Pflügers Arch (1982) 395: 261–270

Pflügers Archiv
European Journal of Physiology
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Neurotransmitter Release and Its Facilitation in Crayfish Muscle

V. Basis for Synapse Differentiation of the Fast and Slow Type in One Axon*

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Abstract. Excitatory postsynaptic currents (EPSCs) were recorded extracellularly from synaptic spots on crayfish opener muscle fibers. Synapses on the proximal fiber bundle were characterized as fast, with a relatively high quantal-release rate m of 0.2–5 and a low twin-pulse facilitation F_s of 1.1–3, at 13.5 mM $[Ca]_o$ and low (0.5/s) repetition rate. Unter the same conditions, distal „slow" synapses had a release rate m of 0.02–0.4 and a facilitation F_s of 2–4. When the $[Ca]_o$ was varied between 1.7 and 27 mM, release and facilitation were much less affected in proximal, fast synapses than in distal, slow ones. The average maximal slope of the log release to log $[Ca]_o$ relation was 1.5 in proximal, and 3.1 in distal synapses, while the average maximal facilitation F_s was 2.5 in proximal and 4.7 in distal synapses, respectively. Assuming saturation kinetics for entry of Ca into the terminal and release of transmitter, possible variations of parameters generating the fast-slow differentiation were explored. Excluding a number of possibilities, it was found that in addition to a higher maximal release level, fast synapses seem to have a higher resting $[Ca]_i$ and/or a lower cooperativity of the release mechanism, as compared to slow synapses.

Key words: Synaptic transmitter release – Facilitation – Fast/slow synaptic differentiation – Intracellular Ca concentration – Cooperativity of transmitter release

Introduction

Many crustacean muscles receive two or a few excitatory axons, which are of the fast or of the slow type. The distinction was made originally from the characteristics of muscle contraction (Wiersma 1961), and is due partly to the membrane and contraction characteristics of the muscle fiber, and partly to differences in synaptic organization (Hoyle and Wiersma 1958; Atwood 1963, 1967, 1973 and 1976; Atwood and Parnas I. 1968; Parnas I. and Atwood 1966; Linder 1973). The synaptic differentiation is mainly at the terminals: fast synapses release many quanta even after single stimuli of low frequency, but they show little facilitation, i.e. increased release on repetitive stimulation. Slow synapses release very few quanta after single stimuli, but release increases dramatically on repetitive stimulation. The fast synapses and muscle fibers are employed for rapid twitches, while the slow system is adapted to exert tonic force. These specializations seem necessary in a motor system which controls movement by means of 2–5 motoneurons per muscle.

Fibers with fast and slow characteristics also occur in muscles innervated by only one excitatory axon. In this case the axon terminals and/or the muscle fibers must show regional differences. A well known example is the opener muscle of the crayfish claw: there is a proximal region with large excitatory postsynaptic potentials (EPSPs) on single stimuli and little facilitation, a central to distal region with very small EPSPs elicited by single stimuli and large facilitation, and in some species a far distal region with properties approaching the proximal one (Iravani 1965; Bittner 1968; Atwood and Bittner 1971; Dudel 1979 and 1981). Bittner (1968) and Atwood and Bittner (1971) showed that the differentiation is mainly presynaptic. Excluding some other possibilities, they proposed that differences in the invasion of terminals by the action potential may be the basis of fast and slow characteristics. In the course of our recent quantitative study of release and facilitation in this muscle we mostly worked with intermediate fibers at the border between the proximal and the distal region and gave quantitative characteristics of this system (Parnas H. et al. 1982a, b; Parnas I. et al., 1982; Dudel et al. 1982). Yet we noticed deviations from the average characteristics in typical proximal and distal fibers; therefore, we set out to study these characteristics systematically, searching for a basic mechanism underlying the striking differences.

Methods

The preparation was the opener muscle of the first walking leg's claw of the crayfish *Astacus leptodactylus*. The animals weighed about 100 g and were freshly imported to Munich from Turkey. They were kept in aerated tap water for not more than one month. This information is given because Bittner (1968) reported loss of differentiation of muscle fibers when the animals were kept in an aquarium for longer periods. The same might occur in the species of crayfish used here.

The details of setting up the preparation and of the recording technique employing modified patch-clamp electrodes are given by Dudel (1981) and Parnas H. et al. (1982). Release was evaluated by counting quanta directly. The modified v. Harreveld solution contained (mM): Na^+ 205, Cl^- 232, K^+ 5.4, Ca^{2+} 13.5, Mg^{2+} 2.5, Tris maleate buffer 10; pH 7.6. Low calcium concentration ($[Ca]_o$) solutions were prepared by mixing this solution with a zero $[Ca]_o$ solution, in

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* Supported by a grant of the Deutsche Forschungsgemeinschaft to J. D. and I. P., and by the Muscular Dystrophy Association to H. P.

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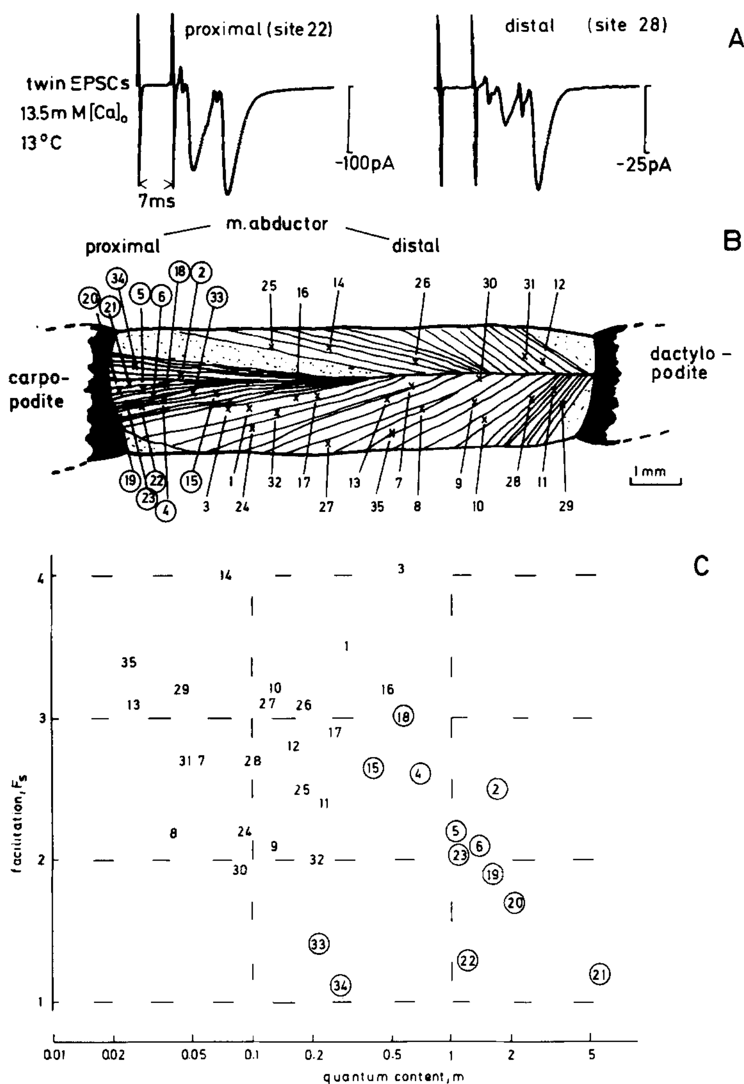

Fig. 1A–C
Characteristics of proximal and distal synapses on the opener muscle. (**A**) Sample twin EPSCs, both at 7 ms stimulation pulse interval, for typical proximal and distal sites. At the proximal site 22, the unit quantum current was $c_1 = 110$ pA, the quantum content of the first EPSC was $m = 1.2$ and facilitation was $F_s = 1.3$. At the distal site 28, the respective values were $c_1 = 145$ pA, $m = 0.10$ and $F_s = 2.6$. Each record average of 256 single measurements, stimuli repeated every 2.2 s. (**B**) Drawing of the surface of the opener muscle (opposite to the closer) from which recordings at the sites 1–20 were registered. The sites 21–35 refer to analogous recording positions on a sister muscle. Recording sites in the proximal fiber bundle display encircled numbering. The stippled areas show inner surface of the shell, free of muscle fibres. (**C**) Relation of quantum content m to short interval (7 ms) twin pulse facilitation F_s for the 35 recording sites of B. The measurements from proximal sites (encircled) show relatively high m and low F_s. All measurements at 13.5 mM $[Ca]_o$ and 13 °C

which $[Na]_o$ was increased to compensate osmotic effects. The bathing solution was cooled to 13° C.

Results

Location of Proximal and Distal Synapses

Figure 1 B is a drawing of an opener muscle, showing the inner surface as viewed through a binocular. Excitatory postsynaptic currents (EPSCs) were recorded from synaptic spots. The spots were selected for clearcut recording of only one quantum size of at least 100 pA which excludes recordings from spots lying close to each other. Otherwise the selection of spots was without bias. In this preparation recordings were taken in the positions marked 1–20. Another set of 15 recordings were made in a sister preparation of similar size and configuration on the next day; the recording sites were entered into the drawing of Fig. 1B at analogous positions marked 21–35. The numbers indicating recording sites from the proximal region are encircled. This proximal region is a bundle of rather opaque, relatively thin fibers located next to the tendon at the proximal end of the muscle. This bundle is often seen to contract vigorously to stimuli of medium rate, e.g. 10/s, to which the rest of the muscle does not visibly respond. The 'distal' fibers are far more numerous than the proximal ones and cover most of the inner surface of the muscle. They are thicker than the proximal fibers and much more translucent.

Figure 1A shows sample records from the proximal and from the distal region: the proximal EPSC has a high quantum content and a low twin-pulse facilitation. The distal EPSC displays a low quantum content and high facilitation. The twin pulses were given at an interval of 7 ms which is the shortest interval at which conduction is reliable in practically all axon branches. At this short interval facilitation is nearly maximal and processes of removal of Ca from the terminal do not contribute significantly (Parnas I. et al. 1982). Quantitatively facilitation is given by the ratio of the amplitudes of the second and the first EPSC; the short interval facilitation measured throughout this study is named F_s.

The amplitude of facilitation F_s was plotted against the quantum content m in Fig. 1C for all recording sites of Fig. 1B. The representations of proximal recordings are grouped at high m and low F_s, while those of the distal ones spread into the low m, high F_s range. The average values were $m = 1.37 \pm 0.38$ (SE) and $F_s = 1.97 \pm 0.17$ for the proximal sites, and $m = 0.16 \pm 0.03$ and $F_s = 2.85 \pm 0.12$ for the distal synapses. The differences for the respective values measured

proximally and distally are highly significant. Even more striking differences would have been observed if the experiments had been done at a lower $[Ca]_o$ (see Figs. 3 and 5). The normal $[Ca]_o$ of 13.5 mM was chosen in order to facilitate comparison with other investigations.

Similar results on differentiation of fibers with different release and facilitation characteristics of the same muscle were also obtained by Bittner (1968) and Atwood and Bittner (1971) who used continuous stimulation at 10/s to show facilitation. They also found the proximal region analogous to ours, but our distal region corresponds to their central one. In addition they described a distal region with characteristics similar to the proximal one (see also Linder 1974). This difference may be due to the different species (*Procambarus clarkii*). Similar rather 'fast' characteristics have also been observed in the far distal group of short and thin fibers in crayfish of the species *Orconectes virilis*, *Orconectes limosus*, and *Astacus fluviatilis* (Dudel, unpublished). In the species employed in this study, *Astacus leptodactylus*, this differentiation was rare and not strongly expressed. In all species investigated by us the proximal fiber bundle was much more strikingly 'fast' than the far distal fibers. Due to difficulties in cleaning the proximal part of the preparation, the small fiber diameter and violent movement it is much more troublesome to record from this section of the muscle than from other parts. This may explain why the proximal fiber bundle has attracted relatively little attention.

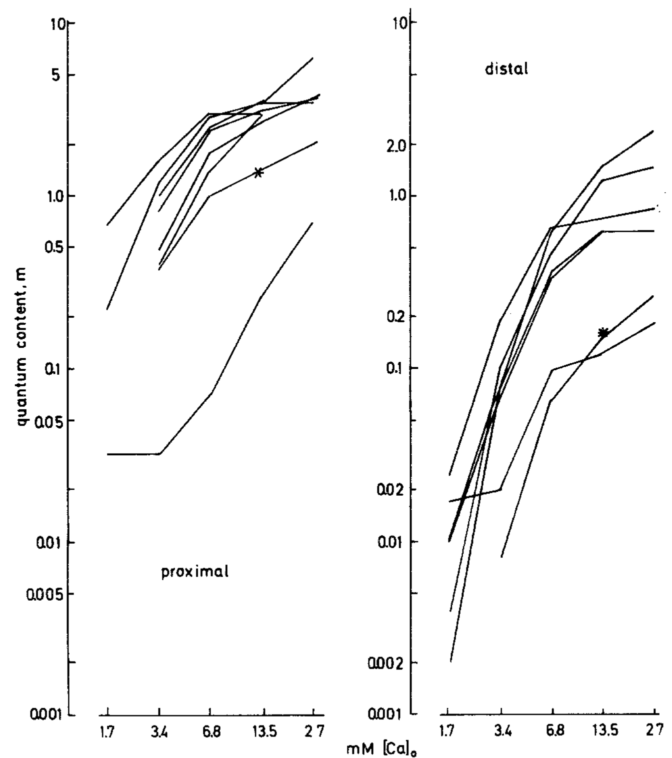

Fig. 2. Dependence of quantum content m of EPSCs (1/2.2 s) on extracellular calcium concentration $[Ca]_o$ for proximal and distal synapses. Logarithmic scales! The curves observed at single synapses in different preparations show the scatter of the observations. The asterisks inserted into the plots at 13.5 mM $[Ca]_o$ represent the average m in proximal and distal synapses, respectively, of Fig. 1C

$[Ca]_o$-*Dependence of Release and Facilitation*

It is generally assumed that release of transmitter and its facilitation are regulated by changes in the intracellular calcium concentration, $[Ca]_i$ (Katz and Miledi 1968 and 1970). Variations in $[Ca]_o$ will affect these changes in $[Ca]_i$ in a predictable manner, and therefore measurements of twin pulse EPSCs at different $[Ca]_o$ were the basis of our non-linear model of synaptic release and facilitation. (Parnas H. et al. 1982a; Parnas I. et al. 1982). We shall present such measurements for a group of proximal and one of distal synapses with the aim of selecting physical parameters which could explain the differences. This selection of parameters will be aided by a discussion of predictions of our model in the next section of this paper; therefore, only the more technical details of the results will be treated here.

Figure 2 shows the dependence of the quantal release m on $[Ca]_o$ for 8 proximal and 7 distal synapses. As can be expected for data compiled from different preparations, both groups of curves show considerable scatter. This scatter also probably represents a real variability in the expression of 'proximal' and 'distal' characteristics; therefore the individual experiments are presented here. The different characteristics of the two groups of curves are even obvious in average curves which level the extreme variations. Because it may not be correct to take averages of relatively few and unevenly scattered values, the release rates were normalized within each group relative to m at 13.5 mM $[Ca]_o$ and then averaged.

The average curves in Fig. 3 differ in the level of m at high $[Ca]_o$, and even more significantly in the slope of decline of m with decreasing $[Ca]_o$ (log-log plot!), the distal synapses being much more sensitive to the reduction of $[Ca]_o$. While at high $[Ca]_o$ the proximal synapses release 3.3 times more transmitter quanta than the distal ones, at the low $[Ca]_o$ of 1.7 mM the average ratio is 16.

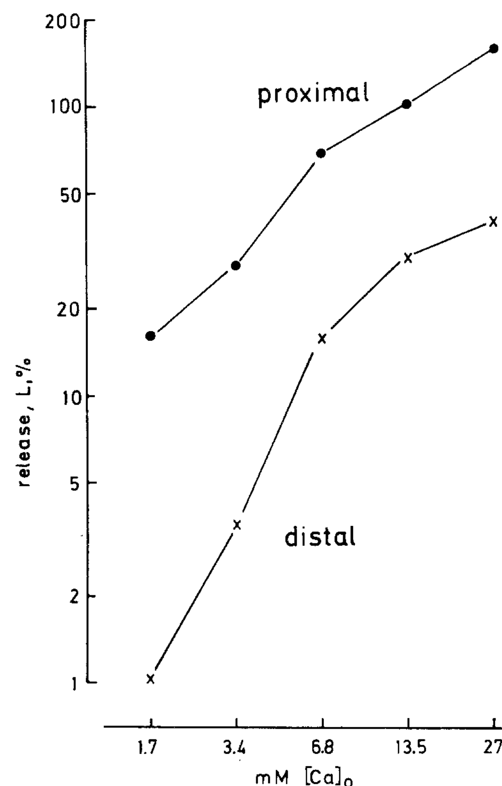

Fig. 3. Average dependence of quantum release on $[Ca]_o$ for proximal (●) and distal (×) synapses. Averages were taken from the normalized values of Fig. 2, as described in the text. Release is given in percent of average quantum content of proximal synapses at 13.5 mM $[Ca]_o$

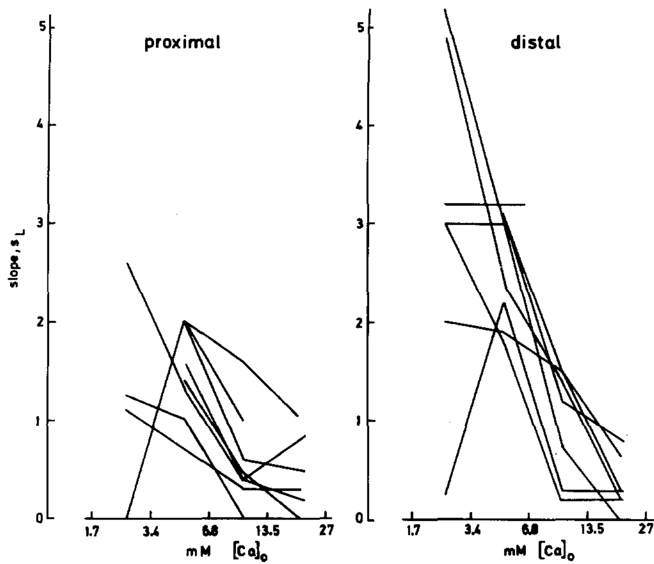

Fig. 4. Slopes s_L of the $[Ca]_o$-dependence of m, $\Delta \log m / \Delta \log [Ca]_o$, for the curves plotted in Fig. 2, for proximal and distal synapses. The slope values are plotted in the middle of the respective $[Ca]_o$ interval

It should be noted that the average m values are higher for both groups in Fig. 2 than in the representative sample taken at 13.5 mM $[Ca]_o$ in Fig. 1: for the proximal fibers average m is 1.7 times higher in Fig. 2 than in Fig. 1, and for the distal fibers the ratio is as high as 4.5. The averages of Fig. 1 are represented by asterisks in Fig. 2. This difference undoubtedly represents a bias in the selection of the sample of Fig. 2, in fact in all the material of this section. Experimentally it is necessary to find 'good releasers' in the different regions of the preparation, if release is known to decrease substantially at low $[Ca]_o$. This is especially true for distal synapses: even in the 'good releasers' sample of Fig. 2 in 4 synapses at 1.7 mM $[Ca]_o$ m was < 0.01. At such low release rates 1000–2000 twin pulses have to be recorded in order to obtain a reasonably accurate value of m, and since the repetition rate was 1/2 s in order to avoid facilitation of the first EPSC, measurement periods of up to more than 1 h were necessary for one determination at a very low value of m. This not only prolonged the experiment, but sometimes led to blocks of the second impulse at long exposure to low $[Ca]_o$ (such experiments had to be excluded from this sample). The results of this section, therefore, still show the differentiation of proximal and distal synapses, but these differences would have been larger in an unbiassed sample.

Significant details of the release curves in Fig. 2 are their slopes which are often used as measure of the cooperativity of release (Dodge and Rahamimoff 1967). In Fig. 4 the logarithmic slopes $s_L = \Delta(\log L)/\Delta(\log [Ca]_o$ are plotted against $[Ca]_o$ for the proximal and the distal synapses. In both groups the slopes are similarly low at high $[Ca]_o$. While they exceed 2 at low $[Ca]_o$ only in one extreme proximal synapse, they reach the high value of 5 in two distal synapses. In both groups the maximal slopes are not found at the lowest $[Ca]_o$ in some curves. This possibly significant feature will be discussed below. The average slopes s_L from the curves in Fig. 4 are given in Fig. 6A.

In addition to the level of release, the $[Ca]_o$ dependence of twin pulse facilitation F_s is the basis for our model of release and facilitation. F_s values are given in Fig. 5 for the same synapses as those in Fig. 2. F_s increases dramatically on reduction of $[Ca]_o$ in distal synapses, while the respective increase is much smaller in the proximal ones. Again in some of these curves maxima occur at $[Ca]_o$ higher than 1.7 mM, which will be discussed below.

Average values of s_L and F_s, taken from Figs. 4 and 5, respectively, are plotted in Fig. 6a and B. These values were not normalized before averaging, since both are relative numbers, s_L being the ratio of two logarithms, and F_s the ratio of two EPSC amplitudes. Both sets of average curves display the features pointed out above; they will be discussed in context of the theoretical model.

Variations in Entry and Release Parameters Possibly Covering Proximal and Distal Characteristics

Based on the data presented in Figs 2–6 we can try to find parameters in which proximal and distal synapses may differ. It will be necessary to present some relevant features of our theoretical model (Parnas, H., et al. 1982a; Parnas, I., et al., 1982) and we also shall make use of the theoretical treatments of Parnas, H., and Segel (1981, 1982).

Release L is given by

$$L = \bar{L}\left(\frac{Y + [Ca]_{ir}}{K_L + Y + [Ca]_{ir}}\right)^l; \tag{1}$$

in which $\bar{L}$ is the saturation level of release, K_L a constant $[Ca]_i$ related to saturation, l the cooperativity of the release process, and $[Ca]_{ir}$ the constant resting $[Ca]_i$. The entry Y during a single pulse is given by

$$Y = \frac{\bar{Y}[Ca]_o^y}{(K_y + [Ca]_o)^y}, \tag{2}$$

in which $\bar{Y}$ is the saturation level of entry, K_y a constant $[Ca]_o$ related to saturation, and y the cooperativity of the entry process which was found to be 1.

A very distinctive feature is the logarithmic slope of the dependence of release on $[Ca]_o$:

$$s_L = \frac{\partial(\log L)}{\partial(\log[Ca]_o)}$$

$$= \frac{y l \bar{Y}[Ca]_o^y K_L K_Y}{(K_y + [Ca]_o)^{y+1}(K_L + Y + [Ca]_{ir})(Y + [Ca]_{ir})}. \tag{3}$$

If removal of Ca from the terminal is disregarded at short pulse intervals, the facilitation is:

$$F_s = \left[\frac{(2Y + [Ca]_{ir})(K_L + Y + [Ca]_{ir})}{(K_L + 2Y + [Ca]_{ir})(Y + [Ca]_{ir})}\right]^l. \tag{4}$$

The object is to adjust each of the parameters in Eq. (1) and (4) in order to achieve changes in release and facilitation which reflect the differences observed in proximal and distal synapses. Effects of changes in $\bar{Y}$ (representing Y), $[Ca]_{ir}$, l and K_L are shown in Fig. 7A to D. For each of these parameters, the curve representing the present set of data (Table 4 in Parnas, H., et al., 1982a, see also legend) is given by a continuous line, increases of the respective parameter are presented by interrupted lines, and decreases by dotted lines. $\bar{L}$ was not included in Fig. 7, since changing it only produces vertical shifts of the release curve. Comparison of Fig. 7 with the

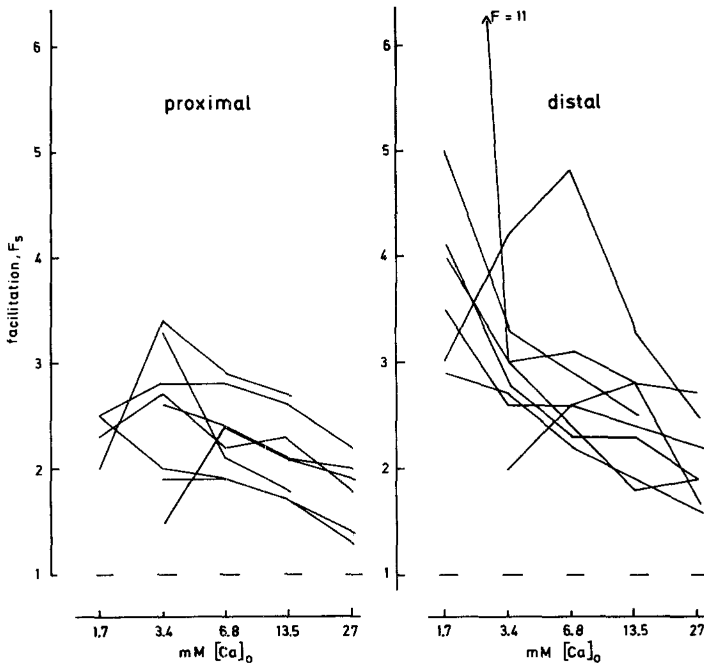

Fig. 5
Dependence of twin pulse, 7 ms interval facilitation F_s on $[Ca]_o$, for the same groups of proximal and distal synapses as contained in Figs. 2, 3 and 4

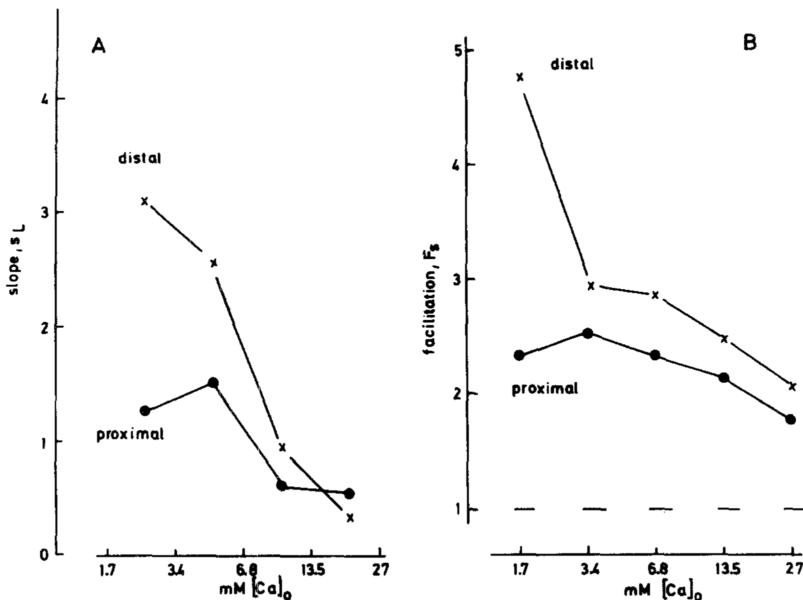

Fig. 6
(A) Dependence of average slope $s_L = \Delta(\log m)/\Delta(\log[Ca]_o)$ of the curves in Figs. 2 and 4 on $[Ca]_o$, for proximal (●) and distal (×) synapses. **(B)** Dependence of average F_s of Fig. 5 on $[Ca]_o$, also for proximal and distal synapses

experimental data summarized in Figs. 3 and 6 leads to the following conclusions: Increased maximum entry ($\bar{Y}$) (Fig. 7A) can predict the large release seen in proximal synapses, but also increases s_L contrary to our findings, and fails to predict the observed shifts in maximal F_s. Other changes in the entry system equivalent to an increase of $\bar{Y}$, i.e. reduction of K_Y or $y > 1$, also cannot account for the experimental findings (Parnas, H., and Segel 1982).

Changes in calcium affinity (K_L) also can approximate the shapes of the release curves for proximal and distal synapses. However, a clear discrepancy is seen in the maxima of F_s: contrary to our experimental findings, in Fig. 7D a maximum of F_s is predicted at higher $[Ca]_o$ for distal synapses than for proximal ones.

Changes in internal resting [Ca], $[Ca]_{ir}$, (Fig. 7B) produce release and facilitation curves which agree well with the experimental ones. The values $[Ca]_{ir} = 0.2$ or 0.01 μM give good approximations to the measured $[Ca]_o$ dependences of L and F for proximal and distal synapses, respectively. A similar good fit is achieved by changes in the cooperativity of the release system (Fig. 7C). With $l = 3$ for proximal synapses and $l = 6$ for distal ones the predictions match the measured $[Ca]_o$ dependences.

Changes in $[Ca]_{ir}$ or l Can Account for the Proximal/Distal Synaptic Differentiation

Thus changes in $[Ca]_{ir}$ or in l could reproduce the shape of the release and of the facilitation curves for proximal and distal fibers. Both do not explain the difference in m at high $[Ca]_o$ (Fig. 3). This difference can be accounted for by adjusting $\bar{L}$ [Eq. (1)]. $\bar{L}$ thus will have to be different by a factor of about

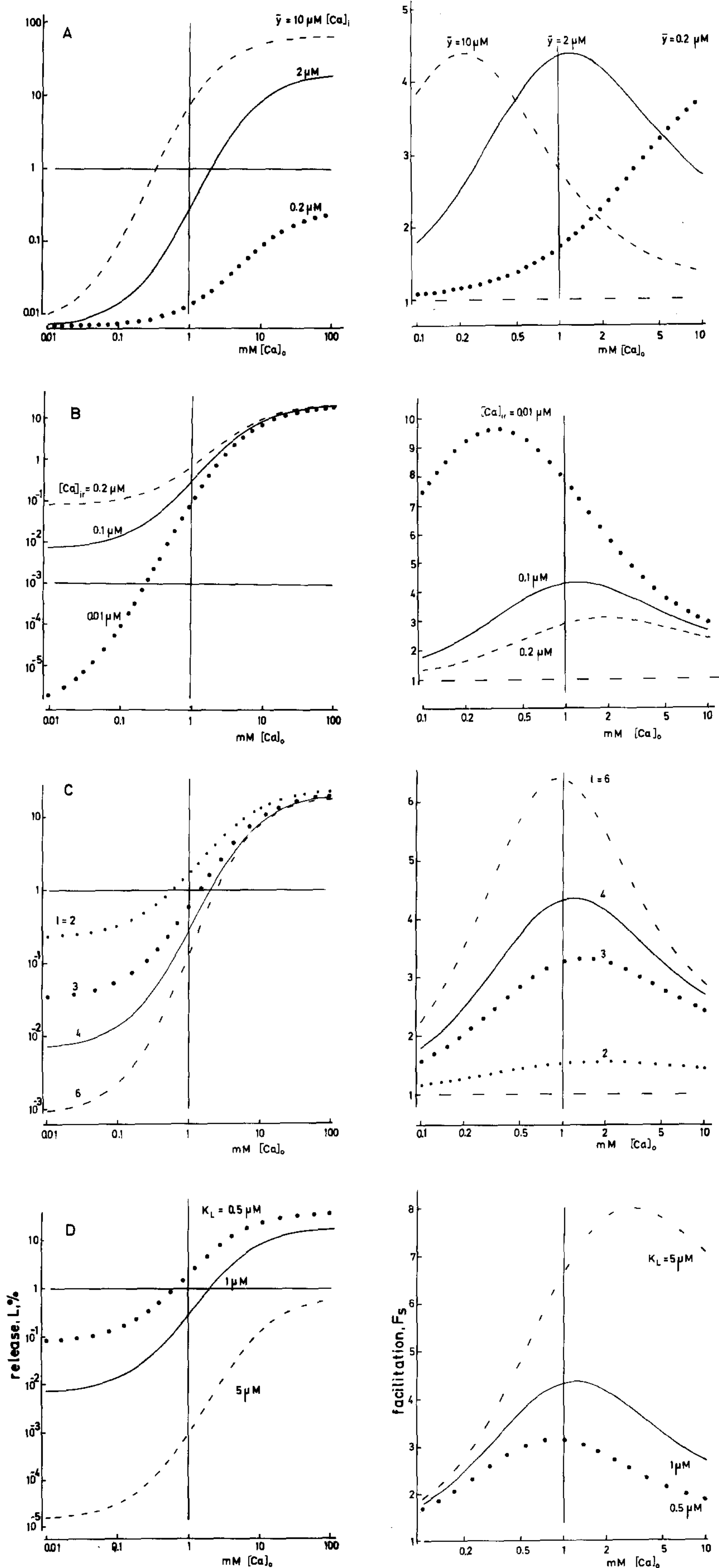

Fig. 7A–D
Effects of changes in parameters on dependence of release (left hand plots) and of short interval facilitation F_s (right hand plots) on $[Ca]_o$, as predicted by Eq. (1), (2) and (4). In the release curves, ordinates and abscissae have logarithmic scales, the abscissae cover the wide range from 0.01 – 100 mM $[Ca]_o$, and release L is given in percent of $\bar{L}$, the maximal saturation level, employing different ranges. In the facilitation curves, only the abscissae have a logarithmic scale with the range from 0.1 – 10 mM $[Ca]_o$, while the range of F_s varies. In **(A)** the parameter $\bar{Y}$, in **(B)** $[Ca]_{ir}$, in **(C)** l and in **(D)** K_L is varied, the specific values are indicated at the different curves. The curve incorporating the 'standard' value is drawn out, the curve showing the effect of an increase of the parameter is dashed, and the curve demonstrating a decrease in the respective parameter is pointed. For each curve, except for the value indicated, the following standard parameters were used: $\bar{Y} = 2$ μM $[Ca]_i$, $K_y = 9$ mM $[Ca]_o$, $y = 1$, $\bar{L} = 100\,\%$, $K_L = 5\,(2^{1/l} - 1)$ μM $[Ca]_i$, $[Ca]_{ir} = 0.1$ μM, $l = 4$

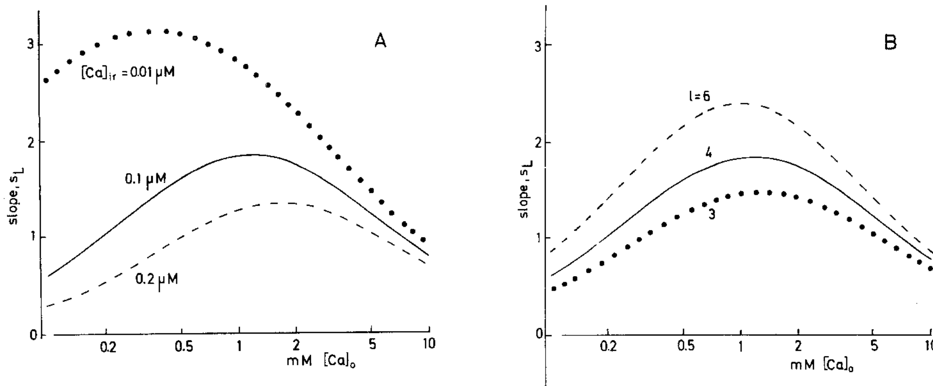

Fig. 8 A and B
Dependence of the logarithmic slope s_L on $[Ca]_o$ as predicted by Eq. (3). (**A**) Effect of the variation of the parameter $[Ca]_{ir}$. (**B**) Effect of the variation of l. With exception of the specific value indicated at each curve, the standard parameters listed in the legend of Fig. 7 were used

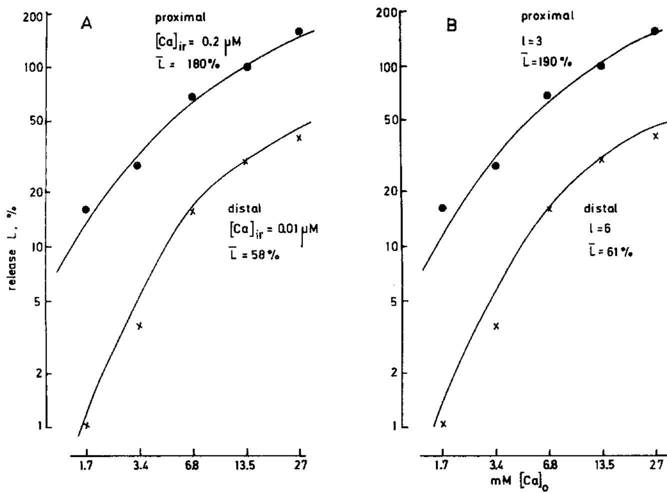

Fig. 9A and B
Average release L measured at proximal (●) and distal (×) synapses in dependence on $[Ca]_o$, like in Fig. 3. Curves given by Eq. (1) and (2), fitting the behaviour of proximal and distal synapses, respectively, obtained in (**A**) by adjusting $[Ca]_{ir}$ and $\bar{L}$, in (**B**) by adjusting l and $\bar{L}$, with specific values indicated at the curves. Other parameter 'standard' as listed in Fig. 7

3 for proximal and distal synapses, in addition to the other variable, $[Ca]_{ir}$ or l. A further discussion with the aim to assess the relative merits of changes in $[Ca]_{ir}$ or l will have to consider in more detail the slopes of the curves and the amplitudes of facilitation.

Figure 8 displays the expected logarithmic slopes s_L [Eq. (3)] against $[Ca]_o$ for changes in $[Ca]_{ir}$ and in l. Both changes in $[Ca]_{ir}$ and in l can easily match the behaviour of s_L at proximal synapses (Fig. 8A and B, lowest curves). One of the single curves in Fig. 4, proximal, peaks already at $[Ca]_o = 5$ mM which would require $[Ca]_{ir} > 0.2$ and could not easily be matched by lowering l below 3. However, for the respective values of $[Ca]_{ir}$ and l, F_s should show a maximum at higher $[Ca]_o$ than s_L (Fig. 7B and C), but in the experiments in which the peak of s_L was at 5 mM $[Ca]_o$ the maximum of F_s was at 3.4 mM $[Ca]_o$. The 'maximum' in the average curve in Fig. 6A which is generated by this one aberrant measurement therefore should not be taken seriously. For the distal synapses, $l = 6$ or $[Ca]_{ir} = 0.01$ µM predict s_L values of nearly 2.4 at 2 mM $[Ca]_o$ (Fig. 8A and B). The average measured s_L at 2 mM $[Ca]_o$ are clearly higher, at 3.2 (Fig. 6A), and individual experiments reach s_L values of 5 (Fig. 4, distal). To account for such high s_L, more extreme values of $[Ca]_{ir}$ and l have to be assumed, or both parameters have to change. The position of maximum s_L seems to be better reproduced by changes in $[Ca]_{ir}$ than in l. In summary, the measured logarithmic slopes require rather large changes of $[Ca]_{ir}$ or l to account for the characteristics of distal and proximal synapses. Changes in $[Ca]_{ir}$ seem to have some advantages over those in l to account for the measurements.

As discussed in the context of Fig. 7C and D, the changes in $[Ca]_{ir}$ or l used above also account for the dependence of F_s on $[Ca]_o$ in proximal and distal fibers. The average measured F_s of 5 at 1.7 mM $[Ca]_o$ is easily matched by both proposed theoretical curves. The maximal facilitation of 11 in Fig. 5, distal, is beyond the scope of each single theoretical relation, requiring both a low $[Ca]_{ir}$ and a high l. Similar to s_L, the observed difference in $[Ca]_o$ at maximum F_s seems to be better reproduced by changes in $[Ca]_{ir}$ than by those in l (compare Fig. 7B and C). This feature again speaks slightly in favour of $[Ca]_{ir}$ as a means to account for the differentiation of proximal and distal synapses.

In conclusion, we want to present the average release and facilitation curves together with curves calculated on the basis of our model, with parameters adjusted as discussed above. We did not try to obtain optimal fits: the changed parameters were selected with only one significant digit accuracy, and the rest of the parameters remained the standard ones selected previously. Only K_L was adjusted when l was altered, because it influences release with the power of l [Eq. (1)]; its effect on the release curve remained constant if $K_L = 5\ (2^{1/l} - 1)$ was assumed. Fig. 9 presents the measured (symbols, see Fig. 3) and calculated (curves) dependence of release on $[Ca]_o$ for the proximal and the distal synapses. Both approximations, by adjusting $[Ca]_i$ or l, give very good fits to the average measurements.

In Fig. 10 the same sets of parameters were used to approximate the dependence of facilitation on $[Ca]_o$ for proximal and distal synapses. The respective measured average F_s values (symbols) are the same as in Fig. 6B. In

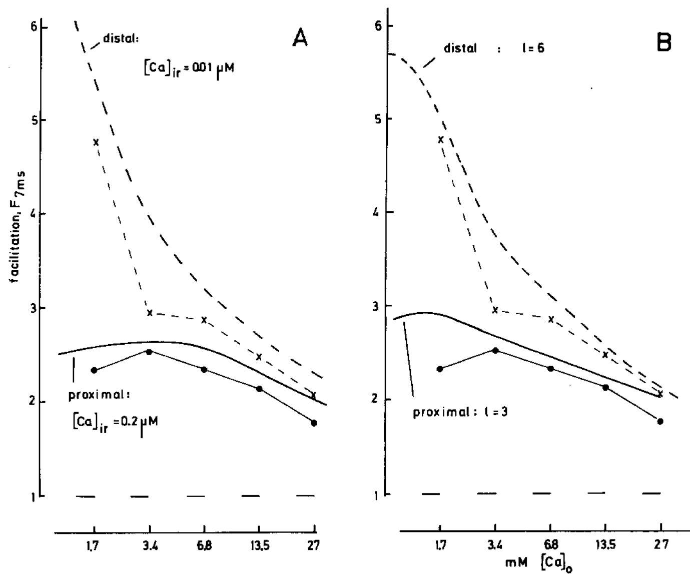

Fig. 10 A and B
Average facilitation measured with 7 ms twin pulse interval, $F_{7\text{ms}}$, for proximal (●) and distal (×) synapses, as in Fig. 6B, as a function of $[\text{Ca}]_o$. Curves approximating the behaviour of proximal and distal synapses, respectively, in (**A**) by adjusting $[\text{Ca}]_{ir}$, in (**B**) by adjusting l, with specific values indicated at the curves (same values as in Fig. 9). Other parameters 'standard' as listed in Fig. 7. The curves were calculated according to Eq. (4), with a correction of '2 Y' for Ca_i-removal during 7 ms, as described in the text

addition to the adjustments of $[\text{Ca}]_{ir}$ or l, in the calculated curves also the underestimate of F_s due to the 7 ms interval between the pulses was corrected: using the standard removal obtained in Parnas, I., et al. (1982), the reduction of $[\text{Ca}]_i$ by removal of Ca after the entry of Ca was calculated and the facilitation $F_{7\text{ms}}$ due to the 'residual $[\text{Ca}]_i$' at this time was determined by an equivalent of Eq. (4). These calculated $F_{7\text{ms}}$ curves agree well with the measured average facilitations, with some qualifications as discussed above. The approximation by adjustment of $[\text{Ca}]_{ir}$ seems to be slightly better, but, within the limits of the present data, both changes in $[\text{Ca}]_{ir}$ or l can account for the different characteristics of proximal and distal synapses.

Discussion

In the previous papers of this series we have set up a model of the synaptic release mechanism trying to cover the experimental results of variations in impulse intervals, or in concentrations of extracellular ions. In the present study, nature did the experiment creating fast and slow synapses, and we have tried to find out which parameters were varied. We had to assume, that there was only one major change in parameters, but this is not necessarily true, since a number of small shifts in several parameters also could achieve the fast/slow differentiation.

Our analysis did not lead to a unique solution: together with $\bar{L}$, both changes in $[\text{Ca}]_{ir}$ and l can explain the differentiation. The resting $[\text{Ca}]_i$ and the cooperativity of release l as such seem *a priori* rather improbable as differentiating characters, and they only could be identified by means of our quantitative non-linear model of release and facilitation. While we could not make a final choice between $[\text{Ca}]_{ir}$ and l on the basis of our present data, the theoretical graphs in Figs. 7 and 8 predict which experimental results could allow a decision. Fig. 7B shows that F_s will peak at about 0.3 μM $[\text{Ca}]_o$ with $[\text{Ca}]_{ir} = 0.01$ μM, while with $l = 6$ the peak in F_s will be at higher $[\text{Ca}]_o$ of about 0.9 mM (Fig. 7C). Similarly, Fig. 8 shows that with $[\text{Ca}]_{ir} = 0.01$ μM s_L should have a maximum at about 0.3 mM $[\text{Ca}]_o$, while with $l = 6$ this maximum will occur above 1 mM $[\text{Ca}]_o$. Therefore, twin pulse measurements as presented here, but extended to very low $[\text{Ca}]_o$ levels of about 0.1 mM should discriminate between the two still open solutions. As mentioned above, measurements at very low $[\text{Ca}]_o$ did not lead to reliable results so far: the release rates become very low resulting in prohibitively long durations of the recordings, and in addition the refractory period of the axon is prolonged which frequently causes block of conduction for the second pulse. More indirect methods could be more promising: one could try to raise the intracellular $[\text{Ca}]_i$ by applying Ca-ionophores, or by blocking the removal of Ca_i, reducing $[\text{Na}]_o$ and/or adding ouabain to block the Na-pump at low $[\text{Ca}]_o$.

Previous interpretations of the differentiation of proximal and distal muscle fibers were mainly given by Bittner (1968) and Atwood and Bittner (1971). They excluded postsynaptic factors as major causes, and also could not find appreciable differences in the distribution and density of terminals or in their morphology. The mechanism of differentiation between the proximal and the distal synapses suggested by Bittner and Atwood (1971) is block of conduction in axon terminals, and this block occurring nearer to the terminal in fast synapses than in slow ones. Such blocking points have been associated with 'bottlenecks' seen in the axons in serial sections (Jahromi and Atwood 1974; Atwood and Pomeranz 1977). Preterminal conduction blocks were also suggested by Dudel (1965a, b) based on measurement of terminal potentials during presynaptic inhibition and facilitation. However, Zucker (1974) reported that large stimuli applied at the terminals elicited antidromically conducted action potentials, which might be seen to exclude the differential block hypothesis of differentiation. On the other hand, the large stimuli may not really have excited the terminal, but closely adjacent axon branches (Dudel 1982). The differential block hypothesis thus may be valid still. However, in the light of our results differential blocks do not seem to be a probable mechanism. A smaller terminal depolarization due to distant conduction block essentially would reduce $\bar{Y}$, and reduction of $\bar{Y}$ could be excluded as an explanation for the characteristics of a distal synapse. Unless the block of conduction itself is much affected by $[\text{Ca}]_o$, conduction blocks at varying distance from the terminal may be disregarded as likely explanation for the differentiation.

The proposed differentiation parameters, $[Ca]_{ir}$ or l, look very different in their physiological context. $[Ca]_{ir}$ must be assumed to be 20 times higher in the proximal terminals than in the distal ones. This does not seem to be too improbable, since $[Ca]_i$ is a typical physiological regulator of cell activity in this concentration range. Linder (1974) and Bittner and Sewell (1976) observed that facilitation after a single pulse decayed much slower in fast synapses than in slow ones. One could conclude from these measurements at 13.5 mM $[Ca]_o$ that the R_2 rate of Ca_i-removal (Parnas, I., et al. 1982; Parnas, H., et al. 1982b) is smaller in the terminals of fast synapses than in those of slow ones. The lower R_2 could result in a higher $[Ca]_{ir}$ in fast synapses. A smaller Ca_i-removal activity could also be correlated to a relatively low content of mitochondria in terminals of a fast synapse noted by Atwood and Jahromi (1978). – The alternative, the cooperativity l would only have to be two times larger in distal than in proximal synapses. The different cooperativities could be assumed to arise by aggregations of releasing sites in the membrane of varying complexity, as has been assumed for postsynaptic receptors (Changeux et al. 1967; Colquhoun 1973). Both, $[Ca]_{ir}$ or l could be under control of hormons or modulators (Glusman and Kravitz 1982) which would be in line with the changes in differentiation observed by Bittner (1968).

Our present results have some bearing on the interpretation of $[Mg]_o$ effects on release given in Dudel et al. 1982. In Fig. 1 of this paper, the dependence of release on $[Ca]_o$ and $[Mg]_o$ was shown for a proximal and for a distal synapse. The behaviour of the proximal synapse was fitted by reducing K_y. As we know now, adjustment of $[Ca]_{ir}$ or l would have been more appropriate. Further, distal fibers were used for the analysis of the $[Mg]_o$-efffect in Fig. 4, since this effect is more impressive in distal fibers. The maximal facilitations $\hat{F}$ shown in this figure were higher than predicted by our model with the 'standard' parameters (Fig. 6 of Dudel et al. 1982). If $[Ca]_{ir} = 0.01$ µM or $l = 6$ had been assumed, the high F values typical for distal fibers would have been predicted. The other quantitative discrepancies between predictions and measurements, concerning $[Mg]_{o\hat{F}}$ and $Y_{\hat{F}}$, would not have been removed by inserting the appropriate values for $[Ca]_{ir}$ or l.

Finally, we would like to comment on the physiological significance of the proximal/distal differentiation. With regard to single pulses, the differentiation of the terminal is complemented by postsynaptic differences: while the input resistance of the proximal fibers is several 100 kΩ to 1 MΩ, the imput resistance of the large distal fibers can be as low as 20 kΩ (Dudel unpublished). These differences are much larger than those reported by Bittner (1968) for the central/far-distal fibers. The relatively much higher input resistance together with the higher quantum content in single EPSCs leads to large EPSPs in the proximal fibers (Iravani 1965) which can pass the threshold of contraction (– 50 mV, Dudel et al. 1968) in single stimuli and sometimes trigger graded spikes (Dudel unpublished). The distal fibers, however, often show single EPSPs of 10 µV amplitude, and threshold for contraction can be reached only in a series of stimuli with large facilitation and summation. The functional significance of postsynaptic differences in the decay of EPSPs, i.e. in ion channel open time, in proximal and distal fibers (Dudel 1979) is unclear.

Acknowledgement. The authors want to thank Dr. Daut for comments on the manuscript, and Miss I. Horstmann and Mrs. M. Griessel for technical and secretarial help.

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Received June 8/Accepted September 15, 1982