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ELECTRICAL PROPERTIES OF NEURONES IN THE OLFACTORY CORTEX SLICE *IN VITRO*

BY C. N. SCHOLFIELD*

From the Department of Pharmacology, School of Pharmacy, 29–39 Brunswich Square, London WC1N 1AX

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SUMMARY

1. Slices of guinea-pig olfactory cortex were maintained *in vitro*. Electrical properties of neurones in the prepyriform region were studied using single high resistance glass micro-electrodes filled with potassium acetate, connected to a resistance-compensating circuit to allow passage of current through the electrode.

2. Neurones showed a high, stable resting membrane potential (75·4 ± 2·7 mV, mean ± S.D.; $n = 47$). Input resistance measured with small depolarizing currents varied over a range of 9–280 MΩ. The time constant for decay of depolarizing potentials was 19·4 ± 7·5 msec (mean ± S.D.).

3. The relationship between membrane potential and $[K]_{out}$ accorded with a value for $P_{Na}:P_K$ of 0·02.

4. Depolarization produced repetitive action potentials (maximum frequency of 85 Hz) having peak amplitudes of + 16 to + 47 mV. The action potential was followed by a depolarizing after potential of about 20 mV positive to the membrane potential.

5. In these and other respects, the prepyriform neurones appear to behave like most other neurones in the mammalian brain, after allowing for the more stable recording conditions in this preparation.

INTRODUCTION

Slices of the olfactory cortex of the guinea-pig can be maintained *in vitro* for long periods. There have been several electrophysiological studies on this preparation using extracellular electrodes (e.g. Yamamoto & McIlwain, 1966; Richards & Sercombe, 1968*b*; Harvey, Scholfield & Brown, 1974) but none to date with intracellular electrodes, although Biedenbach & Stevens (1969) and Haberly (1973) have reported intracellular observations in cat and opossum olfactory cortex *in vivo* respectively.

The present paper describes some properties of neurones in the isolated guinea-pig olfactory cortex recorded with intracellular micro-electrodes. A companion paper (Scholfield, 1978) reports their responses to synaptic activation.

METHODS

Guinea-pigs of either sex and weighing 250–600 g were decapitated, the brain removed and surface slices of olfactory cortex cut using a 0·58 mm thick guide as described previously (Harvey

* Present address: Department of Physiology, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL.

et al. 1974). Such slices were suspended between two nylon meshes and placed in a beaker of Krebs solution for 2 hr at room temperature (24–26 °C). A slice was then transferred to the bath shown in Fig. 1 with the pial surface uppermost. The bath consisted of a fine nylon mesh stretched across an oval-shaped hole in a flat piece of Perspex sheet, 1·5 mm thick. The mesh was clamped into position by another Perspex sheet, 12 mm thick, on top. These were bolted together with another 25 mm thick Perspex block containing a recess to accommodate a Peltier cooling and warming module (De La Rue, Frigistor, 12–15 g) and water cooled heat sink. The temperature-controlling block was electrically isolated from the bathing solution in the bath by a mica sheet and the pressure on the Perspex blocks spread by an electrically floating silver plate.

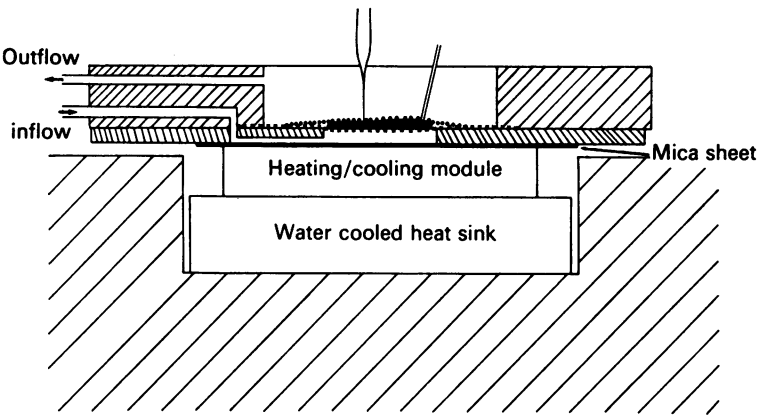

Fig. 1. A section through the recording bath containing a slice of olfactory cortex. The slice is the filled structure sandwiched between the lower (crosses) and upper nylon meshes (dots).

The surfaces of the temperature block and mica sheet were coated with silicon heat-sink compound (having high thermal conductivity) and the Perspex blocks coated with silicon stopcock grease to aid water-tightness. The inflow for the Krebs solution was arranged so that solution passed over a short length of the surface of the temperature controller. The brain slice was positioned onto the nylon mesh such that most of the solution would pass beneath the slice then back over the upper surface to be sucked out via the upper outflow tube. Another nylon mesh was placed over the top of the slice and secured by a stainless-steel circlip. The flow rate through the apparatus was about 2 ml. min^{-1} and was pre-equilibrated with 95% O_2:5% CO_2 at the appropriate temperature. Moistened 95% O_2:5% CO_2 was gently blown over the bath surface. The temperature was controlled manually by regulating the current flowing through the Peltier block. The temperature was read from a thermocouple junction pushed into the middle of the slice about 1 mm away from the area probed by the intracellular recording micro-electrode. The thermocouple was connected to an electronic thermometer and calibrated in Krebs solution against a mercury thermometer, which agreed within 1 °C. Sometimes a tungsten micro-electrode was pushed into the slice so that the uninsulated tip would be at the bottom (cut surface) of the slice where many of the outgoing axons of the neurones pass. Thus occasional cells could be stimulated antidromically. The bath solution was earthed through a Ag/AgCl electrode via an agar bridge. The whole bath assembly was bolted on to a steel base together with a Prior micromanipulator to hold the micro-electrode and was supported in three rubber bungs. This and the support apparatus were placed on a large, 70 kg, reinforced steel plate also resting on rubber bungs.

A single barrelled micro-electrode was used to record membrane potentials and to pass current into the cell. This was connected to an amplifier containing a constant current source and having the facility to compensate for the electrode resistance and capacitance (as detailed in Colburn & Schwartz, 1972). The source resistance of the constant current for injection into the cell was 5×10^8 Ω and the measured amplifier input resistance was more than 10^{11} Ω. The amplifier input current was balanced out on open circuit at the start of the experiment. Command current pulses

to the constant current source were delivered from a previously calibrated Devices type 2533 output unit or purpose built timer. The voltage recordings were fed into a tape recorder (Racal, Store 4) and flat bed recorder.

Electrodes were pulled from 1 mm external and 0·5 mm internal diameter borosilicate glass tubing containing an annealed tubule to aid filling, with a gear type puller giving tip tapers of about 1:20, 1 hr before use. The electrodes were immediately filled with 4 M-K acetate (pH 6). Electrode resistances were 70–400 MΩ (measured with 2×10^{-10} A d.c.) and time constants about 0·05 msec increasing to about 0·5 msec when the tip was lowered to the slice surface (with maximal capacity compensation). These electrodes would pass $0{\cdot}3–2 \times 10^{-9}$ A without substantial changes in electrode resistance and noise.

Properties of the micro-electrodes. The properties of the electrodes are important in assessing the reliability of the potential measurements. When the tip was broken off the electrode (i.e. the tip potential) the electrode potential went positive by 2·5–24 mV whilst bathed in normal solution with or without added 150 mM-KCl, and filled with 4 M-K acetate (or Cl). The electrode potential did not change when the solution was changed from normal solution to 150 mM-KCl in water or added to normal Krebs solution. Usually, when the electrode was pushed through the tissue, the tip became more positive by 0–20 mV associated with an increased tip resistance of up to 10^9 Ω. When the frequency compensation was over-adjusted causing electrical oscillation, the electrode resistance normally returned to the value in free solution. Whilst recording from a cell, an increase in the electrode resistance (as judged by the increased amplitude of the short time-constant part of the current pulse) was observed to be associated with a membrane depolarization of a few millivolts. Careful oscillation by adjustment of the frequency compensation then caused the membrane potential to be apparently restored accompanying a restored electrode resistance. Thus if the electrode resistance increased on penetrating a cell, then the membrane potential would be underestimated by a few millivolts. Some membrane potential values were checked, after enough time for them to stabilize, by withdrawing the electrode upwards with little change in electrode resistance. This membrane potential value agreed to within 5 mV of the initially estimated value where accurately measurable, and where possible, with the reimpalement value. Similar electrodes gave resting potentials in frog sartorius muscle of −75 to −100 mV in 1 mm $[K]_{out}$. These values are accordant with previous estimations (Davson, 1970).

Method of impalements. Recordings were taken entirely from the prepyriform part of the olfactory cortex. The electrode tip was positioned with the aid of a dissecting microscope to avoid touching the nylon mesh. The micro-manipulator was advanced coarsely until the electrode touched the pial surface and then in about 2–5 μm stages with rapid, gentle tapping using a light metal rod to the top of the manipulator directly above the vertical moving faces. Most impalements were obtained from cells 150–400 μm from the surface of the slice. This corresponds to the distribution of superficial layer of neurones within this tissue (Halliwell, 1976).

Impalements were frequently made into cells which had high resting potentials (up to −85 mV). They had a membrane resistance of less than 0·5 MΩ, an unmeasurable time constant and never produced action potentials. They were found at all depths of the slice. In two cells E_m changed by 61 and 42 mV per log $[K]_{out}$ between 1 and 40 mM-$[K]_{out}$. These are the properties of glial cells (Kuffler & Nicholls, 1966).

Solutions. Normal Krebs solution contained (mM): Na, 144; K, 5·9; Ca, 2·5; Mg, 1·3; Cl, 128; HCO_3, 25; SO_4, 1·3; PO_4, 1·2, D-glucose, 11. In Cl-free solution NaCl was substituted by the appropriate amount of Na propionate or isethionate. With different $[K]_{out}$, the KCl was omitted and KH_2PO_4 substituted by 1·2 mM-NaH_2PO_4, and then the appropriate amount of K added as KCl or K_2SO_4. Mannitol solutions were made by omitting the NaCl and using 5 mM-$NaHCO_3$ and 328 mM-mannitol. All solutions were equilibrated with 95% O_2, 5% CO_2 to a pH of 7·3–7·4.

RESULTS

On impalement, an 'injury discharge' of spikes usually ensued, lasting several seconds or minutes. Thereafter, the membrane potential (E_m) either dissipated rapidly or increased over a 5 min period to a stable value in excess of −70 mV. This was then maintained constant over several hours or even (in two cells) overnight for 16 hr. All observations in this paper refer to cells in this stable state.

Fig. 2 shows the distributions of resting membrane potentials and cell input resistances. The average resting potential with respect to pre-impalement electrode potential in forty-seven cells was 75·4 ± 2·74 mV (mean ± S.D.). Values obtained with reference to pre-impalement or post-withdrawal electrode potentials were comparable (within 5 mV in individual cells and insignificantly different on average).

Input resistance was measured from the plateau of the voltage deflexion produced by positive current pulses ($\leqslant$ 80 msec duration) giving $<$ 10 mV depolarization (this being within the linear portion of the current–voltage curves, see below). Resistances ranged from 9 to 280 MΩ (Fig. 2*B*). Greater depolarizations sometimes showed a gradually depolarizing phase presumably due to gradual Na-activation (Fig. 3).

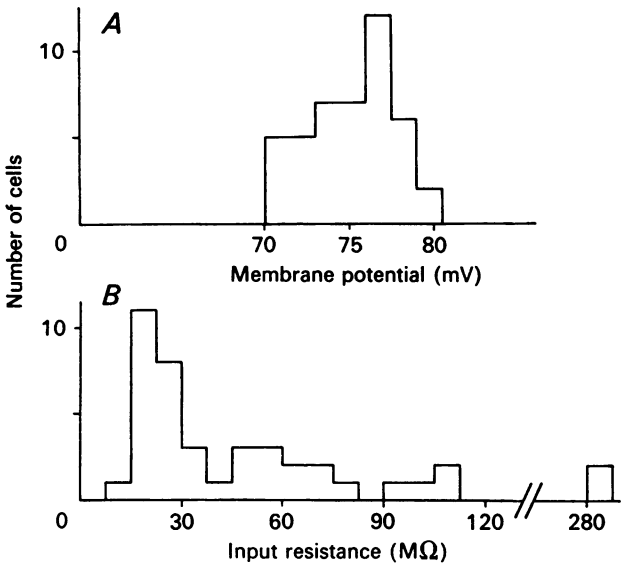

Fig. 2. Frequency histograms for (*A*) membrane potential (mV) and (*B*) input resistance (MΩ).

There was no correlation between input resistance and resting potential in these cells, suggesting that current leaks around the micro-electrode impalement were not serious (cf. Adams & Brown, 1975).

The decline of voltage from the peak deflexion by depolarizing current pulses at the end of the current pulse deviated only slightly from a simple exponential. The average time-constant for the initial rate of decline was 19·4 ± 7·5 msec (mean ± S.D.; n = 25).

Current–voltage curves

Curves were fairly linear up to 20 mV depolarization (Figs. 3 and 4). Greater depolarization produced trains of spikes: thereafter, increasing positive current increased spike frequency without producing further overt depolarization. The current–voltage curve in the hyperpolarizing direction showed a decreasing resistance (anomalous rectification).

Action potentials

A brief (20–30 msec) depolarizing pulse produced a single spike potential at a threshold membrane potential of around −50 mV (Figs. 3 and 5). Such spikes showed a positive overshoot of between 16 and 47 mV, and lasted 4 msec. Focal antidromic stimulation (see Methods) produced spikes of similar amplitude and duration. Both direct and antidromic spikes were followed by a long (50–100 msec) after-depolarization.

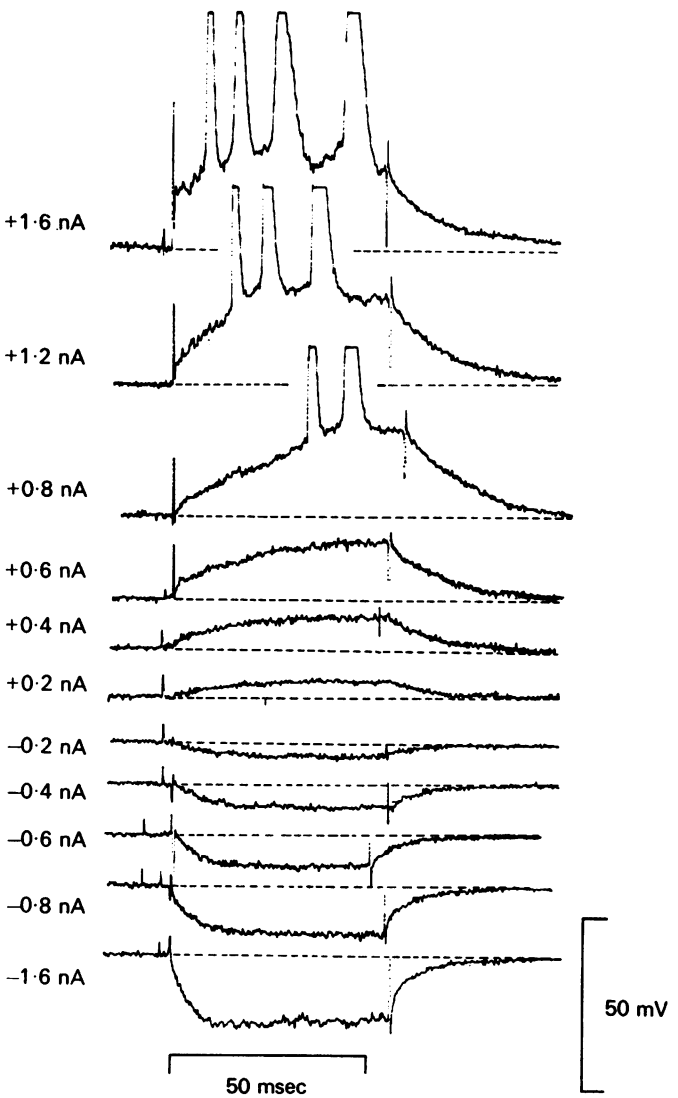

Fig. 3. Potentials produced by passing varying amounts of current through the recording electrode into a single neurone for 50–60 msec. The three highest depolarizing currents produced action potentials which have been truncated in these recordings. The dashed lines are the resting membrane potentials. The initial step with +1·6 nA is an electrode current artefact. These traces were written out on an *X-Y* plotter from a transient recorder. Vertical calibration, 50 mV; horizontal calibration, 50 msec.

Antidromic stimulation in the presence and absence of a depolarizing current pulse (Fig. 5*B*) showed that the after-depolarization was associated with a pronounced fall in input resistance and had a reversal potential of -45 to -70 mV. As $[K^+]_{out}$ was reduced (so increasing E_m, see below) the reversal potential for the after-depolarization partly followed E_m. This after-depolarization could be distinguished from the synaptically-evoked late after-depolarization (see Scholfield, 1978) by its resistance to raised external $[Mg^{2+}]$ (20–40 mM) or reduced external $[Ca^{2+}]$ (0·5 mM). In four out of 150 cells the after-depolarization exceeded the threshold for spike generation, so that a brief current pulse evoked two spikes.

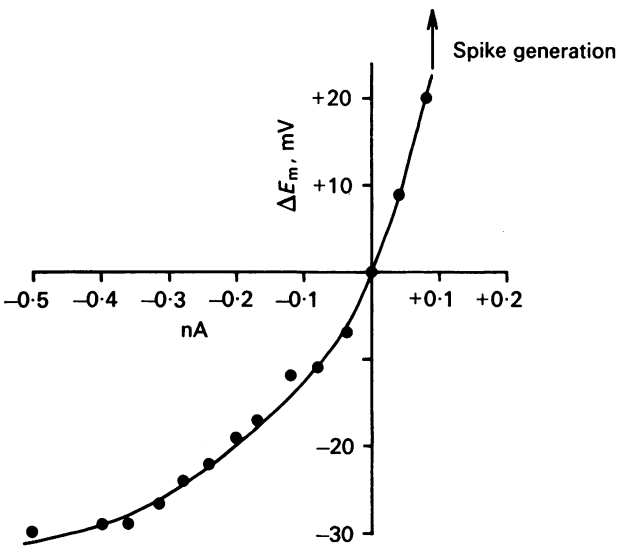

Fig. 4. A current–voltage relationship constructed by passing varying amounts of current into a single neurone and measuring the plateau part of the resultant voltage deflexion. At the point in the curve marked by the arrow, greater amounts of depolarizing current evoked action potentials. The line is drawn 'by eye'. Ordinate: amplitude of the current induced depolarizations (positive values) or hyperpolarizations (negative values) (E_m), mV. Abscissa: current passed through the recording electrode (nA).

With *prolonged* depolarizing current pulses repetitive spikes at an initial frequency of up to 85 Hz were generated (Fig. 3*A*). Second and subsequent spikes of a train showed a reduced positive overshoot and took off from a higher threshold voltage than the initial spike. The discharge frequency declined within 100 msec and discharge stopped after several minutes depolarization.

Action potentials were unaffected by reducing external $[Ca^{2+}]$ to 0·5 mM, or raising external $[Mg^{2+}]$ to 20 or 40 mM, but were blocked by replacing 100 or 128 mM of the external NaCl with isosmotic mannitol or addition or procaine (2–10 mM) or tetrodotoxin (1 μM). Tetra-ethyl ammonium chloride (TEA) 10 mm, prolonged the duration of the action potential by $3{\cdot}0 \pm 0{\cdot}2$-fold and increased the amplitude of the depolarizing after potential (from an E_m -55 ± 5 to -42 ± 2 mV, S.D. of mean of four slices).

Effect of ions on the resting potential

K^+. Fig. 6 shows the relationship between recorded resting potential (E_m) and external K^+ concentration ($[K^+]_{out}$). Responses to high K^+ concentrations were measured after spikes subsided; on reducing $[K]_{out}$, membrane hyperpolarization was slow (up to 1 hr). Over-all, the results accorded with a dependence on $[K]_{out}$ of the form

$$V = 58 \log_{10} \frac{[K^+]_{out} + \alpha[Na^+]_{out}}{[K^+]_{in} + \alpha[Na]_{in}}$$

with $\alpha = 0{\cdot}02$.

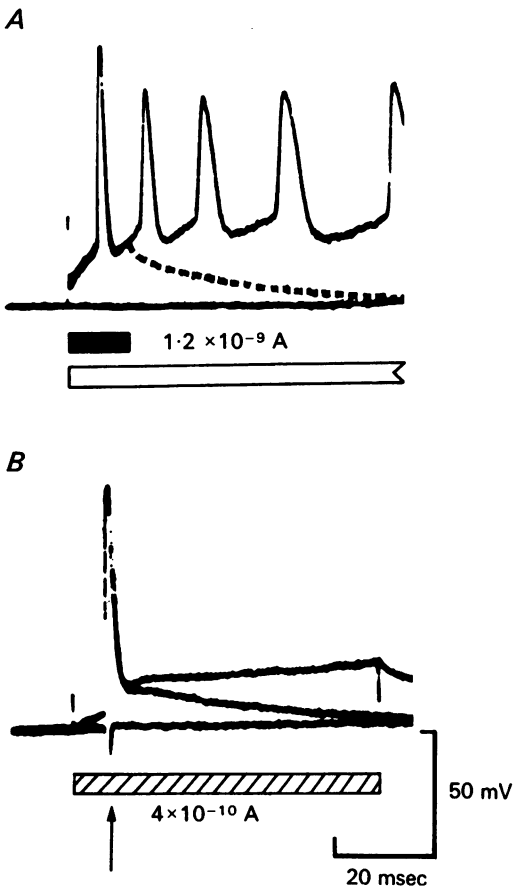

Fig. 5. *A*, a train of action potentials was generated by the injection of $1{\cdot}2 \times 10^{-9}$ A (open bar) through the recording electrode (continuous tracing). Then the cell was injected with the same current for 26 msec (during the filled bar) after which E_m declined to a resting E_m (dashed trace). The action potential generated by this pulse is superimposed in the first one of the train. The jump at the beginning of the current pulse was due to an electrode artifact and not bridge imbalance. *B*, the cell was then stimulated antidromically (↑) at a stimulus voltage just sufficient to fire an action potential (here having a negligible latency). The cell was again stimulated whilst temporarily depolarized by passing $+4 \times 10^{-10}$ A through the recording electrode and marked by the shaded bar (the membrane capacitance is fully charged up by the action potential. Using the same stimulus voltage, the next antidromic stimulus was not quite enough to elicit an action potential and only produced a stimulus artifact. Calibrations: 50 mV and 20 msec.

As expected, input resistance varied with $[K^+]_{out}$. Thus, as a fraction of that at 6 mM-$[K^+]$ input resistance values were: $1{\cdot}76 \pm 0{\cdot}16$ ($n = 4$) at 1 mM and $0{\cdot}64 \pm 0{\cdot}06$ ($n = 7$) at 16 mM-[K]. At 16 and 26 mM-$[K]_{out}$ spikes were initiated for about 0·5 min then ceased.

Cl^-. When external $[Cl^-]$ was reduced below 40 mM (by substitution with isethionate or propionate ions, see Methods) the membrane potential became depolarized by 10–50 mV and unstable. 'Seizure discharges' were seen as described previously (Richards & Sercombe, 1968*a*; Yamamoto & Kawai, 1968). These conditions could be prevented by raising $[Mg^{2+}]$ to 20 mM: then a reduction in external $[Cl^-]$ to 5 mM increased the apparent input resistance of the cells by 10–20 % with little change in membrane potential.

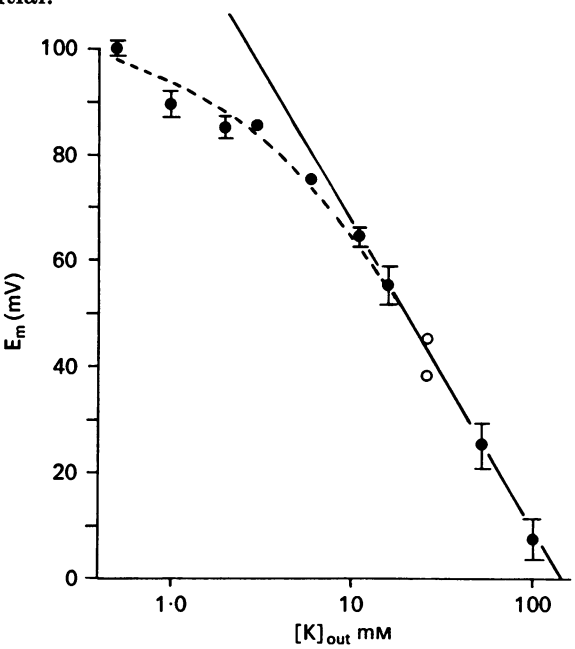

Fig. 6. Effect of various $[K]_{out}$ on E_m. In some experiments, KCl was added to normal Krebs solution. At other times K (as K_2SO_4) replaced equimolar amounts of NaCl. With most of the higher $[K]_{out}$, 20 mM-Mg was added to prevent a sudden depolarization to -10 to 0 mV over 20 sec which sometimes occurred. After such depolarizations, E_m usually completely recovered within 50 min (Mg had no effect on the action of K). Results with the two methods of K application gave similar results and hence were pooled. The added continuous line has a slope of -58 mV per log $[K]_{out}$. The dashed line is according to the Goldman equation:

$$E_m = (RT/F) \log\{([K]_{out} + \alpha[Na]_{out})/([K]_{in} + \alpha[Na]_{in})\}.$$

Values for $[Na]_{in}$ and $[K]_{in}$ were taken from Harvey *et al.* (1974) as 50 and 130 mM respectively. $\alpha = P_{Na}:P_K = 0{\cdot}02$. Ordinate: E_m mV. Abscissa: $[K]_{out}$ on a log scale, mM.

Effect of temperature

Low incubation temperatures (25 °C) were used in the present study since previous experiments have indicated that this helps to maintain the long-term viability of thick slices *in vitro* (Harvey *et al.* 1974). The effects of short-term changes in incubation temperature on the properties of olfactory cortical neurones is shown in Fig. 7.

The resting *membrane potential* remained constant between 24 and 38 °C, but diminished by some 5 mV at 21–13 °C. The input resistance and initial *time constant* changed progressively over the range 13–38 °C with similar Q_{10} values of 1·25 and 1·32 respectively. Thus, the effective capacitance remained fairly constant. The amplitude of the *action potential* fell above 24 °C; its voltage threshold remained constant.

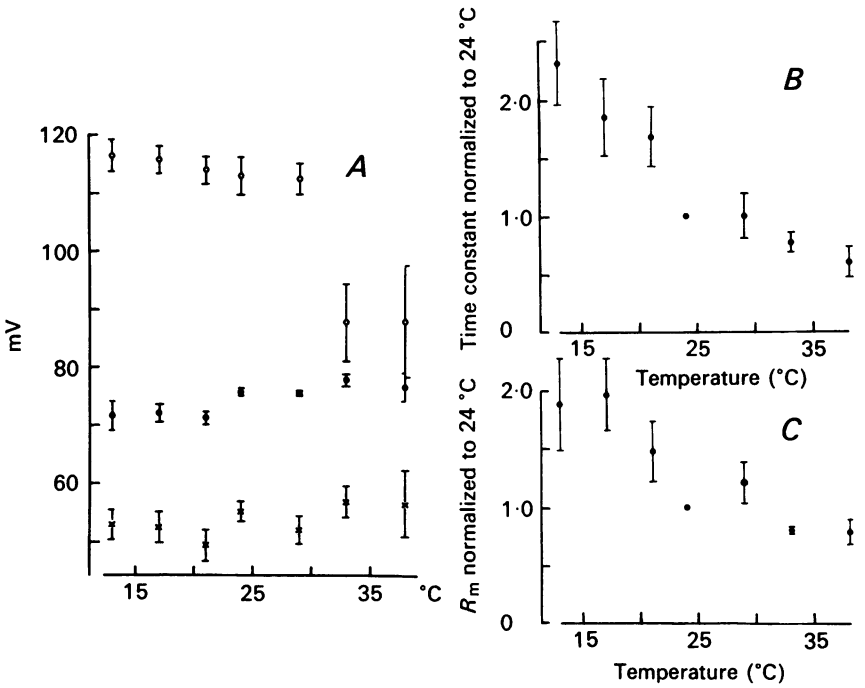

Fig. 7. Effect of temperature on resting membrane properties and excitability. *A*, (i) resting membrane potential (filled circles). (ii) The peak height of the action potential from the resting membrane potential (open circles). (iii) Threshold voltage for the generation of the spike from depolarizing current pulses longer than 80 msec (crosses). Ordinate: potential, mV. *B*, time constant for the decay of membrane voltage after a 10 mV depolarization. Ordinate: normalized to time-constant at 24 °C (= 1·00). *C*, resting input resistance, R_m, measured by a 10 mV depolarization. Ordinate: R_m normalized to that at 24 °C (= 1·00). Abscissae: temperature of the bathing solution, °C. Points are means of measurements from three to ten cells and bars are S.E. of means.

DISCUSSION

The high resting potential in these neurones (−75 mV) is a striking feature, notwithstanding the higher $[K]_{out}$ of 6 mM in the incubating solution (about double the extracellular solution of brain: Leusen, 1972; Katzman, 1976). At the more 'physiological' concentration of 3 mM, the membrane potential of these neurones would be around −85 mV (Fig. 6).

There were several reasons for using this higher $[K]_{out}$: (*a*) solution of this composition gave responses which closely matched those *in vitro* (Harvey *et al.* 1974), (*b*) Na-pump activity is greater at this high concentration (Brown & Scholfield, 1974), (*c*) higher $[K]_{out}$ may be necessary to maintain $[K]_{in}$ similar to that *in vivo* and results in less swelling (Frank, 1970).

The previously-reported value of -40 mV in cat pyriform neurones *in vivo* (Beidenbach & Stevens, 1969) probably reflects a substantial impalement damage (acknowledged by the authors) and so does not provide a good comparison. Nevertheless, the values obtained in the present study are substantially greater than those normally recorded in mammalian brain cells *in vivo*. Four factors might contribute to this discrepancy.

1. *Impalement stability.* One clear advantage of the *in vitro* preparation is the greater mechanical stability permitting the use of finer electrodes. The stability of successful impalements in the present experiments was often impressive. Though difficult to quantitate, it seems plausible that an improved sealing of the membrane around the electrode might contribute to the higher resting potentials.

2. *Lack of spontaneous activity.* Cells in the isolated olfactory cortex showed little evidence of spontaneous synaptic activity. Subthreshold excitatory activity or tonic hormonal influences may lead to a reduced membrane potential *in vivo*.

3. *Temperature.* Subnormal incubation temperatures were used in the present study to provide more suitable conditions for *in vitro* viability (see Harvey *et al.* 1974). This does not itself account for the high resting potential since this is maintained at 38 °C for brief periods. Also, since oxygen requirement varies strongly with temperature, the temperature-insensitivity suggests that anoxia was not a material factor.

4. *Electrode artefacts.* These seem unlikely because the action potential had an appropriate value and the membrane potential was fully dissipated in 100mM-$[K]_{out}$ (see also Methods).

Ionic basis of membrane potential

The relationship between $[K]_{out}$ and membrane potential might reasonably well be attributed to a K^+ diffusion potential with a small Na-contribution ($P_{Na}:P_K = 0{\cdot}02$). The permeability to Cl ions is uncertain owing to the induction of spontaneous activity in low $[Cl]_{out}$. But since reducing $[Cl]_{out}$ in 20 mM-Mg did not produce an abrupt depolarization or a large increase in membrane resistance, it seems that Cl fluxes contribute little to the resting potential.

No evidence was adduced for an 'electrogenic' Na-pumping component to the membrane potential: (*a*) membrane potential *vs.* $[K]_{out}$ did not show a peak at about 3–10 mM-$[K]_{out}$ which might be ascribed to a K-sensitive Na-pump (Marmor & Gorman, 1970); (*b*) the membrane potential was not temperature sensitive suggesting that a metabolic component was not present (cf. Carpenter, 1970; Marmor & Gorman, 1970). Hillman & McIlwain (1961) observed a peak membrane potential of about -50 mV at a $[K]_{out}$ of 5 mM. However, since the membrane resistance at low$[K]_{out}$ increases, this peak might be the result of an impalement leak around the recording electrode.

Action potentials

There is some similarity between these cells and those in the hippocampus described by Kandel & Spencer (1961) and Schwartzkroin (1975) in that individual spikes were succeeded by a pronounced after-depolarization which could generate additional spikes. However, if the K conductance is not very great, then the residual Na conduction may not be completely shunted out as seen in spinal neurones

(Balderserra, 1976), i.e. the spike after potential may not be very near the K equilibrium potential (−82 mV by Fig. 7). Invasion of the dendrites by the short action potential seems less likely because this occurs over a much shorter time course (1 msec) than that of the after depolarization (Halliwell, J. V., personal communication).

The neurones in the isolated olfactory cortex preparation appear to behave qualitatively like other neurones in the mammalian central nervous system. Differences which arise seem to result from the higher membrane potential in the more stable recording conditions.

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