Synaptic vesicle depletion and glutamate uptake in a nerve-muscle preparation of the locust, *Locusta migratoria* L.

R. P. Botham[1], D. J. Beadle, R. J. Hart, C. Potter and R. G. Wilson[2]

School of Biological Sciences, Thames Polytechnic, London SE18 6PF, and the Wellcome Research Laboratories, Berkhamsted Hill, Berkhamsted, Herts HP4 2QE (England), 9 May 1977

Summary. Excitatory terminals, depleted of their synaptic vesicles by stimulation at high frequency, were incubated in the radiolabelled putative neurotransmitter, L-glutamate. Quantitative electron microscope autoradiography revealed that the axoplasm of these recovering terminals had accumulated the ^{3}H L-glutamate.

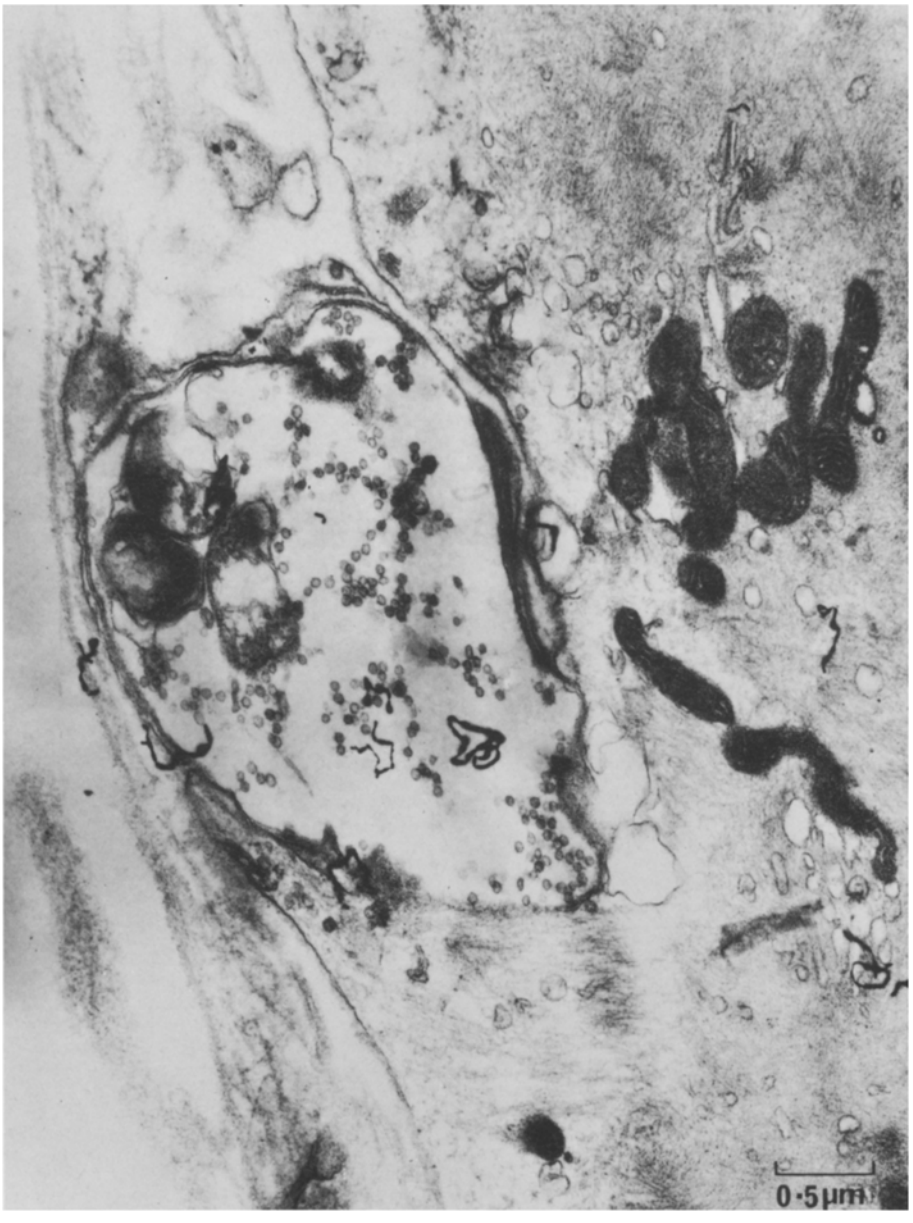
There is now substantial evidence that L-glutamate is the putative neurotransmitter at excitatory terminals on some insect somatic muscle fibres, though the idea of a transmitter role for L-glutamate is not unequivocally established[3]. Stimulation at high frequency has been previously shown to deplete the synaptic vesicle populations and thus presumably exhaust the transmitter stores in fast axon terminals on phasic muscle fibres of locust extensor tibiae muscles[4]. The objective of the

1 Present address: The Wellcome Research Laboratories, Berkhamsted Hill, Berkhamsted, Herts HP4 2QE, England.

2 Acknowledgment. The authors wish to thank Dr N. M. Blackett for running the computer analyses of the autoradiographical results. R. P. Botham gratefully acknowledges the SRC for financial assistance.

3 P. N. R. Usherwood and S. G. Cull-Candy, in: Insect Muscle, p. 207. Academic Press, London 1974.

4 R. P. Botham, D. J. Beadle, R. J. Hart, C. Potter and R. G. Wilson, Experientia *34*, 207 (1978).



Electron autoradiograph of a neuromuscular junction synapsing on a locust extensor tibiae muscle fibre from a preparation stimulated at 100 Hz and incubated in radiolabelled L-glutamate.

Table 1. Distribution of radioactivity within regions of the fast axon branches and terminal in locust extensor tibiae muscles stimulated at 0.5 Hz and rested in radiolabelled L-glutamate for 1 h

Source of radioactivity	Radioactivity/unit area grains/μm^2 n = 30	SE
Axon branch		
Muscle fibre	0.087	0.019
Axon branch (axoplasm)	0.000	0.000
Glial wrappings	0.298	0.105
External	0.182	0.054
Axon terminal		
Muscle fibre	0.019	0.011
Axon terminal (axoplasm)	0.000	0.000
Glial cell	4.007	0.965
External	0.056	0.051

Table 2. Distribution of radioactivity within regions of the fast axon branches and terminals in locust extensor tibiae muscles stimulated at 100 Hz and rested in radiolabelled L-glutamate for 1 h

Source of radioactivity	Radioactivity/unit area grains/μm^2 n = 30	SE
Axon branch		
Muscle fibre	0.207	0.070
Axon branch (axoplasm)	0.076	0.159
Glial wrappings	0.964	0.256
External	0.555	0.250
Axon terminal		
Muscle fibre	0.223	0.025
Axon terminal (axoplasm)	1.848	0.364
Glial cell	2.796	0.806
External	0.194	0.036

SE refers to the variation in the counting procedure in the analyses. External regions include, basement membrane, teacheoles and haemolymph space. Axon terminal resp. (axoplasm) includes axoplasm + organelles.

5 L. G. Caro and R. P. Van Tubergen, J. Cell Biol. *15*, 173 (1962).
6 N. M. Blackett and D. M. Parry, J. Histochem. Cytochem. *25*, 206 (1977).
7 N. M. Blackett and D. M. Parry, J. Cell Biol. *57*, 9 (1973).
8 I. R. Faeder and M. M. Salpeter, J. Cell Biol. *46*, 300 (1970).
9 I. R. Faeder, J. A. Matthews and M. M. Salpeter, Brain Res. *80*, 53 (1974).

present study was to examine the uptake of exogenously applied radiolabelled L-glutamate at 'normal' and depleted excitatory neuromuscular junctions.

Materials and methods. Isolated extensor tibiae nerve-muscle preparations were stimulated and prepared for electron microscopy as described previously[4], except that following stimulation the preparations were incubated in saline containing L-[G-^{3}H]glutamic acid (final concentration 4×10^{-6}M and 125 μCi per ml) or DL-[4,5-^{3}H]-leucine (final concentration 2×10^{-5}M and 20 μCi per ml). Thin sections (pale gold), coated with Ilford L-4-emulsion using a variation of the loop technique of Caro and Van Tubergen[5], were exposed for 20 weeks at 4 °C and developed in Microdol X. Sections were examined in an AEI EM 6B electron microscope and the distribution of silver grains in the electron autoradiographs analyzed using the method described by Blackett and Parry[6]. This method takes into account the cross scatter of grains between neighbouring structures for the actual shapes occurring within the section and no assumptions are required about the size, shape or spatial arrangements of these structures[7].

Results and discussion. Radiochemical experiments indicated that unstimulated whole muscle preparations had a high affinity for L-glutamate relative to L-leucine which was used as a control. Furthermore, fatigued preparations prestimulated at 100 Hz showed an increased uptake of L-glutamate compared to unstimulated control preparations, while there was no change in the uptake of L-leucine. Control muscles incubated in ^{3}H L-glutamate and prepared for light microscope autoradiography indicated that the radioactivity was distributed uniformly over the sections while sections from stimulated muscles displayed 'hot spots' over regions at the muscle fibre surfaces presumed to be sites of nerve endings.

Analysis of the grain distribution in preparations stimulated at 0.5 Hz revealed that the radioactivity was mainly associated with the glial wrappings at the fast axon branches and glial cells at the axon terminals while there was little, if any, radioactivity accumulated by the axoplasm (table 1). This confirms the work of earlier investigators[8,9] who have suggested that the glial cells which cap the terminals play a major role in glutamate uptake at axon terminals and thus provide a means of inactivating the transmitter. In preparations stimulated at 100 Hz to the point of fatigue, radioactivity was again sequestered by the glial cells but in addition substantial radioactivity was found to be present in the axoplasm of the axon terminals (table 2, figure).

These results are considered to support the hypothesis that L-glutamate is the transmitter at the excitatory neuromuscular junctions of the locust extensor tibiae muscle.

Correlation between acidic phospholipids and serotonin and between lysolecithin and dopamine in ganglia of the marine mussel, *Mytilus edulis*[1]

J. E. Haley, G. B. Stefano[2,3] and E. J. Catapane[3,4]
Department of Neurology, Albert Einstein College of Medicine, Bronx (New York 10461, USA), 23 May 1977

Summary. These studies have demonstrated a positive correlation between the acidic phospholipids and the serotonin content and between the lysolecithin and the dopamine content in the cerebral, pedal and visceral ganglia of *Mytilus edulis*. These relationships were further supported by experiments utilizing 6-hydroxydopamine and 5,6-dihydroxytryptamine.

Several recent metabolic studies[5-9] and reviews[10,11] have implicated a functional relationship between neurotransmitters and phospholipids. Hokin[12] and Hokin[13] have generalized this relationship by suggesting that excitatory transmission with acetylcholine, norepinephrine and serotonin (5-HT) results in an increase in the labeling of phosphatidate and phosphatidylinositol; while inhibitory transmissions such as that generally seen with