the length of stride. It is significant that oxygen consumption levels and stride frequencies are relatively constant in *Notomys* at speeds above 3 km per h, the speed at which the gait changes from a walk to a quadrupedal bound. Similar patterns have been obtained during bipedal locomotion in the red kangaroo, this being attributed to storage of energy in elastic elements[6]. Our data also suggest a possible large storage component but in contrast to the kangaroo, occurring during quadrupedal locomotion. The observation that oxygen consumption stabilizes during quadrupedal running has also been made in *Notomys cervinus*[7]. Although our results are qualitatively similar at low and intermediate speeds, we do not find prolonged steady state oxygen consumption levels at higher speeds in the smaller *N. alexis* and find no evidence of a further change in the rate of oxygen consumption as reported in this work.

Given the differences in resting metabolic parameters between eutherians and marsupials, are there differences in aerobic capacity or heat storage between the rodent and the marsupial? If we assume that the plateau of oxygen consumption at high speds represents the maximal oxygen uptake rate (*max* $\dot{V}O_2$), the ratio *max* $\dot{V}O_2$ to standard $\dot{V}O_2$ for *Notomys* is approximately 5.3. We cannot suggest that *Antechinomys* was running at maximal rates of oxygen uptake as the animals are known to run at greater speeds than those at which we could induce in our experiments, however, the ratio of *max* $\dot{V}O_2$ to standard $\dot{V}O_2$ at the highest measured oxygen consumption levels is 7.0 for the lighter animal and 7.1 for the heavier. It appears therefore that the scope for aerobic metabolism is not lower in the marsupial species. The mean resting body temperature for *N. alexis* was 37.8 °C and for *A. spenceri* 36.4 °C. Both groups of animals were run at 2 km h^{-1} for 10 min, a speed which produced oxygen consumption rates of close to 7 ml O_2 g^{-1} h^{-1} in both species. Mean heat storage in *N. alexis* was 0.0399 MJoule (kg h^{-1}) and in *A. spenceri* 0.0405 MJoule (kg h^{-1}). This represents mean values of 28.3 and 29.3% respectively of the total heat production at these speeds; values insignificantly different at the 5% level of confidence. It appears therefore that the lower resting body temperatures in the marsupial confers no advantage in actual heat stored.

Conclusions. We conclude that the apparent specialization of long hind limbs in *N. alexis* results in no marked differences from predicted eutherian locomotory patterns at low running speeds. However, energy demands for locomotion plateau at higher speeds and its appears that elastic storage may become significant. A similar limb specialization occurs in *Antechinomys* but we could not induce steady state running at speeds above 2 km h^{-1}. This difference in maximum voluntary steady state speed, together with the observation that at low speeds the marsupial expends more energy in locomotion than the rodent, provides, even within the limitations of a treadmill experiment, an interesting comparison.

Slow Current Changes Underlying Square Shaped Potential Waves in Warmed *Aplysia* Neurones

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Summary. On warming, the regularly firing L_{11} neurone of *Aplysia* turns into a bursting-type neurone. The bursts of spikes are produced by slow square waves which can also be obtained at room temperature by adding TTX or Co^{++}. Experiments with slow ramp voltage clamp show that warming induces a negative slope (or negative resistance) on the current-voltage characteristic and very slow current variations ($\tau = 10$ to 50 sec) in response to potential changes. The square waves are explained by these two phenomena.

Chalazonitis[1] and Arvanitaki[2] reported that a regularly firing *Aplysia* neurone, denoted 'Gen'[3] or L_{11}[4] generates bursts of spikes when temperature is raised above 25 °C. High frequency bursts are due to slow cyclic potential changes such as in the 'Br' or R_{15} bursting neurones of *Aplysia*[5]. Moreover, voltage clamp experiments indicated that steady current-voltage relations in bursting neurones have a negative slope usually called negative resistance (NR)[6–8], and undoubtedly slow potential waves are due to the instability caused by the NR[9–12]. The present work provides additional evidence for a relationship between steady NR and slow potential changes; also it shows that a slow regenerating mechanism is needed.

Materials and methods. The experiments were performed on the firing cell – 'Gen' or L_{11} – of the parietovisceral ganglion of *Aplysia fasciata*. The ganglion was isolated and pinned in a plexiglass chamber continuously perfused with artificial sea water. Two independent glass microelectrodes filled with 2.5 *M* KCl were inserted in the cell for respectively recording and current injection. The electrical circuit for current or voltage clamping has been already described[13]. To determine i-V relations, a symmetrical triangular ramp pulse was applied to the control amplifier input. The slopes of the positive or negative potential ramps were (±) 0.8 to 3 mV/sec and the potential investigated ranged from –70 mV to 0 mV. The i-V relations obtained with the slow depolarizing ramps can be considered as the steady i-V characteristics of the membrane[11].

Slow permanent records were made on a Philips pen recorder. The temperature was controlled with a thermo-

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electric unit using a Peltier element and measured with a small thermistor placed near the ganglion.

Results. At room temperature (20–22 °C) the L_{11} neurone is continuously firing at a relatively high frequency (1.5 to 4 spikes/sec; Figure 1, B_1). The frequency decreases with cooling and the spiking stops when the temperature is lower than 15 °C. During warming above 22 °C, the spikes group in pairs and around 25 °C the cell generates high frequency discharges separated by long lasting silent periods (Figure 1, B_2). At higher temperature (28 to 35 °C) the spikes are reduced and very often disappear, and the membrane potential alternately passes from a depolarized level (−10 to −20 mV) to a hyperpolarized level (−40 to −50 mV; Figure 1, B_3). These square waves look like the paroxysmal depolarization induced by pentylenetetrazol in molluscan neurones[14].

At low temperature (12–18 °C) the i-V relations obtained with slow potential ramps of positive slope exhibit both anomalous and delayed rectifications, separated by a flattened region with low slope conductance (between −40 and −20 mV; Figure 1 A_1). On warming the L_{11} neurone above 20 °C, a negative slope appears and increases with temperature (Figure 1, A_2 and A_3). The threshold of NR induced by high temperature ranges between −35 and −40 mV. The NR exists in steady-state since with long lasting command potentials ranging from −35 to −15 mV the steady current flows inwardly.

In addition to the induced NR, warming a L_{11} neurone leads to current hysteresis, i.e. the current intensity depends on the potential change direction: the i-V characteristic obtained with a slow repolarizing ramp is still N-shaped, but is shifted towards positive current compared to the i-V characteristic obtained with a depolarizing ramp (Figure 1, A_2 and A_3). Even at the slowest rate which can be used (0.35 mV/sec), the current depends on the potential sweeping direction.

The current hysteresis is conspicuous above 17 °C and increases with temperature. A similar current hysteresis was already observed in bursting neurones of *Aplysia*[10,11]. In L_{11}, the hysteresis is due to a very slowly developing outward current (τ = 10 to 40 sec) at command potentials less negative than −35 mV. Conversely, repolarizing the membrane beyond −35 mV leads to a slowly decreasing current (τ = 10 to 50 sec; Figure 1, D).

Without any external influence, the electrical behaviour of the neurone depends on the potential of its operating

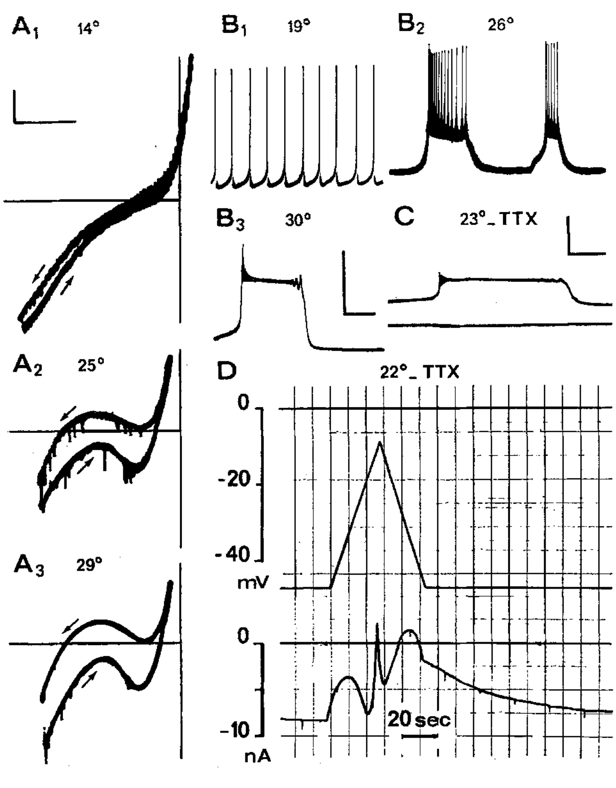

Fig. 1. Current-voltage relations and electrical activity of cell L_{11}. A) i-V characteristics of L_{11}, at 3 different temperatures, obtained by applying slow potential ramps (the sweeping direction is indicated by arrows). Ramp slopes: A_1: 2.8 mV/sec; A_2 and A_3: 2.1 mV/sec. Calibrations: vertical 20 nA, horizontal 25 mV. Outward currents are positive and inward currents negative. The horizontal and vertical lines are, respectively, zero current baseline and zero potential level. B) Spontaneous activity during warming. The cell is firing regularly at 19 °C, displays bursts of spikes at 26 °C and square shaped slow waves at 30 °C. Calibrations: vertical 50 mV, horizontal: 2 sec. C) Spontaneous square wave in L_{11} bathed in TTX 2×10^{-5} *M* for 10 min. Calibrations: vertical: 50 mV; horizontal: 10 sec. D) current time recording (lower trace) in response to applied potential ramps of positive, then negative slope (upper trace). Cell L_{11} bathed in artificial sea water containing TTX 2×10^{-5} *M*. Note the long tail current (τ = 35 sec) after the repolarizing ramp.

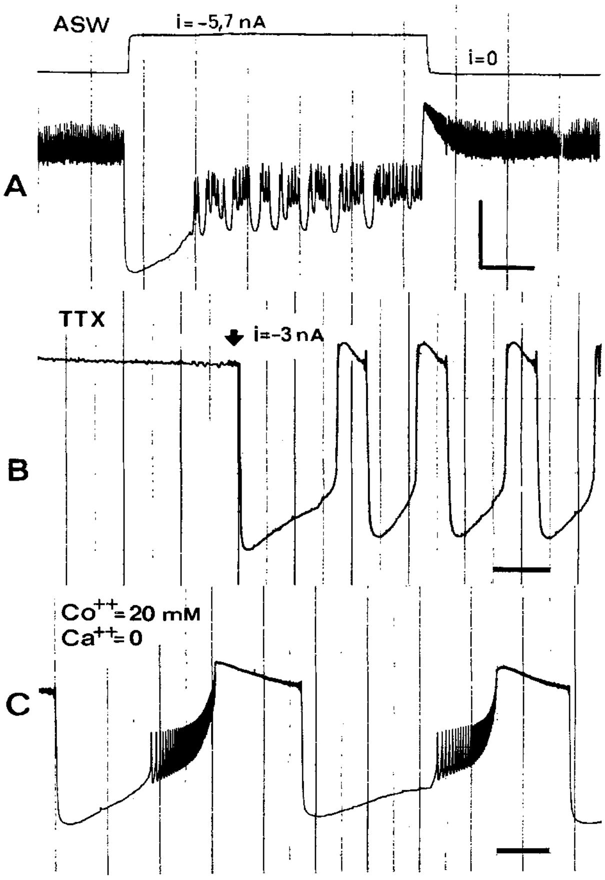

Fig. 2. Slow waves in cell L_{11}. A) Pen recording of spontaneous activity of cell L_{11} bathed in artificial sea water (ASW). Temperature 22 °C. With a 5.7 nA inward current (indicated by upward deflection of top trace) the cell generates irregular bursts of spikes. B) Cell L_{11} in ASW containing TTX 2×10^{-5} *M*. The cell is silent with a low resting potential (−20 mV). A small inward current (arrow) induces long square waves. Temperature 22 °C. C) Spontaneous slow potential waves in L_{11} bathed in artificial sea water deprived of Ca^{++} and containing 20 m*M* Co^{++}. Temperature 25 °C. Small spikes are elicited on the upstroke of the square wave. Vertical calibration: A: 5 mV; B and C: 12.5 mV. Horizontal calibration: A) 7 sec; B) 60 sec; C) 20 sec. In all records, the spike amplitude is strongly attenuated by response time of the pen recorder.

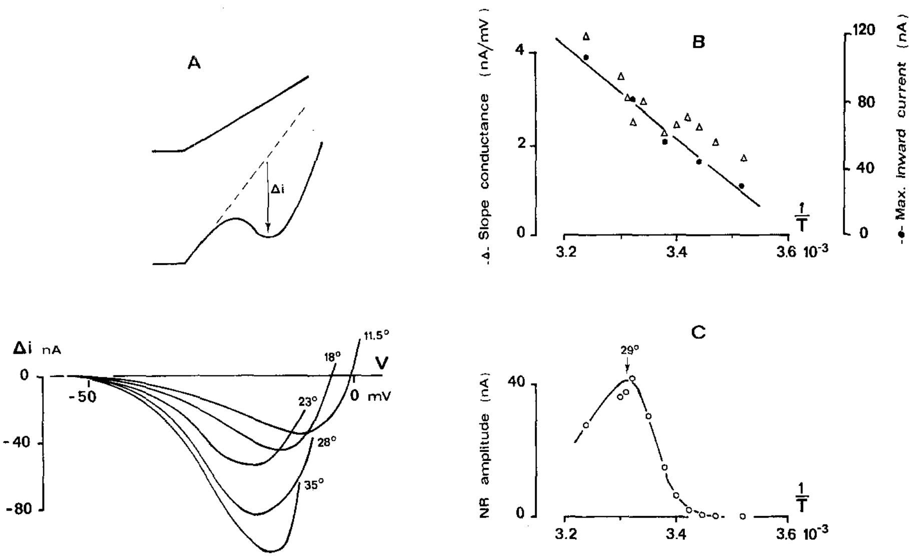

Fig. 3. Steady inward current in warmed L_{11}. A) Inward current versus membrane potential (lower diagram) at different temperatures. The inward current is measured as indicated in top diagram. B) Maximum steady inward current (●), and slope conductance (△) versus 1/T (T = absolute temperature). The slope conductance is the slope of the dashed line in A). The straight line is the inward current-temperature relation. C) Negative resistance amplitude versus 1/T. The maximum at 29 °C results from increase in both inward current and leakage current on warming.

point, defined by the intersection of the $i = 0$ axis with the i-V characteristic. At low temperature, the operating point potential ranges between −25 and −35 mV, whatever the potential slope sign may be. Above 20 °C both NR and current hysteresis lead to the definition of two operating points, corresponding to the characteristics obtained from either a depolarized or a hyperpolarized state. At 22 °C, these two points are on the right-hand positive branch of the characteristic and their potentials are between −15 and −25 mV. Above 25 °C, the enhanced current hysteresis can bring the entire NR into the positive current region and consequently the corresponding operating point passes to the left-hand branch, at about −40 mV. At 30 °C, the two operating points are respectively at −15 and −45 mV and the NR is situated below – depolarizing ramp – or above – repolarizing ramp – the $i = 0$ axis. Thus, when the membrane is previously hyperpolarized, it tends to be depolarized by the low potential level (−15 mV) of its operating point; conversely, when the membrane is depolarized, it tends to be hyperpolarized by the high potential level (−45 mV) of the corresponding operating point. The square waves correspond to the spontaneous potential transitions from the one operating point to the other, according to a mechanism similar to the one proposed for bursting neurones of molluscs[10, 11]. All the necessary elements for this mechanism – steady NR and current hysteresis – already exist at 25 °C. Therefore, it is very likely that the square waves at 30 °C represent the generator potential underlying the burst of spikes at 25 °C, spikes which have been blocked at higher temperature.

The current hysteresis must be large enough to shift the entire NR into the outward current region and to create a high potential operating point. If this condition is not fulfilled, the i-V characteristic of the depolarized membrane has a low potential operating point, such as for a hyperpolarized membrane. At room temperature (20–22 °C) NR and hysteresis are already present but the cell fires regularly. The high frequency firing is thus due to the low potential (−20 mV) of the two operating points: the membrane potential tends to reach the operating point level and the cell fires when the potential reaches the spike threshold (−35 mV). However, with a constant injected inward current (of a few nA), one of the operating points may pass to the left-hand branch of the characteristic and the cell generates bursts of spikes (Figure 2, A). The generator potential of the burst is revealed when the spike mechanism is blocked with TTX (2×10^{-5} *M*; Figure 1, C) or with Co^{++} (substituted for Ca^{++}). In both cases the spikes are strongly reduced or even suppressed and the neurone is silent with a low resting membrane potential (−20 mV). Upon injection of a constant inward current – i_o – the neurone displays long square potential waves (similar to those shown by warmed cells) (Figures 2, B and C). NR amplitude is reduced by TTX or Co^{++} but the current hysteresis is not altered. Consequently, the potentials of the two operating points (under the action of a current i_o) are very slightly modified and correspond well to the bottom and the top of the square waves.

With artificial sea water containing both TTX (2×10^{-5} *M*) and Co^{++} (20 m*M* – $Ca^{++} = 0$), NR is completely suppressed and the cell is depolarized at about −20 mV, but the current hysteresis subsists. These results show that the steady NR is due to a permanent flow of Na^{++} and Ca^{++} ions and that these ions do not interfere with the slow current inducing the hysteresis.

The relation between steady inward current and potential was evaluated taking the leakage current into account as indicated in Figure 3, A. These relations are shown with different temperatures in Figure 3, A. The inward current appears at about −45 mV and is maximum at −20 mV. Due to the outward flow of the slow current, occuring at low potential, the null current points in Figure 3, A are not indicative of the reversal potential of the inward current. The peak inward current increases with temperature but the leakage current is also enhanced (Figure 3, B). Consequently, the induction of NR by the inward current is counterbalanced by the increased outward flow of the leakage current. Since the ability of the cell to generate slow potential waves depends on NR amplitude, it follows that the generation of square waves by L_{11} is optimal at 29 °C (Figure 3, C). This result is comparable with the experiments of Chalazonitis[15] and Salanki et al.[16] who showed that burst generation in R_{15} (or in the corresponding *Helix* neurones) disappears at low and high temperature.

The results obtained on the warmed L_{11} cell strengthen the model set up for bursting neurones[10, 11] and for pentylenetetrazole-induced square waves[17]. They show that two elements are essential for the generation of slow waves: a steady negative resistance and a slow regenerating current.

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