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THERMAL ACCLIMATION OF A CENTRAL NEURONE OF *HELIX ASPERSA*

II. ELECTROPHYSIOLOGICAL RECORDINGS

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SUMMARY

1. The effects of thermal acclimation on the activity of a central autoactive neurone and its temperature dependence were investigated in *Helix aspersa*.

2. Resting membrane potential was changed by acclimation temperature, but not with a simple relationship: cells from both 30 °C-acclimated and 4 °C-acclimated groups were more depolarized at 20 °C than were control cells (acclimated to 19 °C).

3. The input resistance of the neurone decreased as the temperature of acclimation was raised.

4. Rates of change of potential during an action potential decreased with increasing acclimation temperature. Raising the temperature of measurement on the other hand increased dV/dt at a given acclimation temperature. Spike amplitude was little affected.

5. The frequency of spontaneous spike discharge at a given temperature declined with increasing temperature of acclimation but increased with step changes of temperature in a manner suggestive of a compensatory process.

6. All the measured electrical parameters showed a pronounced hysteresis during rewarming after cold block.

7. Upper lethal and cold block temperatures were both significantly raised by acclimation to higher temperatures. Block temperature was much reduced in cold-acclimated individuals, but the upper lethal temperature was less affected.

8. By sampling during the acclimation period of 4 weeks, the above changes were shown to occur progressively. During the initial stages (5–12 days) they could be partially reversed by incubating isolated ganglia at various temperatures for 30–45 min: but after 2 weeks or more the changes could not be reversed by incubations of up to 5 h.

INTRODUCTION

The basic mechanisms underlying long-term temperature acclimation in ectotherms are not well defined (Hazel & Prosser, 1974). Molluscs provide ideal subjects for investigations of the electrophysiological correlates of thermal acclimation, due to the large size of central neurones and the wealth of literature on the electrophysiology of these cells: furthermore, the available studies of the haemolymph of molluscs permit

a more comprehensive picture to be constructed of the acclimation process. In recent years electrophysiological research upon temperature adaptation of neuronal membranes has been largely directed at short-term adaptation (Airapetyan, 1969*a*, *b*; Carpenter, 1967, 1970; Chalazonitis, 1961; Chalazonitis, Romey & Arvanitaki, 1967; Gorman & Marmor, 1970; Kerkut & Ridge, 1962; Marchiafava, 1970; Magura, Valeyev & Zamekhovsky, 1975; Marmor, 1971) although there has been a notable study of long-term adaptation in *Helisoma trivolvis* (Merickel & Kater, 1974).

In the preceding paper (Langley, 1979*a*) it was shown that thermal acclimation of the snail *Helix aspersa* is accompanied by changes in the composition of the haemolymph, most especially in Na and K concentrations. Neuronal excitability is vitally dependent upon the ionic gradients across the cell membrane, and it is, therefore, conceivable that the charges in the chemistry of the haemolymph could be involved in the thermal adaptation of the neuronal membrane.

In the present study, recordings were made from a large, identifiable autoactive neurone of the parietal ganglia – the F1 cell of Kerkut *et al.* (1975). This cell has been implicated by previous workers in neurosecretion, osmoregulation and possibly hibernation. Chronic and acute aspects and the temporal stability of thermal adaptation of this neurone were investigated.

MATERIALS AND METHODS

Experimental animals

Adult specimens of *Helix* were kept under four acclimation regimes, as previously described (Langley, 1979*a*), with control animals being maintained at 19 °C and the others being acclimated for 4 weeks at 4°, 12° or 30 °C. Dissection and preparation of the F1 cell (see Fig. 1) was carried out according to the technique of Walker (1968), usually at 20 °C.

Ringer solutions

The experiments reported in this paper were carried out using Ringer solutions of the following composition: NaCl, 80 mM; KCl, 4 mM; $CaCl_2$, 7 mM; $MgCl_2$, 5 mM; Hepes 5 mM. Hepes was chosen for its lack of effect on thermoacclimated neurones (Langley, 1979*b*). The pH of all solutions was adjusted with Hepes to 7·5 at 20 °C.

Electrophysiological recording

Conventional single-barrelled microelectrodes were used and filled with either 1·5 M potassium acetate or 3 M potassium chloride. Tip potentials (less than 2 mV) and resistance (1–10 MΩ) had only a small temperature sensitivity in the 0°–30°C range. Signals were fed into a 4 channel Telequipment DM63 storage oscilloscope via a microelectrode amplifier with provision for current injection. Permanent records were obtained either with a Polaroid camera or Servoscribe pen-recorder.

Temperature control device

Preparations were maintained in a constantly flowing Ringer solution with continuous aeration.

The temperature control device utilized a Pelcool MSE Cryostat stage with a

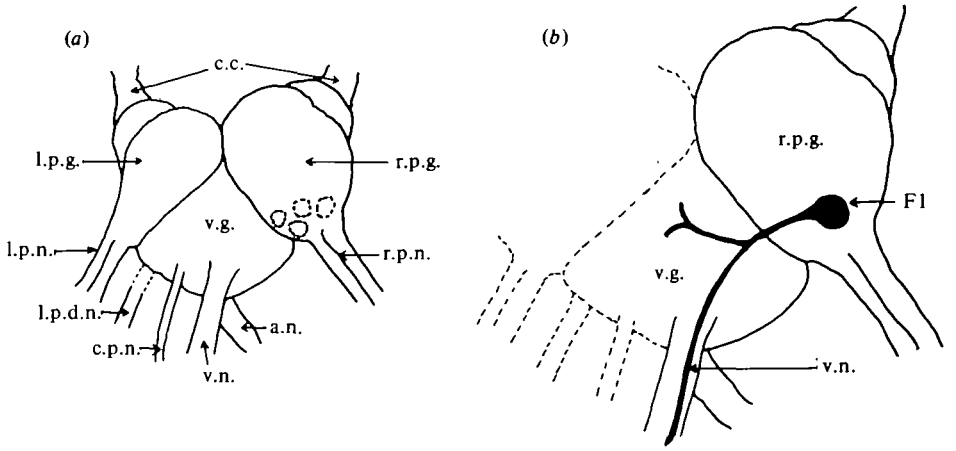

Fig. 1. Adapted from Kerkut *et al.* (1970). (*a*). Diagram to show the various positions of the F1 cell (dotted outline) encountered in both control and acclimated animals. (*b*). The course of the nerve processes running from cell F1 through the visceral nerve (v.n.) and entering the visceral ganglia (v.g.). Other abbreviations: c.c., circumoesophageal connectives; l.p.g., left parietal ganglion; r.p.g., right parietal ganglion; l.p.n., left pallial nerve; r.p.m., right pallial nerve; l.pd. n., left pedal nerve; c.p.n., cutaneous pallial nerve; a.n., anal nerve.

thermister-controlled regulator unit. The temperature of the bathing solution was monitored using a thermistor probe linked to a Philips GM 6020 voltmeter.

RESULTS

(A) *Effects of thermal acclimation, measured at 20 °C*

F1 cells from control (19 °C) animals typically spiked with a regular beat of 1·9–2·1 Hz at 20 °C ('bursting' was never encountered in starved snails from the four acclimation regimes).

(i) *Resting potential* (R.P.) *of the F1 cells*

Compared to the resting potential of control snails (Table 1) the R.P. was depolarized in the warm-acclimated (30 °C) snails, unaffected in the cold-acclimated groups (Table 1). As noted above, dissection was usually at 20 °C. In some experiments the cell was kept at the acclimation temperature until the recordings were made, but the R.P. was no different.

(ii) *Action potential* (A.P.)

The magnitude of the A.P. did not significantly vary between the four groups (Table 1). However, the rise and fall times of the spike were inversely proportional to acclimation temperature, with the fall time being most affected (Table 1).

F1, like most autoactive cells, had small after-potentials, their magnitude being affected by acclimation temperature, such that there was a maximum at 19 °C with a decrease at higher and lower temperatures (Table 1).

Spike duration, when measured at 20 °C, showed an increase with a decrease in the temperature of acclimation (Table 1).

Table 1

Test condn.	Rest. Pot.[1] (mV)	Spike amplitude (mV)	Overshoot (mV)	Spike duration (ms)	Time to Peak (ms)	Input resist. (MΩ)	Positive after potential (mV)	* Rate rise spike (V/S)	* Rate fall spike (V/S)	Freq. spikes per second	Block spont. activity (°C)	Upper lethal Temp. (°C)
Warm acclimated (30 °C)	34·20 ± 0·4	78·8 ± 1·8	22·5 ± 2	11·4 ± 2	10 ± 2	0·856 ± 0·03	8·86 ± 2·4	10·2 ± 1·5	1·86 ± 0·2	1·8 ± 0·2	16 ± 1·8	33 ± 1·9
Control (19 °C)	47·94 ± 3·3	80·2 ± 4·3	32·5 ± 2·5	5·71 ± 1·2	14·3 ± 1·72	3·95 ± 1·05	11·2 ± 3·2	19·5 ± 1·95	9·35 ± 1·4	2·07 ± 0·09	6 ± 1	26 ± 1·0
Cold acclimated (12 °C)	46·5 ± 3·8	78·75 ± 1·5	30·5 ± 2·5	57 ± 5	17 ± 1·52	5·35 ± 0·98	5·71 ± 1·12	28·65 ± 3·4	8·75 ± 1·8	2·2 ± 0·2	4 ± 1·5	24 ± 1·2
Cold acclimated (4 °C)	38·19 ± 0·4	75·5 ± 2·0	35·5 ± 1·5	45 ± 4·2	12·6 ± 1·06	7·06 ± 0·9	4·3 ± 1·85	36·5 ± 1·8	14·10 ± 2	2·9 ± 0·5	−2	22 ± 1·4

Mean of data with S.E. All the above data collected in both morning and afternoon sessions, and at bath temperatures of 20 °C ± 0·5 °C, except for the columns marked • which were collected at a bath temperature of 18° ± 0·5 °C. The differences between the amplitude of the spikes from the four regimes were found to be not significantly different ($P < 0{\cdot}001$); however, F tests carried out on the other spike parameters indicated that there were marked differences between the four acclimation regimes ($P < 0{\cdot}002$). Sample size averaged 40 for each of the four groups of animals.

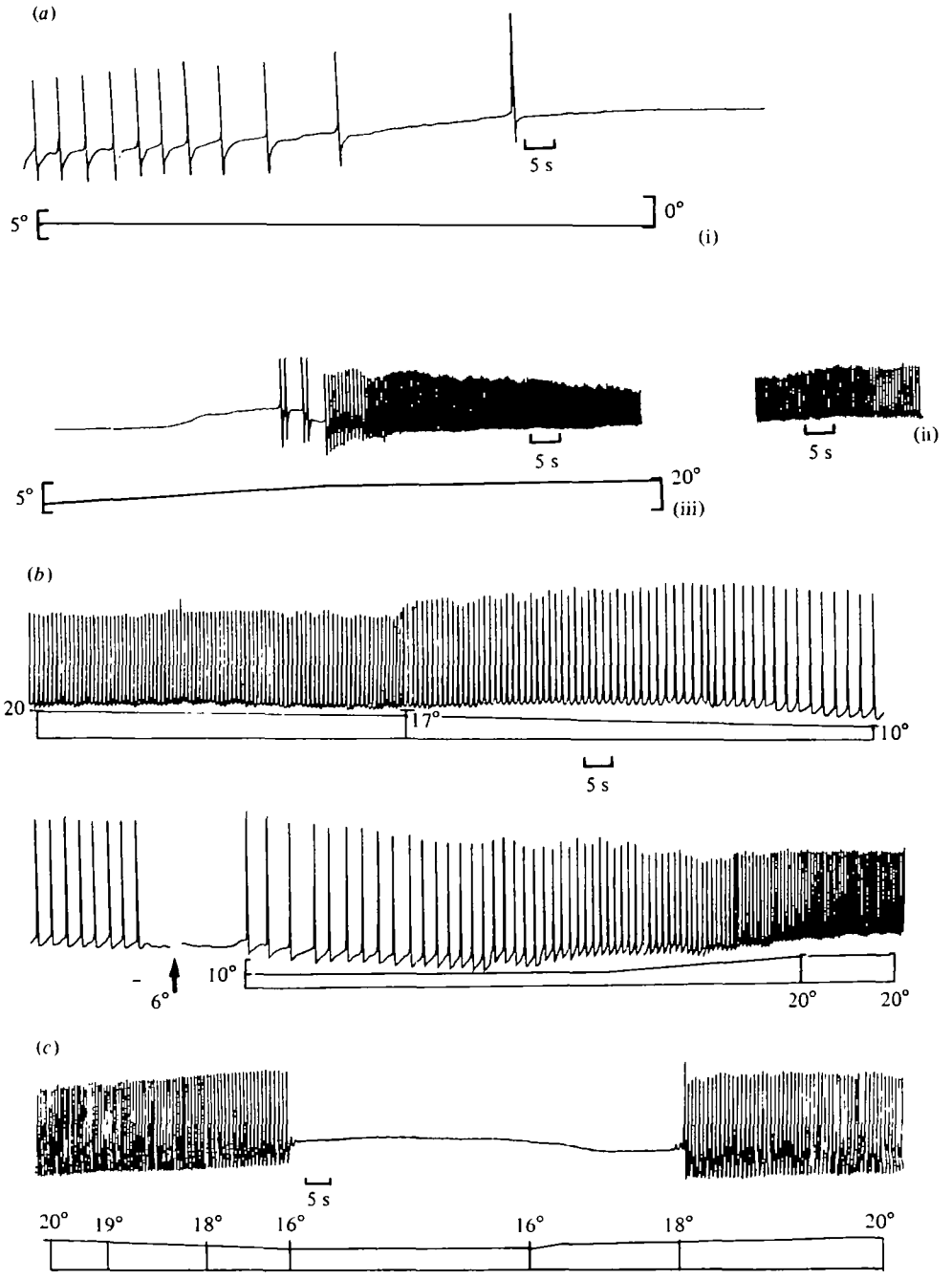

Fig. 2. (*a*). A typical recording from the F1 cell of a cold (4 °C)-acclimated specimen. The record is continuous from (i) through to (iii). (ii) is the record from the same cell at 20 °C 10 min after impalement and prior to the test of temperature sensitivity. The apparent increase in the size of the action potential with cooling is an artifact; since with a drop in temperature the spike becomes slower, and more of its deflexion is traced by the relatively slow pen-recorder. This feature applies to all the pen-recordings illustrated. (*b*) A typical recording from the F1 cell of a cold (12 °C)-acclimated specimen. The arrow indicates where the cell ceased firing spontaneously. The record is continuous. (*c*) A typical recording from the F1 cell of a warm-acclimated specimen. The lower trace in each recording is the output from the temperature sensing device.

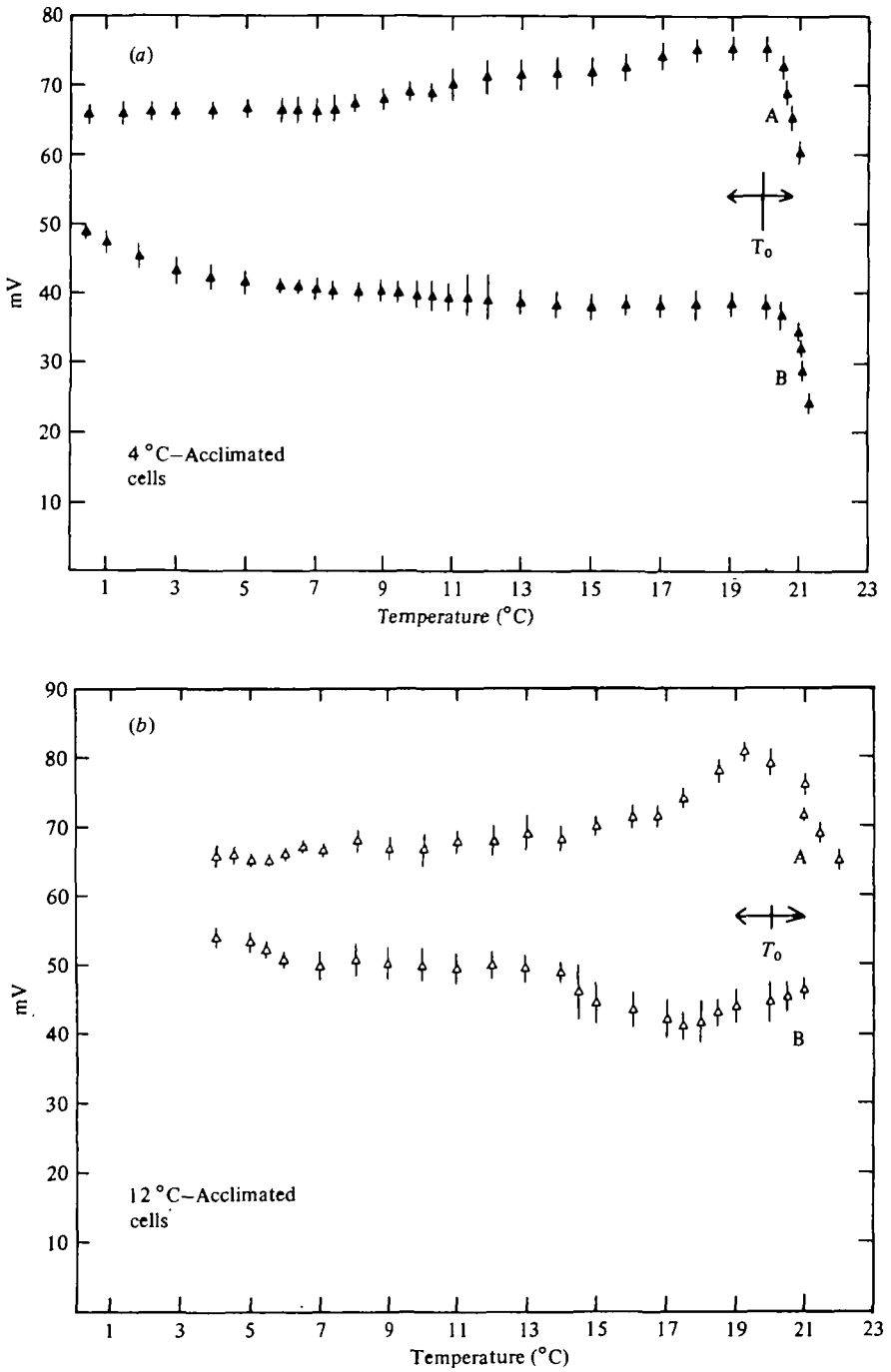

Fig. 3 (*a* and *b*). For caption see opposite.

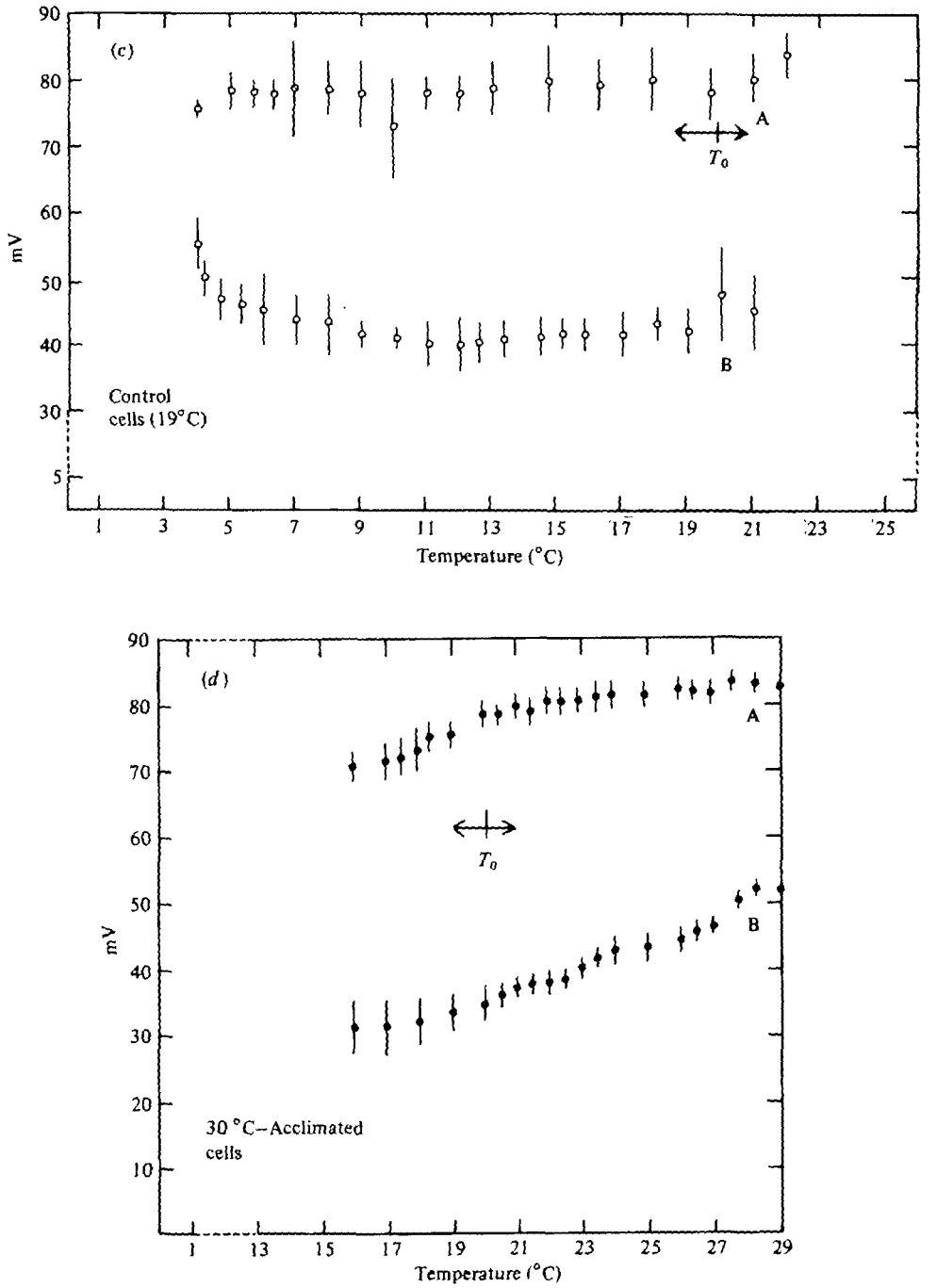

Fig. 3(*a–d*). The effect of temperature on the magnitude of action potential (A) and resting potential (B), vertical bars indicate ± standard errors for the four groups of snails. Arrows indicate the direction in which the temperature was changed (see text). T_0, temperature at which experiment commenced. Note the compression of axes in Figs. 3(*a*) and (*d*).

No observable accommodation of the various spike parameters occurred if the ganglia were maintained for up to 2 h in Ringer solution at temperatures other than those of acclimation.

At least 3 weeks acclimation was required before spike parameters were affected. This proved to be the same time as that required to change block temperature (see below).

(iii) *Frequency of autonomous spike discharge*

Autonomous spike production showed marked acclimation alteration (Fig. 2*a–c*). These changes were not modifiable by incubation in normal Ringer at 20 °C for up to 2 h.

(B) *Effects of thermal acclimation on the lower and upper limits of F1 cell function*

Table 1 shows the effect of acclimation upon block temperature. (In this paper, block temperature refers to the cold-induced cessation of spontaneous activity – the cells were still excitable, as indicated by the injection of short pulses of depolarizing current.) The temperature was lowered by 5 °C/min; upon rewarming the preparations at the same rate there was a marked hysteresis in spike production. The events preceding the cessation of spontaneous activity showed variations between the acclimated groups. In F1 cells from warm-acclimated individuals the cessation was abrupt (Figs. 2*c*, 5), with a steep change in input resistance, whilst in the two cold groups cold-block was preceded by a gradual change in frequency, even with steep changes of temperature, and involved a slower increase in input resistance (Figs. 2*a*, *b*, 5).

The apparent upper-lethal temperature (Table 1) also showed a significant modification as a result of thermal acclimation, ranging from 33 °C in warm-acclimated snails to only 22 °C in the 4 °C-acclimated group. Since the lethal limit depended on both the temperatures recently presented to the cell, and the duration of the exposure to these temperatures, the warming procedure was made as comparable between groups as possible by a gradual approach to the lethal limit.

(C) *Effects of thermal acclimation on the temperature dependence of F1 cell function*

To investigate the acute effects of thermal change on the F1 cells of the four groups, first, recordings were made as temperature was stepped progressively down from 20 °C, and then, after return to 20 °C and the recovery of original data values, recordings were made as temperature was progressively stepped up. Each recording was made after temperature had been held constant for up to 5 min. (The step changes were either completed in 10 s or made at a rate of 10 °C/20–35 s, but no differences were observed between the effects produced with the different rates.)

(i) *Resting potential* (R.P.) *of F1*

In all groups there was a depolarization of resting potential as temperature was raised from a few degrees below the lethal limit to the limit. Fig. 3 shows the effects of temperature upon R.P. but because of the temperature employed, the above depolarization can only be seen for the 4 °C group (Fig. 3*a*). In cold-acclimated and control snails, there was also a hyperpolarization as temperature was brought below

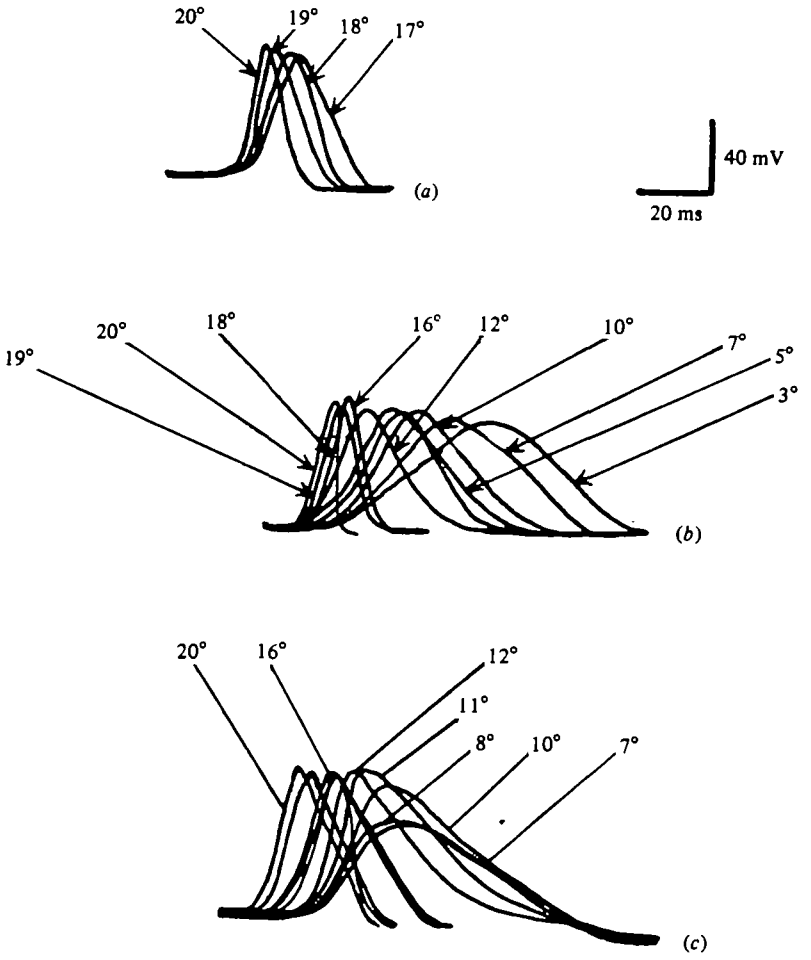

Fig. 4. Typical changes in the shape of the action potential with temperature for (*a*) 30 °C-acclimated, (*b*) 4 °C-acclimated, and (*c*) 12 °C-acclimated animals.

6 °C (Fig. 3*a–c*). In the warm-acclimated group, there was a depolarization with increasing temperature in the range 16°–30 °C.

(ii) *Action potential* (A.P.) *of* F_1

A.P. amplitude was not observed to be affected by temperature in the control group (Fig. 4*c*). In all other groups, however, there was a decrease in amplitude as temperature was lowered from 20 °C. In the 4° and 12° groups there was also a fall as temperature was raised above this point.

Rise and fall times of spikes (especially fall times) showed an inverse relationship to temperature in all four acclimation groups, the effect on the fall phase being most marked in the 30 °C-acclimated group. The effect of these changes was to lengthen the spike as temperature was decreased, with a greater effect in the 4° and 12 °C groups than the other two.

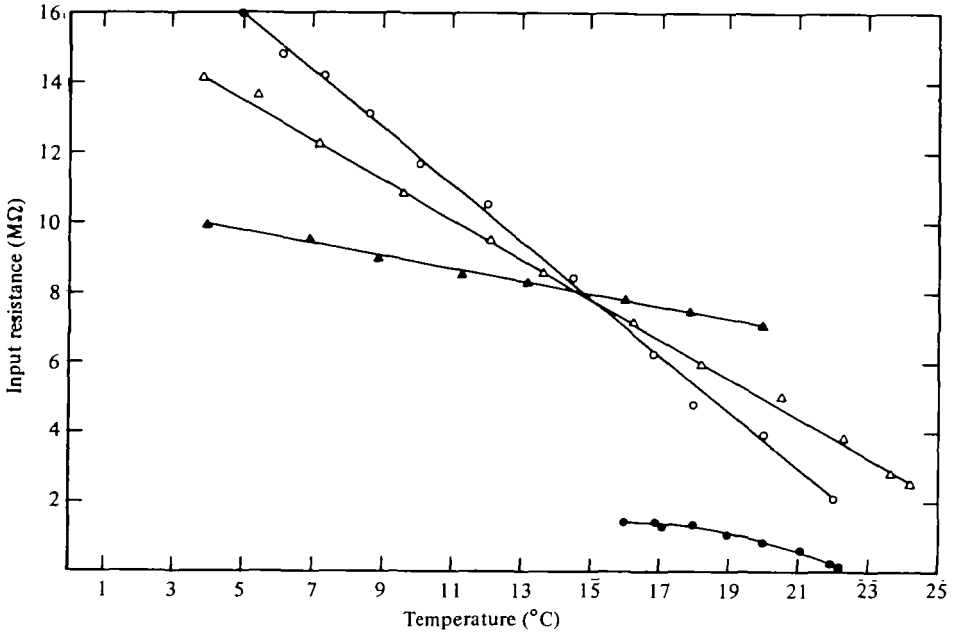

Fig. 5. The effect of temperature upon input resistance. The value of the input resistance was checked ten minutes after impalement and again after all the various temperature changes were completed, and these two values compared. Providing the upper lethal limit for each cell type was not exceeded, the value before and after treatments was found to be almost identical. Standard errors do not show at this scale, being 'hidden' by the data symbols. ▲, 4 °C-acclimated snails; ○, control 19 °C; △, 12 °C-acclimated snails; ●, 30 °C-acclimated snails.

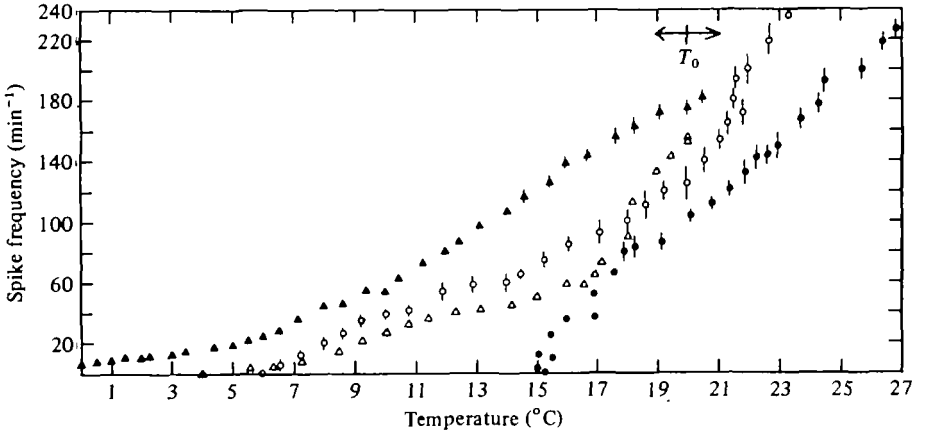

Fig. 6. The effect of temperature on the rate of spike discharge. The arrows indicate the direction of temperature change (see text). ○, Control cells; △, cold, 12 °C-acclimated cells; ●, warm (30 °C)-acclimated cells; ▲, cold 4 °C-acclimated cells.

(iii) *Input resistance of F1*

The input resistance was inversely proportional to temperature in the 4°, 12°, and 19 °C groups, with higher acclimation temperature resulting in a greater sensitivity (Fig. 5). In the 30 °C group, input resistance did not vary much with temperature (Fig. 5).

Such variation in input resistance cannot be attributed to differences in the shape of the F1 cell, since the diameter of cells in the warm- and cold-acclimated groups fell within the 175–200 μm range obtaining in the control group.

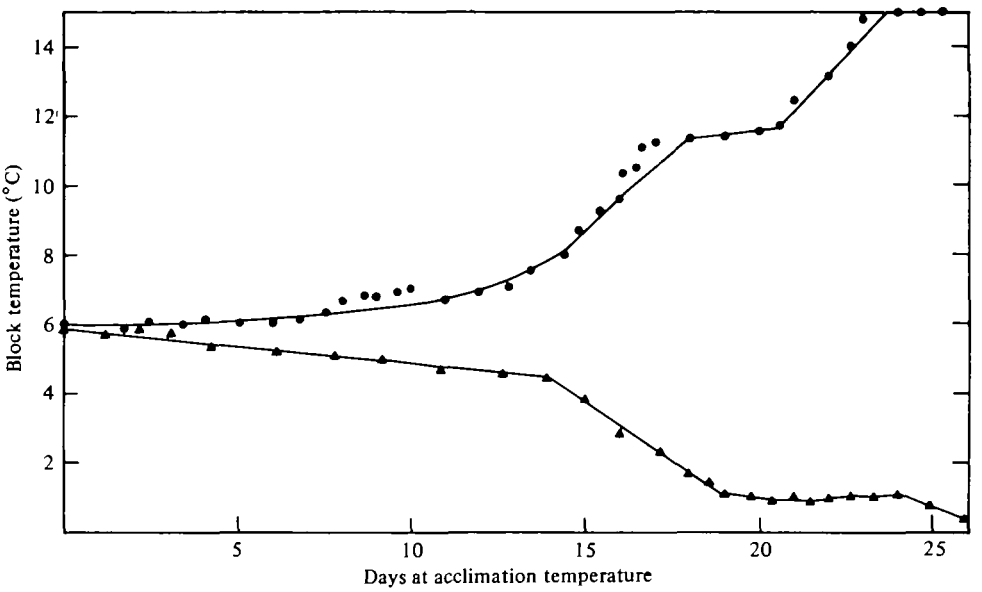

Fig. 7. The time required for the acclimation process in 30° and 4 °C-acclimated snails as evinced by the cold-block temperature. The graph for 12 °C-acclimated group was almost identical to that for the 4 °C group. Each point represents the mean result of at least five experiments where the animals were randomly removed, throughout the year, from the constant temperature rooms and tested for alteration of the block temperature from control values.

(iv) *Frequency of spontaneous F1 spikes*

Fig. 6 shows the relationship between temperature and spontaneous spike frequency for all four groups. In general, the data showed that the lower the acclimation temperature, the lower was the temperature at which a given frequency was observed: acclimation had led to a compensatory change.

(D) *Temporal stability of neuronal acclimation*

The relationship between ambient temperature and the electrical parameters of the F1 cell discussed in Sections A and B could not be modified by incubation of the ganglia at 30 °C for periods up to 4 h.

Some idea of the time course of acclimation is given in Fig. 7 by the change in block temperature during acclimation. Before 12 days acclimation, there was little

change in block temperature, which was reversible by incubation for 30–45 min at the original temperature. After this period of time block temperature changed rapidly and became irreversible.

It was also found that ganglia from snails immediately out of hibernation (March–April) were least able to withstand elevated bath temperatures, and such animals showed higher mortality rates with increasing environmental temperatures than snails collected in mid-summer. Conversely ganglia from summer-aestivated snails showed the best survival at bath temperatures above 19 °C, and readily survived 30 °C environmental temperatures, whereas winter hibernation snails had low tolerance of high temperature.

Shorter acclimation times were required for animals collected in late summer or early autumn months, when placed at 4° or 12 °C than was the case with summer or winter-quiescent snails, as revealed by cold-block. The former, however, required longer periods at 30 °C. As can be seen in Fig. 7, a longer period was required for acclimation to higher than lower temperatures.

DISCUSSION

(i) *Resting potential*

The membrane potential of cells from all four groups varies with temperature in a fashion not predicted by the constant field equation, since if temperature affected only the physico-chemical aspects of the neuronal membrane one would expect an indirect relationship with a change of $-0{\cdot}2$ mV/°C (compare Fig. 2*a–c*). Such a thermally induced change in the electrical parameters of excitable cells has been reported previously in the giant nerve fibres of *Lumbricus terrestris* by Lagerspetz & Talo (1967), Talo & Lagerspetz (1967) and also in the crayfish CNS by Gladwell, Bowler & Duncan (1975).

In view of the data from both chronic and acute thermal change, it appears that acclimation leads to a permanent alteration in the permeability of the cell membrane. Such an irreversible permeability change induced by temperature manipulation has been elucidated before in *Helix* (Gerasimov, Yanishevskii & Skubalyanka, 1967), and in *Aplysia* (Carpenter, 1967; Chalazonitis *et al.* 1964; Marchiafava, 1970; Murray, 1966). Also, Merickel & Kater (1974) showed that the compensatory thermal acclimation of the resting potential of the lateral giant neurone of *Helisoma trivolvis* involved modification of the sodium pump.

(ii) *Action potential*

Thermal change has a more pronounced effect upon spike characteristics than resting potential, partly because there will be an indirect effect through a change in the resting potential. The effects of temperature upon spike frequency is that which is to be expected from the model of repetitive firing in a central neurone of Connor & Stevens (1971*a–c*). In this model, interspike interval is dominated by a potassium conductance, g_A, which has a marked thermal sensitivity (Magura, 1967; Magura & Zamekhovsky, 1973; Magura *et al.* 1975). The effect of acclimation could be to modify this conductance in a permanent fashion. Similarly, moderate thermal modulation of the conventional Hodgkin–Huxley g_K and g_{Na} would lead to the observed changes in

spike profile (see Magura & Zamekhovsky, 1973). Such acclimatory changes are also suggested in the present work by measurement of the input resistance (Fig. 5). The different thermal sensitivities of the rise and fall times of the spike would be explained by different thermal sensitivities of g_{Na} and g_K, as found in other systems, including eel-electroplaque (Ruiz-Manresa & Grundfest, 1976), squid giant axon (Hodgkin & Katz, 1949) and the giant axons of *Lumbricus terrestris* (Dierolf & McDonald, 1969; Dierolf & Brink, 1973).

(iii) *Frequency patterning*

The F1 cell could well be important in a number of physiological process including hibernation, osmoregulation and excretion control (by indirect comparison with analogous cells in other molluscs; Gainer, 1972*a–c*; Kerkut *et al.* 1970; Krause, 1960). It would seem likely that thermal variation will influence such processes by means of a change in the rate of release of neurosecretory material from F1, a relationship between spike frequency and neurosecretory release being well known (Cooke, 1977). Chronic and acute changes in the thermal sensitivity of F1 could permit such control to continue over a wide thermal spectrum.

The mechanisms underlying acclimation-induced alteration of ionic permeability are investigated further in the following paper (Langley, 1979*b*).

I wish to thank the M.R.C. for financial assistance.

REFERENCES

AIRAPETYAN, S. N. (1969*a*). Effect of temperature on membrane potential of giant neurons of the snail. *Biophysics* **14**, 663–665.

AIRAPETYAN, S. N. (1969*b*). Mechanism of regulation of the spontaneous activity of the giant neurons of the snail. *Biophysics* **14**, 866–782.

CARPENTER, D. O. (1967). Temperature effects on pacemaker generator, membrane potential and critical firing threshold in *Aplysia* neurons. *J. gen. Physiol.* **50**, 1469–1484.

CARPENTER, D. O. (1970). Membrane potentials produced directly by the Na^+ pump in *Aplysia* neurons *Comp. Biochem. Physiol.* **35**, 371–385.

CHALAZONITIS, N. (1961). Chemipotentials in giant nerve cells (*Aplysia fasciculata*). In *Nervous Inhibition* (ed. Ernest Florey). London: Pergamon.

CHALAZONITIS, N., ROMEY, G. & ARVANITAKI, A. (1967). Résistance de la neuromembrane en fonction de la température (neurons d'*Aplysia* et d'*Helix*) *C. r. Séanc. Soc. Biol.* **161**, 1625–1628.

CONNOR, J. A. & STEVENS, C. F. (1971*a*). Inward and outward (delayed) membrane currents in isolated neural somata under voltage-clamp. *J. Physiol., Lond.* **213**, 1–19.

CONNOR, J. A. & STEVENS, C. F. (1971*b*). Voltage clamp studies of outward membrane current in gastropod neural somata. *J. Physiol., Lond.* **213**, 21–30.

CONNOR, J. A. & STEVENS, C. F. (1971*c*). Prediction of repetitive firing behaviour from voltage clamp data on an isolated neuron somata. *J. Physiol., Lond.* **213**, 31–53.

COOKE, I. M. (1977). Electrical activity of neurosecretory terminals and control of peptide hormone release. In *Peptides in Neurobiology* (ed. H. Gainer), pp. 345–370. New York: Plenum Press.

DIEROLF, B. M. & BRINK, P. R. (1973). Effects of thermal acclimation on cable constants of the earthworm median giant axon. *Comp. Biochem. Physiol.* **44***a*, 401–406.

DIEROLF, B. M. & MCDONALD, H. S. (1969). Effects of temperature acclimation on electrical properties of earthworm giant axons. *Z. vergl. Physiol.* **62**, 284–290.

GAINER, H. (1972*a*). Patterns of protein synthesis in individual identified molluscan neurons. *Brain Res.* **39**, 369–385.

GAINER, H. (1972*b*). Effects of experimentally induced diapause on the electrophysiology and protein synthesis patterns of identified molluscan neurons. *Brain Res.* **39**, 387–402.

GAINER, H. (1972*c*). Electrophysiological behaviour of an endogenously active neurosecretory cell. *Brain Res.* **39**, 403–418.

GERASIMOV, V. D., YANISHEVSKII, L. & SKUBALYANKA, E. (1967). Rectifying properties of the membrane of the giant nerve cells in solutions of different ionic composition. *Biophysics* **12**, 111–118.

GLADWELL, R. T., BOWLER, K., & DUNCAN, C. J. (1975). Heat death in the crayfish *Austropotamobius pallipes* – ion movements and their effects on excitable tissues during heat death. *J. Thermal. Biology* **1**, 79–94.

GORMAN, A. L. F., & MARMOR, M. F. (1970). Contribution of Na^+ pump and ionic gradients to membrane potentials of molluscan neurons. *J. Physiol., Lond.* **210**, 897–917.

HAZEL, J. R. & PROSSER, C. L. (1974). Molecular mechanisms of thermal compensation in poikilotherms. *Physiol. Rev.* **54**, 620–668.

HODGKIN, A. L. & KATZ, B. (1949). The effect of temperature on the electrical activity of the giant axon of the squid. *J. Physiol. Lond.* **109**, 240–249.

KERKUT, G. A., FRENCH, M. C. & WALKER, R. J. (1970). The location of axonal pathways of identifiable neurons of *Helix aspersa* using the dye Procion yellow M4R. *Comp. Biochem. Physiol.* **32**, 681–690.

KERKUT, G. A., LAMBERT, J. D. C., GAYTON, R. J., LOKER, J. E. & WALKER, R. J. (1975). Mapping of nerve cells in the suboesophageal ganglia of *Helix aspersa*. *Comp. Biochem. Physiol.* **50A**, 1–25.

KERKUT, G. A. & RIDGE, R. M. A. (1972). Effect of temperature changes on the activity of the neurons of the snail *Helix aspersa*. *Comp. Biochem. Physiol.* **5**, 283–295.

KRAUSE, E. (1960). Untersuchungen uder die Neurosekretion in Oschuldring von *Helix pomatia*. *Zellforsch.* **51**, 748–766.

LAGERSPETZ, K. Y. H. & TALO, A. (1967). Temperature acclimation of the functional parameters of the giant nerve fibres in *Lumbricus terrestris* L. I. Conduction velocity and the duration of the rising and falling phase of action potential. *J. exp. Biol.* **47**, 471–480.

LANGLEY, C. K. (1979*a*). Thermal acclimation of a central neurone of *Helix aspersa*. I. Effects of temperature on electrolyte levels in the haemolymph. *J. exp. Biol.* **78**, 181–186.

LANGLEY, C. K. (1979*b*). Thermal acclimation of a central neurone of *Helix aspersa*. III. Ionic substitutions and related studies. *J. exp. Biol.* **78**, 201–211.

MAGURA, I. S. (1967). Quantitative evaluation of ionic currents across the membranes of the soma of the giant neurones of the pulmonate *Plamorbis conversus* during generation of an action potential. *Biophysics* **12**, 521–527.

MAGURA, I. S., VALEYEV, A. E. & ZAMEKHOVSKY, I. Z. (1975). The effect of temperature on the outward currents in the soma of molluscan neurones in voltage clamp conditions. *J. exp. Biol.* **62**, 265–275.

MAGURA, I. S. & ZAMEKHOVSKY, I. Z. (1973). Repetitive firing in molluscan giant neurons. *J. exp. Biol.* **59**, 767–780.

MARCHIAFAVA, P. L. (1970). Effect of temperature changes on membrane potential and conductance in *Aplysia* giant nerve cell. *Comp. Biochem. Physiol.* **34**, 847–852.

MARMOR, M. F. (1971). The effects of temperature and ions on the current–voltage relation and electrical characteristics of a molluscan neurone. *J. Physiol., Lond.* **218**, 573–598.

MERICKEL, M. & KATER, S. B. (1974). Neuronal change: Compensatory acclimation of the contribution of an electrogenic pump to the resting potential. *J. comp. Physiol.* **94**, 195–206.

MURRAY, R. W. (1966). Effect of temperature on the membrane properties of neurons in the visceral ganglia of *Aplysia*. *Comp. Biochem. Physiol.* **18**, 291–303.

RUIZ-MANRESA, F. & GRUNDFEST, H. (1976). Temperature dependence of the 4 ionic processes of spike electrogenesis in eel electroplaques. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3554–3557.

TALO, A. & LANGERSPETZ, K. Y. H. (1967). Temperature acclimation of the functional parameters of the giant nerve fibres in *Lumbricus terrestris* L. II. The refractory period. *J. exp. Biol.* **47**, 481–484.

WALKER, R. J. (1968). Intracellular microelectrode recording from the brain of *Helix*. In *Laboratory Experiments in Physiology and Biochemistry* (ed. G. A. Kerkut), pp. 342–347. London: Academic Press.