J. Physiol. (1968), **199**, *pp.* 205–221
With 8 *text-figures*
Printed in Great Britain

MINIATURE SYNAPTIC POTENTIALS AT FROG SPINAL NEURONES IN THE PRESENCE OF TETRODOTOXIN

BY F. COLOMO* AND S. D. ERULKAR†

From the Department of Biophysics, University College London, Gower Street, London, W.C. 1

(*Received* 18 *June* 1968)

SUMMARY

1. Spontaneous subthreshold potentials have been recorded with an intracellular electrode from neurones of the isolated spinal cord of the frog.

2. Records of both depolarizing and hyperpolarizing potentials were obtained from cells in normal Ringer solution and also when impulse conduction in the cord had been abolished by tetrodotoxin (TTX).

3. Comparisons of results before and after TTX show that the majority of the spontaneous potentials are analogous to miniature end-plate potentials.

INTRODUCTION

There is now a great deal of evidence which shows that at a number of peripheral synapses the nerve endings are in a state of continuous secretory activity (Fatt & Katz, 1950, 1952; Boyd & Martin, 1956; Nishi & Koketsu, 1960; Dudel & Orkand, 1960; Dudel & Kuffler, 1961; Burnstock & Holman, 1962; Katz, 1962, 1965; Blackman, Ginsborg & Ray, 1963; Miledi, 1966, 1967). The transmitter substance is released in 'quanta' which produce local subthreshold potential changes: 'miniature synaptic potentials' (min. syn. pot.s).

Spontaneous subthreshold synaptic potentials or 'synaptic noise' (Eccles, J. C., 1961) have been recorded also from amphibian and mammalian central neurones (Brock, Coombs & Eccles, 1952; Araki, Otani & Furukawa, 1953; Kuno, 1957; Kolmodin & Skoglund, 1958; Li, 1959; Eccles, Eccles, Iggo & Lundberg, 1961). The question is whether these subthreshold synaptic potentials are analogous to the miniature end-plate

Present addresses:

* Istituto di Fisiologia Umana Universitá di Firenze, Impresa di Elettrofisiologia del C.N.R., Firenze, Italy.

† Department of Pharmacology, University of Pennsylvania Medical School, Philadelphia, Pa., U.S.A.

potentials or whether they are due to multiquantal release of transmitter by spontaneous impulses in the afferent pathways. Recently, Katz & Miledi (1963) have presented evidence that a large proportion of the 'synaptic noise' recorded from motoneurones in the isolated spinal cord of the frog is analogous to miniature end-plate potentials (m.e.p.p.s) and that at these synapses the release of the transmitter substance occurs in quantal form. They showed that when synaptic transmission was greatly reduced by adding magnesium to the bathing solution, or when impulse conduction was abolished by increasing the external potassium concentration, spontaneous subthreshold potentials were still present.

Another method of blocking nerve impulse activity is provided by the puffer fish poison tetrodotoxin (TTX). This drug abolishes impulses in nerve and muscle fibres by interfering specifically with the transfer of sodium through the depolarized membrane (Narahashi, Deguchi, Urakawa & Ohkubo, 1960; Nakamura, Nakajima & Grundfest, 1965; Narahashi, Moore & Scott, 1964) without affecting the depolarizing action of the acetylcholine at the end-plate (Furukawa, Sasaoka & Hosoya, 1959) nor the quantal release of transmitter from the nerve terminals (Elmqvist & Feldman, 1965; Katz & Miledi, 1965, 1967*a*, *b*, *c*; Miledi, 1966, 1967). Recently, Hubbard, Stenhouse & Eccles (1967) used TTX on cat spinal cords and reported that after abolition of nerve impulses only a few spontaneous potentials analogous to m.e.p.p.s remained. They questioned the conclusions of Katz & Miledi (1963) and stated that 'most synaptic noise is due to transmitter release evoked by nerve impulses and can be removed by abolition of nerve impulses'.

The aim of the present study was to see whether in the preparation used by Katz & Miledi, viz. the isolated spinal cord of the frog, min. syn. pot.s still remain after all impulse evoked activity has been blocked by TTX and to describe the effects of the drug on the frequency and amplitude distribution of the spontaneous synaptic potentials. It was found that in this preparation abolition of evoked activity by TTX does not affect to any great extent the frequency and amplitude of the spontaneous potentials.

METHODS

Rana temporaria and *R. esculenta* were used. The procedures for removal and mounting of the spinal cords were the same as those described by Katz & Miledi (1963). The pia mater over the lateral surface of the spinal cord was carefully removed. Records were obtained from segments 7–9. The dorsal and ventral roots were stimulated by currents passed through suction electrodes in which the roots were held. Two platinum wires, insulated to the tips by glass, placed directly on the lateral surface of the cord were used for stimulation of the lateral funiculus. All the experiments were performed at 8–12° C and under continuous oxygenation (95 % O_2 + 5 % CO_2). The Ringer solution had the following composition (mM):

NaCl, 114; KCl, 2; $CaCl_2$, 1·8; $NaHCO_3$, 2; and glucose 1 g/l. Micropipettes filled with 3 M potassium chloride or 2 M potassium citrate (slightly acidified with citric acid) were used. Best results were obtained with electrodes of 18–20 MΩ resistance.

Experimental procedure. The motoneurone pool was localized by recording with the micropipette the extracellular response to antidromic stimulation. Penetration of a cell was signalled by a sudden drop of the potential and by the appearance of a large antidromic spike. The responses to dorsal root and lateral funiculus stimulation were also examined. The cathode follower outputs were connected to 2A61 and 3A3 Tektronix amplifiers in a Tektronix 565 oscilloscope. The former amplifier was operated at high gain and recorded, via a capacitative coupling of 0·06 sec, the extracellular responses and, after penetration of a cell, the spontaneous subthreshold activity; the latter amplifier, directly coupled and at lower gain (10 or 20 mV/cm), recorded the level of the resting membrane potential and the spike amplitudes. When spontaneous synaptic activity was present and could clearly be seen above the noise level, the sensitivity of the DC amplifier was increased to 0·5 mV/cm. Records of the spontaneous synaptic activity in normal Ringer solution were taken for approximately 20 min. TTX was then added to the bath. Final concentrations of TTX ranged from 2×10^{-7} to 7×10^{-6} g/ml. in different experiments. Recording was then continued provided that the resting potential remained at a satisfactory level. The responses elicited by lateral funiculus and dorsal root stimulation were periodically examined until no further responses could be evoked.

Since at least 50 min were required for TTX to abolish the responses to lateral funiculus stimulation these experiments were rarely successful. In the majority of cells the resting potentials deteriorated before the abolition of responses to lateral funiculus stimulation was complete. Therefore, a second series of experiments was carried out. After localization of the motoneurone pool, TTX was added to the bath in the concentrations mentioned above and the solution was well stirred. When the extracellular responses to lateral funiculus and dorsal root stimulation had been completely abolished (within 20 min), cell penetrations were attempted. If a stable resting potential was obtained, lateral funiculus and dorsal root stimulation were tested again to verify the absence of responses. Records were taken of any spontaneous activity which was present.

Method of evaluation of spontaneous activity. Measurements of the spontaneous potential amplitudes were made from enlarged records. Because of the low signal/noise ratio many small potentials probably remained undetected, and acceptance or rejection of small synaptic potentials was bound to be somewhat arbitrary. A check was made by having the same series of records examined separately by the two authors. The results of these tests agreed sufficiently well to show that the criteria used were consistent, and that a valid comparison could be made of results obtained before and after TTX paralysis.

In most experiments KCl electrodes were used for intracellular recording. As a result, inhibitory potentials become inverted within a few minutes after cell penetration (see Katz & Miledi, 1963, p. 409) and thereafter cannot be distinguished from excitatory potentials. In general, counting of miniature potentials did not start until this 'inversion' was established.

RESULTS

In any central nerve cell subthreshold synaptic potentials may occur without applied stimulation, either because of spontaneous firing of impulses in other cells or because of spontaneous local release of transmitter from the presynaptic terminals. The extent to which each of these factors operates can be assessed by comparing records from neurones before and after propagated activity has been abolished by TTX. The

test which was used in the present work was the abolition of all detectable electrical responses in motoneurones when stimuli of maximal strength were applied to the ventral and dorsal roots and to the lateral funiculus. Owing to the longer time taken for the TTX to penetrate the cord, the responses to stimulation of the lateral funiculus were always the last to disappear.

Spontaneous activity in normal Ringer solution. Samples of spontaneous synaptic potentials from a motoneurone, before the application of TTX, and the histogram of their amplitude distribution are shown in Fig. 1. This cell responded with large spikes to dorsal root and lateral funiculus stimulation. The average amplitude of the spontaneous potentials was 160 μV; the frequency 21/sec. The distribution of amplitudes is similar to those observed by Katz & Miledi (1963), the peak class merging into the noise level. In five other cells similar results were obtained. The average amplitudes of the spontaneous potentials ranged from 130 to 230 μV and the frequencies from 9·8 to 41/sec (Table 2).

Hyperpolarizing spontaneous potentials resembling those described by Katz & Miledi (1963) could be recorded in motoneurones when potassium citrate micro-electrodes were used. The average amplitude of the hyperpolarizing potentials in the experiment illustrated in Fig. 2 was 140 μV and the frequency 15/sec. The amplitude histogram resembles those of the excitatory potentials from this and other cells.

Spontaneous potentials from the same cell before and after TTX. In one case, the resting potential of the motoneurone remained stable long enough to continue recording while TTX was applied, and after the responses to dorsal root and lateral funiculus stimulation had been abolished. The results from this experiment are illustrated in Figs. 3–5 and listed in Table 1. This motoneurone responded with action potentials to ventral root, dorsal root and lateral funiculus stimulation. Figure 3 shows sample records and the amplitude distribution of the spontaneous potentials before TTX was added to the bathing solution. During this period the average amplitude of the potentials was $160 \pm 3{\cdot}0$ μV and the frequency 18·2/sec. Two minutes after TTX administration, the mean amplitude and frequency of the potentials were slightly reduced. This reduction was associated with a fall in the resting membrane potential which was probably due to the displacement of the electrode during the TTX administration. After this initial reduction, there was no further marked decline in the frequency or amplitude of the spontaneous potentials or in the shapes of the amplitude distribution (Figs. 4 and 5). The responses to dorsal root and lateral funiculus stimulation were abolished at 18 and 50 min respectively; as shown in Table 1, their abolition was not accompanied or followed by any marked change in the characteristics of the spontaneous

potentials. To avoid the ambiguities in measuring the smallest potentials, in columns 3 and 5 of Table 1, potentials greater than 0·2 mV in amplitude were selected. These did show an initial decline in frequency, but not a significant decline after the resting potential had become stable. Calculation of the proportion of the potentials greater than 0·2 mV, relative to the total number of potentials, showed no significant decline so that it is unlikely that many of the large potentials were due to impulses. The experiment shows that after all elicited activity has been abolished by

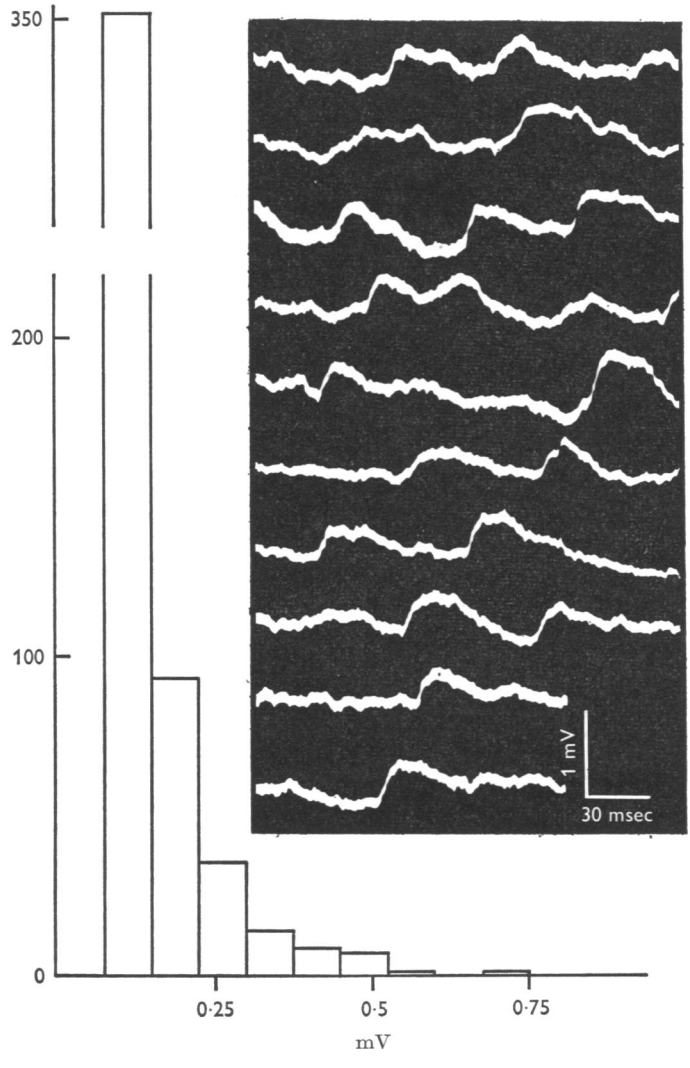

Fig. 1. Amplitude distribution and sample records of spontaneous potentials in a motoneurone. Normal Ringer solution. KCl-pipette. Resting potential (R.P.), 50 mV.

TTX, most of the spontaneous synaptic activity still occurs. If the change in resting potential is taken into account, there is little difference in the mean size and frequency of this activity before or after the addition of the TTX.

A similar result was obtained from another motoneurone (also listed in Table 1) in which the resting potential remained stable for 59 min after the addition of TTX. There was some doubt, however, whether the re-

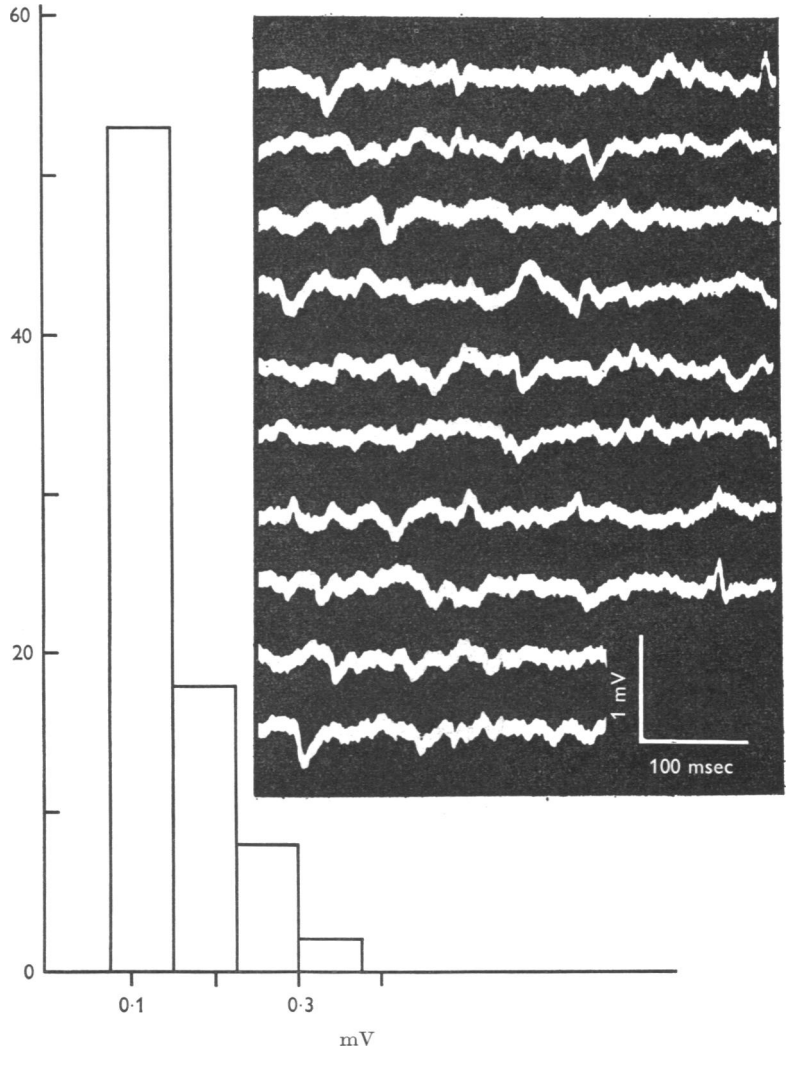

Fig. 2. Amplitude distribution of hyperpolarizing potentials and sample records of spontaneous potentials in a motoneurone. Normal Ringer solution. Citrate-pipette. R.P. 40 mV.

sponse of this cell to lateral funiculus stimulation had been completely abolished before the micro-electrode became dislodged. In two out of twenty-seven trials the stimulus to the lateral funiculus was followed by a small depolarization, and it was impossible to decide whether these were evoked or spontaneous potentials.

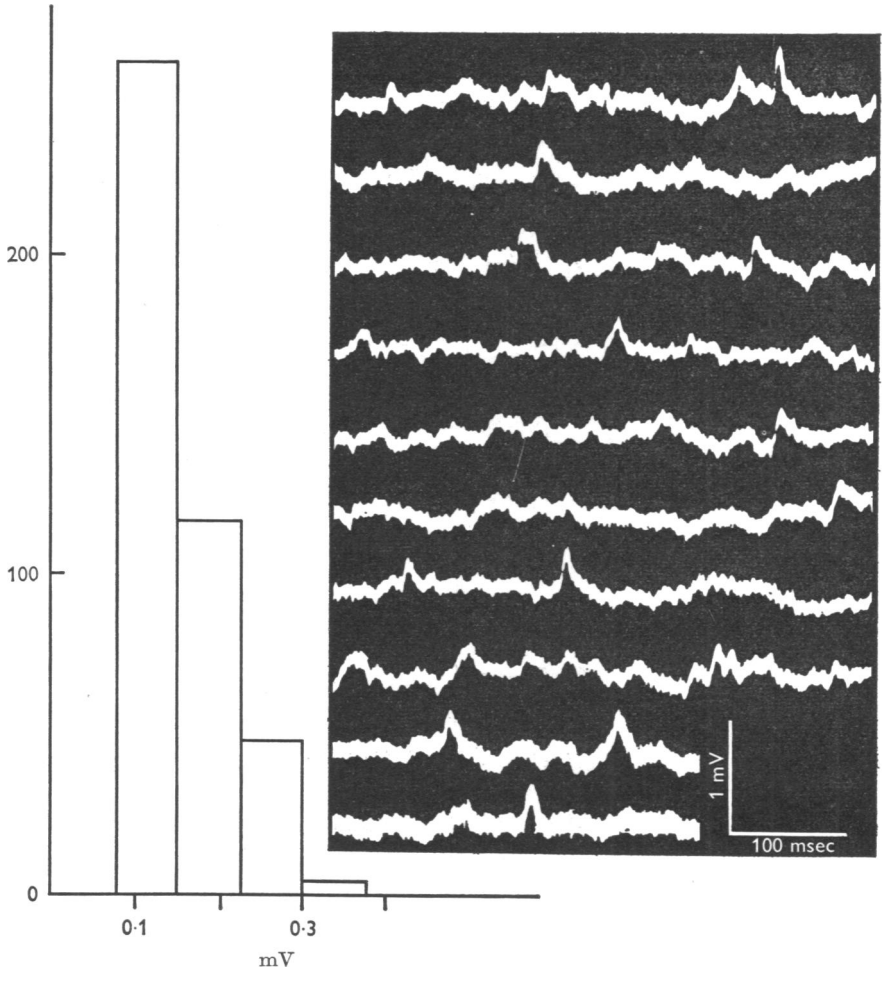

Fig. 3. Amplitude distribution and sample records of spontaneous potentials. Same cell as in Figs. 4 and 5. Normal Ringer. KCl-pipette. R.P. 50 mV.

It might be argued that impulse activity in internuncial neurones which could give rise to some of the potentials may not have been abolished in these experiments. This is possible, but unlikely, for the disappearance of the response to lateral funiculus stimulation shows that TTX had pene-

trated into the cord and had blocked propagation of impulses in tracts which directly impinge on the motoneurone.

Other observations on spontaneous activity in TTX-Ringer. Because of the difficulty in obtaining stable recordings from the same cell both before and after TTX administration, a second series of experiments was made on cells after complete paralysis (see Methods). The results from these experiments are summarized in Table 2. Records of spontaneous potentials from these cells are shown in Figs. 6 and 7, and Fig. 7 also shows the corre-

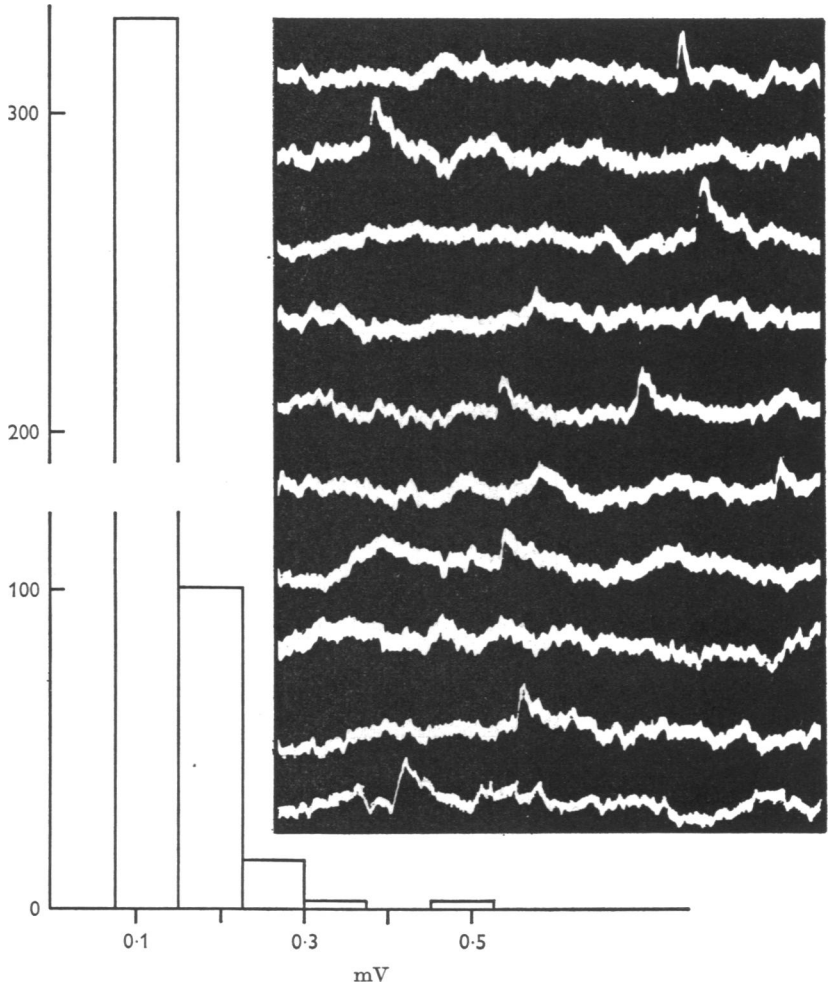

Fig. 4. Amplitude distribution and sample records of spontaneous potentials for same cell as in Fig. 3, but obtained 28 min after TTX administration. At this time, responses to DR stimulation had been eliminated, those to lateral funiculus stimulation were still present. Calibration as in Fig. 3. R.P. 40 mV.

sponding histogram. In ten cells, at times ranging from 9 min to 4 hr after complete block of elicited responses, the frequency of occurrence of the spontaneous potentials ranged from 5·6 to 33·1/sec and the average amplitude from 140 to 250 μV. These results can be compared with those obtained from six cells in normal Ringer solution. For the purpose of a general summary one may calculate an 'over-all' frequency for normal and TTX-treated preparations from the values given in Table 2. In six normal

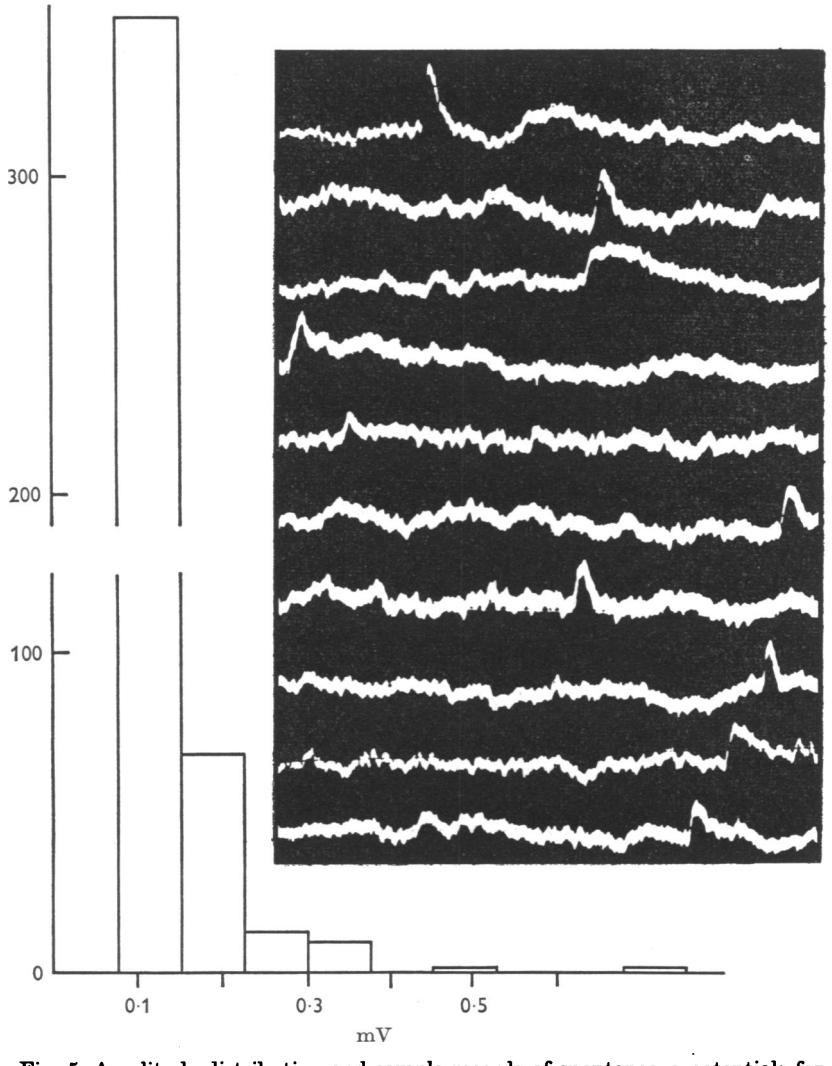

Fig. 5. Amplitude distribution and sample records of spontaneous potentials for same cell as in Figs. 3 and 4, but obtained 61 min after TTX administration and 11 min after responses to lateral funiculus stimulation were abolished. Calibration as in Fig. 3. R.P. 44 mV.

TABLE 1. Summary of the results obtained from two cells in which spontaneous activity was recorded before and after abolition of responses evoked by dorsal root (DR) and lateral funiculus (LF) stimulation

		Total number of potentials n_1	Number of potentials > 0·2 mV n_2	Frequency, per sec n_1/t	Frequency, per sec n_2/t	Mean amplitude of all the potentials (μV)	RP	Response to stimulation DR	Response to stimulation LF
	Before TTX	427	109	18·2	4·6	160 ± 3·0	50	+	+
2 min after		345	63	17·3	3·2	151 ± 3·0	40	+	+
28 min after		450	56	16·1	2·0	144 ± 2·3	40	0	+
45 min after		441	67	15·5	2·4	148 ± 2·8	43	0	+
53 min after		453	58	15·6	2·0	148 ± 3·0	43	0	0
61 min after		437	50	15·9	1·8	148 ± 3·0	44	0	0
	Before TTX	842	406	12·5	6·0	230	53	+	+
10 min after		476	181	17·4	6·6	210	50	+	+
23 min after		496	203	15·1	6·2	200	50	0	+
39 min after		382	139	14·9	5·4	210	50	0	+
59 min after		196	111	10·1	5·7	210	40	0	0

cells, 2348 potentials were counted in a period of 151·1 sec, i.e. an average of 15·5/sec. In ten TTX-treated neurones, 3231 potentials were observed over a period of 281·4 sec, i.e. an average of 11·5/sec. If one eliminates all potentials of less than 0·2 mV size (cf. above), the corresponding average frequencies are 4·6/sec for the six normal, and 3·3/sec for the ten treated neurones. This could mean that some 25–30% of the spontaneous potentials arose from impulses. This calculation, however, disregards the wide

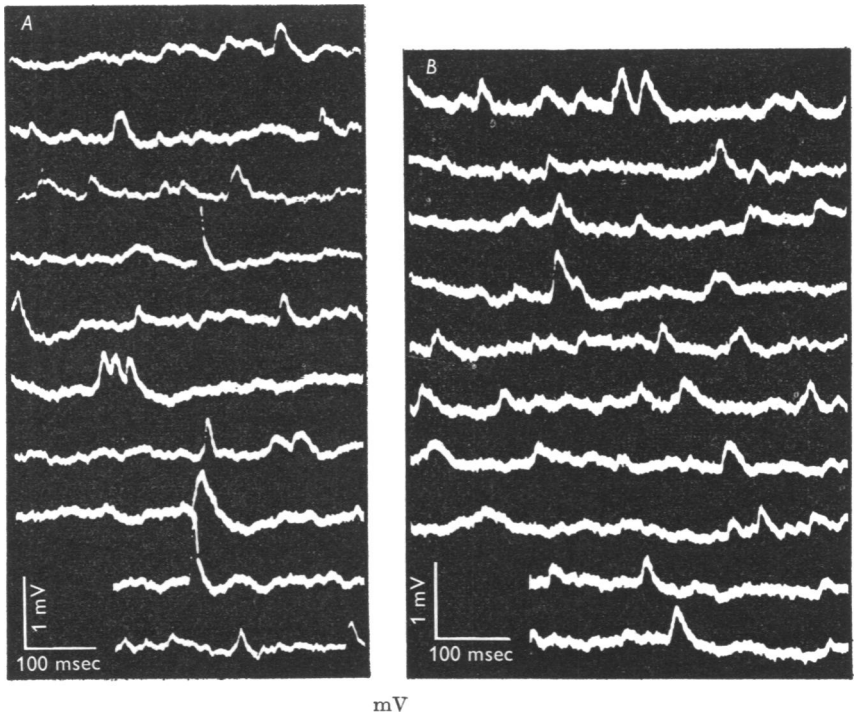

Fig. 6. Spontaneous potentials from two neurones 80 min (*A*) and 150 min (*B*) after abolition of field potential responses to lateral funiculus stimulation by TTX. KCl electrodes. R.P. (*A*) 63 mV; (*B*) 50 mV.

range of frequencies of the spontaneous potentials in the two samples (9·8–41/sec and 0·5–7·4/sec for cells in normal Ringer, 5·6–33·1/sec and 0·6–8·0/sec for TTX-treated cells) so that no great significance can be attached to the difference between these values. If each individual experiment is given equal 'weight' and the arithmetic or (because of the wide range of values) geometric means are calculated, then the mean frequencies for potentials greater than 0·2 mV are actually a little higher for TTX-treated cells than for those in normal Ringer. This difference is again of no significance; what is clear from these results is that the majority of

spontaneous potentials can still be seen after the responses had been completely paralysed by TTX.

On a few occasions when a potassium citrate micro-electrode was used, hyperpolarizing spontaneous potentials were recorded after TTX had blocked impulses. Figure 8 shows records and the amplitude distribution of these potentials from a cell 240 min after abolition of the evoked response by TTX. The experiment suggests that release and action of the

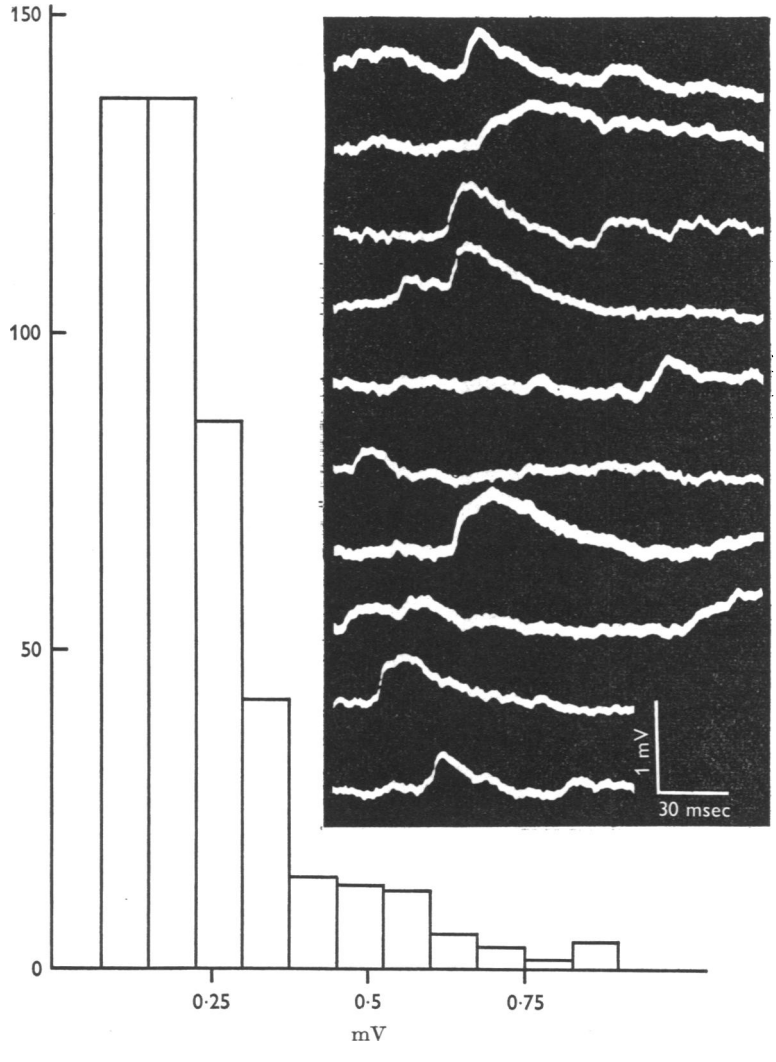

Fig. 7. Amplitude distribution and sample records of spontaneous potentials, 70 min after field potential responses to lateral funiculus stimulation had been abolished by TTX. KCl electrode. R.P. 60 mV.

inhibitory transmitter are not impaired by TTX, in a dose which is sufficient to abolish impulse activity.

TABLE 2. Summary of the results obtained from different cells in normal Ringer and TTX-Ringer

Total number of potentials n_1	Number of potentials > 0·2 mV n_2	Frequency, per sec n_1/t	Frequency, per sec n_2/t	Mean amplitude of all the potentials (μV)	Time after paralysis (min)
Normal Ringer					
222	40	41	7·4	150*	
166	25	9·8	1·5	140	
512	110	21·3	4·6	160	
179	7	12·8	0·5	130	
842	406	12·5	6·0	230	
427	109	18·2	4·6	160	
Σ2348	697				
Arithmetic mean		19·3	4·1	162	
Geometric mean		17·2	3·0	—	
$\Sigma n_1/\Sigma t = 15{\cdot}5$; $\Sigma n_2/\Sigma t = 4{\cdot}6$					
TTX Ringer					
219	85	17·8	6·9	180	240*
437	50	15·9	1·8	148	11
451	252	7·7	4·3	250	70
228	39	19·3	3·3	150	140
243	67	18·7	5·2	160	130
162	46	19·3	5·5	160	150
404	97	33·1	8·0	150	20
196	111	10·1	5·7	210	9
600	150	9·1	2·3	170	80
291	32	5·6	0·6	140	160
Σ3231	929				
Arithmetic mean		15·7	4·4	172	
Geometric mean		13·85	3·55		
$\Sigma n_1/\Sigma t = 11{\cdot}5$; $\Sigma n_2/\Sigma t = 3{\cdot}3$					

* In these cells, both hyperpolarizing and depolarizing potentials were considered.

DISCUSSION

These experiments confirm the existence of miniature synaptic potentials and show that in the isolated spinal cord of the frog spontaneous synaptic activity of motor nerve cells is not reduced to any great extent when impulse-evoked activity is abolished by TTX. Spontaneous subthreshold potentials persisted at frequencies similar to those observed before TTX had been added to the bath. The amplitude histograms and the average amplitudes of the potentials were also not significantly altered and were similar to those reported by Katz & Miledi (1963) for normal Ringer solution.

No definitive proof has been obtained that all spontaneous impulses in afferent and in internuncial axons were abolished by TTX. Nevertheless, the experiments showed that TTX penetrated the spinal cord sufficiently to block excitation in the axons of the lateral funiculus. It is therefore

very probable that during prolonged immersion in the drug solution spontaneous or evoked impulses were abolished generally throughout the cord. It may be emphasized that the preparations had been immersed in the drug solution for periods of up to 4 hr after the field potentials elicited by dorsal root and lateral funiculus stimulation had been abolished. It is conceivable that some spinal neurones are not susceptible to the action

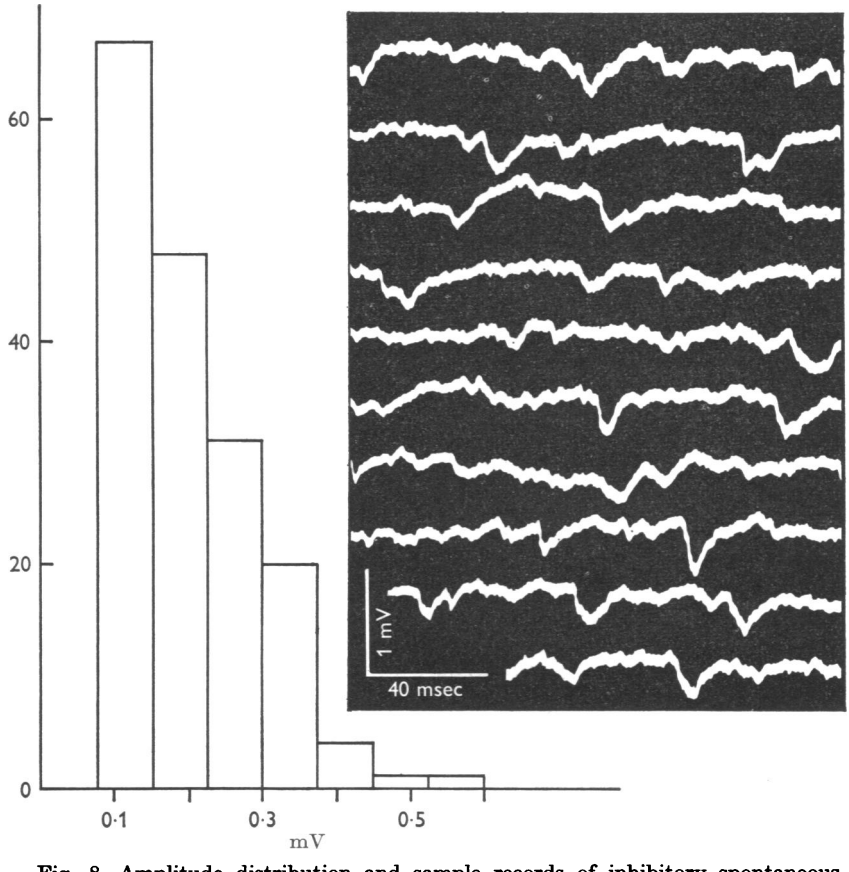

Fig. 8. Amplitude distribution and sample records of inhibitory spontaneous potentials obtained 230 min after field potential responses to lateral funiculus stimulation were abolished by TTX. Citrate-pipette. R.P. 40 mV.

of TTX; Koketsu & Nishi (1968) have found that 'calcium spikes' which may occur in cells of the sympathetic ganglion of the frog are not influenced by TTX. Even if this were the case, the lack of any marked diminution in size and frequency of the spontaneous potentials in the motoneurones strongly indicates, as concluded by Katz & Miledi (1963), that the majority of these potentials are analogous to the m.e.p.p.s and have their origin within the synaptic terminals.

These results differ from those obtained by Hubbard *et al.* (1967). It is not surprising that a mammalian cord, *in situ*, behaves differently from an isolated amphibian cord, and, under the conditions of the experiments of Hubbard *et al.*, spontaneous transmitter release might well be masked by impulse activity. This will be the case particularly if some of the afferent connexions are left intact. Moreover, the size of a quantal synaptic potential depends on the input impedance of the cell (Katz & Thesleff, 1957), and this may be lower in mammalian than amphibian motoneurones because of differences in size, species and temperature.

Hubbard *et al.* (1967) used topical application of TTX to the surface of the circulated spinal cord, in an attempt to distinguish spontaneous transmitter release from impulse-evoked synaptic noise. They concluded that the residual spontaneous activity recorded 216 sec after TTX had been 'squirted from a syringe on to the area around the electrode' was due to local transmitter release, but even this may be questioned. Unless the diffusion of TTX into the kitten's spinal cord is much faster than that into the frog's spinal cord, it is unlikely that impulses in all the relevant pathways could have been blocked in such a short time. It is possible that the residual spontaneous activity recorded by Hubbard *et al.* may have been mainly due to multiquantal release by impulses and that the size of the quantal unit potentials in their cells was too small to be detected. This is a criticism also raised by Blankenship & Kuno (1968) who found that longer times were needed for impulses in the cat spinal cord to be suppressed by TTX.

While there is some doubt whether in the experiments of Hubbard *et al.* (1967) true spontaneous miniature potentials have been recorded, Blankenship (1968) and Blankenship & Kuno (1968) tested this point by following the time course of the TTX effect on individual motoneurones. They observed a decline in the frequency of the spontaneous synaptic activity (to between 5 and 60% of the control values), and disappearance of the large potentials. In some of their experiments, completely deafferented cords were used. It may be that the activity removed by TTX was due to spontaneous firing of interneurones, and that this is more common in the cat spinal cord at 35–38° C than in the frog's isolated cord at 8–12° C.

It is quite likely that some proportion of the potentials which we have recorded did arise from impulse activity (see also Katz & Miledi, 1963, fig. 9*A*) but all our evidence suggests that this proportion must have been small.

In summary, the generalization made by Hubbard *et al.* (1967) that 'most synaptic noise is due to transmitter release evoked by nerve impulses', while it applies to motoneurones in the spinal cord of the cat, does not apply to motoneurones in the isolated cord of the frog.

We are very grateful to Professors B. Katz and R. Miledi for many valuable discussions and criticism during the course of this work, and for their help in the preparation of the manuscript. We express our sincere thanks to Miss Audrey Paintin, Mr Keith Copeland, Mr John Dobbin and Mr Len Ward for their technical assistance.

During the course of this work Dr Colomo held a fellowship from the Wellcome Trust, London; Dr Erulkar was supported by USPHS grants 1F1ONB 1783 and NB 02941.

REFERENCES

ARAKI, T., OTANI, T. & FURUKAWA, T. (1953). The electrical activities of single motoneurones in toad's spinal cord, recorded with intracellular electrodes. *Jap. J. Physiol.* **3**, 254–267.

BLACKMAN, J. G., GINSBORG, B. L. & RAY, C. (1963). Spontaneous synaptic activity in sympathetic ganglion cells of the frog. *J. Physiol.* **167**, 389–401.

BLANKENSHIP, J. E. (1968). Action of tetrodotoxin on spinal motoneurons of the cat. *J. Neurophysiol.* **31**, 186–194.

BLANKENSHIP, J. E. & KUNO, M. (1968). Analysis of spontaneous subthreshold activity in spinal motoneurons of the cat. *J. Neurophysiol.* **31**, 195–209.

BOYD, I. A. & MARTIN, A. R. (1956). Spontaneous subthreshold activity at mammalian neuromuscular junctions. *J. Physiol.* **132**, 61–73.

BROCK, L. G., COOMBS, J. S. & ECCLES, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.* **117**, 431–460.

BURNSTOCK, G. & HOLMAN, M. E. (1962). Spontaneous potentials at sympathetic nerve endings in smooth muscle. *J. Physiol.* **160**, 446–460.

DUDEL, J. & KUFFLER, S. W. (1961). The quantal nature of transmission and spontaneous miniature potentials at the crayfish neuromuscular junction. *J. Physiol.* **155**, 514–529.

DUDEL, J. & ORKAND, R. K. (1960). Spontaneous potential changes at crayfish neuromuscular junctions. *Nature, Lond.* **186**, 476–477.

ECCLES, J. C. (1961). The mechanism of synaptic transmission. *Ergebn. Physiol.* **51**, 299–430.

ECCLES, J. C., ECCLES, R. M., IGGO, A. & LUNDBERG, A. (1961). Electrophysiological investigations on Renshaw cells. *J. Physiol.* **159**, 461–478.

ELMQVIST, D. & FELDMAN, D. S. (1965). Spontaneous activity at a mammalian neuromuscular junction in tetrodotoxin. *Acta physiol. scand.* **64**, 475–476.

FATT, P. & KATZ, B. (1950). Some observations on biological noise. *Nature, Lond.* **166**, 597–598.

FATT, P. & KATZ, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117**, 109–128.

FURUKAWA, T., SASAOKA, T. & HOSOYA, Y. (1959). Effects of tetrodotoxin on the neuromuscular junction. *Jap. J. Physiol.* **9**, 143–152.

HUBBARD, J. I., STENHOUSE, D. & ECCLES, R. M. (1967). Origin of synaptic noise. *Science, N.Y.* **157**, 330–331.

KATZ, B. (1962). The transmission of impulses from nerve to muscle, and the subcellular unit of synaptic action. *Proc. R. Soc.* B **155**, 455–477.

KATZ, B. (1965). The physiology of motor nerve endings. *Proc. XXIIIrd int. Congr. physiol. Sci., Tokyo. Excerpta Medica International Series* **87**, 110–121.

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

KATZ, B. & MILEDI, R. (1965). Release of acetylcholine from a nerve terminal by electrical pulses of variable strength and duration. *Nature, Lond.* **207**, 1097–1098.

KATZ, B. & MILEDI, R. (1967*a*). Tetrodotoxin and neuromuscular transmission. *Proc. R. Soc.* B **167**, 8–22.

KATZ, B. & MILEDI, R. (1967*b*). The release of acetylcholine from nerve endings by graded electric pulses. *Proc. R. Soc.* B **167**, 23–38.

KATZ, B. & MILEDI, R. (1967*c*). A study of synaptic transmission in the absence of nerve impulses. *J. Physiol.* **192**, 407–436.

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

KOKETSU, K. & NISHI, S. (1968). Calcium spikes of nerve cell membrane: role of calcium in the production of action potentials. *Nature, Lond.* **217**, 468–469.

KOLMODIN, G. M. & SKOGLUND, C. R. (1958). Slow membrane potential changes accompanying excitation and inhibition in spinal moto- and inter-neurones in the cat during natural activation. *Acta physiol. scand.* **44**, 11–54.

KUNO, M. (1957). Effects of strychnine on the intracellular potentials of spinal motoneurons of the toad. *Jap. J. Physiol.* **7**, 42–50.

LI, C-L. (1959). Cortical intracellular potentials and their response to strychnine. *J. Neurophysiol.* **22**, 436–450.

MILEDI, R. (1966). Miniature synaptic potentials in squid nerve cells. *Nature, Lond.* **212**, 1240–1242.

MILEDI, R. (1967). Spontaneous synaptic potentials and quantal release of transmitter in the stellate ganglion of the squid. *J. Physiol.* **192**, 379–406.

NAKAMURA, Y., NAKAJIMA, S. & GRUNDFEST, H. (1965). The action of tetrodotoxin on electrogenic components of squid giant axons. *J. gen. Physiol.* **48**, 985–996.

NARAHASHI, T., DEGUCHI, T., URAKAWA, N. & OHKUBO, Y. (1960). Stabilization and rectification of muscle fiber membrane by tetrodotoxin. *Am. J. Physiol.* **198**, 934–938.

NARAHASHI, T., MOORE, J. W. & SCOTT, W. R. (1964). Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. gen. Physiol.* **47**, 965–974.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.