J. exp. Biol. ***183****, 323–339 (1993)*
Printed in Great Britain © The Company of Biologists Limited 1993

LOCUST MEDIAL NEUROSECRETORY CELLS *IN VITRO*: MORPHOLOGY, ELECTROPHYSIOLOGICAL PROPERTIES AND EFFECTS OF TEMPERATURE

WOLFGANG RÖSSLER

AG Neurobiologie, FB Biologie-Zoologie, Philipps Universität, Marburg, Postfach 1929, 35032 Marburg, Germany

AND ULF BICKMEYER

Abteilung Neurotoxikologie, Medizinisches Institut für Umwelthygiene an der Heinrich-Heine Universität Düsseldorf, Auf'm Hennekamp 50, 40225 Düsseldorf, Germany

Accepted 23 June 1993

Summary

The medial neurosecretory cells of the pars intercerebralis in the protocerebrum of larval and adult locusts (*Locusta migratoria*) were cultured in a chemically defined serum-free culture medium. The morphology of the cells was investigated by light microscopy and the electrophysiological properties were studied using the patch-clamp technique in the whole-cell configuration. The dissociated neurosecretory cells grew new processes under these conditions and were maintained in culture for up to 2 months. The percentage of cells showing outgrowth was significantly higher in third-instar larvae than in instars 4 and 5 and adults. A primary axonal stump promoted a unipolar cell morphology; in other cases, most neurosecretory cells became multipolar. The presence of glial cells in undissociated groups of neurosecretory cells improved outgrowth and the formation of neurite bundles. A considerable number of the recorded cells showed spiking activity in response to depolarization. The influences of temperature on spike frequency, duration and amplitude as well as on membrane potential and ionic currents were investigated. The results suggest that temperature may directly affect the function of neurosecretory cells.

Introduction

The medial neurosecretory cells (MNSC) of the pars intercerebralis of insects influence a variety of physiological activities ranging from moulting (Wigglesworth, 1940) and reproduction (Girardie, 1966) to the regulation of metabolism (Goldsworthy, 1969; Goldsworthy *et al.* 1977) and they are therefore of considerable biological significance. The important role of this neurosecretory system was demonstrated by Kopec (1917) with the discovery of a brain hormonal effect in a caterpillar. Wigglesworth (1940, 1964) demonstrated the release of a brain hormone, from the pars intercerebralis, which regulates moulting in the insect *Rhodnius prolixus*. The morphology and anatomy of the

Key words: cell culture, medial neurosecretory cells, patch-clamp recordings, *Locusta migratoria*, insect.

pars intercerebralis and the retrocerebral neuroendocrine complex of locusts has been investigated in detail by Girardie and Girardie (1967) and Mason (1973).

Dissociated primary cell cultures provide a useful system to investigate the morphology, function and pharmacology of neurones in a controlled environment. Chen and Levi-Montalcini (1969) introduced an *in vitro* system able to promote axonal outgrowth and cell migration from the embryonic nervous system of cockroaches. Subsequent research on insect nerve cell cultures mainly focused on embryonic growth of the nervous system (for a review, see Beadle and Hicks, 1985). Seshan *et al.* (1974) established a culture system for larval and adult cockroach medial neurosecretory cells in combination with embryonic organs or ovaries of the same species. Recently, it has also been demonstrated that the presence of haemolymph in the culture medium can significantly improve neurite outgrowth in dissociated neuronal cell cultures of adult *Periplaneta americana* (Howes *et al.* 1991), *Locusta migratoria* and *Schistocerca gregaria* (Kirchhof and Bicker, 1992).

The release of neurohormones from neurosecretory cells *in vivo* is elicited by the arrival of action potentials in the terminals; *in vitro* it can be elicited by depolarization with high external potassium levels (Maddrell and Gee, 1974). Thus, the electrophysiological properties of neurosecretory cells are of great importance for their function. Neurosecretory activity is known to be affected by environmental temperature and light conditions (Lees, 1963, 1964). Cook and Milligan (1972) found that light affects the firing frequency and membrane potential of medial neurosecretory cells in *Periplaneta americana*. However, very little is known about the function of the pathways mediating between the environmental and internal inputs and the activity of neurosecretory cells.

The aim of the present work was to establish a primary culture system for larval and adult locust medial neurosecretory cells in order to facilitate electrophysiological investigations. The factors contributing to cell growth and morphology were studied. The function of isolated medial neurosecretory cells was investigated with electrophysiological recordings using the patch-clamp technique. Since temperature is an important variable affecting the release of neurohormones, we examined the direct temperature-dependence of some electrophysiological properties of medial neurosecretory cells.

Materials and methods

All experiments were performed using both larvae (third to fifth instar) and adults of *Locusta migratoria* L. of both sexes reared in a crowded colony under constant conditions in the laboratory. The animals were kept at 35˚C (day)/25˚C (night) and 30% relative humidity with a 13h:11h light:dark cycle.

Preparation of cultures

After the animals had been anaesthetized with CO_2, the head was severed from the body and fixed on a plate. The head and plate were then surface-sterilised by rinsing in

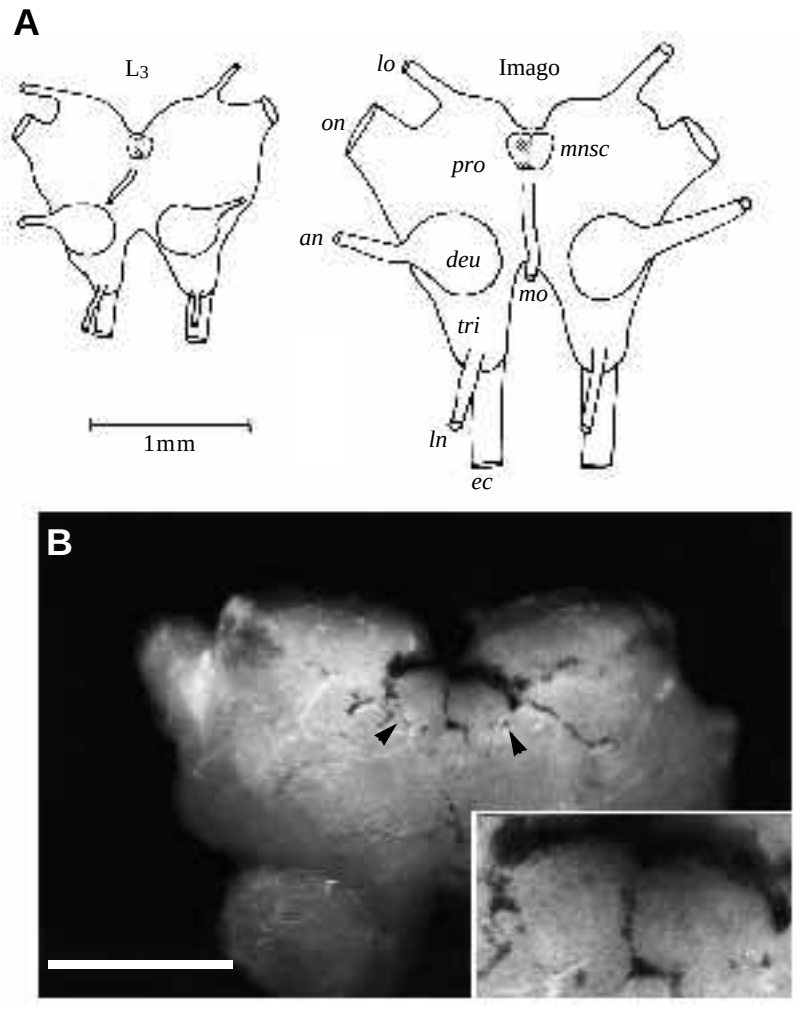

Fig. 1. Location of medial neurosecretory cells (MNSCs) in the pars intercerebralis of the supraoesophageal ganglion of *Locusta migratoria*. (A) Diagram of the supraoesophageal ganglion of the third-instar larva (L_3) and adult (imago) in a dorsal view. The nerves and parts of the ganglion are lettered: antennal nerve (*an*); deuterocerebrum (*deu*); oesophageal connective (*ec*); labial nerve (*ln*); lateral ocellar nerve (*lo*); medial neurosecretory cells (*mnsc*); medial ocellar nerve (*mo*); optic nerve (*on*); protocerebrum (*pro*); tritocerebrum (*tri*). Anterior is upwards. (B) Microphotograph of a desheathed supraoesophageal ganglion of an adult animal under dark-field illumination. The two clusters of medial neurosecretory cells in the pars intercerebralis are marked by arrowheads. Scale bar, 0.5mm. The inset shows the two clusters of MNSCs at higher magnification.

70% alcohol. Working in a laminar flow cabinet and using sterile dissecting techniques, a piece of cuticle was dissected away from between the compound eyes. The supraoesophageal ganglion (Fig. 1) was removed from the head and immediately transferred to a dish (Nunc organ culture dish, 35mm×10mm) containing fresh medium. The medium was similar to that used by Howes *et al.* (1991), consisting of five parts of

Schneider's *Drosophila* medium and four parts of Minimum Essential Medium (MEM) with Hanks' salts (5+4 medium) and containing 25mmol l^{-1} Hepes (Gibco). The only additives were 100i.u.ml^{-1} penicillin/streptomycin and 0.25 $\mu g\,ml^{-1}$ Fungizone (Gibco). The ganglia were dissected free from adhering fat and tracheae, and the ganglion sheath was removed with very fine forceps. After rinsing, the ganglia were transferred to another dish containing fresh medium. In the desheathed ganglia, the two large groups of medial neurosecretory cells (MNSCs) in the pars intercerebralis become very distinct under dark-field illumination (Thomsen and Thomsen, 1954) (Fig. 1). The protocerebral lobes were carefully stretched to expose the pars intercerebralis. The two clusters of MNSCs were dissected out by cutting the fibre tracts with very fine forceps. The ganglionic debris was removed from the dish with forceps. The average size of one group of MNSCs in adults was 0.22mm in the longitudinal axis and the maximum lateral extension was 0.17mm; in third-instar larvae, a length of 0.15–0.17mm and a width of 0.1mm was measured. Similar values are described in histological investigations of the pars intercerebralis of *Locusta migratoria* carried out by Girardie and Girardie (1967), who counted a total of 570 medial neurosecretory cells in adult locusts and 492 in the fourth larval instar. The two groups of MNSCs were mechanically dissociated in the centre of the dish using very fine sterile insect mounting pins. The dishes were then transferred to a humid incubator chamber at 30°C; the cultures were incubated either in air or with 5% CO_2 in 95% air. The medium was partly changed for fresh medium every 7 days, in some cases every 14 days.

Electrophysiology

Electrophysiological recordings were carried out using the patch-clamp technique in the whole-cell configuration (Hamill *et al.* 1981) and a conventional patch-clamp tower (Luigs and Neumann Co.). We used fire-polished electrodes with a resistance of 2–5 MΩ. Measurements were performed in culture medium (five parts of Schneider's *Drosophila* medium and four parts of MEM with Hanks' salts containing 25mmol l^{-1} Hepes obtained from Gibco). Our standard pipette solution (I) contained (in mmol l^{-1}): potassium gluconate, 180; NaCl, 10; $CaCl_2$, 1; MgATP, 2; EGTA, 10; $MgCl_2$, 2; and Hepes, 10; adjusted to pH7.3. In some experiments, we used a second pipette solution (II) containing in (mmol l^{-1}): KCl, 145; MgATP, 2; EGTA, 2; and Hepes, 10. The CED system hardware and software packages were used in combination with the EPC7 patch-clamp amplifier (List Electronics) for stimulus generation and for storage and analysis of data. For current–voltage relationships, voltage steps of 10mV were applied from different holding potentials. Current-clamp measurements were carried out in the current-clamp mode of the EPC7. For depolarization of cells, we injected currents of 0.1–0.5nA for 50ms. Membrane capacity was determined using the compensation circuit of the EPC7. Some experiments were performed with intracellular electrodes filled with 3 mol l^{-1} KCl (resistance 10 MΩ). Temperature was controlled and regulated by a 'Temperature Controller' (Luigs and Neumann Co.) provided with two Peltier heat-exchange elements. The temperature was automatically regulated to a preset value by a feedback loop. The temperature controller was connected to a tape recorder (Sony instrumentation tape recorder) for permanent recording of the temperature.

Results

Morphology and growth of medial neurosecretory cells in primary cultures

Depending on the efficiency of the dissociation process, the MNSCs were either completely isolated or formed smaller or larger groups. The proportion of isolated cells was usually high. After dissociation, the soma were spherical and some of the cells retained a primary axonal stump (10–20% of the isolated cells). Short primary axonal stumps often retracted within the first 4 days in culture, while longer primary neurites often started to sprout new processes (Figs 2A and 3B). The majority of the cells adhered to the culture dish within the first hour of culture. In larvae (third to fifth instar) and adults, the diameter of the cell bodies ranged from 10 to 70 μm. Diameters between 60 and 70 μm were found in only a few cases; most of the cells had diameters between 15 and 30 μm (mean value 26 μm, N=58). The cells were maintained in culture for up to 2 months.

New processes started to sprout from the somata or from primary neurite stumps within the first 2 days in culture. The percentage of isolated cells showing outgrowth was around 80% in the third larval instar and about 60% in instars 4 and 5 and in adults. Since this was the only obvious difference between larvae and adults that we observed, most morphological observations on larval and adult tissue are considered together.

Incubation with 5% CO_2 in the atmosphere significantly improved viability and outgrowth of the cells in culture. The effect of CO_2 was compared in identically treated cultures of MNSCs from third-instar larvae observed for 30 days. The two samples were kept simultaneously in two incubators. During the first 5 days in culture, the percentage of cells showing outgrowth was around 80% in both cases. After 2 weeks in culture, about 90% of the isolated cells in the samples without CO_2 started to deteriorate and retract their processes. In the presence of CO_2, most of the isolated cells retained their processes over the whole observation period. In undissociated groups of MNSCs, survival of the cells and processes was better, even without CO_2 in the atmosphere.

Cultured MNSCs showed a variety of morphologies. When a long primary axonal stump was retained after the dissociation process, a unipolar cell morphology was often promoted (Figs 2A and 3B; see also Kirchhof and Bicker, 1992); in other cases, most MNSCs tended to become multipolar (Figs 2B,C and 3A,C,D). Cells with short primary neurites usually became multipolar (Fig. 3C). After 4 days in culture, varicosities appeared on some of the processes (Figs 2D, 3D and 4). In many cases, the number and size of these axonal swellings increased with further growth. Morphological contacts between isolated MNSCs were often observed after about 3 days in culture (Figs 2B,E,F and 3D). Contacts occurred more frequently within clusters of MNSCs (Figs 2E,F and 3D). Processes of isolated cells were usually thin, while cells within or between groups of MNSCs formed larger neurite bundles. In undissociated tissue, cells with a flattened appearance and with granular inclusions were observed within cell groups and along large processes. Since any adhering tissue was carefully removed from the ganglion during dissection, we propose that these cells were of glial origin (Fig. 2I,J). In the presence of such glial cells, growth of large neurite bundles was promoted; the growth rate could be fast (up to 150 μm in a single day) and the overall length of processes was

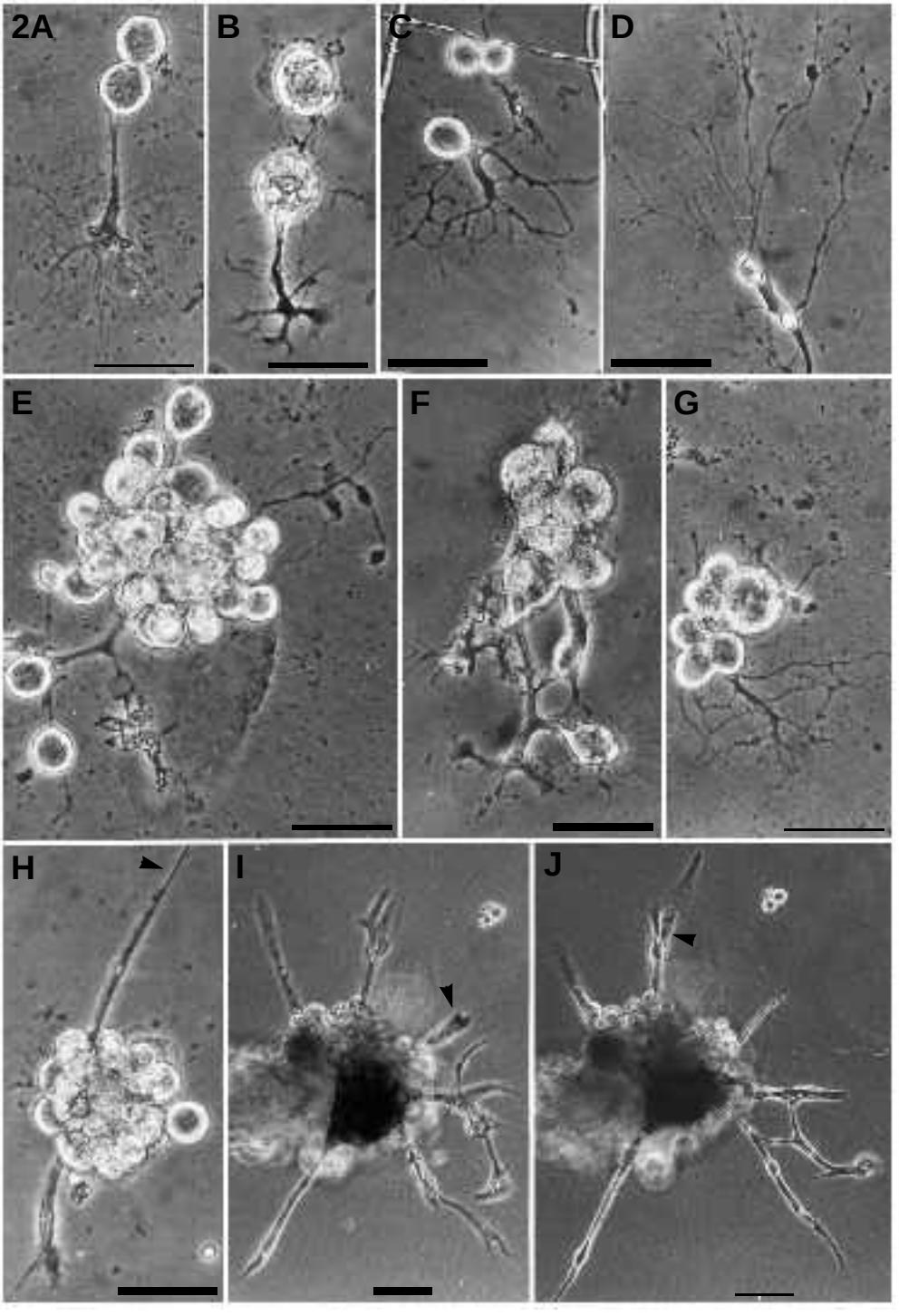
2A
B
C
D
E
F
G
H
I
J

Fig. 2. Phase-contrast light micrographs showing outgrowth from isolated MNSCs and groups of MNSCs maintained in primary culture. (A) Two cells after 4 days in culture, one of them showing multiple-process outgrowth from a primary neurite stump that remained after dissociation (third instar). (B) Two isolated MNSCs making contact after 3 days in culture (adult). (C) MNSC after 8 days in culture with a multipolar morphology (fourth instar). (D) Branching of a large process of a small group of MNSCs after 35 days in culture showing varicosities along the fine branches (third instar). (E,F,G) Clusters of MNSCs showing connections and outgrowth *via* large neurite bundles and single processes after 3 days (F, adult), 7 days (E, fourth instar) and 8 days (G, fourth instar) in culture. (H) A group of MNSCs with a process that orientates along a scratch (arrowhead) on the bottom of the dish caused by an insect mounting pin after 7 days in culture (third instar). (I,J) A large group of MNSCs grown together with cells of probable glial origin (see arrowheads) showing large-diameter processes and fast outgrowth at day 8 (I) and day 9 (J) in culture (fourth instar). Scale bars, 50 μm.

high (up to 800 μm). Groups of MNSCs containing these putative glial cells also survived for longer in culture (Fig. 4E). In one case, the two large processes emerging from a small group of MNSCs oriented along a scratch caused by an insect mounting pin on the bottom of the dish (Fig. 2H). The pattern of growth from a group of about 30MNSCs from day 7 to day 32 is shown in Fig. 4A–D. In isolated cells and groups of MNSCs, the growth rate was highest during the first 5 days in culture (up to 150 $\mu m\,day^{-1}$). After 5 days in culture, some of the processes continued to grow, while some higher-order side branches shortened. During this phase, the rate of growth was usually lower. In a group of MNSCs observed for 25 days after the initial growth phase, the average rate of growth was $10\pm5\ \mu m\,day^{-1}$.

Electrophysiological properties and the effects of temperature

To study the electrophysiological properties of single MNSCs, we performed recordings from isolated cells with the patch-clamp technique in the whole-cell configuration (Hamill *et al.* 1981). All experiments were carried out with isolated MNSCs of adult locusts in order to exclude variations caused by functional changes during larval development. After establishment of a giga-seal on the soma of the cell, the patch membrane was disrupted by suction. Successful recordings were obtained from 77 cells. Cells could be held for 1h or more. Most recordings were carried out between day 3 and day 7 in culture. The stability of the electrode potentials was checked outside the cell before and after recording. Measurements were performed directly in the medium. Most recordings were made with pipette solution I.

Both spiking cells and nonspiking cells were found. Immediately after dissociation and during the first 2 days in culture, most MNSCs did not show spiking activity and some had very low membrane potentials. Between day 3 and day 7, the ‘resting’ membrane potential was in most cases between −15 and −50mV and the percentage of cells showing spiking activity in response to a depolarizing current rose to become significantly higher (approximately 50% of the recorded cells). Membrane capacities between 6pF (small-diameter cells) and 100pF (large-diameter cells) were measured (N=21; 36±21pF). No obvious correlation between the membrane capacity (and the corresponding diameter of the cells) and spiking activity could be observed.

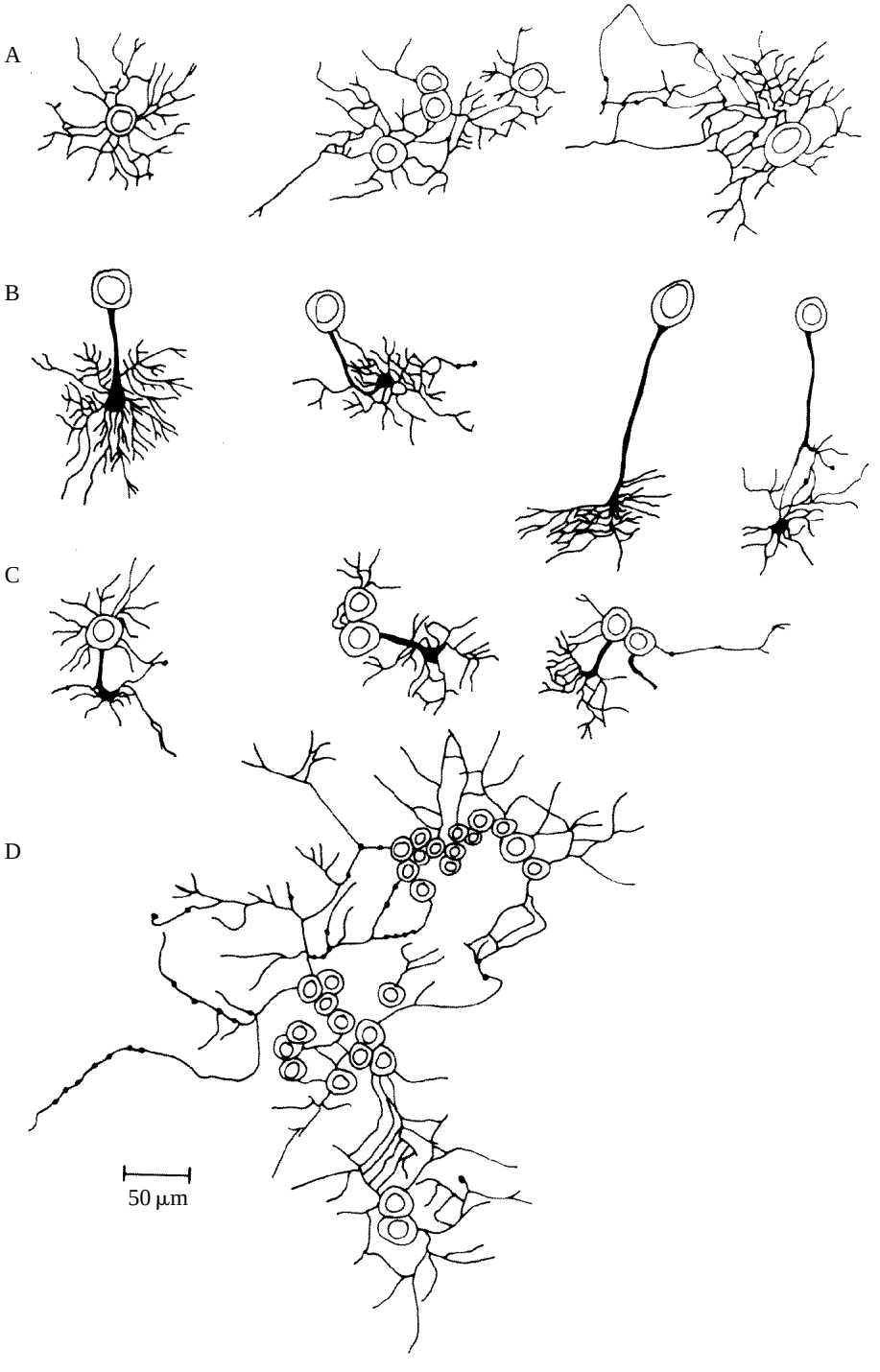

Fig. 3

Fig. 3. Variety of morphologies of isolated MNSCs *in vitro* shown as *camera lucida* drawings. (A) Multipolar cells after 4 days in culture. (B) Cells that retained a primary neurite stump after the dissociation process show unipolar morphology and multiple processes on the neurite stump after 5 days in culture. (C) After 5 days in culture, cells with a short primary axonal stump also tend to become multipolar. (D) Multiple processes, cell contacts and expression of varicosities within a cluster of MNSCs after 6 days in culture.

In spiking cells, the action potentials typically had long durations (between 3 and 12ms, depending on the temperature; see below), which is known to be a characteristic of neurosecretory cells in general (Gosbee *et al.* 1968; Cook and Milligan, 1972; Seshan *et al.* 1974; Rowell, 1976; Raabe, 1983). Spike amplitudes between 10mV and 80mV were recorded.

Fig. 5 demonstrates typical spike responses in current-clamp recordings from isolated MNSCs. The membrane potential was in all cases set to −30mV and the cells were stimulated with a depolarizing current pulse of approximately 0.5nA and of 50ms duration. About 50% of the spiking cells responded with a low-amplitude action potential (10–20mV; Fig. 5A), others discharged with a single high-amplitude spike (up to 80mV; Fig. 5B), and a number of cells showed continuous spiking activity when the membrane potential was set to −30mV (Fig. 5C). These cells often responded with more than one action potential to the stimulus pulse.

Since temperature is an important variable affecting neurosecretory activity (Lees, 1963), we examined its effect on the electrophysiological properties of MNSCs. In Fig. 6A,B, examples of current-clamp recordings from an MNSC are shown under similar stimulus conditions at temperatures of 20˚C and 34˚C. The 'resting' membrane potential of this cell was −40mV and the membrane potential was set to between −50mV and −20mV. A depolarizing current pulse of approximately 0.5nA with a duration of 50ms was applied. At 34˚C, the cell discharged with 2–5 spikes per stimulus, depending on the membrane potential, and became spontaneously active at a potential of −30mV (spike frequency about 15Hz). The duration of action potentials at the 'resting' membrane potential level (−40mV) was between 3 and 4ms at this temperature. The time interval between the beginning of the fast rising phase and the corresponding point on the repolarizing phase of the action potentials was determined. At −20mV, the fast inward current for the generation of action potentials became inactivated (complete inactivation was between −15mV and −10mV in all cases). At 20˚C, spike duration was increased to 12ms at a membrane potential of −40mV and the cell always responded to the current pulse with a single action potential. No spontaneous discharge was observed at a potential of −30mV. In Fig. 6B, the responses of the cell starting from the 'resting' membrane potential (−40mV) at the two temperatures are shown enlarged and superimposed. At 20˚C, the spike number was lower, the spike amplitude was higher and the passive and active components of the response were clearly slower.

With a change of temperature, there was also a consistent membrane potential change. The cells hyperpolarized on heating and depolarized on cooling (Fig. 7A,B). In Fig. 7A, the change in membrane potential with a change in temperature was recorded in a single experiment over a period of 20min. The change in potential with temperature was on

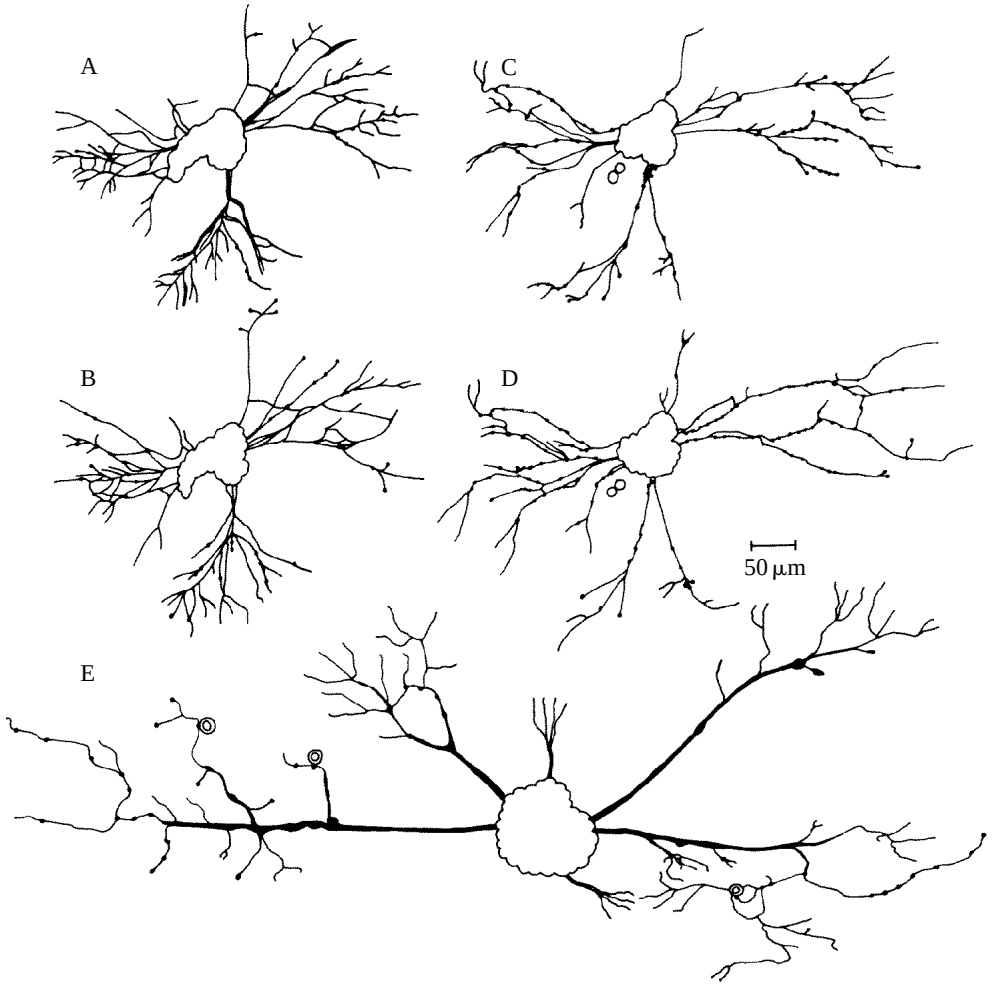

Fig. 4. Morphology of groups of MNSCs shown as *camera lucida* drawings. (A–D) Outgrowth from a group of about 30 MNSCs after 7 (A), 10 (B), 22 (C) and 33 (D) days in culture. Note the reduction of higher-order side branches during growth and the increase in the number of varicosities along the processes (third instar). (E) A large group of MNSCs grown together with cells of probable glial origin after 46 days in culture, showing large-diameter processes and contacts to single isolated cells (fourth instar).

average $0.78+0.2\,mV^{\circ}C^{-1}$ (N=9). Fig. 7B demonstrates the distribution of membrane potentials recorded at constant temperatures of 20°C and 34°C. The electrode potential was checked outside the cell before and after recording. The mean membrane potential of the recorded MNSCs was -19.4 ± 8.1mV at 20°C (N=34) and -30.8 ± 10.3mV at 34°C (N=24). The change of potential with temperature calculated from the difference between these values is $0.8\,mV^{\circ}C^{-1}$. We found similar results using intracellular electrodes filled with $3\,mol\,l^{-1}$ KCl and with a resistance of 10 MΩ.

In Fig. 8, a typical whole-cell voltage-clamp recording shows the inward and outward

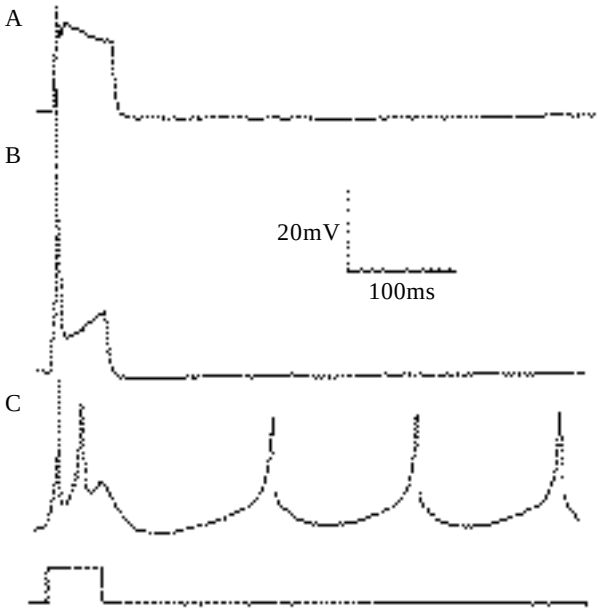

Fig. 5. Whole-cell current-clamp recordings showing typical spike forms from MNSCs isolated from adult *Locusta migratoria* and recorded between days 3 and 7 in culture. (A) A single low-amplitude spike. (B) A single large-amplitude spike. (C) Two medium-amplitude spikes and a continuous discharge. The membrane potential was in all cases set to −30mV ('resting' membrane potentials in A and B were about −30mV, in C about −40mV). Stimulation was with a depolarizing current pulse (indicated below the traces) of approximately 0.5nA and 50ms duration.

currents elicited in an MNSC producing high-amplitude action potentials at two temperatures (20˚C and 34˚C). The holding potential was −60mV and the membrane potential was set to between −40mV and +30mV for 100ms in 10mV voltage steps. The time course of the currents was clearly slower at the lower temperature.

Discussion

Medial neurosecretory cells of the pars intercerebralis of larval and adult *Locusta migratoria* can be isolated from the supraoesophageal ganglion purely by mechanical dissociation and without treatment with enzymes. Depending on the efficiency of the dissociation process, the resulting population of isolated cells is relatively numerous. The culture medium described by Howes *et al.* (1991) for *in vitro* growth of differentiated neurones from the thoracic ganglia of *Periplaneta americana* also proved to be suitable for larval and adult locust MNSCs. MNSCs prepared in this manner are well-suited for patch-clamp recordings. When established, the culture system facilitates investigations on the electrophysiological properties of isolated neurosecretory cells.

In contrast to MNSCs *in situ* (Mason, 1973), many cells *in vitro* become multipolar. As has been reported by Kirchhof and Bicker (1992) for neurones isolated from meso- and

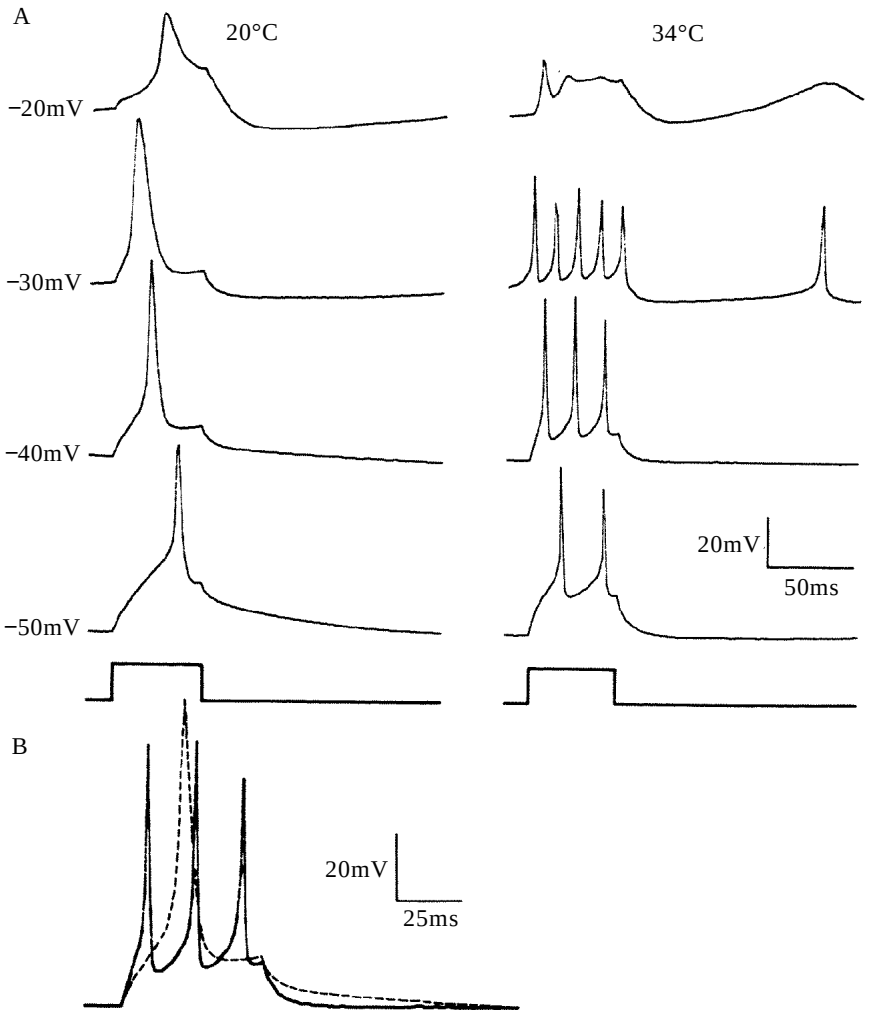

Fig. 6. Temperature-dependence of spike activity and spike variables of MNSCs. (A) Example of whole-cell current-clamp recordings of a single MNSC at 20˚C (left-hand side) and 34˚C (right-hand side). The 'resting' membrane potential of the cell was −40mV. The membrane potential was set to potentials between −50 and −20mV in steps of 10mV and stimulated with a depolarizing current pulse of 0.5nA with a duration of 50ms (indicated below A). (B) Enlarged and superimposed responses of the cell starting from the 'resting' membrane potential (−40mV) at 20˚C (dashed line) and 34˚C. Stimulation as in A.

metathoracic ganglia of *Locusta migratoria*, the presence of a primary neurite stump influenced the morphology of the cells in culture. We found that the percentage of cultured MNSCs showing outgrowth was significantly higher in third-instar larvae than in fourth and fifth instars and adults. Outgrowth and survival were promoted by incubation with CO_2 and also when large groups of MNSCs were grown together with cells of probable glial origin. In contrast to our present findings in *Locusta migratoria*, Seshan *et al.* (1974) demonstrated that significant outgrowth from groups of MNSCs from

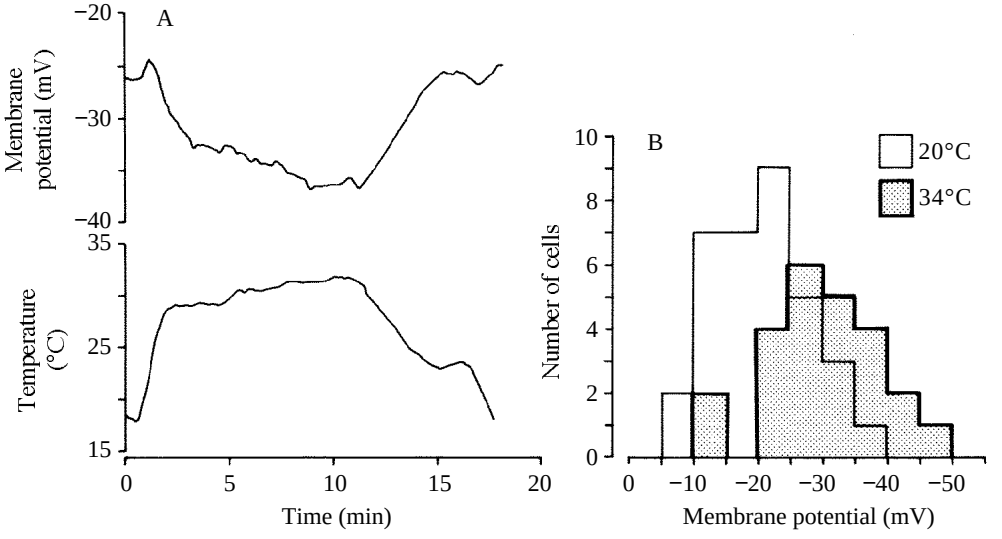

Fig. 7. Change in the membrane potential of MNSCs with a change in the temperature. (A) The membrane potential of MNSCs hyperpolarizes on heating and depolarizes on cooling. As an example, a typical membrane potential of one MNSC over a period of about 20min is shown in the upper trace; the temperature is shown in the lower trace. (B) Histogram of the distribution of membrane potentials recorded from MNSCs at constant temperatures of 20˚C (open columns, *N*=34) and 34˚C (shaded columns, *N*=24) (class width is 5mV).

cockroaches was only achieved when MNSCs were co-cultured with embryonic nervous tissue, embryonic foregut explants or ovarian follicles. Kirchhof and Bicker (1992) also reported that, in cultured neurones from adult *Schistocerca gregaria*, less outgrowth occurred than for *Locusta migratoria* neurones and that growth was promoted by a haemolymph-conditioned medium, as previously described by Howes *et al.* (1991). In preliminary experiments, we found that haemolymph-conditioning of the medium using the method described by Howes *et al.* (1991) also seemed to promote growth of MNSCs isolated from adult locusts.

The electrophysiological results obtained from cultured MNSCs are in several ways consistent with reported findings from *in situ* recordings of insect MNSCs. After an initial phase of 3 days in culture, a considerable number of MNSCs produced action potentials (amplitudes between 10 and 80mV) with a typically long duration (3–4ms at 34˚C, up to 12ms at 20˚C) and some of the cells started to discharge continuously when the membrane potential was set to −30mV. ‘Resting’ membrane potentials between −15mV and −50mV were most frequent in our recordings. Cook and Milligan (1972) found spontaneous spiking activity in more than 50% of the MNSCs recorded *in situ* in *Periplaneta americana* (intracellular recordings from the soma) and reported ‘resting’ membrane potentials between −12mV and about −50mV (when the neuronal sheath potential was substracted). ‘Resting’ membrane potentials between −20mV and −50mV and spike amplitudes of about 60mV are mentioned by Raabe (1983). Action potentials with a relatively long duration and slow rise time are characteristic of neurosecretory cells (3–6ms and more compared with 0.6–2.5ms for other neurones)

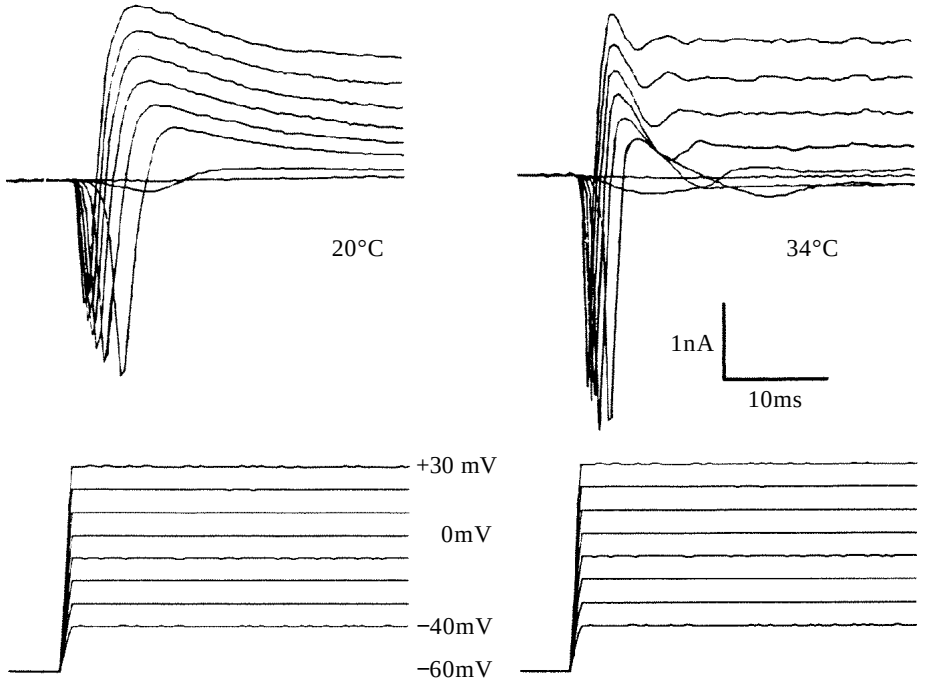

Fig. 8. Whole-cell voltage-clamp recordings showing inward and outward currents of an MNSC at 20˚C (left-hand side) and 34˚C (right-hand side). Holding potential was −60mV; the membrane potential was set to potentials between −40mV and +30mV for 100ms in 10mV voltage steps (indicated below); stimulation was at 1Hz.

(Gosbee *et al.* 1968; Cook and Milligan, 1972; Normann, 1973; Rowell, 1976; Raabe, 1983). Seshan *et al.* (1974) also observed long spike durations (2–7ms at a temperature of 25˚C) in extracellular recordings from large groups of cultured MNSCs from cockroaches; however, attempts to record from isolated MNSCs *in vitro* have been unsuccessful.

The electrophysiological results indicate that MNSCs from the pars intercerebralis of *Locusta migratoria* are rather heterogeneous with respect to membrane potential and excitability. This seems also to be the case for MNSCs of *Periplaneta americana* as shown in *in situ* recordings from the somata (Cook and Milligan, 1972). It may be that our pipette solution (I) containing calcium and EGTA was not appropriate for buffering intracellular calcium to an optimal level in all recordings. This is especially important in the context of the investigations of Pitman (1979) and Thomas (1984), who demonstrated that the properties of some insect neurones are very dependent upon intracellular calcium concentration. Chelation of intracellular calcium by injection of citrate or EGTA can enable somata that are normally passive to generate calcium-dependent action potentials. Experiments are in progress to investigate in detail the ionic current components of the action potentials of locust MNSCs.

The influence of temperature on electrophysiological properties was also investigated in locust motoneurones by Heitler *et al.* (1977). The reported effects of temperature on

spike variables and membrane potential are consistent with our results. Heitler *et al.* (1977) calculated slopes of potential *versus* temperature of between $0.46 mV^{\circ}C^{-1}$ and $0.57 mV^{\circ}C^{-1}$, depending on the type of neurone. In locust MNSCs, we measured a mean value of $0.78 \pm 0.2 mV^{\circ}C^{-1}$ when the temperature was shifted while recording from single MNSCs. The mean value calculated from two samples of membrane potentials recorded at constant temperatures ($0.8 mV^{\circ}C^{-1}$) corresponds well with the value found in single recordings. However, the large spread of 'resting' membrane potentials may indicate that the cells cannot be regarded as a single population and the calculation of a mean value from such a sample may, therefore, be inappropriate. The effects on the spike variables and the membrane potential indicate that an increase in temperature mediates an increase in the excitability of the MNSCs. In cells showing spiking activity at low temperature, the spike number is increased with increasing temperature (Fig. 6). The spread of 'resting' membrane potentials in different cells may play an important role in this context. MNSCs that have a very low 'resting' membrane potential ($-10 mV$ to $-20 mV$) and that are non-excitable at low temperatures may become excitable at higher temperatures. At low membrane potentials, all fast inward currents become inactivated. Complete inactivation occurs between $-15 mV$ and $-10 mV$. An increase in temperature of $15^{\circ}C$ causes an increase in the membrane potential of $-12 mV$, which would be high enough to activate the fast inward currents. The total number of cells in the pars intercerebralis capable of generating action potentials may therefore be significantly increased by raising the temperature.

Since the release of neurohormones is elicited by spike activity of neurosecretory cells, the dependence of the electrophysiological properties of MNSCs on temperature is especially important for poikilotherms such as insects. Temperature and photoperiod are known to have a decisive role in insect growth and reproduction, two processes that are triggered by the activity of neurosecretory cells (Lees, 1963, 1964). Lees (1964) has suggested that neurosecretory cells in the pars intercerebralis may be implicated as both photoperiodic receptors and humoral effectors. Cook and Milligan (1972) demonstrated that light affects both the 'resting' and spike potentials of MNSCs of cockroaches, suggesting that MNSCs receive synaptic inputs. However, the effects of light may also be enhanced by transformation of light into heat and the change in temperature might contribute to the change in activity of neurosecretory cells. The pars intercerebralis and neighbouring regions in the supraoesophageal ganglion of larval and adult *Locusta migratoria* are extensively covered by a red-brown pigment which might play a role in this context. Our results indicate that, in addition to synaptic inputs of sensory information, the direct influence of temperature on the electrophysiological properties of MNSCs may contribute to changes in the release of neurohormones with changes in temperature.

We thank Professor Dr K. Kalmring and Professor Dr H. Wiegand for supporting this work. We are grateful to Professor Dr G. Heldmaier for the use of his cell culture laboratory, to D. Encke for help with the cell culture technique and to Mrs E. Schäfer and L. Koschubat for technical help. Two anonymous referees improved the paper with their

constructive criticism. This work was supported by the DFG, Sonderforschungsbereich Ökophysiologie 305, A7.

References

BEADLE, D. J. AND HICKS, D. (1985). Insect nerve culture. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 5 (ed. G. A. Kerkut and L. I. Gilbert), pp. 213–298. Oxford: Pergamon Press.

CHEN, J. S. AND LEVI-MONTALCINI, R. (1969). Axonal outgrowth and cell migration *in vitro* from nervous system of cockroach embryos. *Science* **166**, 631–632.

COOK, D. J. AND MILLIGAN, J. V. (1972). Electrophysiology and histology of the medial neurosecretory cells in adult male cockroaches, *Periplaneta americana. J. Insect Physiol.* **18**, 1197–1214.

GIRARDIE, A. (1966). Contrôle de l'activité génitale chez *Locusta migratoria*. Mise en évidence d'un facteur gonadotrope et d'un facteur allatotrope dans la pars intercerebralis. *Bull. Soc. zool. Fr*. **91**, 423–439.

GIRARDIE, A. AND GIRARDIE, J. (1967). Etude histologique, histochimique et ultrastructurale de la pars intercerebralis chez *Locusta migratoria* L. *(Orthoptere). Z. Zellforsch. mikrosk. Anat.* **78**, 54–75.

GOLDSWORTHY, G. J.(1969). Hyperglycaemic factors from the corpus cardiacum of *Locusta migratoria. J. Insect Physiol.* **15**, 2131–2140.

GOLDSWORTHY, G. J., LEE, S. S. AND JUTSUM, A. R.(1977). Cerebral neurosecretory cells and flight in the locust. *J. Insect Physiol.* **23**, 717–721.

GOSBEE, J. L., MILLIGAN, J. V. AND SMALLMAN, B. N. (1968). Neural properties of the protocerebral neurosecretory cells of the adult cockroach *Periplaneta americana. J. Insect Physiol.* **14**, 1785–1792.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMAN, B. AND SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch*. **391**, 85–100.

HEITLER, W. J., GOODMAN, C. S. AND ROWELL, C. H. F. (1977). The effects of temperature on the threshold of identified neurons in the locust. *J. comp. Physiol.* **117**, 163–182.

HOWES, E. A., CHEEK, T. R. AND SMITH, P. J. S. (1991). Long-term growth *in vitro* of isolated, fully differentiated neurones from the central nervous system of an adult insect. *J. exp. Biol.* **156**, 591–605.

KIRCHHOF, B. AND BICKER, G. (1992). Growth properties of larval and adult locust neurons in primary cell culture. *J. comp. Neurol.* **323**, 411–422.

KOPEC, S.(1917). Experiments on metamorphosis in insects. *Bull. Acad. Sci. Cracovie* **3**, 57–60.

LEES, A. D.(1963). The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton. III. Further observations on the maternal switching mechanism in apterous aphids. *J. Insect Physiol.* **9**, 153–164.

LEES, A. D.(1964). The location of photoperiodic receptors in the aphid *Megoura viciae* Buckton. *J. exp. Biol.* **41**, 119–133.

MADDRELL, S. H. P. AND GEE, J. D. (1974). Potassium-induced release of the diuretic hormones of *Rhodnius prolixus* and *Glossina austeni*: Ca dependence, time course and localization of neurohaemal areas. *J. exp. Biol.* **61**, 155–171.

MASON, C. A. (1973). New features of the brain–retrocerebral complex of the locust *Schistocerca vaga* (Scudder). *Z. Zellforsch. mikrosk. Anat.* **141**, 19–32.

NORMANN, T. C. (1973). Membrane potential of the corpus cardiacum neurosecretory cells of the blowfly, *Calliphora erythrocephala. J. Insect Physiol.* **19**, 303–318.

PITMAN, R. M. (1979). Intracellular citrate or externally applied tetraethylammonium ions produce calcium-dependent action potentials in an insect motoneurone cell body. *J. Physiol., Lond.* **291**, 327–337.

RAABE, M. (1983). The neurosecretory–neurohaemal system of insects; anatomical, structural and physiological data. In *Advances in Insect Physiology*, vol. 17 (ed. M. J. Berridge, J. E. Treherne and V. B. Wigglesworth), pp. 205–303. London: Academic Press.

ROWELL, H. F. (1976). The cells of the insect neurosecretory system: constancy, variability and the concept of the unique identifiable neuron. In *Advances in Insect Physiology*, vol. 12 (ed. J. E. Treherne, M. J. Berridge and V. B. Wigglesworth), pp. 63–123. London: Academic Press.

SESHAN, K. R., PROVINE, R. R. AND LEVI-MONTALCINI, R. (1974). Structural and electrophysiological properties of nymphal and adult insect medial neurosecretory cells: an *in vitro* analysis. *Brain Res.* **78**, 359–376.

THOMAS, M. V. (1984). Voltage-clamp analysis of a calcium-mediated potassium conductance in cockroach (*Periplaneta americana*) central neurones. *J. Physiol., Lond.* **350**, 159–178.

THOMSEN, E. AND THOMSEN, M. (1954). Dark field microscopy of living neurosecretory cells. *Experientia* **10**, 206.

WIGGLESWORTH, V. B. (1940). The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera). *J. exp. Biol.* **17**, 201–222.

WIGGLESWORTH, V. B. (1964). The hormonal regulation of growth and reproduction in insects. In *Advances in Insect Physiology*, vol. 2 (ed. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth), pp. 247–335. London: Academic Press.