Stimulation induced depletion of the synaptic vesicles in excitatory motor nerve terminals of the locust, *Locusta migratoria* L.

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Summary. Neural stimulation at high frequency induced gross changes in the ultrastructural properties and depleted the synaptic vesicles in fast axon terminals of locust extensor tibiae muscles. After a 1-h-rest the synaptic vesicle populations recovered and the ultrastructural properties of these recovering neuromuscular junctions closely resembled those of the controls.

The release of transmitter from axon terminals is generally considered to be a quantal process which has long been correlated with the existence of presynaptically located vesicles[3,4]. This correlation has been partly confirmed by investigations which have indicated that the terminal synaptic vesicle population could be depleted by electrical stimulation[5]. Information from studies of this nature appear to be lacking in insects and results of the few published studies are conflicting[6,7]. The objective of the present study was to determine the conditions required for stimulation induced depletion of the synaptic vesicles in neuromuscular junctions of locust extensor tibiae nerve-muscle preparations.

Materials and methods. Isolated extensor tibiae muscles, stimulated via N5 at frequencies varying from 0.5 to 100 Hz, were fixed in glutaraldehyde (2%, $2^1/_2$ h) either immediately after stimulation or following a 1-h-rest. The tissues were post-fixed in osmium tetroxide (1%, 1 h), dehydrated in graded alcohols and embedded in Araldite resin. Thin sections ($\simeq 70$ nm) stained with uranyl acetate and lead citrate were examined in an AEI EM 6B electron microscope.

Quantitative estimates of synaptic vesicle densities were determined from micrographs depicting axon terminals, using a version of the morphometric analysis described by Usherwood and Rees[8]. Axon terminals were divided

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Table 1. Density of synaptic vesicles per μm^2 of axoplasm in specific areas of normal, stimulated and recovering axon terminals from control, stimulated and recovering preparations respectively

Treatment	Preparation	Specific area SA Mean	SD	NSA_1 Mean	SD	NSA_2 Mean	SD	Remainder Mean	SD	Inner Mean	SD
	CONT	196	138	111	71	125	61	71	23	115	54
100 Hz	STIM	116	62	66	36	19	13	12	12	52	59
	REST	145	50	59	29	76	9	33	21	49	17

Number of observations per mean = 5. Mean and SD given to nearest whole number.

Table 2. Comparisons of densities of synaptic vesicles in similar areas of normal (control), stimulated and recovering axon terminals

Comparison	Specific area	Difference of 2 means ($\log_e$ scale)	Comparison	Specific area	Difference of 2 means ($\log_e$ scale)
CONT with STIM		0.506	CONT with STIM		2.209*
CONT with REST	SA	0.133	CONT with REST	Remainder	0.848
STIM with REST		−0.373	STIM with REST		−1.361
CONT with STIM		0.380	CONT with STIM		1.313*
CONT with REST	NSA_1	0.427	CONT with REST	Inner	0.801
STIM with REST		0.047	STIM with REST		−0.512
CONT with STIM		2.174*			
CONT with REST	NSA_2	0.374			
STIM with REST		−1.797*			

CONT = Control, STIM = Stimulated, REST = Rested. Number of observations per mean = 5. Critical difference between any two means = 1.228. Significant differences denoted by asterisks.

to give 5 specific areas (figure 1) and vesicle densities determined for each of the specific areas. A plot of the means versus the SD of the densities revealed that the SD was proportional to the mean, indicating that the variance was not homogeneous. The data was transformed to the $\log_e$ scale and the corresponding scatter diagram showed that this transformation removed any relationship between the mean $\log_e$ and SD $\log_e$ of the densities. The variance was analyzed and Scheffé's S-test[9] used to determine the significance of any differences.

Results and discussion. The ultrastructural properties of the fast excitatory terminals from unstimulated control preparations (see figure 2a) resembled those described previously for a wide variety of insect neuromuscular junctions[10]. No noticeable changes were observed in the ultrastructure of excitatory neuromuscular junctions stimulated at the lower frequencies but gross differences were apparent following stimulation at 50 Hz for 5 min or 100 Hz for 1 min (figure 2b). The terminals in these fatigued preparations contained distended mitochondria with indistinct cristae and large irregular shaped cisternae. These frequencies induced tetanic muscle responses and rapid fatigue. Normal responses resumed after a 1-h-rest. Neuromuscular junctions in fatigued muscles either appeared to have fewer vesicles in areas away from the synaptic sites, relative to normal junctions (figure 2b) or were almost devoid of vesicles. Neuromuscular junctions on muscle fibres stimulated at 50 or 100 Hz and allowed a 1-h-rest, were similar in ultrastructural appearance to the control junctions; the synaptic vesicles in these recovering terminals being more dispersed than those of the fatigued terminals. In normal, stimulated and recovering axon terminals the highest vesicle densities were always found in the synaptic areas adjacent to the presynaptic membranes (table 1). Neuromuscular junctions stimulated at 100 Hz showed a significant reduction in the population densities of the vesicles in nearly all the nonsynaptic areas (NSA_2, Remainder and Inner) relative to normal terminals (table 2). If fatigued terminals were allowed to rest there was a recovery of the vesicle populations (table 1 and 2). We suggest that these results support the idea that the synaptic vesicles contain the transmitter, since their depletion at the axon terminal was correlated with loss of mechanical function, which returned as the vesicle population recovered.

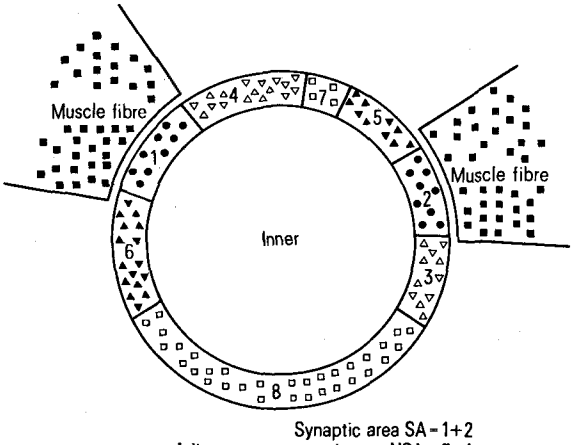
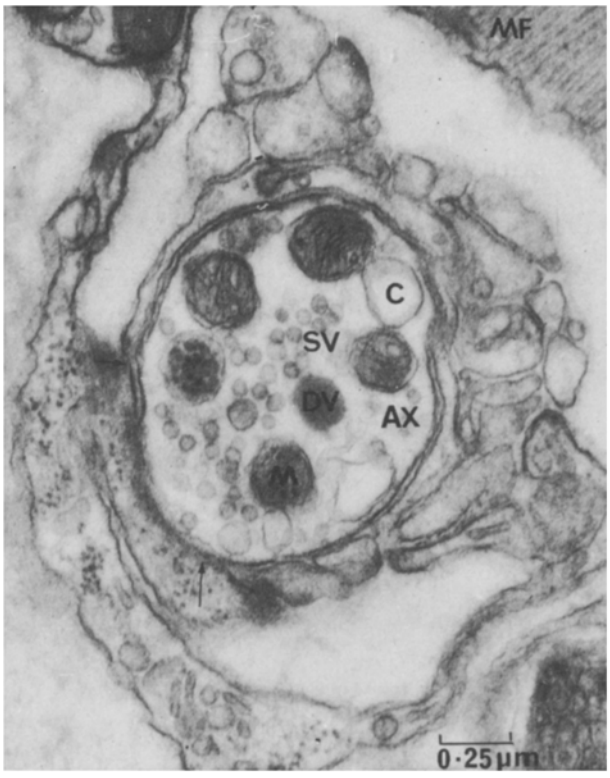


Fig. 1. Diagram of section through an axon terminal to illustrate specific areas of the terminal used to determine the population densities of the synaptic vesicles per μm^2 of axoplasm.



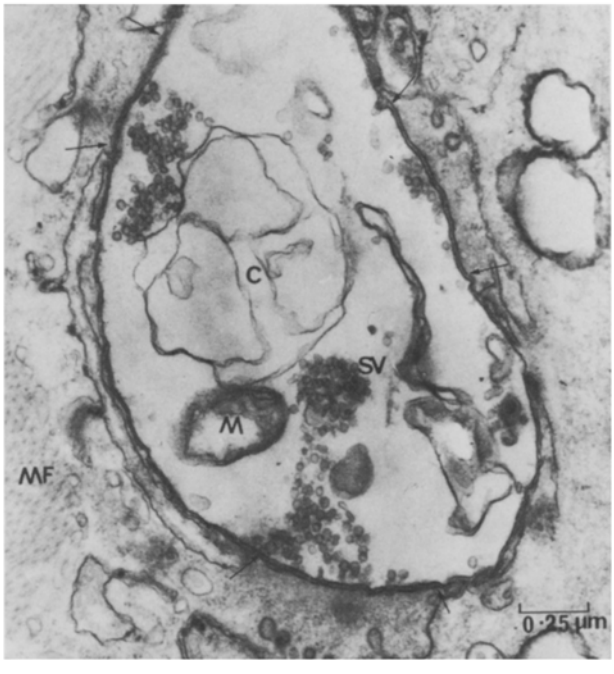


Fig. 2. *a* Electron micrograph depicting the general structure of a control fast axon neuromuscular junction synapsing (arrows) on a phasic muscle fibre. The axon terminal (AX) contains synaptic vesicles (SV), dense core vesicles (DV) mitochondria (M) and cisternae (C). *b* Electron micrograph of a neuromuscular junction from a preparation stimulated at 100 Hz for 1 min prior to fixation. Note the aggregated clumps of vesicles, distended mitochondria with indistinct cristae and large irregular cisternae. Arrows indicate synapses.

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