J. Physiol. (1953) 121, 539–547

NEUROMUSCULAR TRANSMISSION IN A LOCUST

By J. del CASTILLO, G. HOYLE and XENIA MACHNE

From the Departments of Physiology and Zoology, University College, London

(*Received* 12 *February* 1953)

This paper gives a description of some of the electrical events associated with the neuromuscular transmission of impulses in the jumping leg of the locust, as recorded with intracellular capillary micro-electrodes.

METHODS

Fibres of the flexor tibialis muscle of the jumping leg of the locust (*Locusta migratoria migratorioides* R. & F.) were used. These fibres, of about 8 mm length, are attached at one end to the cuticle of the curved border of the femur near the trochantero-femoral joint, and to the flexor tibialis apodeme at the distal end. The muscle is divided into separate bundles or units of about 7–20 muscle fibres, each provided with a common tracheal system and nerve supply. They are innervated by a branch of the crural nerve which arises at the metathoracic ganglion.

The jumping leg, attached to the metathoracic segment, was isolated, leaving intact the nervous connexions with the central ganglia, as shown by the presence of reflexes. The preparation was brought into a chamber consisting of a glass dish divided into two separate troughs by a paraffin wall 2 mm thick (see Fig. 1). The metathoracic segment was placed in one trough and the femur in the other, whereas the coxa and trochanter were laid in a slot of appropriate size cut in the partition wall, where they were sealed by gently warming the surrounding paraffin. This provided a good fixation for the preparation and the electrical insulation necessary for stimulation. The joints between femur and tibia, and coxa and metathorax were fixed so that movements of the leg were prevented. The main ridge of the ventral surface of the femur was arranged to project upwards. The dish containing the preparation was fixed to a Perspex block sliding on a Perspex plate. The whole was mounted on the base plate of a Brown-Schuster myograph; its vertical column was used to hold the microelectrodes and the micrometer drive controlling them.

Once the preparation was fixed, the chamber was brought under a binocular-dissecting microscope and a portion of the cuticle of the femur about 3 mm long and 1 mm wide was cut away with a sharp knife exposing parallel fibres of the flexor tibialis muscle. A second area of cuticle together with some attached muscle fibres, was also dissected from the dorsal surface of the limb so that it was possible to illuminate the preparation from underneath.

The troughs on either side of the paraffin wall were then filled with saline; this was of the same composition as that used by Hoyle (1953). Its ionic content expressed in m.mole/l. was as follows: K 10; Na 140; Ca 2; Mg 2; H_2PO_4 6; HCO_3 4; Cl 148.

The crural nerve was stimulated by applying an electric pulse of about 0·5 msec duration across the partition wall by means of platinum electrodes immersed in the bathing fluid. 0·1 μF condensers were placed at each stimulating electrode.

The electrical activity of single muscle fibres was recorded with the aid of intracellular capillary micro-electrodes of external tip diameter less than about 0·5 μ. Both the micro-electrodes and the recording circuit of high input impedance were conventional and similar to those described by previous authors. In some experiments a polarizing current was made to pass through the membrane with a micro-electrode situated at a distance less than 50 μ from the recording one. Both electrodes were cemented together and controlled by the same micrometer drive. A square pulse of current of variable duration was delivered through the polarizing electrode via a 0·5 μF condenser. In these experiments the bath electrode was directly connected to the second grid of the input stage. Another bath electrode connected to earth through a resistance closed the polarizing circuit, the resistance being used to measure the applied current.

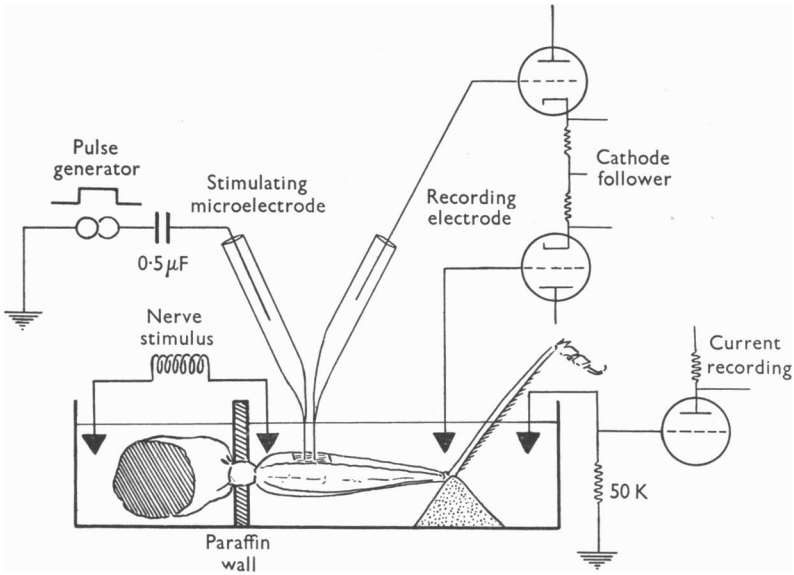

Fig. 1. Diagram of experimental arrangement.

RESULTS

Muscle responses to nerve stimulation

When the electric pulse applied across the partition wall reaches threshold strength, the first sign of neuromuscular activity is the appearance of a brisk twitch of the tarsus. If the strength is now increased, and the leg is free to move, a twitch of the flexor tibialis muscle is observed. With higher stimuli, a twitch and frequently a tetanic contraction of the extensor tibialis muscle is seen following each single shock.

If a micro-electrode is inserted into a muscle fibre a resting membrane potential of negative sign is observed. Its average value measured by Hoyle (1953) under the same experimental conditions, is of about 60 mV. Now, when the crural nerve is stimulated, a potential change in opposite direction is observed, which will be referred to as a 'muscle potential'. Its presence is

always associated with a contraction of the muscle fibre. This was determined by simple visual inspection, and no attempt has been made to record the mechanical responses of the muscle. In some preparations, and with supra-maximal stimulation, the applied pulse may be followed by a repetitive response; this seems to be due to the presence of the intact metathoracic ganglion, as it could be prevented by severing its peripheral connexions. In some cases it is possible to record the first three or four muscle potentials before the electrode breaks or comes out of the fibre. A small potential which may be attributed to the activity of intramuscular nerve branches is observed in some cases (see Fig. 3*A*) preceding the muscle potential. At room temperature there is no appreciable latency between the nerve potential and the initiation of the electrical activity of the muscle. At lower temperatures, however, there

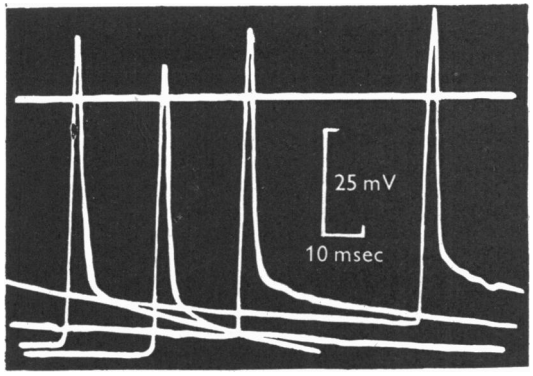

Fig. 2. Repetitive activity evoked by the impalement of a locust muscle fibre recorded on a stationary frame. The upper horizontal trace represents the zero base-line.

is a marked delay between both events, which may reach the value of about 5 msec as can be observed in Fig. 3*C*. At 20–23° C the muscle potential begins about 3–5 msec after the initiation of the stimulus artifact and reaches its peak in about 3–4 msec; its half-decay time is also of the same order of magnitude.

The amplitude of the muscle potential is roughly the same as that of the corresponding resting potential. In many fibres, however, a reversal of the resting potential during activity has been observed, i.e. the muscle potential overshoots the zero base-line. The magnitude of this overshoot is smaller than that recorded in frog muscle (cf. Nastuk & Hodgkin, 1950) and in the present experiments never reached more than 20 mV. The size of the overshoot seems to be dependent upon the condition of the preparation. Its absence is one of the earliest signs of deterioration. It is possible, therefore, that in the intact animal the overshoot reaches higher values than those observed. Fig. 2 shows

repetitive muscle potentials elicited by the impalement of a muscle fibre in a fresh preparation; these were the highest values of overshoot which have been recorded.

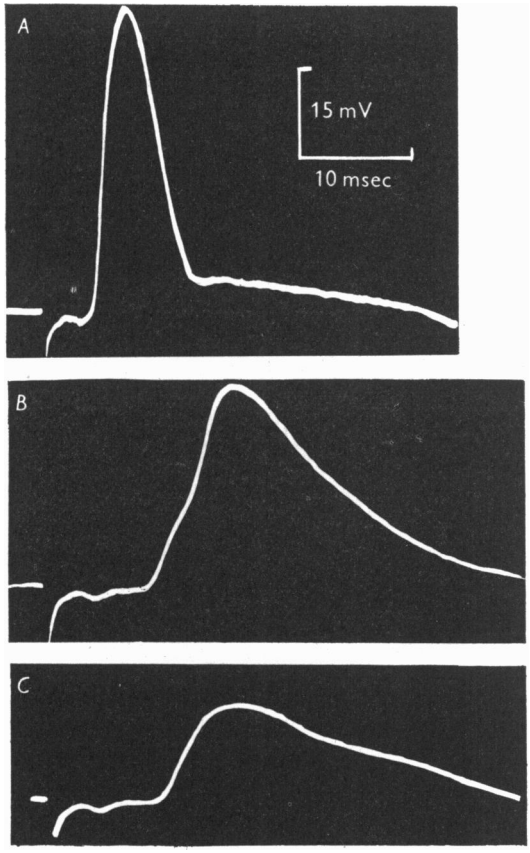

Fig. 3. Electrical activity of a locust muscle fibre at three different temperatures as recorded without removing the micro-electrode. The temperatures of the bath fluid were, in records *A*, *B* and *C*, 18·5, 10·5 and 6·0° C respectively.

Influence of temperature on the muscle responses to indirect stimulation

The influence of temperature on the muscle potential has been examined. The temperature of the surrounding fluid was made to vary between 25 and 5° C. In some cases, this could be done without removing the electrode from a fibre. As Fig. 3 shows, not only does lowering the temperature result in a change of the time course and amplitude of the muscle potentials, but also some details of their nature become apparent. The most interesting change is the appearance of a deflexion, or step, in their rising phase. This suggests that

the described muscle potential is not a single event but the sum of two different processes: a basic depolarization reinforced by a superimposed potential change.

By lowering the temperature below 10° C the superimposed potentials are considerably reduced in magnitude and eventually disappear. Only the basic depolarization can then be observed. This remaining potential change seems to be analogous to the end-plate potential (e.p.p.) recorded at the neuromuscular junction of curarized vertebrate muscle, and in non-curarized crustacean muscle (Katz & Kuffler, 1946). The superimposed potentials do not seem to be essential for muscular contraction and brisk twitching of the muscle fibres could be seen in their absence. The effects of lowering the temperature are fully reversible.

No significant changes in the size or time course of muscle potentials and pure e.p.p.'s due to variations in the frequency of stimulation could be demonstrated, their size being constant from the first impulse. The fact that the initial amplitude of the e.p.p. is large and that there are no signs of facilitation indicates that the nerve fibres stimulated in the present experimental conditions behave in a similar manner to those of the 'fast' systems, as described in other species.

The size of the e.p.p. did not vary in size by more than about 10–15% when recorded at different points of the same muscle fibre, i.e. no evidence of a spatial decrement of the e.p.p. in relation to a fixed focus could be obtained. This suggests that the locust muscle fibres are provided with nerve endings extending over a great length of the fibre, rather than with a discrete focal innervation, as in the case of vertebrate muscle. This fact supports the earlier histological findings of Marcu (1929) in other insects.

Relationship between end-plate potential and resting potential

As Fatt & Katz (1951) have indicated, it is possible to get further information on the nature of the membrane process responsible for the production of the e.p.p. by studying the relationship between its magnitude and that of the corresponding resting potential. By passing an inward current through the membrane with the aid of a second internal electrode, the resting potential can be made to increase above its normal value. The e.p.p. can then be elicited and measured in this new condition. This has been done in ten fibres. Fig. 4 illustrates one experiment. In Fig. 5 the values of the e.p.p.'s have been plotted against the corresponding resting potentials; the size of the former being approximately proportional to that of the latter. It appears, therefore, that in the locust muscle fibre, the end-plate potential may arise by a mechanism similar to the one suggested by Fatt & Katz in the case of frog muscle, namely by the establishment of a fixed leak resistance across the membrane.

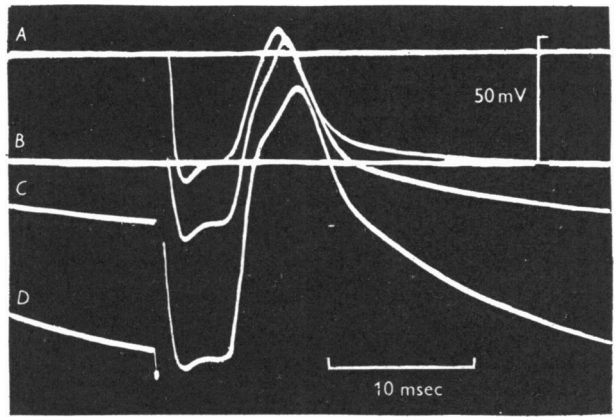

Fig. 4. End-plate potentials and superimposed responses obtained with three different values of resting potential (see text). Its original value (*B*) was raised at *C* and *D*, *A* representing the zero base-line.

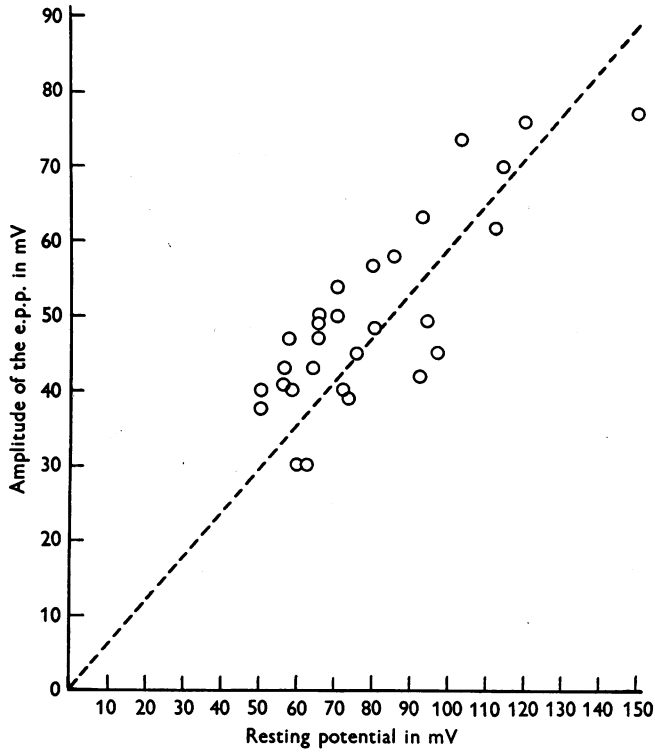

Fig. 5. Magnitude of the e.p.p. in mV plotted against value of resting potential in mV. Dotted line indicates direct proportionality (see text).

Direct stimulation of the muscle fibre membrane

In order to obtain more information about the nature of the potential change reinforcing the e.p.p., experiments were performed in which the muscle fibre membrane was directly stimulated by applying current with a second microelectrode. The relationships between the magnitude of the current made to flow through the membrane in both directions, and the resulting potential changes were first studied. In the case of an inward current, an approximately linear relationship between voltage and current was found, whereas with an

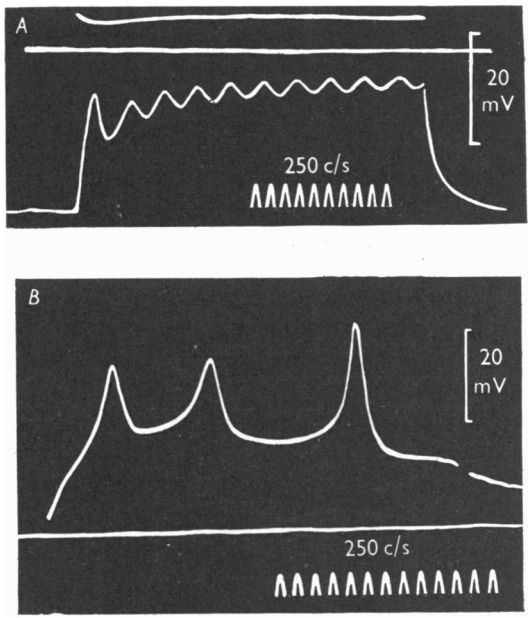

Fig. 6. Oscillatory potential changes evoked in a locust muscle fibre by applying a steady outward current. The upper record in *A* shows the voltage drop produced by the current flowing through the membrane across a monitor resistance (see Fig. 1).

outward current, and in fresh preparations, a departure from linearity has been observed when the depolarization exceeded a value of about 20–25 mV due to the appearance of active membrane responses, particularly evident when the duration of the applied current was of about 10 msec or more. The characteristics of these potential changes are variable and seem to be dependent upon the condition of the preparation. In fresh preparations they took the form of potential oscillations superimposed on the electrotonic potential generated by the steady flow of current (Fig. 6). Their frequency varied from a few to over 100 per sec, showing little or no accommodation and occasionally

a spike-like character, see Fig. 6*B*. The maximal amplitude reached was of about 25 mV above the steady electrotonic potential. They were always, however, graded events and one may regard them as local responses.

A deterioration of muscle excitability was invariably observed during the course of the experiments. The size of the oscillatory potential changes diminishes, and accommodation becomes apparent. Eventually, only a small deflexion at the beginning of the flow of current can be observed, usually for 2–3 hr. In the preparation employed, the normal oxygen supply to the muscle fibres, i.e. the direct pumping of air through the tracheal system, is interrupted. The normal excitability of the membrane may be dependent upon the maintenance of this air supply. In addition, it is possible that the composition of the saline used does not provide an adequate medium. However, the nerve excitability and the e.p.p. did not seem to be appreciably impaired during the course of the experiment. Many preparations continued to exhibit e.p.p.'s as a result of nerve stimulation and reflexes after 24 hr.

DISCUSSION

The results of the experiments described indicate the existence in locust muscle of a neuromuscular transmission mechanism which resembles in some features that described in vertebrates and crustacea. The arrival of the nervous impulse at the terminals elicits an e.p.p. which is reinforced by a superimposed potential change due to an active response of the muscle membrane. The experiments in which the relationship between size of the e.p.p. and resting potential have been determined indicate that the e.p.p. in locust muscle fibres may be due to the establishment of a resistance leak across the membrane, in a similar way to that suggested by Fatt & Katz (1951) in frog muscle.

It has also been shown that the membrane of locust muscle fibres is capable of showing active responses as a result of the depolarization elicited by an applied current. The size of the observed responses never reached more than 25 mV and they appear to be graded events. This, however, does not exclude the occurrence of all-or-nothing responses in the intact animal.

The behaviour of the muscle fibres of the jumping leg of the locust differs, apparently, from that shown by the second tergal muscles of the trochantin in another insect, *Periplaneta americana* (L.), according to the results obtained by Roeder & Weiant (1950). The stimulation of the nerve branch supplying motor fibres to this muscle elicits electrical changes which spread in the muscle very rapidly from the point of nerve entry, so that all the parts of the muscle were involved within 0·5 msec. They concluded that the recorded potentials were due to the sum of local (end-plate) potentials developing at the muscle fibres, and no evidence was found of their transition into active membrane responses.

SUMMARY

1. The neuromuscular transmission of impulses in the jumping leg of the locust was studied with the aid of intracellular micro-electrodes.

2. Indirect stimulation of the muscle fibres results in an electrical change consisting of an e.p.p. reinforced by an active membrane response. The second of these two events is decreased by lowering the temperature and is absent below 10° C.

3. The size of the e.p.p. is approximately proportional to the value of the resting potential. This suggests that in these fibres the e.p.p. may be due to a mechanism similar to that postulated for the frog striated muscle.

4. The membrane of locust muscle fibres shows also active responses as a result of the depolarization produced by an applied current.

Our thanks are due to Mr P. M. G. Bell for his unfailing technical assistance.

REFERENCES

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. *J. Physiol.* **115**, 320–370.

HOYLE, G. (1953). Potassium ions and insect nerve-muscle. *J. exp. Biol.* **30**, 121–135.

KATZ, B. & KUFFLER, S. W. (1946). Excitation of the nerve-muscle system in Crustacea. *Proc. Roy. Soc.* B, **153**, 374–389.

MARCU, O. (1929). Nervendingungen an den Muskelfasern von Insekten. *Anat. Anz.* **67**, 369–380.

NASTUK, W. L. & HODGKIN, A. L. (1950). The electrical activity of single muscle fibres. *J. cell. comp. Physiol.* **35**, 39–74.

ROEDER, K. D. & WEIANT, E. A. (1950). The electrical and mechanical events of neuromuscular transmission in the cockroach, *Periplaneta americana* (L.). *J. exp. Biol.* **27**, 1–13.