J. exp. Biol. (1982), 96, 435–438
With 1 figure
Printed in Great Britain

A SIMPLE METHOD FOR STUDYING THE INNERVATION OF A COMPLEX MUSCLE

BY G. THEOPHILIDIS

Laboratory of Animal Physiology, School of Physics and Mathematics, Aristotelian University of Thessaloniki, Thessaloniki, Greece

(*Received* 8 *June* 1981)

The innervation of an insect muscle can be studied by correlating the evoked spikes in neuronal cell bodies with extracellular or intracellularly recorded events in various parts of the muscle (Burrows & Hoyle, 1973; Hoyle & Burrows, 1973; Phillips, 1980). However, when a muscle is innervated by a large number of neurones, difficulties may occur: first, in the reliable identification of the neurones (especially when their cell bodies are closely clustered) and secondly, in maintaining stable records from the cell bodies for a sufficient period of time to permit reliable investigation of the muscle.

To eliminate these difficulties, a new method of identifying the neurones was developed, mainly based on the distribution of the axons on the muscle surface, the size of the EPSP or IPSP which they produce on the muscle fibres, and the size of the action potential recorded from the motor nerves of the muscle.

The method was first applied to study the innervation of the mesothoracic flexor tibiae muscle of the locust. Not only is this muscle innervated by a large number of neurones with their cell bodies lying very close together (Theophilidis, 1979; Wilson & Hoyle, 1978) but its motor axons were also found, in preliminary studies, to innervate only certain areas of the muscle. The flexor tibiae is a pinnate muscle and its muscle bundles are divided into three areas (Fig. 1*c*): proximal, middle and distal (Theophilidis, 1979). To investigate the distribution of the axons on the muscle, records were obtained from these three parts of an isolated muscle or from the flexor muscle bundles of an inact locust.

RECORDS FROM AN ISOLATED MUSCLE

The mesothoracic femur of a locust was cut from the coxa-femur joint, fixed in a watch glass (ventral side down) and dissected to expose the flexor muscle and its motor nerves. The whole preparation was bathed in oxygenated saline (Usherwood & Grundfest, 1965). The flexor motor axons were excited by stimulating N 5 (Campbell, 1961) through a suction electrode. The activity of these axons was simultaneously monitored in the three parts of the muscle. Changes in muscle tension were recorded from the distal parts by cutting the tendon between the middle and distal parts (see arrow in Fig. 1*c*). At the same time intracellular records were obtained

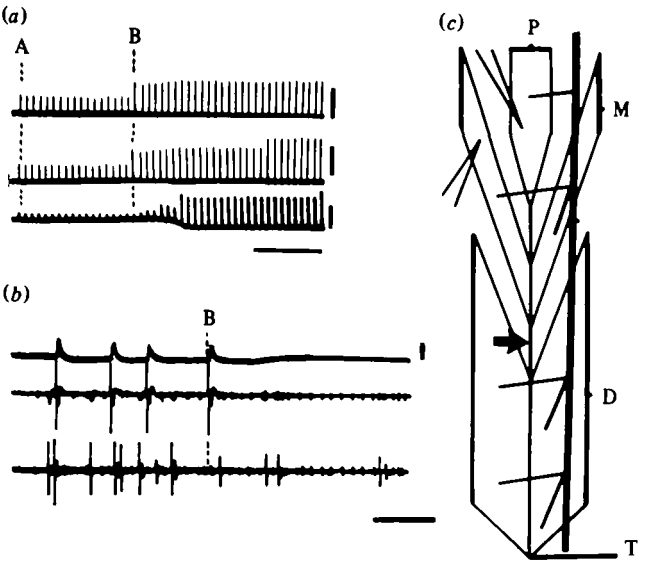

Fig. 1. (*a*) Records from mesothoracic flexor tibiae muscle isolated in saline. (1) Intracellular recording from the proximal part. For the location of the electrode positions see (*c*). (2) Intracellular recordings from the middle part. Vertical scale bars in 1 and 2 are 30 mV. (3) Muscle tension monitored from the distal flexor using a silicon strain gauge transducer. Vertical scale bar: 0·3 g. Horizontal scale bar 10 s. (*b*) Records from the flexor muscle of an intact locust. (1) As in (*a*). Vertical scale bar 30 mV. Horizontal scale bar 100 ms. (2, 3) Extracellular records from the nerve branches innervating the middle and distal parts of the muscle. (*c*) Diagrammatic representation of the mesothoracic flexor tibiae muscle. P, Proximal part. M, middle part. D, distal part. T, tension transducer.

from the proximal and middle parts. When the intensity of the electrical stimulus was gradually increased, the monitored increments in active tension and the recorded EPSPs increased in jumps which reflected the threshold of the axons innervating the muscle (Fig. 1*a*). When a relatively small EPSP occurred in the proximal and middle parts (Fig. 1*a*, first and second traces), a small muscle contraction was simultaneously recorded from the distal part (third trace), suggesting that there is an axon A innervating all three parts of the muscle. However, when a large EPSP occurred in the proximal and middle muscle fibres (see B in Fig. 1*a*) there was no change in the muscle contraction recorded from the distal flexor. This shows that a relatively large axon B innervates the proximal and middle flexor, but not the distal one.

RECORDING FROM AN INTACT ANIMAL

The locust was fixed (ventral side down) in the middle of a Plasticine platform with a small drop of cyanocrylate adhesive (Advelbond No. 3). The legs were horizontally mounted on the platform to allow free movement of the tibia in the vertical plane. The femur of the leg to be investigated was dissected and care was taken to leave most of the femoral proprioceptors intact. The mesothoracic flexor tibiae motoneurones were reflexively excited by extending the tibiae (resistance reflexes) with a constant-velocity movement, and activity from the motoneurones

was monitored extracellularly, from the nerve branches of the middle and distal flexor (using fine hook electrodes), and intracellularly from the proximal part. From the records in Fig. 1*b* it is obvious that the large axon B innervates only the proximal and middle parts (common action potential and EPSP in the first and second traces), and does not innervate the distal part, since no equivalent action potential occurred in the distal nerve branch (third trace). In the distal nerve branches action potentials were recorded which did not correspond with spikes in the middle nerve branches (second and third trace, Fig. 1*b*). This shows that there are some flexor motoneurones innervating only the distal part of this muscle.

Some problems were encountered when N 5 was electrically stimulated to excite the flexor motor axons. First, the inhibitory axons were also activated, thus affecting the recorded muscle tension and the recorded EPSPs. Their effects were eliminated by using one of the properties of saline to gradually diminish the mechanical response (relaxation) to inhibitory stimulation. In the leg muscles of the locust Usherwood (1968) found that after 100 min in 10 K saline, no relaxation can be recorded during inhibitory stimulation. The IPSPs are chloride potentials and can be converted to depolarizing responses by changing either the external or internal chloride concentration of the muscle fibres. This technique, although it was not always sufficient, was preferable to eliminating relaxation with picrotoxin perfusion, since picrotoxin does not always perfuse properly between the muscle fibres and may affect the condition of the muscle itself. The second problem was that in the first 40 min. of immersion in saline the large flexor axons had the lowest threshold and so the smaller axons could not be separately activated. However, after 60–90 min. in saline the relative threshold changes and records like those in Fig. 1*a* (third trace) were easily obtained.

Using the above method, it was possible to investigate in detail the innervation of the flexor tibiae muscle of the locust (Theophilidis & Burns, in preparation) and to define the number of muscle fibres which are innervated by the same axon and vice versa. In the same preparation, this method can be combined with intracellular recordings of the cell bodies to provide a better and more reliable identification of the flexor motoneurones. This method, with some modifications, can also be applied to study the innervation of other complex insect muscles like the metathoracic flexor tibiae (Phillips, 1980), the locust neck (Shepheard, 1973) and abdominal (Tyrer, 1971) muscles, the flexor tibiae muscle of the cockroach (Dresden & Nijenhuis, 1958) and others.

REFERENCES

Burrows, M. & Hoyle, G. (1973). Neural mechanisms underlying behavior in the locust *Schistoceria gragaria*. III. Topography of limp motoneurons in the metathoracic ganglion. *J. Neurobiol.* **4**, 167–186.

Campbell, J. I. (1961). The anatomy of the nervous system of the mesothorax of *Locusta migratoria migratoroides*. R. and F. *Proc. Zool. Soc. Lond.* **137**, 403–432.

Dresden, D. & Nijenhuis, E. D. (1958). Fibre analysis of the nerves of the second thoracic leg in *Periplaneta americana*. *Proc. K. ned. Akad. Wet.* **61**, 213–223.

Hoyle, G. & Burrows, M. (1973). Neural mechanisms underlying behavior in the locust, *Schistocerca gregaria*. I. Physiology of indentified motoneurons in the metathoracic ganglion. *J. Neurobiol.* **4**, 3–41.

Phillips, C. E. (1980). An arthropod muscle is innervated by nine excitatory motor neurons. *J. exp. Biol.* **88**, 249–258.

SHEPHEARD, P. (1973). Musculature and innervation of the neck of the desert locust, *Schistocerca gregaria* (Forskål). *J. Morph.* **139**, 439–464.

THEOPHILIDIS, G. (1979). The neuronal control of the mesothoracic flexor tibiae muscle of the locust. Ph.D. Thesis, University of Glasgow.

TYRER, N. M. (1971). Innervation of the abdominal intersegmental muscle in the grasshopper. I. Physiological analysis. *J. exp. Biol.* **55**, 315–324.

USHERWOOD, P. N. R. (1968). A critical study of the evidence for peripheral inhibitory axons in insects. *J. exp. Biol.* **49**, 201–222.

USHERWOOD, P. N. R. & GRUNDFEST, H. (1965). Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol.* **28**, 497–518.

WILKENS, L. A. & WOLFE, G. E. (1974). A new electrode design for en passant recording, stimulation and intracellular dye infusion. *Comp. Biochem. Physiol.* **48**, 217–220.

WILSON, J. A. & HOYLE, G. (1978). Serially homologous neurons as concomitants of function specialization. *Nature*, **274**, 377–379.