CHANGES OF EXTRACELLULAR POTASSIUM ACTIVITY INDUCED BY ELECTRIC CURRENT THROUGH BRAIN TISSUE IN THE RAT

By A. R. GARDNER-MEDWIN* and C. NICHOLSON

From the Department of Physiology and Biophysics, New York University Medical Center, New York, NY 10016, U.S.A.

SUMMARY

1. Ion-selective micro-electrodes have been used to measure K^+ and Ca^{2+} activity changes in extracellular space beneath the surface of the neocortex and cerebellar cortex during current flow across the tissue surface in anaesthetized rats.

2. Inward currents produced decreases of $[K^+]_o$ and outward currents produced increases, with insignificant changes in $[Ca^{2+}]_o$.

3. Changes of $[K^+]_o$ were largest just under the surface of the tissue, but were detectable down to depths of *ca.* 1 mm. With appropriate siting of electrodes in the cerebellar cortex, currents of 22 μA mm^{-2} for 400 sec produced changes averaging -42% for inward current and $+66\%$ for outward current.

4. The $[K^+]_o$ changes near the surface were most rapid immediately after the onset of current and more gradual after some tens of seconds. Deeper within the tissue the rate of change was more uniform and after the end of stimulation the return to base line was slower.

5. The amplitude, depth dependence and time course of the $[K^+]_o$ changes were in reasonable agreement with the results calculated for a model in which K^+ moves partly through extracellular space but primarily through membranes and cytoplasm within the tissue.

6. The $[K^+]_o$ changes were not attributable to variations in neuronal activity, although unit activity could be modified by current, since alternating currents failed to produce $[K^+]_o$ changes and neither 0·1 mM-tetrodotoxin nor 5 mM-Mn^{2+} abolished the changes.

7. The $[K^+]_o$ changes were not abolished by topically applied ouabain (4×10^{-4} M), 2,4-dinitrophenol (20 mM) or iodoacetate (10 mM), or by asphyxiation. Consequently the $[K^+]_o$ changes are not dependent on metabolism.

8. The data suggest that there is a selective mechanism for passive K^+ transport in an electrochemical gradient within brain tissue that results in higher K^+ fluxes than could be supported by ionic mobility in the extracellular fluid. This mechanism exists not only at the surface but within the brain parenchyma and may involve current flow through glial cells.

* Permanent address: Department of Physiology, University College London, London WC1E 6BT.

INTRODUCTION

The preceding paper (Gardner-Medwin, 1983a) provides two kinds of data about movement of ions across the surface of the rat brain. Measurements of the K^+ flux associated with current flow revealed larger K^+ fluxes than could be attributable to extracellular flux, while the size of the potential difference developing across the tissue when there is an imposed gradient of extracellular K^+ concentration ($[K^+]_o$) suggests a flux of K^+ ions for a given concentration gradient that is also larger than for other ions.

The explanation of these phenomena may be that there is substantial movement of K^+ through cells in brain tissue, as well as through extracellular space. If this is correct, there must be changes of $[K^+]_o$ resulting from the movement of potassium into and out of the cells. These changes are studied in the present paper.

Transcellular flux might explain these results even if it were restricted to the superficial cells associated with the pia or arachnoid mater. In this way the results might reveal simply a property of these barriers rather than of the brain tissue as a whole. Quantitative consideration of the build-up and depletion that would result if this explanation were correct made it appear unlikely (Gardner-Medwin, 1983a) but a more crucial test comes in the present experiments. Suppose that a selective barrier at the brain surface passes a higher K^+ flux for a given current than the K^+ flux through the bulk of the brain tissue. Then for current flow out of the brain surface there should be a depletion of tissue potassium in the region of brain beneath the superficial barrier (Fig. 1A). If, on the other hand, the bulk of the tissue supports a high K^+ flux per unit current due to passage of some of the current through cell membranes and cytoplasm, then there should be a build-up of K^+ in the superficial tissue (Fig. 1B). The changes described here are consistent with the second hypothesis and not the first.

Another issue stems from the comparison made by Gardner-Medwin (1983a) of the observed K^+ flux during current flow with the flux expected for purely extracellular transport. This comparison rests on the assumption that the brain extracellular space has approximately the normal composition attributed to it in the literature, with $[K^+]$ approximately 3 mM as in cerebrospinal fluid (Somjen, 1979). It is possible that the experimental procedures employed in these experiments might have sufficiently increased $[K^+]_o$ so that the high observed transport number for K^+ was simply a result of an abnormal extracellular fluid composition. The direct measurements of K^+ activity in the present experiments permit this hypothesis to be tested.

The measurements of K^+ flux during current passage (Gardner-Medwin, 1983a) were performed on the rat neocortex. This gave a large enough area of relatively flat brain surface to accommodate a 5 mm diameter cup designed to house several electrodes and a stirring device. The present experiments did not require such a large cup and were performed both on the neocortex and on the cerebellum of anaesthetized rats. The cerebellum offered the advantage that the effects of current flow could be studied along with the characteristic field potentials at different depths generated by surface stimulation. This gave an electrophysiological index of the condition of the preparation and also a sensitive indication of the depth of the recording electrode relative to the brain surface. Once it was established that the effects of current flow
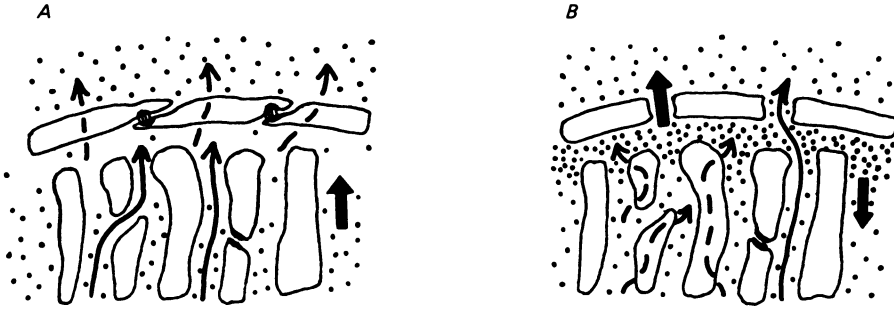
A B

Fig. 1. Two possible mechanisms by which transcellular K^+ flux could account for a high K^+ flux carried by current across the brain surface. The diagrams represent cup fluid (at the top), a layer of cells constituting the brain surface and cells within the bulk of the tissue. Thin arrows represent current flow: those which are broken represent current carried substantially by K^+. Heavy arrows represent K^+ diffusion in the resultant concentration gradients. *A*, there is no significant transcellular K^+ flux in the bulk of the tissue, but the surface presents a barrier with a higher K^+ transport number than extracellular fluid. The result is K^+ depletion in extracellular space beneath the surface. *B*, current through cells in the bulk of the tissue carries a substantial K^+ flux, producing K^+ build-up in extracellular space near the surface (high concentration of dots). Entry of K^+ into the cup is largely by diffusion, with no selectively permeable surface barrier.

on K^+ activity were similar in the cortex and cerebellum, most of the further experiments were carried out on the cerebellum.

Some experiments were performed using Ca^{2+}-sensitive instead of K^+-sensitive micro-electrodes, to see whether the observed effects were specific to K^+. We had no reason to expect changes in $[Ca^{2+}]_o$ during current passage, since the Ca^{2+} movements caused by current through brain tissue would normally be expected to be largely extracellular. But a change in $[Ca^{2+}]_o$, such as is caused by neuronal activity (Nicholson, ten Bruggencate, Stöckle & Steinberg, 1978) might have indicated that there was a general disturbance of the brain cell micro-environment due to current flow, requiring a more complex explanation than we have envisioned for the K^+ results.

Some of the results and conclusions described here have been published in abstracts (Gardner-Medwin & Nicholson, 1978; Gardner-Medwin, 1981).

METHODS

Adult Sprague–Dawley rats were anaesthetized with urethane (1·4 g/kg) and a tracheostomy was performed to assist breathing. A surgical exposure was made either of the parietal neocortex on one side or of the posterior cerebellum over the mid line, and the dura was removed. For experiments on the neocortex the cup and procedures described by Gardner-Medwin, (1981) were employed. For experiments on the cerebellum a small Perspex cup (i.d. 2·9 mm; Fig. 2) was lowered onto the brain surface by the use of a micromanipulator. Warm agar (3 % w/v) in 300 mM-sucrose was used to cover the brain outside the cup and to ensure a seal preventing the cup fluid from running out. The cup was filled with warm saline (140 mM-NaCl, 3 mM-KCl, 1·6 mM-CaCl₂, 2 mM-NaH₂PO₄, 4 mM-Na₂HPO₄; pH 7) which for most experiments was continuously circulated with a peristaltic pump through a large reservoir (100 ml.). The fluid entering the cup passed through a heat exchanger at 37 °C. Rectal temperature was monitored and kept at 36–38 °C with a heating pad.

Current was passed from a chlorided silver wire in the cup to a similar remote electrode in the mouth of the animal. A potentiometer in the circuit for controlling the current (Fig. 2) was operated
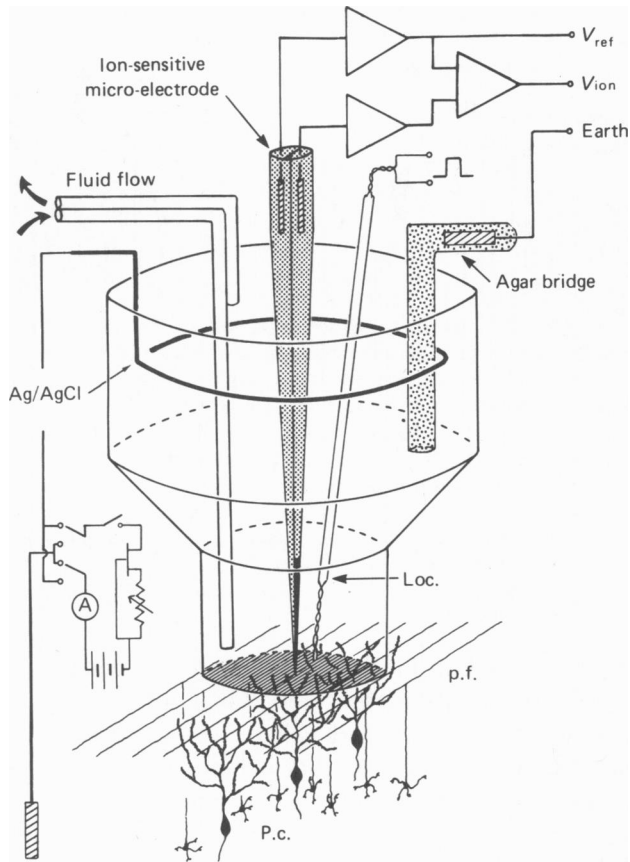
Fig. 2. Diagram showing the apparatus in a Perspex cup (diameter 2·9 mm at the cortical surface) positioned on the pia-arachnoid surface of the cerebellar cortex. The circuit for control of current is described by Gardner-Medwin (1981). Bipolar local surface stimulation (Loc.) activates parallel fibres (p.f.) which make synaptic contact on Purkinje cells (P.c.). Field potential responses were recorded with the reference barrel of the ion-selective micro-electrode.

manually to increase and decrease the current gradually over about 0·5 sec at the beginning and end of current passage to minimize the amount of transient neuronal firing associated with sudden changes of current. The current was constantly monitored with a microammeter. The animal and current-passing circuitry were electrically isolated from earth, except through a Ag/AgCl electrode connected to the cup fluid through a bridge of 3 % agar dissolved in the standard saline.

Extracellular ionic concentrations were inferred from measurements with doubled-barrelled ion-sensitive micro-electrodes comprising an ion-sensing channel and a reference channel. The ion-sensing channel contained either Corning 477317 K^+-exchanger or a neutral carrier Ca^{2+}-exchanger provided by Professor W. Simon, Zurich. Details of the electrodes, calibration procedures and amplifiers are described elsewhere (Nicholson *et al.* 1978). Calibration was performed before and after experiments. The sensitivity of the electrodes (mV per 10-fold change of $[K^+]$ or $[Ca^{2+}]$) were consistent within 10 % during an experiment. Base-line shifts up to *ca.* 2 mV occurred and corrections for these were made by reference to frequent measurements in the cup fluid during an experiment. The indicated ionic concentrations are the molar concentrations of KCl or $CaCl_2$ which gave equivalent readings in solutions made up with 150 mM-NaCl. The buffered saline contained 1·6 mM-$CaCl_2$ and gave readings equivalent to 1·2 mM in the calibration solutions,

possibly because the free Ca^{2+} activity was reduced in the buffered saline by interaction with phosphate ions. There was no such discrepancy for K^+ between buffered and unbuffered saline.

In experiments on the cerebellum, the surface was stimulated with a bipolar electrode and the characteristic field potentials were used to locate the recording tip at the cerebellar surface. Subsequent depth changes were measured with the micromanipulator, with the laminar field potentials providing a control against movement of the brain and dimpling or tenting of the surface. Ion and reference signals were recorded on a four-channel chart recorder after filtering to remove fast potentials. Field potentials, derived from the reference channel of the electrode, were displayed on an oscilloscope and photographed. In some experiments single-barrelled glass micropipettes (*ca.* 2 μm tip diameter), filled with 1 M-NaCl, were used to record extracellular action potentials from individual cells in the cerebellar cortex. The signal was filtered and a continuous monitor of spike frequency was made with a Schmitt trigger and a low-pass filter fed from a pulse shaper.

RESULTS

When the tip of a K^+ micro-electrode was lowered beneath the surface of either the rat neocortex or cerebellum approximately steady measurements were normally obtained, corresponding to $[K^+]$ levels of 3·0–3·5 mM in calibration solutions. This is consistent with earlier observations with micro-electrodes and with values for $[K^+]_0$ inferred from other types of data (see Somjen, 1979, for review). The electrodes used in the present study (*ca.* 2 μm tip diameter) never showed signs of penetration of cell membranes without cellular damage, since with advancement of an electrode only transient negative resting potentials and increases of K^+ concentration or activity were registered, lasting at most for some tens of seconds. The experiments required that measurements should be made from sites that were in diffusional equilibrium with extracellular clefts. When the electrode was moved to a new depth and gave an unstable base line we moved it down and up a few tens of μm to achieve steady readings. On progressive withdrawal of an electrode along its track transient increases in $[K^+]$ were not observed and the measurements agreed with those made from stable sites during lowering of the electrode. These measurements were interpreted as extracellular. The region around the electrode tip must include damaged tissue or an enlarged zone of extracellular space, so it is likely that these measurements would show a small lag in their response to rapid changes of $[K^+]_0$. Slow changes and those lasting for several seconds or more should be recorded faithfully (Lux & Neher, 1973; Lothman & Somjen, 1975). The measurements are described for conciseness here as $[K^+]_0$ measurements, which must strictly be taken to mean the K^+ concentration in calibration solutions (see Methods) that register the same K^+ activity as the environment of electrode tips positioned in one of the ways described above.

Passage of current across the surface of the brain raised or lowered $[K^+]_0$ from its resting or pre-existing level, according to whether the current was passed respectively outward or inward across the exposed brain surface (Fig. 3A). This was true with only one exception (referred to below) in several observations on each of twenty animals, with penetration depths up to 3 mm beneath the exposed surface of the neocortex (four animals) or cerebellum (sixteen animals). Current was passed between an electrode in a cup (2·9 mm or 5 mm diameter) at the exposed surface and an indifferent electrode elsewhere, usually in the mouth. Outward current at the cup (with the cup negative: hence described as negative current) is associated, of course, with inward current across some other regions of the brain surface. This must be
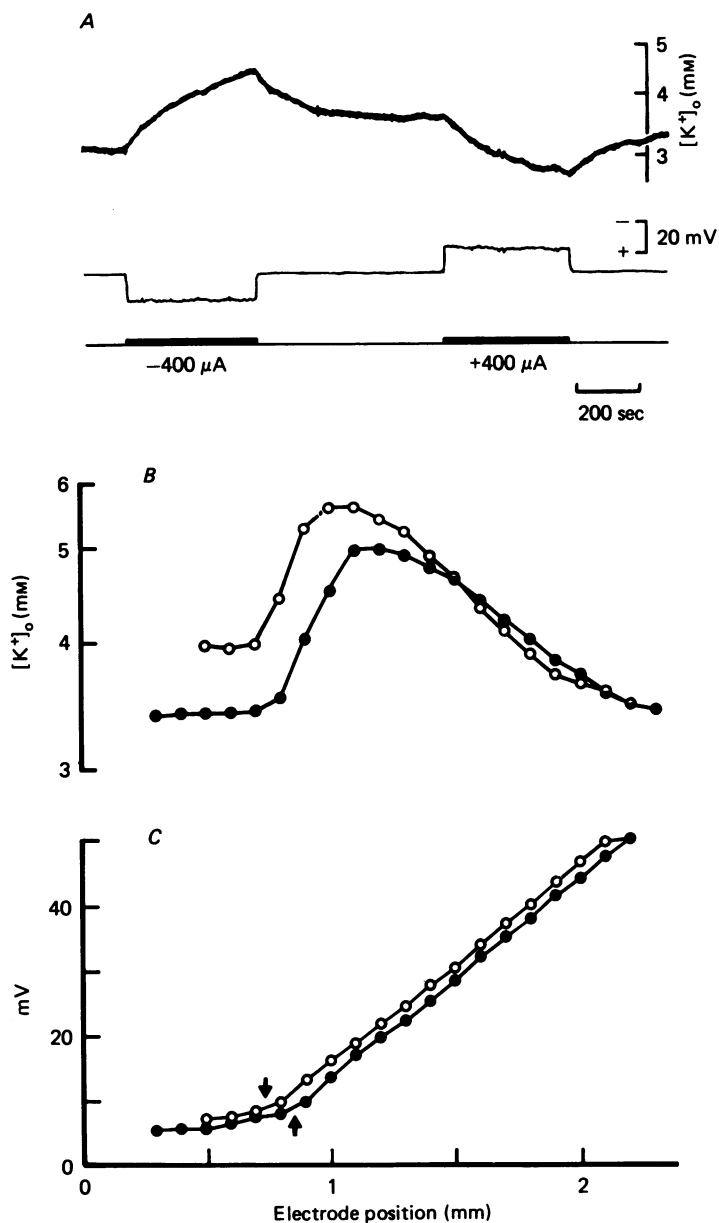
Fig. 3. Changes of $[K^+]_o$ recorded with a cup (5 mm diameter) on the pia-arachnoid surface of the neocortex. *A*, measurements of $[K^+]_o$ *ca.* 200 μm beneath the cortical surface during current flow from the brain to the cup (-400 μA) and in the opposite direction ($+400$ μA). *B*, measurements of $[K^+]_o$ in a different animal during current passage (-400 μA) as the electrode was first lowered (●) and then raised (○) in 100 μm steps, starting *ca.* 0·5 mm above the tissue surface. Each observation took *ca.* 10 sec; the series began 300 sec after the onset of current and finished 700 sec after onset. *C*, the potential difference between the reference barrel of the micro-electrode and the earth electrode in the cup, relative to the base line in the cup before onset of current, measured at the same time as *B*. Arrows indicate the approximate positions of the tissue surface on penetration and withdrawal, inferred from the change of voltage gradient.

presumed to have led to $[K^+]_0$ changes in the opposite sense close to the surface in these regions. These remote changes, which will generally have been small because of the much larger area of brain surface through which the current was passing, were not studied systematically. The one occasion on which a change in the abnormal direction was observed was at a depth of 700–800 μm in the cerebellar cortex and may have been due to the approach of the recording electrode to the inverted edge of a folium through which current passed in the opposite sense to the current at the surface.

Control experiments with the same techniques were made with agar blocks in place of brain tissue. These blocks (2 % and 3½ % agar dissolved in the saline solution used for superfusion and equilibrated in this solution overnight) revealed no detectable change of $[K^+]$ induced by current flow. This is to be expected since an agar gel has little effect on the mobility of small ions (Nicholson & Phillips, 1981) and the flux of K^+ for any given current should therefore be the same as for the saline with which it is in equilibrium.

Fig. 3A shows the change of $[K^+]_0$ recorded approximately 200 μm beneath the surface of the neocortex with a current of 400 μA passed first outward ($-400\ \mu$A) and then inward ($+400\ \mu$A) across the surface of the brain. The cup on the brain surface and the conditions were the same as for measurements of K^+ flux across the neocortical surface in earlier work (Gardner-Medwin, 1983a). The current density at the brain surface was 20 μA mm^{-2}, the largest normally used for the flux measurements. The changes of $[K^+]_0$ were most rapid initially after the onset or termination of current flow and became more gradual after some tens of seconds. This contrasts with the measurements of $[K^+]$ in the cup at the cortical surface under these circumstances (Gardner-Medwin, 1983a), which changed slowly in the first 30–100 sec during current flow.

A crude but direct indication of the depth dependence of the $[K^+]_0$ changes induced by current flow was obtained by moving the K^+-sensitive electrode during the flow of current. Fig. 3B shows $[K^+]_0$ and extracellular voltage plotted against the position of the micro-electrode along its penetration track during outward current flow ($-400\ \mu$A) as for Fig. 3A, but in a different animal. After the current was turned on the electrode was first lowered and then withdrawn in steps of 100 μm. During such measurements $[K^+]_0$ was gradually increasing at any one site, as shown in Fig. 3A. Consequently the measurements of $[K^+]_0$ on withdrawal were higher than those on penetration. The depth dependence of $[K^+]_0$ at any one instant would presumably be intermediate between the curves for penetration and withdrawal. The measurements of voltage at the reference barrel (lower Fig. 3B) show the relative small extent of tissue displacement relative to the electrode co-ordinates, due to the electrode pushing the tissue down on penetration and lifting it on withdrawal. The site at which the electrode tip entered or left the surface was not readily visible, but is indicated electrically by the change of gradient of the measured extracellular voltage (arrows, Fig. 3B) due to the high resistivity of brain tissue compared with saline (Van Harreveld, 1972). The largest increases of $[K^+]_0$ were evident close to the tissue surface, within a range of a few hundred μm. Even outside the tissue in these experiments there was a rise of $[K^+]$ in the cup (Fig. 3B) due to the build-up of K^+ in the surface fluid which was stirred but not replenished. This build-up was studied in experiments by Gardner-Medwin (1983a) to infer K^+ flux across the tissue surface.

For more detailed study of the effects of current flow, the cerebellum offered certain practical advantages over the neocortex. In particular, the responses to local surface stimulation are well characterized in relation to underlying physiological mechanisms and provide a reliable index of the depth of a recording electrode close to the surface (Eccles, Ito & Szentagothai, 1967). Furthermore the electrical and $[K^+]_0$ base lines
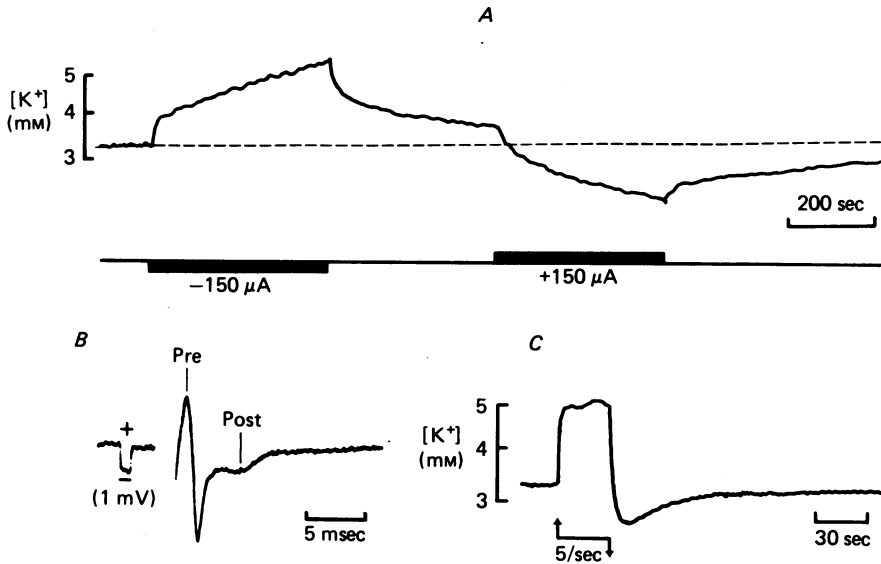

Fig. 4. Records from a single micro-electrode site less than 100 μm beneath the surface of the cerebellar cortex. A, changes of $[K^+]_0$ induced by current outward ($-150\ \mu$A) and inward ($+150\ \mu$A) across the tissue surface. B, responses to local surface stimulation ca. 1 mm from the recording site, showing components due to both presynaptic parallel fibre activity (Pre) and post-synaptic Purkinje cell activation (Post). C, changes of $[K^+]_0$ produced by repetitive local surface stimulation (5/sec) for 30 sec at the same site.

were found to be more stable in the cerebellum than the neocortex. Neither in these experiments nor in experiments on the electrical response to superfusion with altered $[K^+]$ (Gardner-Medwin, 1983a) was there evidence for substantial differences between the K^+ dynamics in the neocortex and cerebellum. A smaller cup (2·9 mm diameter) was used on the cerebellar surface and this was continuously perfused with warmed saline of constant composition (see Methods), to prevent build-up or depletion of K^+ in the cup. This simplified the interpretation of $[K^+]_0$ changes near the tissue surface. Smaller currents were passed (usually 150 μA instead of 400 μA) to give approximately the same current density as with the larger neocortical cup (5 mm diameter).

Fig. 4A shows $[K^+]_0$ changes recorded less than 100 μm beneath the cerebellar surface, with current passed first outward ($-150\ \mu$A) and then inward ($+150\ \mu$A) across the surface. At the same recording site the electrical response to local surface stimulation showed clear presynaptic and post-synaptic components (Fig. 3B), though with these recording conditions (earthed saline at the tissue surface) the negative post-synaptic component close to the surface is small in amplitude compared

with the presynaptic component. Repetitive stimulation (Fig. 4C) led to a rapid rise of $[K^+]_0$ with a subsequent undershoot. These changes are indicative of a preparation in good condition and have been previously described for the rat cerebellum by Nicholson, ten Bruggencate, Steinberg & Stöckle (1977). Surface stimuli were applied during and after periods with current flow for up to 400 sec and individual responses were not changed significantly. When pairs of stimuli were delivered with a 20 msec interval the presynaptic response to the second stimulus was normally 15–20 % earlier than the first response, due to a supernormal conduction velocity in the parallel fibres (Gardner-Medwin, 1972a). After prolonged current flow outward across the cerebellar surface, when $[K^+]_0$ was above about 5 mM, the latency of the second response was no longer reduced. This is probably an indirect effect due to the $[K^+]_0$ changes, since similar observations have been made *in vitro* with altered bath $[K^+]$ (Gardner-Medwin, 1972b).

In five animals current (150 μA) was passed for 400 sec in each direction with electrodes positioned on the cerebellar cortex in sites similar to that for Fig. 4. The average change of $[K^+]_0$ from base-line levels was $+65\cdot8$ ($\pm9\cdot0$ S.E., $n = 5$) % for outward current ($-150\,\mu$A) and $-42\cdot2$ ($\pm3\cdot9$ S.E., $n = 5$) % for inward current ($+150\,\mu$A). The area of the base of the cup was 6·7 mm², giving an average current density of 22 μA mm^{-2} for these observations. The current density at the tissue surface may have been less than this because of the slight convexity of its surface and because of possible current leakage under the rim of the cup. In one experiment outward current ($-150\,\mu$A) was maintained for 2000 sec (33 min), by which time $[K^+]_0$ had reached a nearly steady level of 11·5 mM. It may be significant that this steady level was close to the 'ceiling' for $[K^+]_0$ rises seen in several other preparations under a variety of conditions (Somjen, 1981).

Fig. 5 shows the results for an experiment in which an electrode was positioned at a number of different depths in the cerebellar cortex as current was passed for 100 sec at a time, first inward ($+150\,\mu$A) and then outward ($-150\,\mu$A). The site of the tissue surface was judged with a probable accuracy of about $\pm50\,\mu$m from the most superficial response to local surface stimulation (Fig. 5B) and the depths relative to this site of the various recordings are indicated at the left (Fig. 5A). The induced $[K^+]_0$ changes were largest at a site 100 μm beneath the estimated surface. At deeper sites the $[K^+]_0$ changes were smaller and had a different time course: they developed at a more constant rate and declined less after the end of the initial period of current flow. In Fig. 5C these results are superimposed with calculated $[K^+]_0$ changes (dashed lines) based on a model of K^+ uptake and release associated with current flow through a syncytium of cells in brain tissue (Gardner-Medwin, 1983b). The parameters for the tissue model are the same as those employed for comparisons with other types of data elsewhere (Gardner-Medwin, 1983a), with an assumed current density at the tissue surface of 16 μA mm^{-2} (see below). Each of the calculated curves for Fig. 5C is for the same depth as the corresponding data (Fig. 5A), except at the very surface. The boundary condition for the calculations assumes no $[K^+]$ changes at the surface or in the fluid outside, corresponding to perfect stirring and replenishment of the surface fluid. Such replenishment is only approximated in an experiment. The dashed line superimposed with the data from the estimated tissue surface has the time course of the calculated K^+ flux across the surface. Changes of $[K^+]_0$ close to the surface
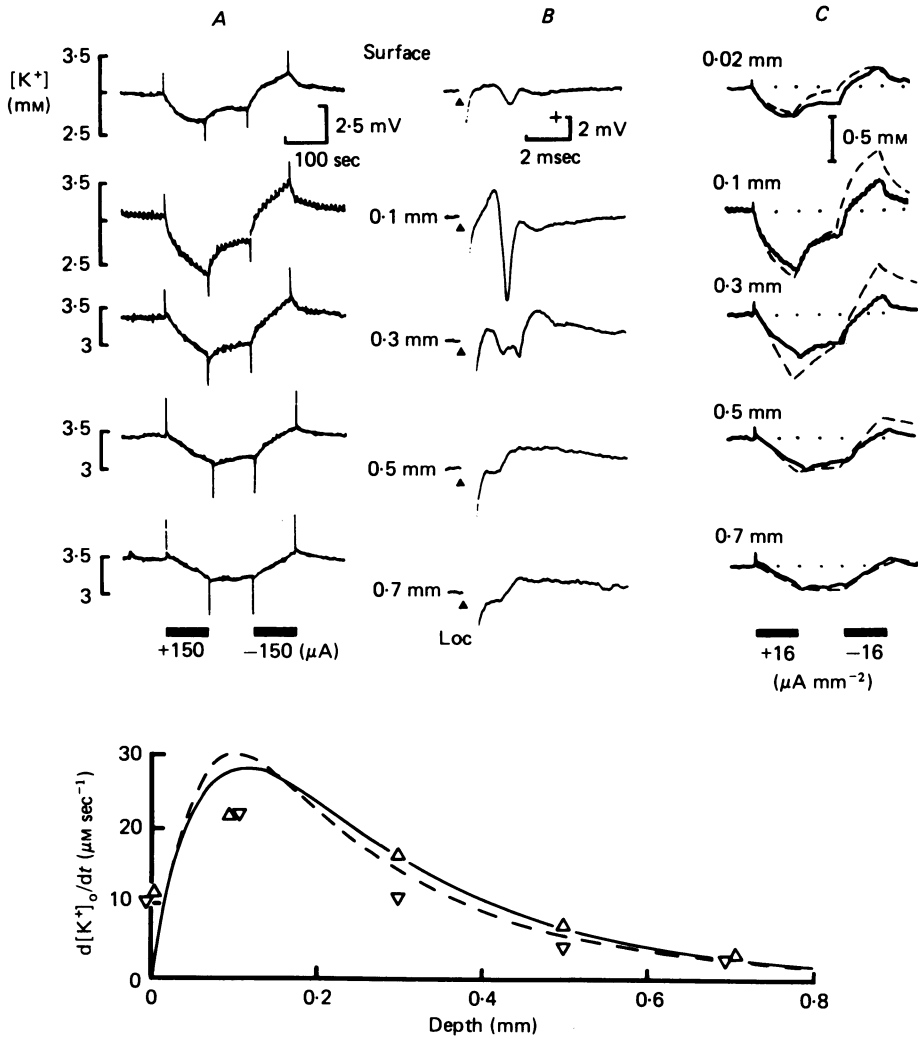
Fig. 5. Changes of $[K^+]_o$ produced by current flow at different depths in the cerebellar cortex. *A*, measurements during successive periods with current passed into the brain $(+150\,\mu A)$ and out of the brain $(-150\,\mu A)$, each for 100 sec, with the K^+-electrode withdrawn in multiples of 0·1 mm between records. Depths are indicated relative to the surface as judged from field potential responses (*B*). Cup diameter: 2·9 mm. *B*, responses to local surface stimulation at each position, recorded between the records in *A*. *C*, records from *A* superimposed with $[K^+]_o$ changes (dashed lines) calculated for a tissue model with simultaneous movement of K^+ through extracellular space and through cells (Gardner-Medwin, 1981). A surface current density of $16\,\mu A\,mm^{-2}$ is assumed, with parameters otherwise as for Figs. 3, 4 and 6 in Gardner-Medwin (1981): $\Lambda = 0{\cdot}2$ mm, $T = 220$ sec. $\beta = 5$. *D*, the average rate of change of $[K^+]_o$ during the first 20 sec after current onset, plotted as a function of depth for positive current (∇) and negative current ($\triangle$) from the data in *A*. Curves are predictions for a current density of $22\,\mu A\,mm^{-2}$ based on the tissue model using linear equations (dashed line) and non-linear equations (continuous line, for positive current only).

are, in the model, proportional to this flux and with the scale employed here would correspond roughly with data expected at 20 μm beneath the surface in the model. If there was a stagnant layer of fluid above the tissue surface, such changes proportional to the flux could be expected slightly above the surface.

Fig. 5D shows the rate of change of $[K^+]_0$ during the first 20 sec of current flow plotted as a function of recording depth for the data of Fig. 5A. This is compared with curves derived from calculations with the model referred to above, for a current density of 22 μA mm^{-2}, equal to the average current density at the bottom of the experimental cup. The calculations were made with both the linear equations for the model (dashed line) and the non-linear equations (continuous line) that are more accurate if there are large disturbances of the tissue parameters from resting levels. The differences between the two sets of calculated curves are slight, as might be expected since the changes of $[K^+]_0$ do not exceed about 20 % (see Gardner-Medwin, 1983b, for fuller discussion). The largest observed changes in Fig. 5D are about 75 % of the calculated changes. In order to compare the time course of the calculated changes with the data (Fig. 5C) the calculated results with the linear calculations were scaled down by this amount and therefore correspond to a currrent density in the model of 16 μA mm^{-2}.

The data and calculations in Fig. 5 show similarities in both depth dependence and time course. The accuracy of comparisons is limited by uncertainties about the data that are hard to quantify: the accuracy of the depth estimates, the thickness of a possible stagnant layer at the fluid surface and the proportion of the current flow that may have leaked under the rim of the cup instead of passing through the tissue. It seems appropriate to conclude only that the principal qualitative features of the data are ones that can be expected to arise through the mechanisms for current-mediated K^+ transport incorporated in the model and that there is broad quantitative agreement (within a few tens of percent) between the data and that expected with the same model parameters as are required to fit other data (Gardner-Medwin, 1983a).

In order to eliminate the possibility that the $[K^+]_0$ changes were the result of neuronal activity induced by the current, several control experiments were made. Measurements of $[K^+]_0$ were made with alternating current pulses in two animals. A recording electrode was positioned in the cerebellar cortex within 100 μm of the surface, approximately at the site of the largest changes observed with steady currents (Figs. 4 and 5). A current of 150 μA was passed for up to 400 sec and the polarity reversed, using a relay, ten times per second so that there was no net transfer of electric charge. Transient artifacts due to the switching prevented reliable measurements of $[K^+]_0$ being made during such current flow, but there was no detectable change of $[K^+]_0$ measured after the current was switched off. This suggests that the transfer of charge through the tissue is important rather than any possible activation of neurones with current flow.

The effect of current flow on the firing pattern of Purkinje cells in the cerebellar cortex was studied in four animals. Extracellularly recorded action potentials were isolated from spontaneously firing neurones at a depth of 200–400 μm beneath the cortical surface with the cup positioned in the normal way. In some experiments, for better mechanical stability, the cup was filled with agar (3 % in saline) without superfusion of the brain surface. Electrode penetrations were made approximately

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in line along a folium from the local surface stimulating electrode. The influence of local surface stimulation on the neuronal firing was examined (Fig. 6B). A compound action potential was always elicited by sufficiently strong local surface stimulation in this way, but it was not possible to identify whether the activated cells included the particular one from which spontaneous action potentials were recorded. After the
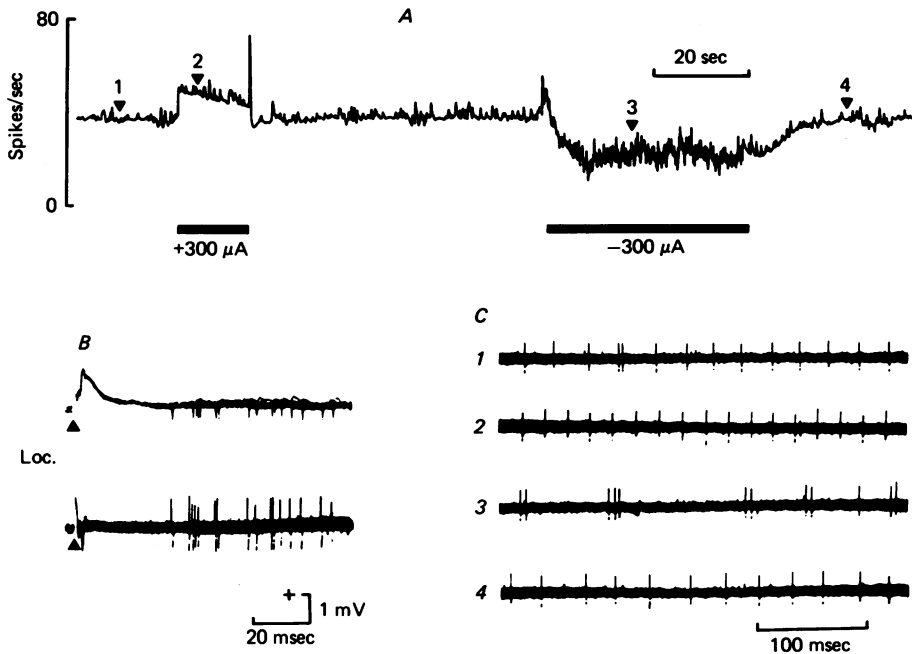

Fig. 6. The effect of current passage on Purkinje cell firing. A, the rate of action potentials recorded with a micro-electrode 0·3 mm beneath the surface is shown as current was passed inward (+300 μA) and outward (−300 μA) across the tissue surface. B, superimposed oscilloscope records of the response from the same electrode site following local surface stimulation (Loc.), recorded directly (upper trace) and with high-pass filtering (lower trace). Note the silent period lasting ca. 30 msec. C, filtered oscilloscope recordings showing the firing pattern recorded at times indicated by 1–4 in A.

excitation, however, there was a cessation of firing for 10–50 msec, revealed clearly when several oscilloscope sweeps were superimposed (Fig. 6B). This is characteristic of the response of Purkinje cells (Eccles et al. 1967). In eleven cells studied, the effects of current flow on spontaneous firing were variable. In five there was no evident influence with currents of 300 μA (twice the strength used for studying $[K^+]_o$ changes) maintained for 30 sec. Five out of six of those that were affected showed an increase of firing with current passed into the brain (+300 μA in Fig. 6A) and a decrease of firing rate with a tendency to clustering of action potentials (3 in Fig. 6A and C) with current in the opposite direction. This result agrees with the results of Brookhart & Blachly (1952) and is to be expected through direct depolarization of the Purkinje cell somata with current inward across the cerebellar surface. The neurone that behaved in the opposite fashion exhibited irregular spontaneous firing and the effect of current may have been due to indirect neuronal influences.
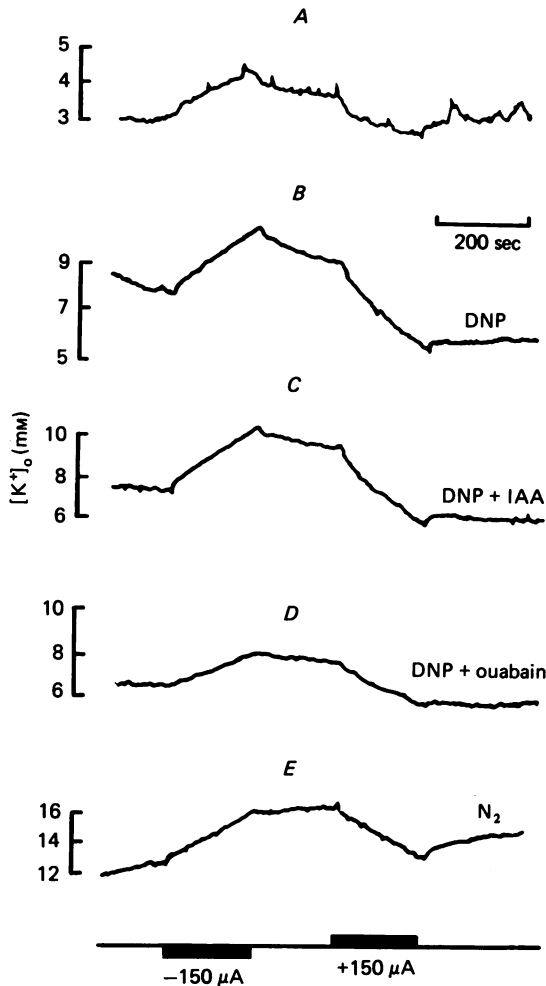
Fig. 7. Changes in $[K^+]_o$ recorded with outward and inward current flow with an electrode *ca.* 200 μm beneath the surface of the cerebellar cortex under normal conditions (*A*) and successively following application of cup fluid containing drugs: *B*, 2,4-dinitrophenol (DNP: 20 mM); *C*, DNP + sodium iodoacetate (IAA: 10 mM); *D*, DNP + ouabain (0·1 mM) and *E* following asphyxiation for 20 min with the tracheal cannula connected to a supply of pure N_2. In *B–D* the drugs were administered for at least 15 min before the records were made.

Tetrodotoxin (10^{-4} M) and manganese (5 mM-Mn^{2+}) had no clear effect on either the base-line $[K^+]_o$ levels or the changes induced by current, even after 1 hr or more. Manganese abolished all signs of post-synaptic responses to local surface stimulation, while with tetrodotoxin there was no electrical activity elicited with local surface stimulation after *ca.* 5 min, and searches with a micro-electrode after about 15 min revealed no cells in the cerebellar cortex from which action potentials could be recorded.

Measurements of $[Ca^{2+}]_o$ during current flow were made in five animals. The Ca^{2+} electrodes (see Methods) were positioned *ca.* 100 μm beneath the cerebellar surface

at sites giving large electrical responses to local surface stimulation, where the largest $[K^+]_0$ changes were normally observed (Figs. 4 and 5). In these sites there was a clear and rapid fall of $[Ca^{2+}]_0$ by 10–30 % during repetitive local surface stimulation (20/sec) from a base line of ca. 1·2 mM, as described by Nicholson et al. (1977). During current passage across the brain surface (150 μA for 400 sec) there were no consistent changes in $[Ca^{2+}]_0$. For each direction of current flow the average change after 400 sec was calculated from the most stable records obtained in each animal. This gave $-6·9$ ($\pm 3·0$ s.e., $n = 5$) % for inward current ($+150$ μA) and $+1·4$ ($\pm 0·9$, $n = 5$) % for outward current (-150 μA). Neither change is significantly different from zero and both are significantly less ($P < 0·1$ %, one-tailed t test) than the average $[K^+]_0$ changes under the same conditions (see above), which were $-42·2$ % ($+150$ μA) and $+65·8$ % (-150 μA) in five animals, including two of the same animals used for Ca^{2+} measurements.

We tested whether the $[K^+]_0$ changes could be abolished by interfering with active transport and metabolic processes in the tissue. Ouabain (0·4 mM) or 2,4-dinitrophenol (20 mM) added to the superfusion solution or asphyxiation with a N_2 supply connected to the tracheal cannula each produced increases in base line $[K^+]_0$ to at least 6 mM after ca. 30 min. The animal died soon after asphyxiation and $[K^+]_0$ rose at about the time of cardiac arrest to levels of 30 mM or more. Changes of $[K^+]_0$ produced by current flow were not abolished with any of these interventions, or with 2,4-dinitrophenol in combination with 10 mM-sodium iodoacetate (Fig. 7). The absolute magnitude of $[K^+]_0$ changes was sometimes increased, probably due to a reduction in size of the extracellular space. We concluded that the mechanism of the $[K^+]_0$ changes, though influenced by the metabolic state of the tissue, did not require metabolic processes or the integrity of neuronal or synaptic function.

DISCUSSION

These results show that current passed through brain tissue can induce changes of $[K^+]_0$ near the tissue surface. Decreases of $[K^+]_0$ were observed with inward current and increases with current in the opposite direction. The effects in the neocortex and cerebellar cortex of anaesthetized rats were broadly similar, though for technical reasons most of the quantitative studies and experiments to examine the effects of drugs were carried out on the cerebellum.

Structures responsible for the $[K^+]_0$ changes

Changes in level of neuronal activity can produce changes of $[K^+]_0$ in brain tissue (see Somjen, 1979, for review). Various lines of evidence suggest that such effects were not significant in contributing to the $[K^+]_0$ changes in the present study. The increases of $[K^+]_0$ were not accompanied by falls of $[Ca^{2+}]_0$, characteristic of the response to stimulation of neuronal activity (Nicholson et al. 1978). The changes of $[K^+]_0$ and of the firing rate of Purkinje cells would be expected to be in the same direction if either effect were directly the cause of the other, whereas in fact they were generally in opposite directions. Lastly, all signs of neuronal activity were abolished with tetrodotoxin (10^{-4} M) in the surface fluid, while the $[K^+]_0$ changes during current flow persisted.

The passage of current through cells can be expected to cause changes of $[K^+]_o$, since the proportion of transmembrane current carried by K^+ is much larger than the corresponding proportion for current in extracellular space. Increases of $[K^+]_o$ are expected wherever there is principally outward current across cell membranes and decreases where the current is principally inward. Fig. 1 shows how transcellular current within the bulk of the tissue (Fig. 1 *B*) and restricted to surface structures (Fig. 1 *A*) would lead to expected changes of $[K^+]_o$ in opposite directions beneath the tissue surface, though both hypotheses could explain the high surface flux of K^+ measured by Gardner-Medwin (1983*a*). The results are qualitatively in agreement with Fig. 1 *B* and inconsistent with Fig. 1 *A*, which rules out an explanation of the results in terms of properties of the surface structures alone.

The results might be explained by some special characteristic of extracellular ion transport, leading to a higher extracellular transport number for K^+ deep within the bulk of the tissue than at the surface. Outward current would then cause K^+ build-up near the surface because of the greater K^+ flux brought up from the depths than transported across the surface. On this hypothesis the distribution of K^+ build-up early during current flow (before diffusion could act to disperse it significantly) would correspond to the zone of transition between the low and high K^+ transport numbers. On the basis of the results (Fig. 5), the transition would have to be gradual over a range of several hundred μm rather than an abrupt transition at the tissue surface. This would be a wholly unexpected characteristic for a property of the extracellular space. Since the K^+ distribution is adequately explained by the hypothesis that $[K^+]_o$ changes are due to current flow through cells in the bulk of the tissue (see below) and since the extracellular transport number for K^+, from quite separate evidence, is unlikely to differ substantially from that in the surface fluid (Gardner-Medwin, 1983*a*) it seems unlikely that an explanation for the results should be sought in extracellular mechanisms.

Current flow through both neurones and glia within the bulk of the tissue will have contributed to the observed effects. Calculations with a simple model involving a single cell type (Gardner-Medwin, 1983*b*) give reasonable agreement with both the time course and the distribution of the $[K^+]_o$ changes (Fig. 5). At the same time, with identical parameters involving five times as much K^+ transfer through cells as through extracellular space ($\beta = 5$) and a space constant for the transfer cells (Λ) of 0·2 mm, the model gives satisfactory agreement for flux and voltage measurements (Gardner-Medwin, 1983*a*). This favours the conclusion that the results are due to K^+ transfer through cells, but it does not identify the relative importance of neurones and glia. In the retina of the honeybee drone it has been possible with the aid of intracellular measurements of K^+ activity (Coles & Tsacopoulos, 1979) to identify glial cells as providing a significant pathway for current-mediated K^+ flux (Gardner-Medwin, Coles & Tsacopoulos, 1981). A contribution of glial cells to the present results is suggested by work of U. Heinemann and I. Dietzel (cited by Nicholson, 1980, p. 241) in which qualitatively similar effects have been observed in gliotic scar tissue largely devoid of neurones. Two other arguments also favour a glial contribution. The principal neuronal current carriers for current perpendicular to the surface of the cerebellar cortex are the dendrites of the Purkinje cells. These terminate (apart from thin axons) 200–300 μm beneath the surface (Palay & Chan-Palay, 1974). The effects

on $[K^+]_0$ of current flow through these cells should be in opposite directions at the two ends of these finite terminated cables. The lack of such a reversal suggests longer cables are involved, or a syncytium of electrically coupled cells such as is postulated for glial cells in mammalian brain (Kuffler & Nicholls, 1976). Lastly, several lines of evidence (reviewed recently by Somjen, 1973, 1981) suggest that glial cells are responsible for causing extracellular current and negativity in regions of central nervous tissue with raised $[K^+]_0$. The present work shows that this negativity (discussed by Gardner-Medwin, 1983 a) can be related quantitatively both to the high K^+ flux per unit current observed in brain tissue (Gardner-Medwin, 1983 a) and to the current-induced $[K^+]_0$ changes described here and that they are all predicted by a single model for transcellular K^+ flux (Gardner-Medwin, 1983 b). It would be surprising if they had their basis in different cell types.

The mechanism of the induced $[K^+]_0$ changes seems to be purely passive in view of their persistence, albeit modified by changed base-line levels of $[K^+]_0$, after application of ouabain and metabolic blockers and after asphyxia. This is consistent with their explanation in terms of the passive transcellular K^+ flux incorporated in the tissue model and the 'spatial buffer' concept for passive glial involvement in K^+ dynamics suggested by Orkand, Nicholls & Kuffler (1966).

The quantitative significance of the results

Extracellular ion-selective electrodes probably underestimate rapid changes of ion activity because of the time required for diffusional equilibration between true extracellular space and a dead space around the electrode tip. Lux & Neher (1973) suggested that the equilibration time would be in the range 8–70 msec on the basis of free diffusion within a dead space of radius 5–15 μm. This supposes that the true concentration to be measured is always maintained at the outer limit of the dead space. If diffusion through the surrounding interstitial clefts is included, with parameters for extracellular diffusion inferred by Nicholson & Phillips (1981), the time for 95 % equilibration is 0·2–1·7 sec for radii of 5–15 μm using equations from Carslaw & Jaeger (1959, section 13·9 (IV)). Even a lag of this order is too small to have affected significantly the $[K^+]_0$ measurements in this paper, since the changes developed and were maintained over tens or hundreds of seconds. The ion activities in the damaged zone around an electrode tip might differ from true extracellular activities even with steady measurements if there is a Donnan equilibrium with a fixed charge matrix in interstitial space; however such effects are probably small in mammalian brain (Nicholson, 1980; Gardner-Medwin, 1983 a).

The most likely interpretation of the results is in terms of K^+ transfer through cells in the tissue (see above). The electrotonic space constant (Λ) of the transfer cells is then reflected fairly directly in the depth distribution of $[K^+]_0$ changes early during current passage and fits with a value of about 0·2 mm (Fig. 5 D). The time course of the $[K^+]_0$ changes at each depth agrees reasonably well with calculations based on the tissue model (Fig 5 C) while their size is within about 20 % of that expected on the basis of the nominal current density at the tissue surface ·(Fig. 5 D). These aspects of the results, though consistent with the inferred parameters of K^+ dynamics, would probably not provide the best data from which to estimate these parameters since the inter-relationships are complex (Gardner-Medwin, 1983 b: Fig. 3 C).

The ratio of tissue K^+ flux to that expected for purely extracellular transport has been estimated as *ca.* 5:1 from measurements of K^+ flux induced by current flow at the brain surface (Gardner-Medwin, 1983*a*). The changes of $[K^+]_0$ described here suggest that the tissue flux at depths of a few hundred μm must have been greater than at the surface to account for K^+ build-up (with outward current) or depletion (with inward current). A rough estimate of the discrepancy can be derived from Fig. 3*B* (filled circles), which shows the $[K^+]_0$ distribution under approximately the conditions after a period of flux measurement with outward current (-20 μA mm^{-2}, 400 sec). Integrating the excess of $[K^+]_0$ over the cup level, from the surface to 1·5 mm depth, gives *ca.* 0·75 mM . mm. The change of tissue K^+ content, in m-mole per litre of tissue, under such circumstances is probably about equal to the change of $[K^+]_0$ in mM (Gardner-Medwin 1983*b*: $\xi = 1$). We can estimate therefore that the superficial build-up of K^+ within the tissue was roughly 0·75 n-mole per mm^2 of surface. When added to the observed surface flux (5 n-mole mm^{-2}, corresponding to a K^+ transport number of 0·06: Gardner-Medwin, 1983*a*) this indicates that the deep flux was probably about 15% greater than the surface flux, or 5·7 times the expected extracellular flux. The more rigorous analysis of the equations for transport suggests that the total flux deep in the tissue must be about six times the expected extracellular flux (i.e. $\beta = 5$: Gardner-Medwin, 1983*b*) to account for the observed flux.

The significance under more general circumstances of these inferred characteristics of K^+ transport in brain tissue is discussed elsewhere (Gardner-Medwin, 1981; 1983*b*).

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