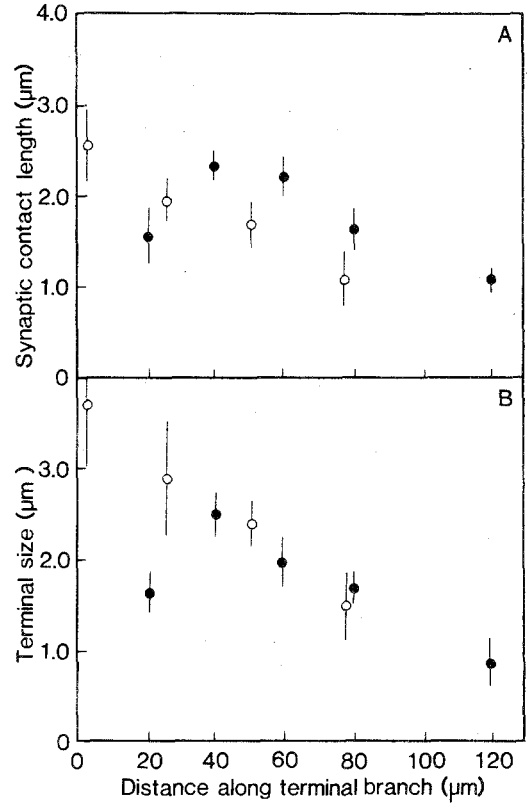


Fig. 6. Comparison between the release site parameters measured in the present study (based on sections through release sites which showed the longest synaptic contacts) and that in Davey & Bennett (1982) (based on sections through release sites identified as having the greatest concentration of horseradish peroxidase-labelled vesicles). (A) Synaptic contact length; (B) terminal size. The open circles are the mean (± S.E.M.) values given in Table 2 for long terminal branches; the filled circles are for the mature terminal branches given in Tables 1 and 2 in Davey & Bennett (1982). There is no significant difference in the changes in synaptic contact length or terminal size using the two different criteria beyond 20 μm from the beginning of branches.

muscles contralateral to a nerve crush, in which the quantal release per unit length of terminal is elevated compared with controls (Herrera *et al.*, 1985b). Both sets of observations indicate that synaptic contact length is correlated with quantal release. Given that most long terminal branches show a decline in the probability of secretion (Bennett *et al.*, 1986a, b), it would then be anticipated that this change should be correlated with a decline in synaptic contact length, as has been observed in the present work. Alternatively, release site characteristics not directly measured in this study, such as the length of the active zone and the arrangement of its intramembranous particles, may change along release sites and provide the basis for the decline in secretion probability.

There was no significant change in terminal apposition length along terminal branches, so that terminals did not show different degrees of indentation into the synaptic gutter except at the very end. The ratio of synaptic contact length to terminal apposition length therefore declined along the long terminal branches. This effect was due to the progressive intercalation of Schwann cell processes between the presynaptic and postsynaptic membranes for release sites progressively more distal along the terminal branches (see Fig. 7). There is then

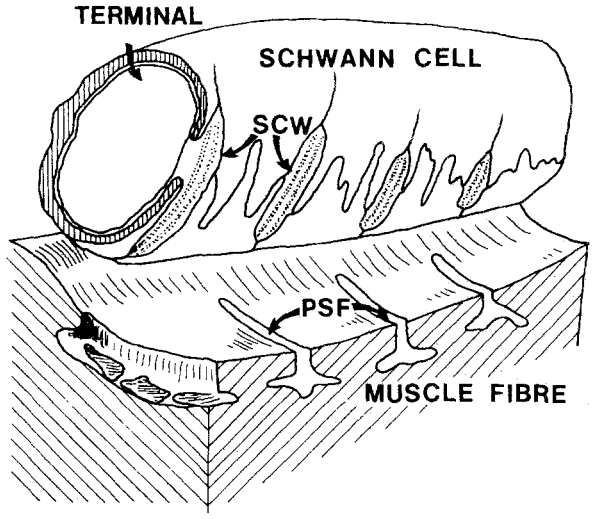


Fig. 7. Diagram illustrating the hypothesis that the decrease in length of the synaptic contacts compared with the length of the terminal apposition is due to the intercalation of Schwann cell membranes between the presynaptic and postsynaptic membranes at release sites. It is now known whether the active zone extends beyond the synaptic contact length, and is therefore partly occluded by Schwann cell, or not. SCW, Schwann cell window; PSF, postsynaptic fold.

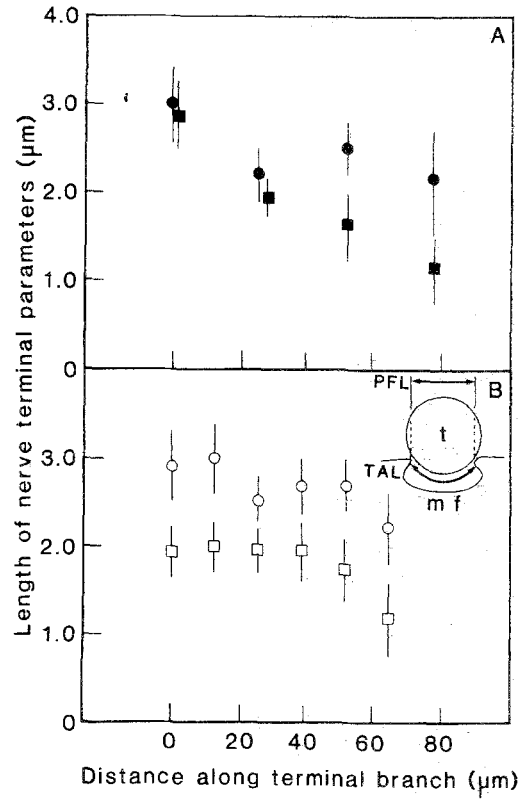


Fig. 8. Comparison of the size of release site parameters determined from transmission and scanning electron microscopy. (A) Average (± S.E.M.) synaptic contact length (filled squares) and terminal apposition length (filled circles) from sections viewed with the transmission electron microscope. (B) Average (± S.E.M.) width of the synaptic gutter (open circles) and the length of postsynaptic folds (open squares). Values in (A) are from Table 2 and in (B) from Table 3. The diagrammatic insert shows a transverse section through a release site to illustrate why the terminal apposition length (TAL), determined from transmission electron microscopy, appears to be longer than the postsynaptic fold length (PFL), determined from scanning electron microscopy; t, terminal; mf, muscle fibre.

an inverse relationship between the probability of secretion at release sites and the extent to which these are enclosed by Schwann cell processes. A similar correlation exists between the extent to which release sites are enclosed by Schwann cell processes in the frog cutaneous pectoris and the sartorius muscles and their respective transmitter secretion capacity per unit length of terminal (Herrera *et al.*, 1985a). It will be of great interest to determine whether the active zones at progressively more distal release sites along terminal branches are progressively more occluded by the intercalation of Schwann cell processes or whether the active zones are shorter at progressively more distal release sites.

Estimates of both changes in synaptic contact length and terminal size along the length of terminal branches greater than 80 μm agree reasonably well with those determined previously (Fig. 6A, B; Davey & Bennett, 1982). In the present work synaptic contact length and terminal size were measured on the transverse section through a release site which showed the maximum synaptic contact out of 15 or so sections through the site. In previous work (Davey & Bennett, 1982) these parameters were measured on the transverse section through a release site which showed the highest vesicle density out of 10 sections through the site; both procedures give similar results for release sites 26 μm beyond the beginning of long terminal branches (Fig. 6A, B). However, it should be noted that in the previous study (Davey & Bennett, 1982) the preparation was first bathed in HRP for 1 h in Krebs solution and the nerve stimulated at 1 Hz for 1 h in order to label the vesicles with HRP. This procedure could lead to changes in the size of release site dimensions and may be responsible for differences between the observations in the previous study and those of the present work at distances less than 26 μm (Fig. 6).

Changes in postsynaptic folds along terminal branches

Not in this study or others has any significant change been observed along terminal branches in the width of the terminal gutter (Shotton *et al.*, 1979), the length of postsynaptic folds (Desaki & Uehara, 1981) or the frequency of the folds (Verma & Reese, 1984). All three parameters eventually decline, but only at the very ends of the terminal branches. Active zones generally appear opposite postsynaptic folds, where they can extend over much of the fold length

(Pumplin, 1983). However, it is not known whether this is the case for active zones at progressively more distal release sites along terminal branches.

Fig. 8 shows the relationship between average values for synaptic contact length, terminal apposition length, length of postsynaptic fold and width of synaptic gutter. All four parameters decrease along the length of long terminal branches, but only synaptic contact length decreases significantly. The difference between synaptic contact length and terminal apposition length, which becomes progressively larger along the terminal branches, is, as we have seen, due to the intercalation of Schwann cell processes between the presynaptic and postsynaptic membranes. Surprisingly, the terminal apposition length is always greater than the length of the postsynaptic fold. This is because the former was measured along the length of the apposition as viewed in transmission electron micrographs, whereas the latter was measured *'en face'* in scanning electron micrographs of synaptic gutters; this does not allow for the curvature of the postsynaptic folds and therefore gives an underestimation of their length (see insert in Fig. 8B).

Factors determining the probability of secretion at release sites

A correlation exists between transmitter secretion and total length of nerve terminal at neuromuscular junctions, but it shows considerable variation (Kuno *et al.*, 1971; Bennett & Raftos, 1977; Angaut-Petit & Mallart, 1979; Harris & Ribchester, 1979; Grinnel & Herrera, 1980). This may be attributed to the discovery that short terminal branches (less than 78 μm) show very little variation in the probability of secretion along their length whereas, in general, the probability of secretion declines for long terminal branches (greater than 78 μm; Bennett *et al.*, 1986a, b). This may explain why the amount of transmitter secreted per unit length of terminal can be inversely related to the total length of nerve terminal at endplates on fibres with the same input resistance (Nudell & Grinnell, 1982, 1983). If terminals at these endplates grow by the extension of long terminal branches, then these will add very little to the amount secreted by the terminal while at the same time substantially increasing the total length of nerve terminal at the endplate.

It appears that the probability of secretion averaged over all release sites of a terminal is correlated with the average length of its synaptic contact (Herrera *et al.*, 1985a, b); furthermore, as shown previously (Davey & Bennett, 1982) and in the present work, the probability of secretion at different release sites of long terminal branches within an endplate is correlated with the length of the synaptic contacts at these sites. The synaptic contact probably represents the extent of active zone, indicating that the probability of secretion at a release site is determined by the length of the active zone. However, if the active zone is occluded from directly apposing the postsynaptic fold by Schwann cell processes, then the synaptic contact will underestimate the length of the active zone. If this is the case, then it is likely that transmitter secreted by synaptic vesicles will not reach the receptors on postsynaptic folds in regions of the active zone which are occluded by Schwann cell. If such transmitter does diffuse to the receptors it is unlikely to give rise to a quantized event and may contribute to the non-quantal transmitter release at the endplate (Katz & Miledi, 1977, 1981). There is evidence to suggest that a normal nerve impulse will only give rise to secretion from a single synaptic vesicle at an active zone (Bennett *et al.*, 1977; Triller & Korn, 1982), although impulses of long duration after treatment with 4-aminopyridine can give rise to secretion from several vesicles at a release site (see for example Katz & Miledi, 1979; Miller & Heuser, 1984; Torri-Tarelli *et al.*, 1985). It seems that once a vesicle commences secretion at an active zone, the rest of the zone becomes refractory for secretion over a short period of time. If this is so, then occlusion of part of the active zone by Schwann cell processes would change the probability of secretion; vesicles secreting in occluded parts of the zone would not only have no quantal effect on the postsynaptic membrane but would ensure that no other quantal event was generated by the active zone. In this context it will be of interest to see whether the active zone is partly occluded by Schwann cells or not.

References

ANGAUT-PETIT, D. & MALLART, A. G. (1979) Dual innervation of endplate sites and its consequences for neuromuscular transmission in muscles of adult Xenopus laevis. *Journal of Physiology* **289**, 203–18

BENNETT, M. R. & FISHER, C. (1977) The effect of calcium ions on the binomial parameters that control acetylcholine release during trains of nerve impulses at amphibian neuromuscular synapses. *Journal of Physiology* **271**, 673–98.

BENNETT, M. R., FISHER, C., FLORIN, T., QUINE, M. & ROBINSON, J. (1977) The effect of calcium ions and temperature on the binomial parameters that control acetylcholine release by a nerve impulse at amphibian neuromuscular synapses. *Journal of Physiology* **271**, 641–72.

BENNETT, M. R. & FLORIN, T. (1974) A statistical analysis of the release of acetylcholine at newly formed synapses in striated muscle. *Journal of Physiology* **238**, 93–107.

BENNETT, M. R., JONES, P. & LAVIDIS, N. A. (1986a) Transmitter secretion varies between visualized release sites at amphibian neuromuscular junctions. *Neuroscience Letters* **65**, 311–15.

BENNETT, M. R., JONES, P. & LAVIDIS, N. A. (1986b) The probability of quantal secretion along visualized terminal branches at amphibian (*Bufo marinus*) neuromuscular junction. *Journal of Physiology* **379**, 257–74.

BENNETT, M. R. & LAVIDIS, N. A. (1979) The effect of Ca ions on the secretion of quanta evoked by an impulse at nerve terminal release sites. *Journal of General Physiology* **74**, 429–56.

BENNETT, M. R. & LAVIDIS, N. A. (1982) Variation in quantal secretion at different release sites along developing and mature motor terminal branches. *Developmental Brain Research* **5**, 1–9.

BENNETT, M. R. & RAFTOS, J. (1977) The formation and regression of synapses during the re-innervation of axolotl striated muscles. *Journal of Physiology* **265**, 261–95.

COUTEAUX, R. & PECOT-DECHAVASSINE, M. (1970) Vesicules synaptiques et poches au niveau de les 'zones actives' de la junction neuromusculaire. *Comptes rendus hebdomadaire des seances de l'Academic des Sciences* **271**, 2346–9.

D'ALONZO, A. J. & GRINNELL, A. D. (1985) Profiles of evoked release along the length of frog motor nerve terminals. *Journal of Physiology* **359**, 235–58.

DAVEY, D. F. & BENNETT, M. R. (1982) Variation in the size of synaptic contacts along developing and mature motor terminal brances. *Developmental Brain Research* **5**, 11–22.

DE CINO, P. (1981) Transmitter release properties along regenerated nerve processes at the frog neuromuscular junction. *Journal of Neuroscience* **1**, 308–17.

DESAKI, J. & UEHARA, Y. (1981) The overall morphology of neuromuscular junctions as revealed by scanning electron microscopy. *Journal of Neurocytology* **10**, 101–10.

DREYER, F., PEPER, K. M., AKERT, D. M., SANDRI, C. & MOORE, H. (1973) Ultrastructure of the 'active zone' in the frog neuromuscular junction. *Brain Research* **62**, 373–80.

FUKUNAGA, H., ENGEL, A. G., OSAME, M. & LAMBERT, E. H. (1982) Paucity and disorganization of presynaptic membrane active zones in the Lambert–Eaton myasthenic syndrome. *Muscle and Nerve* **5**, 686–7.

GRINNELL, A. D. & HERRERA, A. A. (1980) Physiological regulation of synaptic effectiveness at frog neuromuscular junctions. *Journal of Physiology* **307**, 301–17.

HARRIS, J. B. & RIBCHESTER, R. R. (1979) The relationship between endplate size and transmitter release in normal and dystrophic muscles of the mouse. *Journal of Physiology* **296**, 245–65.

HERRERA, A. A., GRINNELL, A. D. & WOLOWSKE, B. (1985a) Ultrastructural correlates of naturally occurring differences in transmitter release efficacy in frog motor nerve terminals. *Journal of Neurocytology* **14**, 193–202.

HERRERA, A. A., GRINNELL, A. D. & WOLOWSKE, B. (1985b) Ultrastructural correlates of experimentally altered transmitter release efficacy in frog motor nerve terminals. *Neuroscience* **16**, 491–500.

HEUSER, J. E., REESE, T. S. & LANDIS, D. M. (1974) Functional changes in frog neuromuscular junction studied with freeze-fracture. *Journal of Neurocytology* **3**, 108–31.

KARNOVSKY, M. J. (1964) The localization of cholinesterase activity in rat cardiac muscle by electron microscopy. *Journal of Cell Biology* **23**, 217–32.

KATZ, B. & MILEDI, R. (1977) Transmitter leakage from motor nerve endings. *Proceedings of the Royal Society of London, Series B* **196**, 59–72.

KATZ, B. & MILEDI, R. (1979) Estimates of quantal content during 'chemical potentiation' of transmitter release. *Proceedings of the Royal Society of London, Series B* **205**, 369–78.

KATZ, B. & MILEDI, R. (1981) Does the motor nerve impulse evoke 'non-quantal' transmitter release? *Proceedings of the Royal Society of London, Series B* **212**, 131–7.

KO, C-P. (1984) Regeneration of the active zone at the frog neuromuscular junction. *Journal of Cell Biology* **98**, 1685–95.

KO, C-P. (1985) Formation of the active zone at developing neuromuscular junctions in larval and adult bullfrogs. *Journal of Neurocytology* **14**, 487–512.

KO, C-P., PROPST, J. W. & HERRERA, A. A. (1985) A comparison of active zone ultrastructure in two muscles with markedly different synaptic efficacy. *Society for Neuroscience Abstracts* **11**, 302, 88.4.

KUNO, M., TURKANIS, S. A. & WEAKLEY, J. N. (1971) Correlation between nerve terminal size and transmitter release at the neuromuscular junction of the frog. *Journal of Physiology* **213**, 545–56.

LLINAS, R. R. (1982) Calcium in synaptic transmission. *Scientific American* **247**, 56–65.

MCMAHAN, U. J., SPITZER, N. L. & PEPER, K. (1972) Visual identification of nerve terminals in living isolated skeletal muscle. *Proceedings of the Royal Society of London, Series B* **181**, 421–30.

MILLER, T. M. & HEUSER, J. E. (1984) Endocytosis of synaptic vesicle membrane at the frog neuromuscular junction. *Journal of Cell Biology* **98**, 685–98.

NUDELL, B. M. & GRINNELL, A. D. (1982) Inverse relationship between transmitter release and terminal length in synapses on frog muscle fibres of uniform input resistance. *Journal of Neuroscience* **2**, 216–24.

NUDELL, B. M. & GRINNELL, A. D. (1982) Regulation of synaptic position, size, and strength in anuran skeletal muscle. *Journal of Neuroscience* **3**, 161–76.

PROPST, J. W. & KO, C-P. (1985) The correlation between synaptic efficacy and active zone ultrastructure studied with freeze fracture of identified neuromuscular junctions. *Society for Neuroscience Abstracts* **11**, 303, 88.5.

PUMPLIN, D. W. (1983) Normal variations in presynaptic active zones of frog neuromuscular junctions. *Journal of Neurocytology* **12**, 317–23.

REYNOLDS, E. S. (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–12.

SHOTTON, D. M., HEUSER, J. E., REESE, B. F. & REESE, T. S. (1979) Post-synaptic membrane folds of the frog neuromuscular junction visualized by scanning electron microscopy. *Neuroscience* **4**, 427–35.

SPURR, A. R. (1969) A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **26**, 31–43.

TORRI-TARELLI, F., GROHOVAZ, F., FESCE, R. & CECCARELLI, B. (1985) Temporal coincidence between synaptic vesicle fusion and quantal secretion of acetylcholine. *Journal of Cell Biology* **101**, 1386–99.

TREMBLAY, J. D., ROBITAILLE, R. & GRENON, G. (1984) Distribution of spontaneous release along the frog neuromuscular junction. *Neuroscience Letters* **51**, 247–52.

TRILLER, A. & KORN, H. (1982) Transmission at a central inhibitory synapse. III. Ultrastructure of physiologically identified and stained terminals. *Journal of Neurophysiology* **48**, 708–36.

VERMA, V. & REESE, T. S. (1984) Structure and distribution of neuromuscular junctions on slow muscle fibres in the frog. *Neuroscience* **12**, 647.

WALROND, J. P. & REESE, T. S. (1985) Structure of axon terminals and active zones at synapses on lizard twitch and tonic muscle fibers. *Journal of Neuroscience* **5**, 1118–31.

WERLE, M. J., HERRERA, A. A. & GRINNELL, A. D. (1984) Ultrastructural uniformity along branches of frog motor nerve terminals. *Neuroscience Abstracts* **10**, 919.

WERNIG, A. (1975) Estimates of statistical release parameters from crayfish and frog neuromuscular junctions. *Journal of Physiology* **244**, 107–221.

YOSHIKAMI, D. & OKUN, L. M. (1984) Staining of living presynaptic nerve terminals with selective fluorescent dyes. *Nature* **310**, 53–6.