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PRIMARY CULTURES OF MUSCLE FROM EMBRYONIC LOCUSTS (*LOCUSTA MIGRATORIA*, *SCHISTOCERCA GREGARIA*): DEVELOPMENTAL, ELECTROPHYSIOLOGICAL AND PATCH-CLAMP STUDIES

BY J. A. DUCE* AND P. N. R. USHERWOOD

Department of Zoology, University of Nottingham, Nottingham NG7 2RD, UK

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SUMMARY

Primary cultures of muscle tissue from 10-day-old embryos of *Locusta migratoria* and 11-day-old embryos of *Schistocerca gregaria* have been grown and maintained in 5+4 insect medium and Grace's insect medium. Myofibres grown in 5+4 medium reached maximum size after about 5 weeks in culture and could be maintained for 2–3 months. They were often branched and rarely striated in appearance. Those grown in Grace's medium reached maximum size within 3 weeks and could be maintained for about 4 weeks *in toto*. They were striated and resembled, at least superficially, locust myofibres *in vivo*. Patch-clamp recordings from myofibre cultures grown in 5+4 medium, either on the myofibres or from excised membrane patches, indicated the presence of a diffusely-distributed population of receptors for L-glutamate. 10^{-6} mol l^{-1} concanavalin A blocked the desensitization of these receptors. The glutamate receptors gated large conductance channels which had reversal potentials of about 0 mV. The amplitude of the channel current was sensitive to the concentration of calcium in the membrane environments.

INTRODUCTION

It is well-established that L-glutamic acid is the transmitter at excitatory nerve–muscle junctions on adult insect skeletal muscle (Usherwood & Cull-Candy, 1975; Usherwood, 1978; Piek, 1985) and that γ-aminobutyric acid transmits at the inhibitory junctions (Usherwood & Grundfest, 1965; Duce & Scott, 1984). Glutamate receptors (D-receptors), with similar pharmacological and physiological characteristics to the glutamate receptors present postjunctionally at excitatory synapses, are also widely distributed extrajunctionally on locust leg muscle (Cull-Candy & Usherwood, 1973; Usherwood & Cull-Candy, 1974). These extrajunctional receptors have been extensively studied during the past few years using patch-clamp techniques, and through single channel analysis a detailed account of their channel gating kinetics is now available (Patlak, Gration & Usherwood, 1979; Usherwood,

* Formerly Miss J. A. Cook.

Key words: locust muscle, glutamate receptors, single-channel recordings, tissue culture.

Clark, Gration & Patlak, 1979; Gration, Lambert, Ramsey & Usherwood, 1980; Gration & Usherwood, 1980; Gration *et al.* 1981, 1982; Cull-Candy, Miledi & Parker, 1981; Cull-Candy & Parker, 1981; Ashford *et al.* 1984*a*,*b*; Kerry *et al.* 1986). Advances made in understanding the properties of the glutamate receptor channel complex have been facilitated by the use of concanavalin A to block desensitization (Mathers & Usherwood, 1976, 1978). However, further progress in understanding the gating kinetics and ligand binding properties of this membrane protein is impeded by the inability to obtain giga-ohm seals routinely between patch-clamp pipettes and adult locust muscle membranes. This may be due to the properties of the thick basal lamina which characterizes the leg muscles of adult locusts (Usherwood, 1967; Rees & Usherwood, 1972) and which may restrict access of the patch pipette to the muscle plasma membrane. Enzymatic treatment of the muscle to remove connective tissue has been undertaken but with unsatisfactory results (Gration, 1982). Although Franke & Dudel (1985) have reported successful single-channel recordings from crayfish muscle using giga-ohm seals, these are not possible if the muscle is pretreated with concanavalin A (J. Dudel, personal communication).

Developing muscle is often characterized by a thin basal lamina which may account for the ease with which giga-ohm seal techniques can be employed for patch-clamping membrane receptors and other channel proteins of embryonic vertebrate skeletal muscle in culture (e.g. Sakmann & Neher, 1983). This paper describes the methods employed to establish primary cultures of locust muscle tissue and contains a preliminary account of patch-clamp recordings, using giga-ohm seals, from single glutamate receptor channel complexes on the cultured myofibres. An abstract of this work has been published previously (Cook, Ramsey & Usherwood, 1985).

MATERIALS AND METHODS

The growth and viability of embryonic locust and embryonic cockroach excitable tissue *in vitro* depend upon the age of the material from which the cultures are prepared, the temperature and humidity at which they are maintained and the medium in which they are grown. Based on studies extending over the past 8 years (Giles, Joy & Usherwood, 1978; Lynch, 1980; Hicks, Beadle, Giles & Usherwood, 1981; Giles, Usherwood, Hicks & Beadle, 1983), we have developed the following techniques for obtaining primary cultures of excitable tissues from embryonic locust (and cockroach).

Developing eggs were obtained from either 10-day-old (*Locusta migratoria*) or 11-day-old (*Schistocerca gregaria*) egg pods and the material from these was divided equally to prepare 4–5 primary cultures. Most pods contained 20–30 eggs. When fewer eggs were present embryos from a number of pods were pooled to produce sufficient material for 4–5 cultures. An important feature of the eggs at this stage is that the cuticle of the embryo is still untanned and this facilitates dissociation of the embryos for culturing. Cultures prepared from eggs older than 10(11) days were rarely successful; those from younger eggs gave lower yields of muscle. After removing the eggs from their pods they were washed with distilled water and then

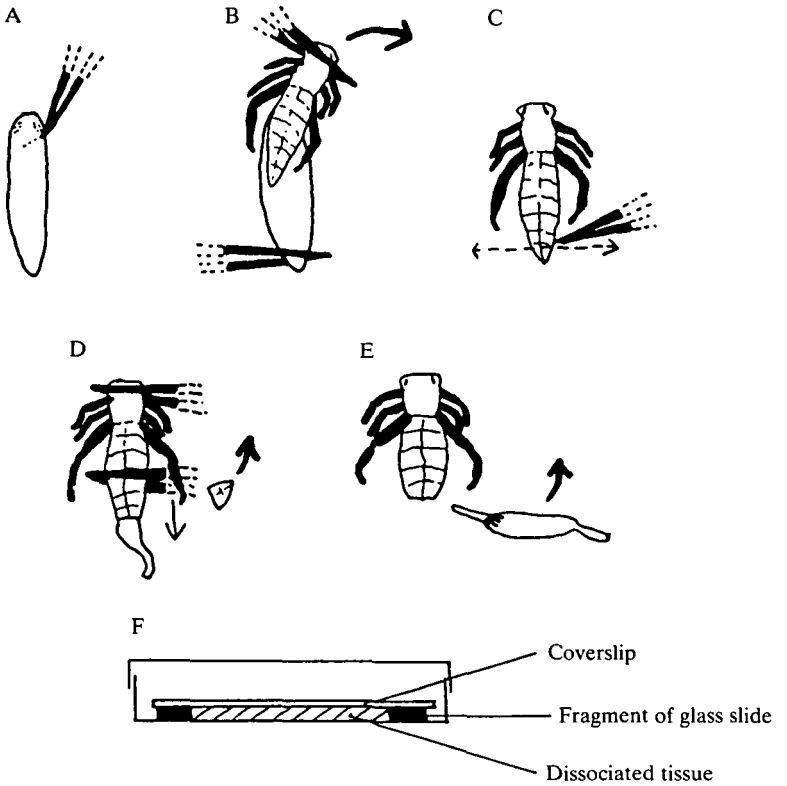


Fig. 1. Preparation of locust embryos from muscle culture. (A),(B) Removal of embryo from its egg case. (C)–(E) Removal of tip of abdomen and entire gut of embryo using fine forceps. (F) Culture dish with hanging column of medium containing dissociated embryonic tissue, excluding gut.

immersed in Methcol for 20 min for surface sterilization. All subsequent operations were performed under aseptic conditions, mostly in a laminar flow cabinet. The eggs were transferred to a Petri dish containing sterile saline (in mmol l^{-1}: NaCl, 180; KCl, 10; $CaCl_2$, 2; NaH_2PO_4, 6; Na_2HPO_4, 4; pH 6·8; Usherwood & Grundfest, 1965) and the embryos were separated from the egg cases (Fig. 1A,B) after rupturing the latter with fine forceps (no. 5). The entire gut was then removed from each embryo (Fig. 1C–E). The remainder of the embryo was transferred to a dish of fresh saline, where it was mechanically dissociated with fine forceps. The dissociated material was centrifuged at 250 ***g*** for 5 min, resuspended in 5+4 medium (Chen & Levi-Montalchini, 1969) and dissociated further by trituration using a Pasteur pipette. The dissociated material, suspended in 5+4 medium, was cultured in 50×9 mm Falcon dishes using a modified version of the hanging column technique described by Shields, Dubendorfer & Sang (1975). Two pieces of sterile, broken glass slide, coated in sterile Vaseline, were placed in the bottom of each sterile Petri dish as supports. The dissociated tissue was then plated out between the supports. Finally, sterile coverslips were gently lowered onto the supports and pressed down until the medium containing the dissociated material filled the whole area between

the coverslips and the Petri dish (Fig. 1F). The dishes were stored under high humidity at 28°C. Medium changes were made initially after 2 weeks *in vitro* and thereafter *ad lib.* Apart from removing debris, changes of medium also initiated further development of the cultures.

Cultures of dissociated material from 10-day-old *Locusta* embryos were also grown successfully in Grace's insect medium (GIBCO 041-1590) (Grace, 1962) using the above procedures.

Unless otherwise stated similar results were obtained from *Schistocerca* and *Locusta* cultures.

Electrophysiological techniques

Cultures of different ages were equilibrated in saline (in $mmol\,l^{-1}$: NaCl, 180; KCl, 10; $CaCl_2$, 2; Hepes, 3; pH 6·8) for 30 min before being mounted on the stage of an inverted, phase contrast microscope.

Membrane potentials of the myofibres were determined using intracellular, glass microelectrodes filled with $3\,mol\,l^{-1}$ KCl. The sensitivity of the myofibres to L-glutamic acid was tested by applying $10^{-6}\,mol\,l^{-1}$ sodium L-glutamate, in saline, under pressure from micropipettes (tip diameter about 2 μm). For single-channel studies, patch-clamp micropipettes with fine tips (diameter 1–2 μm), either polished or unpolished and filled with saline containing sodium L-glutamate (5×10^{-9}–10^{-6} $mol\,l^{-1}$), were used. When approaching a myofibre a slight positive pressure was applied to the inside of the pipette to keep its tip free of debris. The pressure was removed when contact with the myofibre was made and a giga-ohm seal then often formed spontaneously, although sometimes it was necessary to apply a small negative pressure to the inside of the pipette to achieve a good seal. During the latter part of these studies patch recordings were made after treatment of the muscle cultures for 30 min with $10^{-6}\,mol\,l^{-1}$ concanavalin A (Mathers & Usherwood, 1976, 1978) and these were compared with recordings made in the absence of this lectin. Single-channel currents were recorded on a Racal FM tape recorder at a bandwidth of d.c. to 1–3 kHz. Full details of the procedures employed for analysing the channel data can be found in previous publications from our laboratory (Gration *et al.* 1982; Kerry *et al.* 1985).

RESULTS

Growth of muscle tissue in culture

5+4 medium

When first plated out the cultures contained mainly rounded cells, but after only about 30 min *in vitro* some of these had become spindle-shaped with polar processes and had attached to the base of the culture dish. During the first 7 days in culture there was a lot of cell death, particularly of isolated cells, but by the end of this period many spindle-shaped cells remained, particularly in the vicinity of tissue clumps. Careful observation of their later development showed clearly that these remaining

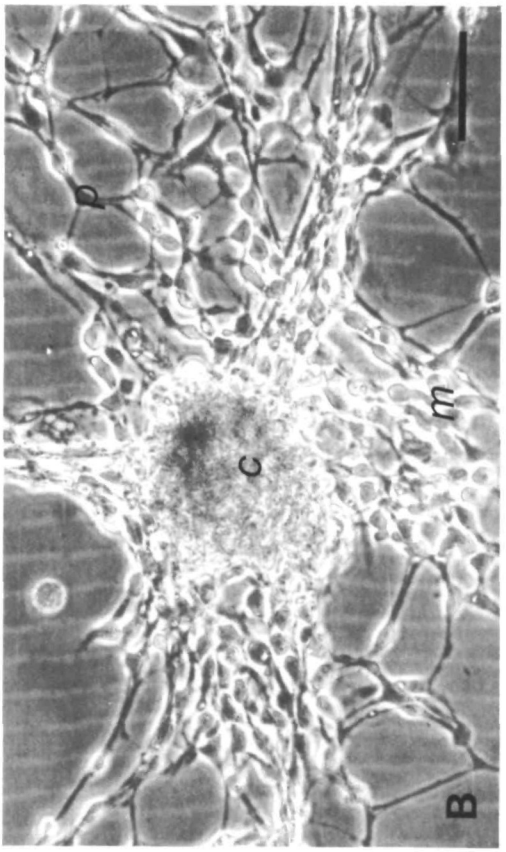
cells were myocytes. Either through extending their polar processes, to make contact with adjacent myocytes (Fig. 2A), or by associating laterally with other myocytes they eventually formed reticular arrays or chains of cells (Fig. 2B), before giving rise to multinucleated myofibres (Fig. 2C). By about 10 days a mixture of branched and unbranched myofibres was present in the cultures, the latter often stretched between tissue clumps (Figs 2D, 3A). After 2 weeks *in vitro* the myofibres were the most abundant structures and it was at this stage that spontaneous contractile activity appeared in the *Locusta* cultures. (Myofibres in *Schistocerca* cultures rarely contracted spontaneously.) Application of 10^{-6} mol l^{-1} L-glutamate in saline by pressure from a micropipette increased the frequency of these contractions. The cultures also contained neurones, glial cells and tracheolar tissue (Fig. 2D). The neurones were usually aggregated into groups and were readily distinguishable from muscle tissue by their fine processes. The neurone processes were often closely associated with myofibres but it remains to be established whether functional connections develop between nerve and muscle tissue in these cultures.

A change of medium after about 4 weeks *in vitro* precipitated further release of myocytes from tissue clumps and the development of more myofibres (Fig. 2D). The myofibres were fully grown after about 5 weeks *in vitro*, when they were up to 400 μm in length and 15 μm in diameter (Fig. 3A). Myofibres of this size were usually detached from the base of the culture dish for a greater part of their length. Unlike other tissues in the cultures, the myofibres contained a granular and fibrous cytoplasm, but they were rarely striated (Fig. 3A,B). Nervous tissue could still be readily distinguished from the muscle tissue at this stage by its highly branched, fine, recurrent processes radiating from clumps of loosely-packed, round cell bodies (Fig. 3C).

The myofibres continued to develop for a further 2–3 months during which time any remaining clumps of cells and most non-muscular tissues were lost (Fig. 3D). The myofibres no longer contracted spontaneously but many, particularly those of *Locusta* cultures, still contracted in response to a challenge with sodium L-glutamate (10^{-6} mol l^{-1}). Although the development of the cultures was similar for *Locusta* and *Schistocerca* tissue, a larger area of growth was usually obtained with the *Schistocerca* cultures.

Grace's medium

Freshly-dissociated material from 10-day-old *Locusta* embryos was also grown in modified Grace's insect medium. After about 30 min *in vitro* the majority of the cells in these cultures had attached to the base of the culture dish. At this stage and for the next few hours, most of the attached cells had a rounded outline although by 4 h some spindle-shaped cells were present (Fig. 4A). By 17 h most of the myocytes had polar processes with many cells aggregated into groups with their processes aligned. By 24 h the myocyte groups had fused to form multinucleate structures, whilst isolated myocytes were now characterized by thickened processes (Fig. 4B). After 7 days myofibres with a fibrous appearance had developed either as mononucleate structures from single myocytes or as multinucleate structures from the groups of muscle cells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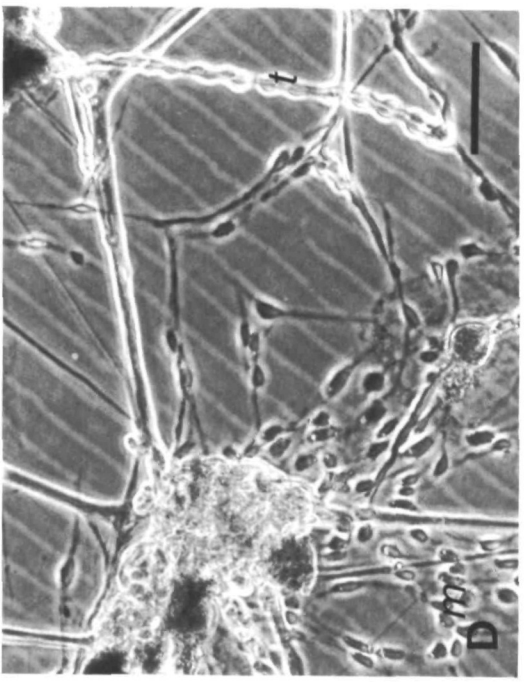
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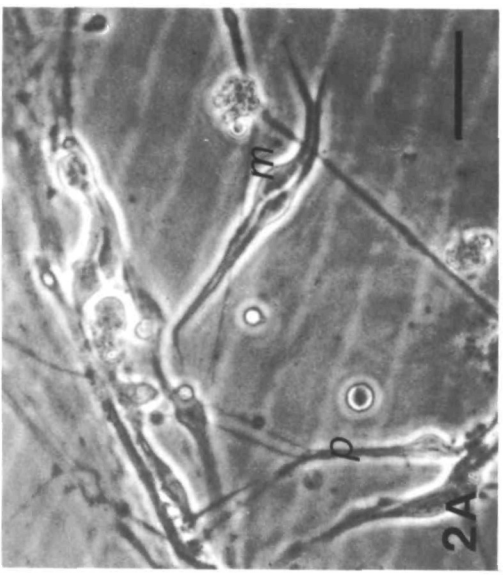
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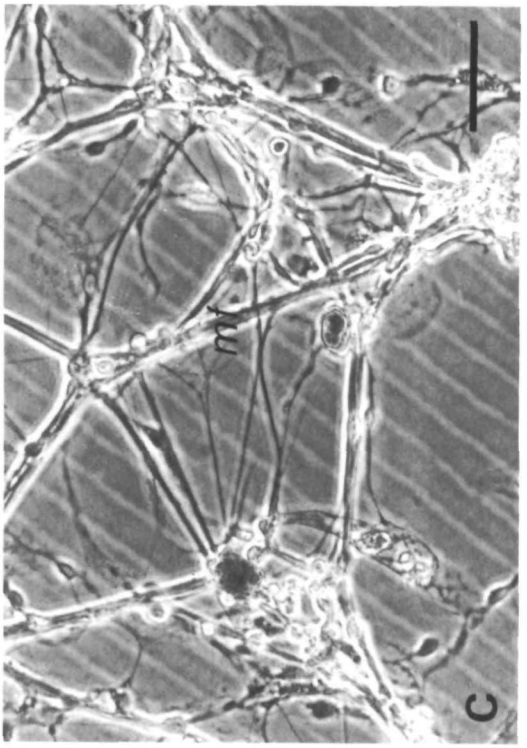
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(Fig. 4C). At this time they were up to 30 μm in length and 13 μm in diameter. After 3 weeks many of the myofibres were distinctly striated (Fig. 4D), even those with only one nucleus. Their length had increased to over 75 μm and their diameter to over 20 μm, and they bore a striking resemblance to myofibres which develop *in vivo*. They survived for up to 1 month in culture but showed no further signs of growth. They were never observed to contract either spontaneously or in response to a challenge with sodium L-glutamate (10^{-6} mol l^{-1}).

Electrophysiological data

Our studies of the physiology and pharmacology of both *Locusta* and *Schistocerca* muscle cultures have so far been mainly restricted to those grown in 5+4 medium, despite the fact that myofibres grown in Grace's medium looked more like those found *in vivo*. Unfortunately, myofibres grown in Grace's medium usually became detached from the base of the culture dish, which effectively precluded routine studies using patch-clamp techniques.

Resting potentials

Intracellular recordings using KCl electrodes from two *Locusta* cultures and two *Schistocerca* cultures, all of which had been more than 4 weeks *in vitro*, gave a mean resting potential of $-33{\cdot}4 \pm 13{\cdot}5$ mV (S.D.; $N = 19$). Unfortunately, because the myofibres were not attached to the base of the culture dish for much of their length and often contracted when impaled by a microelectrode, they were easily damaged. For this reason only those potentials which were maintained for more than 1 min after impalement are included in the above result. It is accepted that the true resting potential of the myofibre may be somewhat higher than the recorded value, i.e. closer to the −60 mV which characterizes adult locust leg muscle after equilibration in locust saline (Usherwood, 1969).

When 10^{-6} mol l^{-1} L-glutamate was ejected onto the surface of the myofibre there was a transitory fall in membrane potential, sometimes accompanied by a contraction. Application of saline alone had no effect.

Spike-like potentials

When patch pipettes were used to form mega-ohm seals with the surface membrane of myofibres present in cultures 3–8 weeks old, spontaneous spike-like events were recorded from about 5% of the myofibres in both *Locusta* (Fig. 5) and

Fig. 2. (A) Closely associated myocytes (*m*) with extended processes (*p*) seen in a culture 7 days *in vitro*. Scale bar, 50 μm. (B) Myocytes migrating from a clump (*c*) of embryonic tissue in a culture 12 days *in vitro*. Many of the muscle cells have fused to form either reticular structures or chains of cells (*p*). Scale bar, 80 μm. (C) Myofibres (*mf*) seen in a culture 21 days *in vitro*. Scale bar, 80 μm. (D) Addition of fresh medium to a culture 27 days *in vitro* was seen to have caused a secondary release of myocytes from tissue clumps when the culture was examined 24 h later. This culture contained tracheolar tissue (*t*). Scale bar, 80 μm. All of these photographs are of *Locusta* cultures maintained in 5+4 medium.

Schistocerca cultures, sometimes associated with contractions. These spike-like events were most frequently recorded from myofibres closely associated with nerve fibres. The effects of L-glutamate on the spike-like events have not yet been investigated.

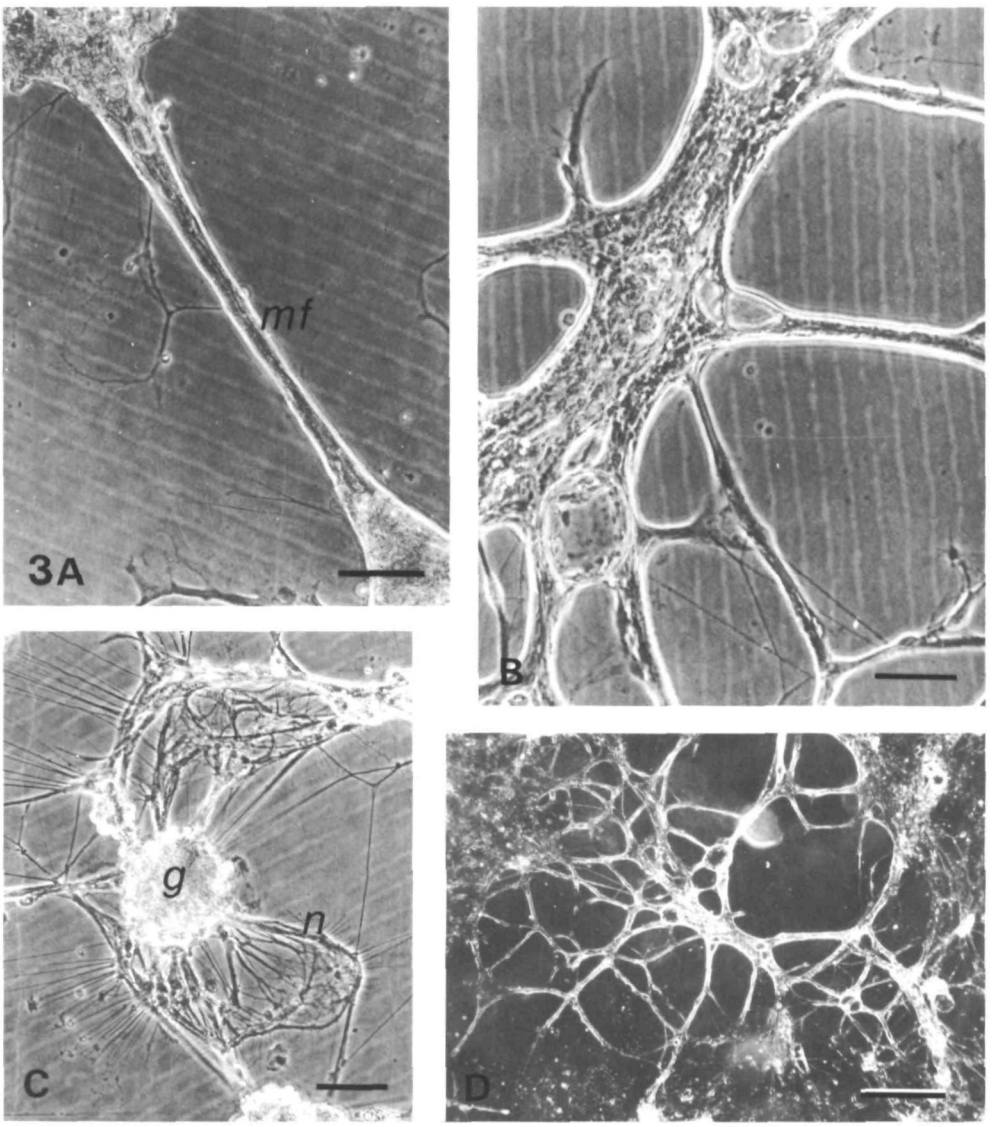

Fig. 3. (A) A large, unbranched myofibre (*mf*) seen in culture 35 days *in vitro*. Scale bar, 80 μm. (B) Part of a large, branched myofibre, 56 days *in vitro*, containing granular, fibrous cytoplasm. Scale bar, 80 μm. (C) Neurone processes (*n*) emanating from a clump of neurones (*g*) and forming a network of fibres in a culture 42 days *in vitro*. Scale bar, 80 μm. (D) Low power photographs of a branching myofibre seen in a culture 56 days *in vitro*. Most other tissues have disappeared by this time. Scale bar, 420 μm. All of these photographs are of *Locusta* cultures maintained in 5+4 medium.

Single-channel studies

Giga-ohm recordings of glutamate channel currents have been obtained from patches of myofibre membrane either on intact myofibres or as excised, cell-free, outside-out and inside-out patches. With patch pipettes containing 10^{-3} mol l^{-1} Ca^{2+} and sodium L-glutamate at a concentration as low as 5×10^{-9} mol l^{-1}, inward channel currents were usually seen for a brief period immediately after formation of a giga-ohm seal and then rarely thereafter. These channel currents, which were about 4 pA in amplitude in cell-attached patches when the patch pipette potential was held at 0 mV, were recorded from cultured myofibres as early as 3 weeks *in vitro*. The impression gained was of a homogeneous glutamate receptor channel population diffusely distributed over the surface of the myofibre and exhibiting rapid desensitization. This impression was subsequently confirmed when myofibres pretreated with

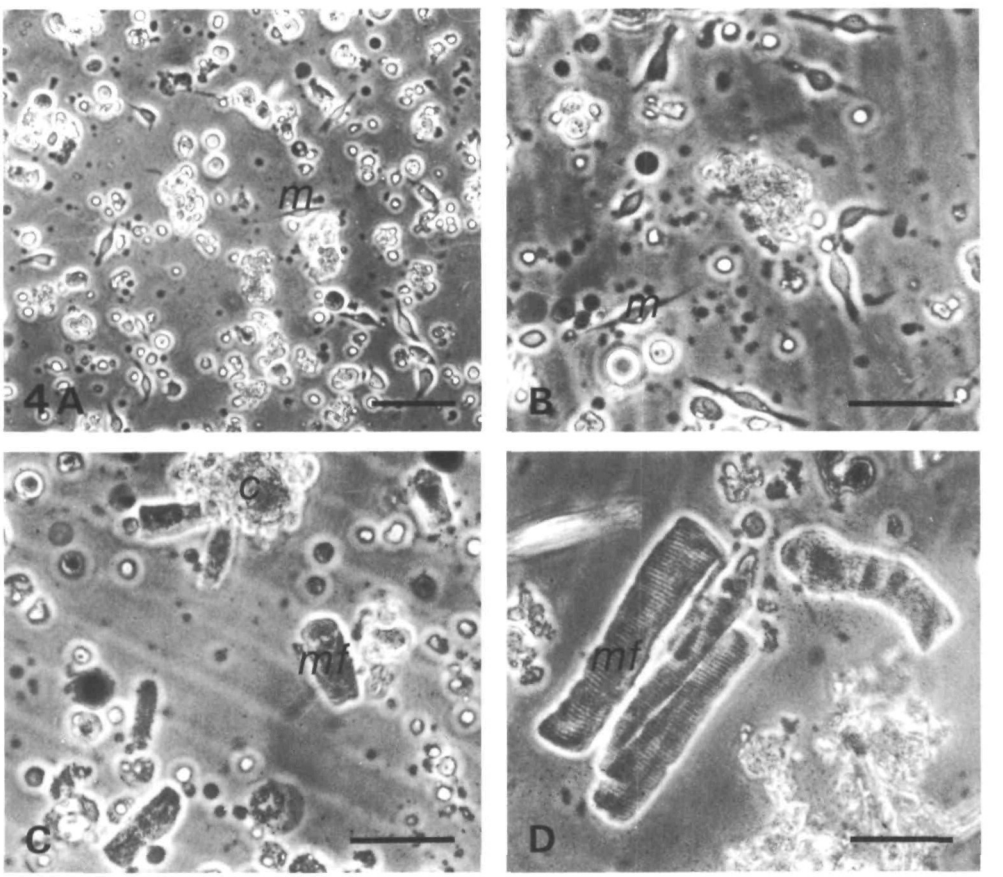

Fig. 4. (A) Spindle-shaped myocytes (*m*) seen in a culture 4 h after plating. Scale bar, 100 μm. (B) Myocytes with extended and thickened processes seen in a culture 24 h *in vitro*. Scale bar, 50 μm. (C) Myofibres (*mf*), which have either developed from isolated myocytes or have migrated from tissue clumps (*c*), seen in a culture 7 days *in vitro*. Scale bar, 50 μm. (D) Large myofibres seen 21 days *in vitro*. Scale bar, 50 μm. All of these photographs were of *Locusta* cultures maintained in Grace's medium.

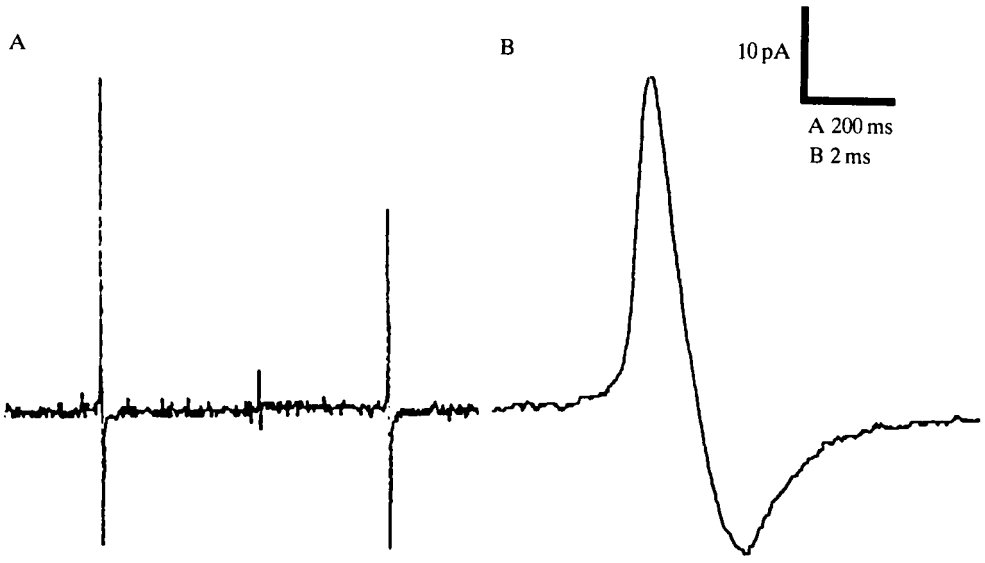

Fig. 5. Spike-like currents recorded from a patch of membrane on a *Locusta* myofibre, 35 days *in vitro* using a patch pipette with a mega-ohm seal.

concanavalin A were studied. In the presence of this lectin these large conductance channels were found to be present over the entire surface of a myofibre and channel events occurred throughout the period of maintenance of a giga-ohm seal. The latter observation suggests that concanavalin A blocks desensitization of glutamate receptors on embryonic muscle. The channel currents were never seen when sodium L-glutamate was omitted from the patch pipette.

Most of the single-channel recordings, both on myofibres and from excised patches, including those made with only 5×10^{-9} mol l^{-1} sodium L-glutamate in the patch pipette, were characterized by channel events exhibiting two equal, open conductance states (Fig. 6A). It seems likely that these represent the openings of two receptor channels but there remains the possibility that they are sub-conductances of a single receptor channel, since sudden transitions from the closed state to the double open state and *vice versa* were sometimes seen. Studies are currently in progress to clarify this issue. The noise level exhibited by a channel in its open state is usually greater than that seen when the channel is closed (Fig. 6A). During the simultaneous occurrence of more than one open conductance state the open noise level is further increased.

The amplitude and polarity of the glutamate channel current was dependent upon the magnitude and polarity of the pipette potential. A reversal potential of about 0 mV was routinely obtained for the single-channel current recorded with cell-attached patches (assuming a resting potential of about −35 mV), which suggests that it may be a cation-selective channel like its counterpart on adult locust muscle (Anwyl & Usherwood, 1974). However, further studies are required before this can be firmly established.

The open–closed kinetics of the glutamate-gated channel are complex and vary according to the recording mode, i.e. whether recordings are made from a patch of membrane on a myofibre, from an excised inside-out patch or from an excised outside-out patch. Examples of the channel dwell-time distributions (open and closed) are presented in Fig. 6C,D for data obtained from an inside-out patch. The histogram of channel open times indicates the presence of at least three open states for the channel. The corresponding histogram for channel closed times is less easy to interpret because of the presence of either more than one channel or, perhaps, sub-conductance open states, in the recording from which the data were obtained, but it suggests that the glutamate channel is characterized by multiple closed states. A fuller account of the channel kinetics for inside-out patches and other recording modes will be presented in due course.

The conductance of the channel varied with the Ca^{2+} environment of the membrane patch. With 10^{-3} mol l^{-1} Ca^{2+} in the patch pipette, 2×10^{-3} mol l^{-1} Ca^{2+} in the bathing medium and symmetrical distributions of Na^+ and K^+, the current–voltage characteristic for channels recorded from an inside-out patch was linear for pipette potentials between +80 mV and −80 mV. The slope conductance under these conditions was about 60 pS (Fig. 7). However, when the pipette Ca^{2+} concentration was reduced to 10^{-7} mol l^{-1} (buffered with EGTA) the slope conductance for inward currents (i.e. those flowing from pipette to bathing medium) was twice (about 120 pS) that for outward currents (Fig. 7). This is similar to the conductance of the channel gated by extrajunctional D-receptors on adult locust leg muscle (Patlak *et al.* 1979).

DISCUSSION

Significant advances in understanding the properties of vertebrate skeletal muscle have arisen from studies of primary cultures of muscle (Lake, 1916; Lewis & Lewis, 1917; Firkett, 1967; Bischoff & Holtzer, 1969; Larson, Jenkinson & Hudgson, 1970; Shimada, 1971; Nelson, 1975; Fambrough, 1978), most of which have been made on embryonic tissues. Primary cultures of insect muscle have been prepared using tissue from butterfly (Kurtti & Brooks, 1970), fruitfly (Seecof, Alleaume, Teplitz & Gerson, 1971; Dubendorfer, Blumer & Deak, 1978) and cockroach (Lynch, 1980) embryos, but their physiology and pharmacology have not been investigated. In this paper we have presented techniques for the establishment of primary cultures of muscle from embryonic locust together with a description of *in vitro* myogenesis. In addition we have shown that the cultured myofibres express receptors for L-glutamic acid and that recordings of channels gated by these receptors can be obtained using giga-ohm seal, patch-clamp techniques.

The development of locust muscle in culture displays many of the characteristics already described for cultured muscle of other insects (Kurtti & Brooks, 1970; Lynch, 1980) and, to a lesser extent, of vertebrates (Nelson, 1975; Bachmann, 1980). However, there are differences in the time course of myogenesis between insect species (Seecof *et al.* 1971), and between insect and vertebrate species,

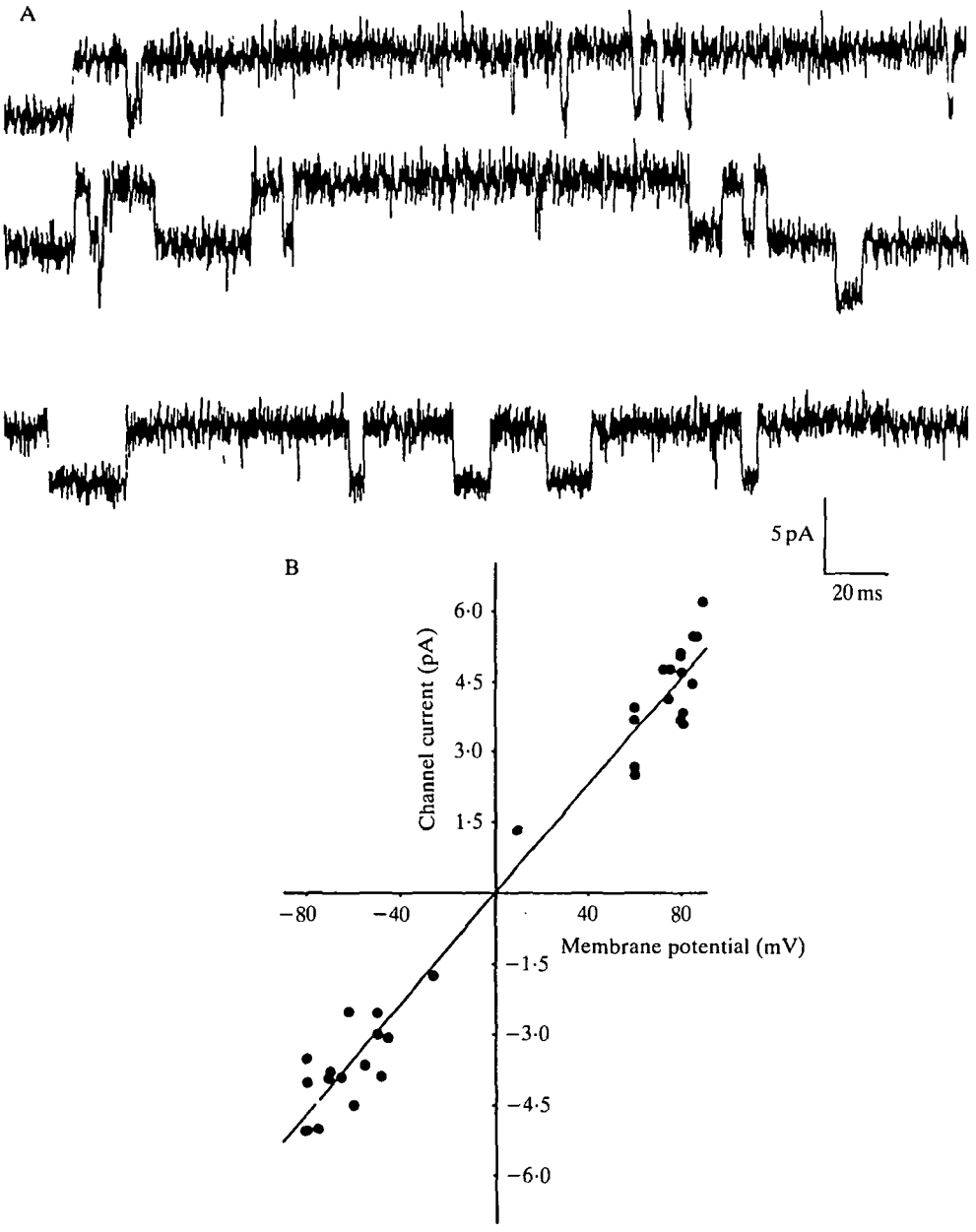

Fig. 6. (A) Outward (bulk saline to pipette), single glutamate-gated channel currents (upward deflections) recorded from an excised, inside-out membrane patch from a myofibre in a *Locusta* culture 55 days *in vitro*. The patch pipette was filled with 10^{-7} mol l^{-1} L-glutamate and 10^{-3} mol l^{-1} Ca^{2+} and was held at a potential of −90 mV. The myofibre was pretreated with 10^{-6} mol l^{-1} concanavalin A to inhibit glutamate receptor desensitization. Note the two equal, open channel conductance states, which may represent either activity of more than one glutamate receptor channel or the presence of sub-conductance states of a single channel, and the increased noise level of the open channel. (B) Current–voltage characteristic for channels of the type illustrated in A. The mean conductance of about 60 pS was determined from the slope of a linear least-squares fit to data points which were obtained from 23 inside-out patches (*Locusta* and *Schistocerca* cultures; 21–60 days *in vitro*).

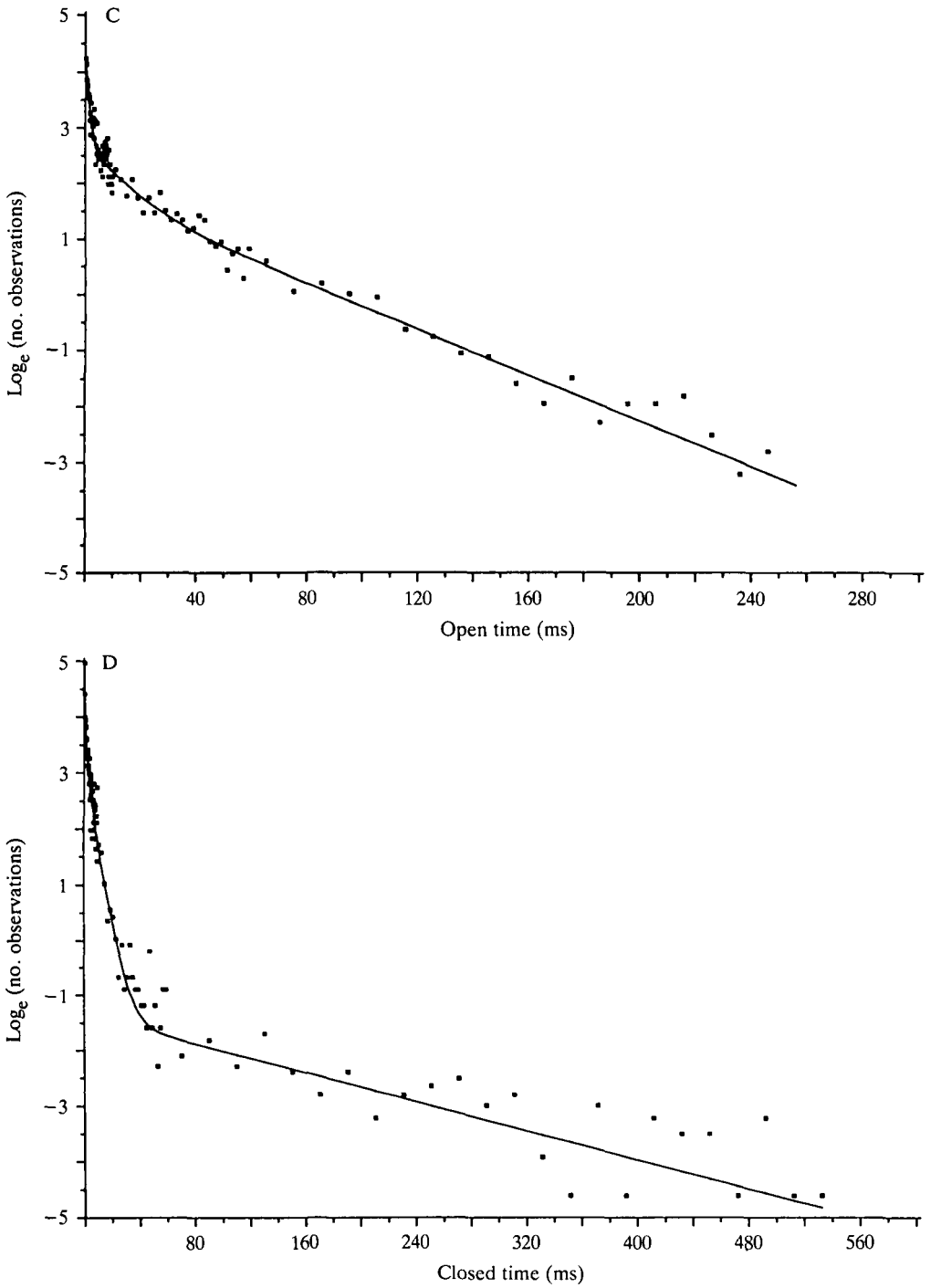

Fig. 6. (C),(D) Open time (C) and closed time (D) histograms for data of the type illustrated in (A). The open time distribution is fitted (Kerry *et al.* 1986) by the sum of three exponentials with time constants of 1·2 ms, 12·0 ms and 49·2 ms. The closed time distribution is fitted by the sum of four exponentials with time constants of 0·259 ms, 1·90 ms, 6·74 ms and 154 ms.

which tend to complicate such comparisons. Further complications arise because of methodological differences in terms of media used, the temperatures at which the cultures are maintained and the conditions under which the embryonic tissues are dissociated. The growth medium is obviously an important factor in determining the fate of the cultures, since one major result of this study is the discovery of striking differences in the morphological appearance of muscle tissues grown in 5+4 medium compared with Grace's insect medium. Cultures grown in 5+4 medium express glutamate receptors resembling those found on adult muscle, but the morphology of the *in vitro* myofibres bears little resemblance to mature muscle tissue found *in vivo*. Myofibres grown in Grace's insect medium are morphologically similar to mature muscle *in vivo*, but it remains to be established whether they exhibit similar physiological and pharmacological characteristics. One main advantage of the techniques we have developed is that they involve rather simple procedures. Also, once a culture has been established, only one change of medium is normally required for 'full' development of the muscle tissue. In 5+4 medium, myofibres survived for up to 3 months *in vivo* under these conditions.

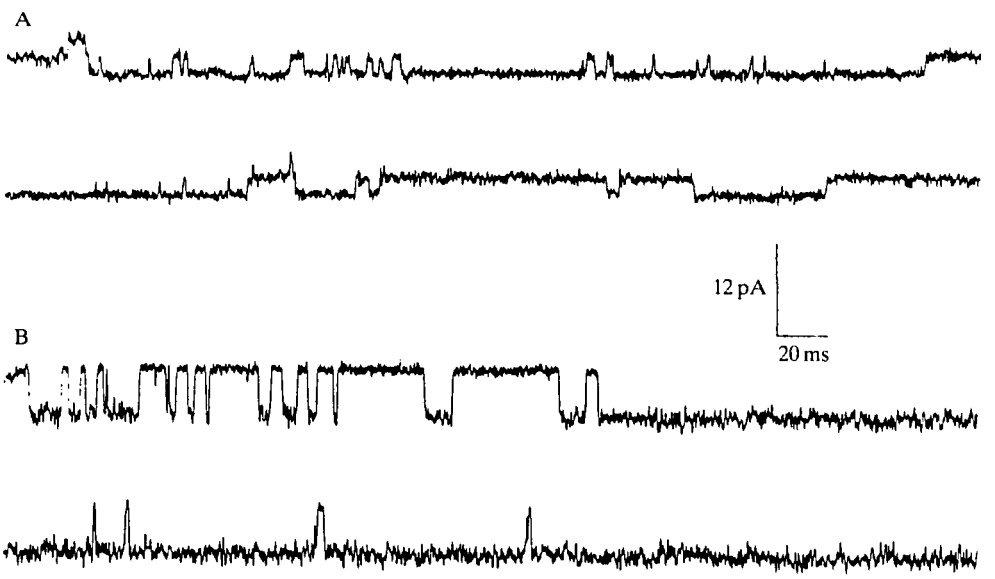

Fig. 7. Glutamate-gated channels recorded from an excised inside-out membrane patch taken from a myofibre of a *Schistocerca* culture 60 days *in vitro*. The patch pipette was filled with 10^{-6} mol l^{-1} L-glutamate and 10^{-7} mol l^{-1} Ca^{2+} (buffered with EGTA). The Ca^{2+} content of the bulk saline was 2 mmol l^{-1}. Concentrations of Na^{+} (180 mmol l^{-1}) and K^{+} (10 mmol l^{-1}) were identical on both sides of membrane patch. The myofibre was pretreated with 10^{-6} mol l^{-1} concanavalin A. In (A) the patch pipette was held at a potential of −80 mV. The presence of 'outward' channel currents is indicated by the upward deflections. In (B) the patch pipette was held at +80 mV. Inward channel currents are downward deflections. The channel current amplitude was zero when the potential difference across the membrane patch was set at 0 mV. Note increased noise when channel was open, especially in (B), and smaller channels for the outward currents in (A) compared with the inward currents in (B).

The discoveries that receptors for L-glutamic acid are widely distributed over the surface of myofibres grown for 3 weeks or more in 5+4 medium, that desensitization of these receptors is blocked by concanavalin A, that the receptors can be studied using giga-ohm seal recording techniques and that the channel conductance is large provide ample opportunities for studying the channel gating kinetics of these receptors at high recording resolution.

The influence of Ca^{2+} on the conductance of the channel is interesting because, in adult locust muscle, Ca^{2+} blocks the channel gated by junctional glutamate receptor channels (Cull-Candy, 1982) and extrajunctional glutamate D-receptor channels (Kits & Usherwood, 1984), although this only occurs with concentrations of Ca^{2+} in excess of 10^{-2} mol l^{-1}. In terms of their interactions with Ca^{2+}, there thus appears to be a quantitative difference between the glutamate channel *in vivo* and *in vitro* which merits further investigation. This is not the only difference, of course. The sensitivity of the glutamate receptor of cultured locust muscle to L-glutamate seems to be at least a thousand times greater than that of the glutamate D-receptor of adult locust muscle, since the frequency of channel openings is similar for 5×10^{-9} mol l^{-1} L-glutamate in the case of the cultured muscle and 5×10^{-5} mol l^{-1} L-glutamate in the case of the adult muscle. The apparent dissociation constant (K_d) for L-glutamate interaction with the adult extrajunctional D-receptor is 5×10^{-4} mol l^{-1} (Gration *et al.* 1980, 1981). It remains to be established whether the equivalent K_d of the cultured muscle receptor is three orders of magnitude smaller than this.

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