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A STUDY OF THE MECHANISMS BY WHICH POTASSIUM MOVES THROUGH BRAIN TISSUE IN THE RAT

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SUMMARY

1. The flux of K^+ produced by electric current across the pia-arachnoid surface of the neocortex of anaesthetized rats has been studied with K^+-selective electrodes in a cup at the surface and with flame photometry.

2. The potential differences developed across three regions of the rat brain (neocortex, cerebellum, hippocampus) have been measured as $[K^+]$ was altered in fluid at the surface.

3. The experimental results have been related to those that would be expected (i) if K^+ moved principally by diffusion in extracellular space and (ii) if current flow through cells makes a significant contribution to K^+ transfer.

4. K movement produced by current across the neocortical surface accounted for 0·06 of the transfer of electric charge with small currents in either direction (*ca.* 5 μA mm^{-2}) and with larger currents out of the tissue. Large currents (*ca.* 20 μA mm^{-2}) into the tissue produced less K^+ movement, but still more than the fraction 0·012 expected for purely extracellular flux.

5. Alternating current pulses (5 Hz) with zero net transfer of charge produced no flux of K^+ across the surface, while alternation with unequal durations produced the same effects as the equivalent steady charge transfer.

6. The K^+ flux lagged behind the onset and cessation of current with a time constant *ca.* 45 sec, approximately as expected from calculations with a model of the tissue.

7. A surface-negative potential shift averaging 2 mV was observed when $[K^+]$ at the brain surface was increased from 3 to 12 mM. The time for development of half of the full potential change was 20 sec, with the solution changes complete in less than 4 sec.

8. These results are inconsistent with the hypothesis that K^+ movement through brain tissue occurs principally through intercellular clefts, except where these movements involve very localized gradients. They are consistent with the conclusion that *ca.* 5 times as much K^+ flux passes through cells (probably largely glial cells) as through extracellular space, with fluxes driven by either extracellular voltage or concentration gradients.

INTRODUCTION

The movement of potassium in brain tissue from one region of extracellular space to another and the uptake of potassium into cytoplasm are important factors affecting potassium concentration changes around active nerve cells (Nicholson, 1980). This and the following paper (Gardner-Medwin & Nicholson, 1983) are concerned with mechanisms of potassium movement, in particular with the relative importance of the K^+ fluxes through cells and directly through the extracellular space. This has proved a controversial issue ever since it was first considered by Kuffler and his colleagues (Kuffler & Nicholls, 1966). The substantial contribution due to transcellular flux suggested in these papers goes against the interpretation suggested by other recent work (Lux & Neher, 1973; Fisher, Pedley & Prince, 1976; Somjen & Trachtenberg, 1979). In a separate analysis (Gardner-Medwin, 1983) the relationships between several critical types of data on K^+ dynamics are treated using diffusion theory and a specific model for ion transport and cytoplasmic uptake.

The release of K^+ during action potentials and the consequent build-up of K^+ in extracellular space were established for the squid axon by Frankenhaeuser & Hodgkin (1956). In brain tissue the substantial increases of extracellular K^+ concentration ($[K^+]_o$) that occur with neural activity are probably due partly to fluxes during action potentials and partly to increases of permeability produced by synaptic transmitters (see Somjen, 1979, for review). These changes of $[K^+]_o$ constitute disturbances of the brain cell microenvironment that may significantly affect the balance of interactions between neurones, even with normal physiological activity (Somjen, 1979). If $[K^+]_o$ rises to pathological levels it can result in a further regenerative release of K^+, probably involving non-specific transmitter release, in a process known as 'spreading depression' (Leão, 1944; Bures, Buresová & Krivánek, 1974; Nicholson & Kraig, 1981). Spreading depression may be the underlying mechanism for the profound cortical dysfunction during certain migraine attacks (Milner, 1958; Gardner-Medwin, 1981*a*, *b*). Buffering processes that reduce or disperse a potassium build-up may affect the threshold for spreading depression as well as normal neural interactions and are therefore of considerable interest.

Potassium ions will move passively between different regions of extracellular space whenever there is an electrochemical gradient for K^+. The experiments in this paper are of two sorts, dealing with situations with principally electrical and chemical gradients. Information about the relative roles of transcellular and extracellular K^+ movement is inferred most simply from the experiments involving electrical gradients (i.e. those involving current flow through the tissue). The proportion of current flow associated with K^+ flux (the K^+ *transport number*) can be inferred with reasonable confidence for the extracellular space (see Discussion) and any contribution due to transcellular K^+ flux will tend to increase this transport number for the tissue as a whole. The first experiments, therefore, involve estimation of the K^+ transport number for rat cortex, from measurements of the net K^+ flux produced by current flow across the tissue surface. These observations are discussed in relation to measurements of $[K^+]_o$ beneath the surface made under similar conditions with K^+-selective micro-electrodes (Gardner-Medwin & Nicholson, 1983).

A potassium flux through cells when there is a $[K^+]_o$ gradient leads to a pattern

of current flow in and around the cells. The transcellular K^+ flux represents current flow through the cell membranes and cytoplasm in the direction of the flux, with the return current path through extracellular space. Such currents, probably flowing principally through glial cells, have long been accepted as the likely explanation for the negativity in extracellular space commonly observed in association with raised $[K^+]_o$ (Somjen, 1975). In principle one might measure the current flow to estimate transcellular K^+ flux and the extracellular diffusion parameters to estimate K^+ diffusion in extracellular space and compare the two. In practice the extracellular voltage gradient can be more directly measured than the extracellular current flow. Since the extracellular resistivity is closely related to the extracellular diffusion parameters, it is possible to argue quite directly from the observed voltage gradients to the ratio of transcellular and extracellular fluxes (see Discussion). Thus the second group of experiments involves the measurement of the voltage differences developed across brain tissue with controlled changes of $[K^+]$ at the tissue surface.

In both groups of experiments the interpretation based on the simple arguments outlined above is also supported by calculations based on a numerical model involving a single class of K^+ transfer cells and specific assumptions about cytoplasmic uptake of K^+ (Gardner-Medwin, 1983). Theoretical curves based on these calculations are presented here for comparison with the data. The theoretical analysis is necessary if a full assessment is to be made of the extent to which a single model can account for different types of data (Gardner-Medwin, 1983); however the present paper is self-contained in drawing the principal conclusions about the relative magnitude of transcellular and extracellular K^+ fluxes.

Particular interest attaches to the possibility that neuroglia may be involved in the buffering of $[K^+]_o$ in neural tissue (Varon & Somjen, 1979; Treherne, 1981). The possible role of glial cells in providing current-mediated transcellular K^+ flux is known as the 'spatial buffer' hypothesis, proposed by Orkand, Nicholls & Kuffler (1966). The differential depolarization of glial cells in a gradient of $[K^+]_o$ leads to current flow and K^+ transfer through the cells and their electrical junctions, with the return current carried largely by movement of sodium and chloride. The quantitative significance of this mechanism compared to extracellular diffusion has not been clear. Support comes from experiments by Trachtenberg & Pollen (1969), showing that mammalian glial cells have a short input time constant suggestive of a high glial membrane conductance, but this interpretation is open to question when the branching nature of glial cell processes is considered (Gardner-Medwin, 1983). Evidence that glial cells may contribute to the K^+ transfer involved in the present experiments is discussed by Gardner-Medwin & Nicholson (1983).

Preliminary results and conclusions from this work have been published (Gardner-Medwin, 1977, 1980; Gardner-Medwin, Gibson & Willshaw, 1979).

METHODS

Animals. Rats (200–350 g) were anaesthetized with urethane I.P. (5·7 ml./kg: 25 % w/v in 0·9 % NaCl). Rectal temperature was maintained at 35–38 °C by heat from above and below. The trachea was cannulated and the head was held in a frame, with precautions to ensure electrical isolation from earth. One of three regions of brain tissue was exposed: the pia-arachnoid surface of the parietal neocortex (through a 6 mm diameter opening in the left parietal bone, extending to within

1 mm of the mid line), the pia-arachnoid surface of the cerebellum (through a 4 mm diameter mid-line opening in occipital bone, with the head tilted forward), or the dorsal ventricular surface of the left hippocampus with parietal neocortex and corpus callosum partially removed by suction (Bliss & Gardner-Medwin, 1973). In each case the final exposure (i.e. removal of the dura or of the tissue overlying the lateral ventricle) was performed carefully under warmed saline and was immediately followed by positioning of a Perspex or glass cup (described below) on the brain surface. The cup was filled with saline, and adjustments were made until the fluid would not run out. On removal

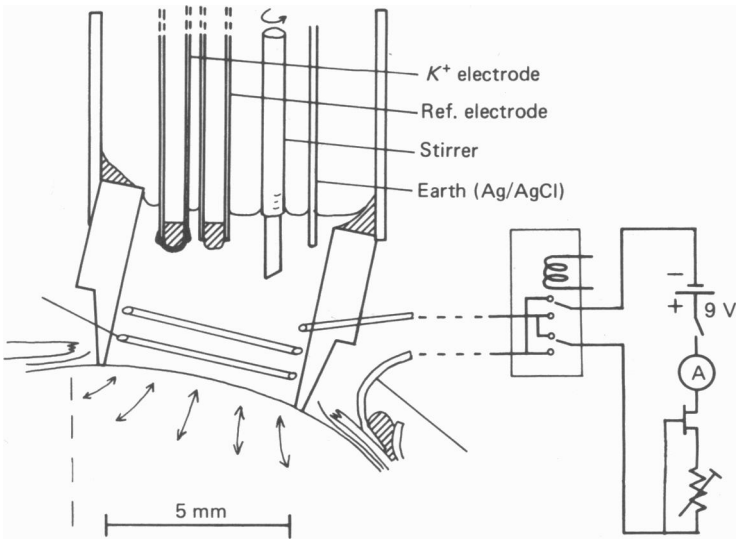

Fig. 1. The Perspex cup and associated equipment positioned on the pia-arachnoid surface of the rat neocortex. The transistor (FET 2N3819) and variable resistor (100 kΩ) permit a constant current to be set, which is unaffected by electrode polarization.

of the cup after several hours a circular dimpling was usually evident at the rim of the cup, but the blood vessels both then and during an experiment looked normal over the whole area. Preparations were rejected if bleeding was seen within the cup. In some experiments on the neocortex, electrode penetrations were made with 3 M-NaCl-filled micropipettes, but to ensure that the cortex was undamaged these were never made before a period of flux measurements.

Apparatus for K^+ flux measurement during current passage. The flux of K^+ across the neocortical surface was deduced from measurements of changes of $[K^+]$ within a Perspex cup (Fig. 1: 5 mm i.d., 6 mm o.d.; area 20 mm^2) on the cortical surface. The cup was filled to a standard depth, at which it contained 130 μl. (measured by absorbing the fluid with cotton wool and weighing it). A 50 mm length of Ag wire (0·3 mm diameter) was formed into a coil within the cup and sealed through its wall with acrylic cement; it was then heavily chlorided by passage of 0·5 mA for an hour in a solution of NaCl. A draught shield was attached to the cup to reduce evaporation. The fluid was stirred constantly with a rotating nylon paddle (1 mm across, *ca.* 500 rev/min).

In some experiments an extra mixing device was used to displace stagnant fluid at the bottom of the cup near the brain surface. This consisted of a glass tube into which 20 μl. fluid was alternately sucked and expelled. The tube was made from 2 mm electrode glass, tapering to a 0·5 mm nozzle near the brain surface. The cycle of fluid movement (once per 1·5 sec) was produced by connecting the tube simultaneously to the inlet and exhaust ports of a Palmer respiration pump, with a pipe connecting the other two ports together. This gave a slow intake of fluid with a rapid expulsion.

During experiments in which electrical measurements were made in the cup the animal was earthed solely through a chlorided Ag wire dipped in the cup. Currents, controlled with a field-effect transistor (2N3819) and potentiometer (Fig. 1) were passed between the chlorided Ag coil in the

cup and a similar wire in muscle on the contralateral side of the skull. A pair of reed-switch relays permitted reversal of the direction of the current. For some experiments in which rapid alternation of the current direction was required, the relays were operated with pulses *ca.* 100 msec in duration every 200 msec. The precise duration was adjusted to give the required mean current (commonly zero), measured with a moving-coil DC ammeter (Avometer).

Measurements of $[K^+]$ in the cup were monitored continuously in most experiments with a K^+-selective PVC membrane electrode containing valinomycin and potassium tetraphenylborate (Band, Kratochvil & Treasure, 1977). The membrane was moulded on the outside surface of a porous ceramic plug inserted in a length of PVC tube (Fig. 1). The potential difference was measured between two calomel electrodes connected through saline bridges to the inside of this membrane and to a similar reference junction with a ceramic plug and without the K^+-selective membrane. The saline used in the bridges was the same as in the cup, with $[K^+] = 3$ or 3·5 mM. The electrodes lasted for many experiments and for several weeks, during which time the fluid would be replaced several times, especially when bubbles developed. Calibration tests using solutions made up with $[KCl] = 1{\cdot}5-12$ mM and $[NaCl]+[KCl] = 153$ mM yielded an accurate linear dependence of the potential difference on the logarithm of $[K^+]$, with a slope of 53–56 mV per $\log_{10}$ unit at room temperature (20–22 °C). The slope never changed detectably between the beginning and end of an experiment, while changes in DC level were less than 1 mV over a period of several hours. Measurements were recorded to 0·1 mV accuracy with a FET input differential amplifier, a digital voltmeter and a printer. When measurements were made during a period with passage of current through the cup, the current was switched off for 5 sec around the time of the measurement to eliminate any errors from voltage gradients within the cup.

In some experiments, K^+ concentration in the cup was measured with flame photometry. Samples of fluid were removed with a 50 μl. Lang-Levy micro-pipette and were diluted with 5 ml. or 10 ml. distilled water for flame photometry with a Corning 100 or 150 Photometer. This method proved less sensitive and accurate than the use of K^+-sensitive electrodes and suffered from the drawback that the cup contents were destroyed for each measurement.

Apparatus for measurement of voltage changes induced by changes of superfusing fluid. Simpler cups were used in these experiments than that shown in Fig. 1, with inside diameters 1·03, 2·1 and 3·2 mm at the brain surface. With a light smear of silicone grease on the lower rim, these formed a good seal on the limited area available on the dorsal surface of the hippocampus or on a single folium of the cerebellar vermis, as well as on the neocortex. Fluid entered through a fine tube (0·5 mm diameter) with its outlet positioned near the brain surface. This led from a small two-way tap constructed of stainless-steel tubing and Teflon. The total inflow dead space (tap + tube + 3·2 mm diameter cup) was 120 μl. The supplies to the tap were from two bottles containing saline (see below) at room temperature at a pressure head of 50 cm. The flow rate was *ca.* 4 ml./min, producing a change of fluid composition after changing the tap, with a latency of 1–1·5 sec. The change was complete within a further 4 sec at all points within the largest cup, assessed by changing the fluid between normal and half-strength saline and observing the conductivity change of the fluid by measuring the voltage shifts produced in one barrel of a θ-tubing micro-electrode with current pulses passed down the second barrel. Fluid changes at the brain surface and in the smaller cups were probably more rapid. The arrangements for outflow differed in the three cups. The largest cup was open to the atmosphere with outflow upwards through a 1·2 mm diameter nozzle into a 3 mm diameter flexible siphon pipe to an overflow, adjusted to maintain a fluid depth *ca.* 3 mm within the cup. The smaller cups widened at the top to form a sealed connexion to the siphon pipe. For these cups the overflow was adjusted to give a subatmospheric pressure of a few mm H_2O within the cup, to aid the seal of the cup to the tissue.

Voltage differences were measured between two chlorided Ag wires connected through saline bridges to the preparation. The reference bridge was filled with 3 M-KCl with a 0·5 mm diameter cotton wick in a pool of saline surrounding the cup. The second electrode was a glass micro-electrode (3 M-KCl, 2 μm diameter) in the open cup or a nylon tube (0·5 mm diameter) filled with the normal saline solution, with its tip sealed into the chamber of the closed cups. The animal was earthed through a Ag wire embedded in neck muscle.

Solutions. Most experiments were performed with a simplified artificial cerebrospinal fluid, consisting of 150 mM-NaCl + 3 or 3·5 mM-KCl. In some flux measurements and in all of the experiments involving continuous superfusion of the brain surface, 1 mM-$CaCl_2$ was added and

10 mM-NaCl was replaced by 4 mM-Na_2HPO_4 + 2 mM-NaH_2PO_4, with pH adjusted to 7·0 or 7·4. The differences of composition had no apparent effect on the flux measurements. Alterations of $[K^+]$ in the saline were produced by NaCl/KCl substitution.

Calculation of predicted results. A simple model for transport of K^+ by diffusion and current-mediated transfer through cells is set out in a separate paper (Gardner-Medwin, 1983). The principal results in the present paper are compared with calculated predictions employing (a) the linear equations (15, 22 in the analysis) that accurately express the model behaviour for small deviations from resting conditions (dashed lines in Figs. 3, 4 and 6) and (b) the non-linear equations (14, 21) for the model under more general conditions (continuous lines). The continuous lines refer more accurately to the conditions of the experiments, which sometimes involve substantial deviations from resting levels. The linear calculations (dashed lines) are included to show to what extent the qualitative features of the predictions arise from only the basic aspects of the model, ignoring second-order effects such as the non-linear dependence of membrane potential on $[K^+]_o$. The linear solutions require the specification of only three parameters in the model: $\beta = 5$, $T = 220$ sec, $n_B = 0{\cdot}012$. The full solutions require additional parameters: $V_r = -80$ mV and, in the current-passing experiments (Figs. 2 and 4), $\Lambda = 0{\cdot}2$ mm. Imposed currents are assumed to be uniformly distributed over a tissue area equal to the area of the cup (20 mm^2). These extra assumptions are not critical, since the differences between the results of the linear and non-linear calculations are minor in most cases. The significance of the assumed parameters is discussed by Gardner-Medwin (1983).

RESULTS

Changes of $[K^+]$ in the cortical cup without passage of current

Even without current passage there were slow $[K^+]$ changes that had to be taken into account in deducing the effects of current flow. These $[K^+]$ base-line changes were probably due to two factors: concentration of the cup contents due to evaporation of fluid and K^+ exchange with the brain. The measurements described here were made with the valinomycin-based K^+-sensitive electrodes (see Methods).

With fluids in the cup having $[K^+] = 3$–$3{\cdot}5$ mM there was often a gradual upward drift of $[K^+]$: at 3·5 mM varying from zero to 0·3 μM sec^{-1} (i.e. up to 0·01 % sec^{-1}). During current flow these changes were treated as base-line drifts and differences were measured from the extrapolated base line. There was some tendency for the base-line drift to become larger over a period of several hours during an experiment, though such changes were smaller than the variation between animals. There was no relationship between the size of changes seen with current and the extent of the base-line drift.

The changes seen without passage of current depended on $[K^+]$ in the cup. A level of 3·5 mM was most commonly used since the drifts were then low enough to be satisfactory for the measurement of induced changes. In two experiments systematic changes of $[K^+]$ were made to study how the rate of change of concentration depended on the concentration itself, in the range 1·5–12 mM. The relationship was closely linear, with a rate of rise of $[K^+]$ given by an expression of the form $\frac{1}{T_0}(c_0 - c)$, where c is the concentration in the cup and T_0, c_0 are constants. In the two experiments the values of T_0 and c_0 giving the best fit were: $T_0 = 4500$, 3900 sec; $c_0 = 3{\cdot}7$ and 4·4 mM respectively.

The concentration dependence of the $[K^+]$ drift is presumably due mainly to net flux of K^+ between the brain tissue and the surface fluid (Bradbury & Davson, 1965). Since the area of exposed brain surface (20 mm^2) and the volume of fluid in the cup

(130 μl.) are known, it is possible to calculate an exchange constant for the clearance of K^+ from the cup. Expressed as a volume of cup fluid cleared of excess K^+ per unit time over unit area of the cortical surface, this is 8–10 μl. min^{-1} cm^{-2}. The changes of $[K^+]$ were so slow (T_0 represents the time constant for approach of the cup $[K^+]$ to a steady level: more than 1 hr) that the results are probably affected somewhat by evaporation losses from the cup. These were minimized by use of a draught shield attached to the cup (Fig. 1); but the apparatus was designed to measure rapid fluxes with a good time resolution and some evaporation (*ca.* 20 % per hr) resulted from the stirring arrangements. The effect of this would be to over-estimate the concentration required for zero K^+ exchange and to underestimate the exchange constant, each by the same factor. Judging from the discrepancy between the values of c_0 (3·7–4·4 mM) and the concentration found by Bradbury & Davson (1965) for zero net flux (2·9 mM), it seems that the error factor could be of the order of 1·5 and the true constant for exchange of K^+ with the brain might be up to 50 % higher than stated above, i.e. up to 15 μl. min^{-1} cm^{-2}. There appear to be no previous measurements in the literature of the net exchange of K^+ with a known area of brain surface, but these figures are consistent with the estimate of 40–60 μl. min^{-1} obtained using different techniques for the entire ventriculo-cisternal system of the rabbit, probably involving a few cm^2 of ependymal surface (Bradbury & Davson, 1965; Bradbury & Štulcová, 1970).

A steep rise of $[K^+]$ in the cup took place when an animal died or was killed with an overdose of urethane (I.P.). Within 1–2 min of the cessation of breathing, $[K^+]$ in the cup fluid rose abruptly at a rate of 1–2 μM sec^{-1}, corresponding to an efflux of K^+ from the brain of the order of 1 n-mole cm^{-2} sec^{-1}. This efflux is presumably caused by the increase of extracellular $[K^+]$ known to occur in the central nervous system during anoxia (see Hansen, 1981, for review).

K^+ flux produced by current flow

Fig. 2 shows the effects on $[K^+]$ measured in the cortical cup of currents passed across the brain surface for periods of 440 sec at a time. During each such period the current was switched off for 5 sec every 50 sec to allow $[K^+]$ to be measured without any bias from voltage gradients within the cup (open circles). The current thus flowed for 400 sec during the total of 440 sec. Periods with current in each direction were normally alternated to minimize any cumulative effects. Currents passing out of the brain (described as negative currents, since for these the cup electrode was the cathode) produced increases of $[K^+]$ in the cup. The rate of increase did not change abruptly as the current was switched on and off, but lagged behind significantly, so that the total transfer of K^+ into the cup was not complete until about 300 sec after the end of the current passage (Fig. 2). Currents passed into the brain produced similar, though smaller, changes in the opposite direction. The effects of alternating currents ($\pm$0·4 mA) are described below.

The changes produced with steady currents were assessed by measurement of the total change of $[K^+]$ between the base line before passage of current and the steady level attained afterwards. When there were significant base-line drifts a measurement was made between the two extrapolated base lines, which were normally sufficiently parallel for no uncertainties greater than about 10 % to be introduced. The total

change in $[K^+]$ depended on the size of the current and on its duration. The total charge flow across the brain surface was calculated as the product current × duration, and the change in $[K^+]$ is plotted against this in Fig. 3 for forty-two periods of current passage in eleven animals, each with the same time course as for Fig. 3.

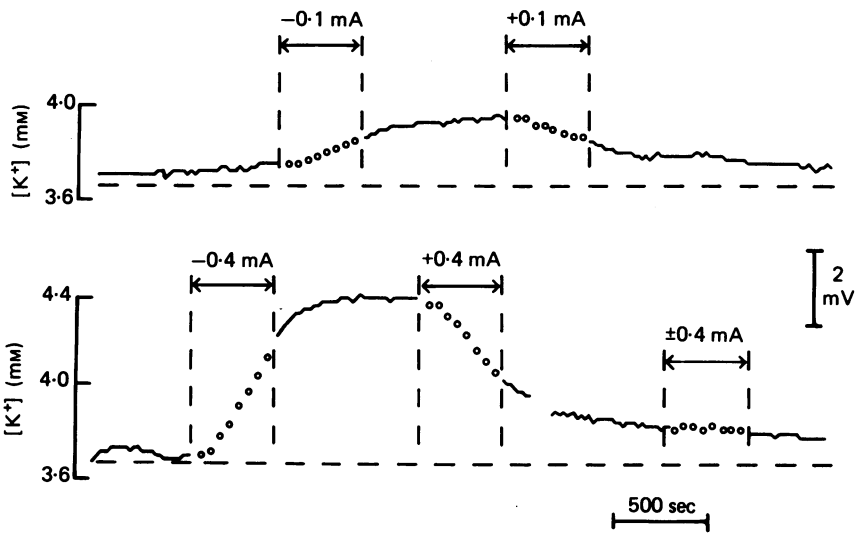

Fig. 2. Measurements of $[K^+]$ in a cup on the cortical surface, made with a K^+-sensitive electrode. Changes were induced by passage of currents from the brain to the cup (−ve currents) and from the cup to the brain (+ve currents) while there was no change with alternating current pulses (±0·4 mA, 5 Hz) with no net passage of charge. Circles: measurements during the period (440 sec) with current flow, with the current briefly switched off (see Methods).

The data in Fig. 3 can be used to estimate the effective transport number for K^+ movement across the tissue surface:

$$K^+ \text{ transport number} = \frac{\text{change of } [K^+] \text{ in cup} \times \text{fluid volume (130 } \mu\text{l.)} \times F}{\text{current passed} \times \text{duration}}$$

The dotted line (Fig. 3) shows the results that would correspond to a transport number of 0·012, as expected for current flowing in the brain extracellular fluid (see Discussion). For all values of current in each direction the transport number was greater than this figure. The mean transport number for current out of the brain (negative) was 0·061 (±0·012 S.D., $n = 28$) and for current into the brain (positive) it was 0·039 (±0·013 S.D., $n = 18$). The continuous and broken lines in Fig. 3 show results that are calculated as described under Methods for a model with both extracellular and transcellular flux through a network of transfer cells (Gardner-Medwin, 1983). The parameters correspond to a ratio transcellular: extracellular flux deep within the tissue of 5:1. The linear and non-linear calculations (broken and continuous lines respectively) give curves that do not differ substantially. Agreement of the data is satisfactory for negative (outward) currents but the flux is significantly

less than predicted ($P > 99{\cdot}6\%$) for the largest positive (inward) current. This discrepancy with inward current may be due to the substantial fall in $[K^+]_o$ near the surface (Gardner-Medwin & Nicholson, 1983) causing a loss of cell K^+ through reduced Na–K pumping: an effect not incorporated in the model (Gardner-Medwin, 1983).

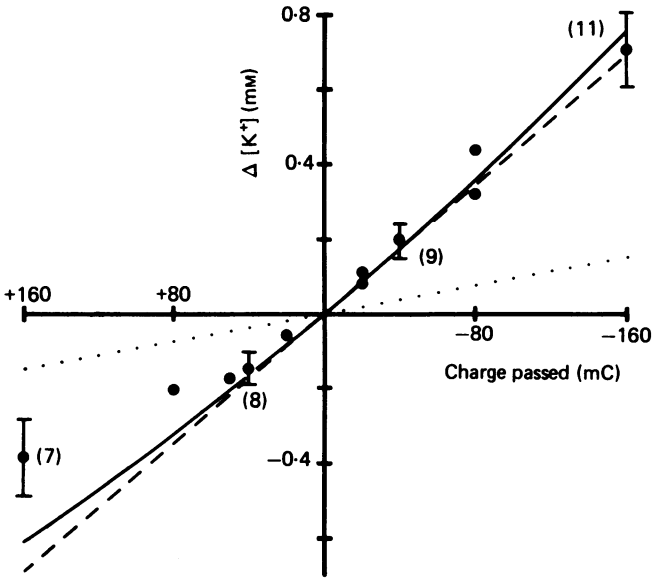

Fig. 3. Total changes of $[K^+]$ in the cup plotted as a function of the total charge passed in periods of current flow as shown in Fig. 2. The data are pooled from eleven animals, with mean ± S.D. indicated at values of charge for which there were several observations (the number indicated). Duration of current flow: 400 sec in 440 sec with intermittent breaks for measurements. Dotted line: expected results if K^+ flux accounted for 0·012 of the current (i.e. expected if there were only extracellular flux). Curves: results expected on the basis of a simple model of the tissue (Gardner-Medwin, 1983) employing the full equations (continuous line) and linear approximations (dashed line), using parameters stated in Methods.

An alternative means of calculating the K^+ transport number was to measure the rate of change of $[K^+]$ in the cup once this had reached a steady level after current onset (Fig. 2). The base-line drift was subtracted and the gradient was compared with the current in the same manner as above. These results lay within the same range as those from the total K^+ movement and the total charge passed. This agreement corresponds to the fact that approximately the same amount of K^+ continued to move after the cessation of current as had seemingly failed to move early on during current passage.

Flux produced with square-wave alternating currents

In some experiments the direction of current flow was reversed 10 times per second, with the current set to 0·4 mA. The average d.c. current could be adjusted and measured (see Methods). This provided a means of stimulating the tissue without any

net passage of current. As with steady currents, the alternating current was turned off for periods of 5 sec every 50 sec for measurements during the period of stimulation. In experiments on five animals the alternating regime was employed with a zero mean current: in no case was there any detectable change of $[K^+]$ in the cup attributable to the stimulation over a period of 400 sec (Fig. 2). In two animals the mean current was set to non-zero values. Mean currents of 100–125 μA from brain to cup were employed and produced changes with the same time course and magnitude as the effects of steady currents of the same size. Thus it is the total charge passed through the tissue that accounts for the results, irrespective of whether the experimental procedure involves large intermittent currents.

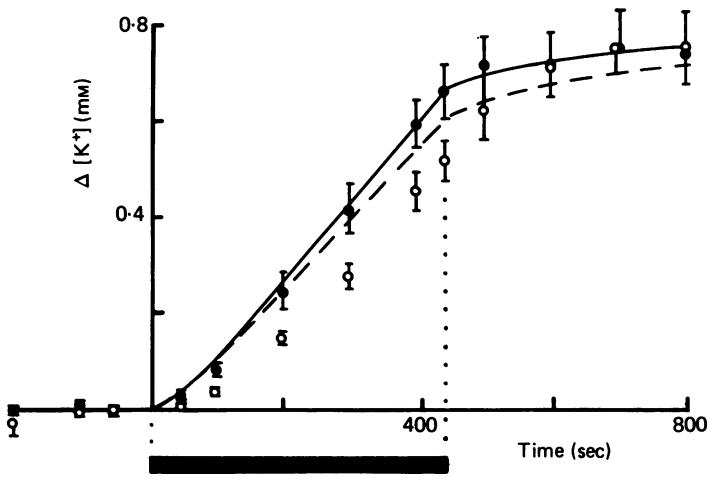

Fig. 4. Comparison of the time course of $[K^+]$ changes measured in the cortical cup with stirring only (○) and with, in addition, intermittent displacement of fluid from the cortical surface (●). Horizontal bar: period during which current (−0·4 mA) was passed for 400 sec. Vertical bars indicate mean ± S.D. ($n = 4$). Concentration differences are referred to the values at the onset of current, with correction for base-line drift of 0·013 mM min^{-1}. Curves: results expected from calculations using the simple tissue model (see Fig. 3 and Methods).

The time course of K^+ flux associated with prolonged current flow

As is evident in Fig. 2, the K^+ concentration in the cup did not start to change immediately at a steady rate when the current was switched on or off. One component of this lag might be due to inadequate stirring of the cup contents. All the data were obtained using a rotating stirrer in the cup (Fig. 1, see Methods). Since no flux lag was evident in experiments with agar blocks in place of brain tissue (see below) it was originally thought that this stirring must be adequate. This conclusion was wrong, however, since tests with small amounts of dye ejected from a micropipette revealed regions near the brain surface where the dye took several tens of seconds to disperse. Since more K^+ is carried through brain tissue than is carried by current through the cup saline, substantial gradients of concentration might be expected to

develop near the surface. These would be absent with an agar block, where the K^+ build up would occur initially close to the cathode in the cup before it was dispersed through stirring action. In order to measure the time course of the K^+ flux more accurately it was necessary to displace the stagnant fluid layer at the brain surface. This was done with a device that sucked up fluid and expelled it in a stream across the brain surface (see Methods), in addition to the normal stirring. Tests showed that dye ejected at sites close to the majority of the brain surface (except the very edge of the cup) was dispersed within 5 sec.

Fig. 4 shows results from measurements made with and without the additional mixing arrangements. Two sets of measurements under each condition were made on each of two animals. The electrical measurements were converted to concentrations using the calibration curve for the K^+-sensitive electrodes and the differences in cup concentration from the time of onset of current were averaged and plotted for the two conditions. With extra mixing (filled circles), a lag of the $[K^+]$ change in the cup was still evident but was less than with stirring alone (open circles). An index of the lag can be obtained by extrapolation of the approximately linear rise of $[K^+]$, late during current passage, back to the base line and measurement of the intercept relative to the time of current onset. With stirring alone this gave 95 sec and with the improved mixing 45 sec. The time course obtained with improved mixing was close to that expected from calculations with the numerical model involving transcellular flux (continuous line, Fig. 4). The total eventual increase in $[K^+]$ in the cup was not affected significantly by the improved mixing and was equivalent in these experiments to a K^+ transport number 0·064 ($\pm$0·013 S.D., $n = 8$).

Flame photometer data

Measurements of $[K^+]$ with flame photometry were used in two ways. In preliminary experiments flame photometry was the only technique used to measure the change in $[K^+]$ when currents up to 1 mA were passed for various times (60–1200 sec). The changes in $[K^+]$ seen when the cup was stirred for an equal length of time without passage of current were subtracted. The samples were taken within 15 sec after cessation of current and will thus have underestimated the total K^+ movement somewhat because of the lag of the K^+ flux described in the previous section. The cup fluid was mixed by stirring only in these experiments. For outward (negative) current the average K^+ transport number from these measurements was 0·059 ($\pm$0·035 S.D., $n = 8$), close to the value (0·061) from electrode measurements. With positive currents the flame photometer data gave 0·023 ($\pm$0·009 S.D., $n = 6$), significantly less ($P > 95\%$) than the electrode measurements. In view of the non-linear relation between current and flux revealed with the electrode measurements (Fig. 3) the low transport number for inward current in these experiments was probably due to the high currents employed, averaging 0·72 mA compared with a maximum of 0·4 mA for the experiments with electrode measurements. With both current directions the transport numbers measured with the flame photometer were significantly higher than the value (0·012) expected for extracellular transport ($P > 98\%$, 95% for negative and positive currents respectively).

In two experiments flame photometry was used to analyse the cup fluid after continuous monitoring of changes with K^+-sensitive electrodes. Agreement of $[K^+]$

measurements within 5 % was always obtained, despite changes of $[K^+]$ up to a factor of two. Thus the measurements cannot be ascribed to changes of other ion concentrations or to biological substances in the cup that might have affected the electrodes.

Voltage changes associated with current flow

The voltage gradients within the superficial cortical tissue near the centre of the cup were measured in three animals during current passage. Current was switched on and off and the voltage changes were recorded with a 3 M-NaCl-filled micro-electrode

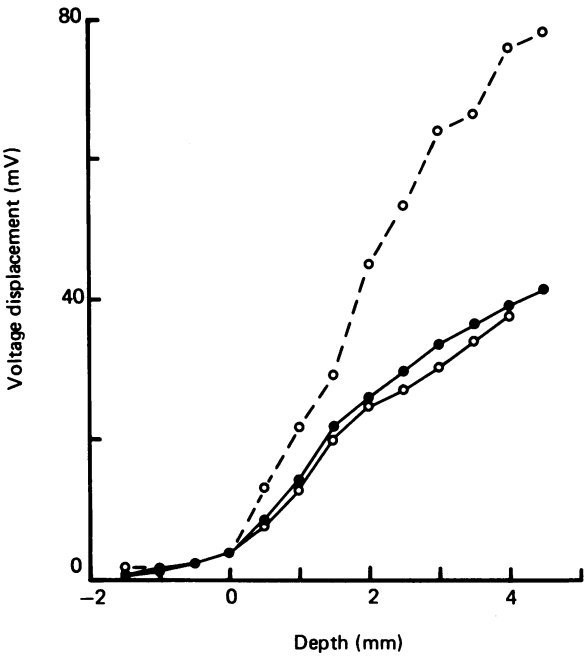

Fig. 5. Potential changes (referred to an earth electrode in the cup) produced by a current of 0·1 mA across the cortical surface, as a function of the depth of a micro-electrode in the tissue. Electrode tracks were perpendicular to the surface in the centre of the cup (○) and less than 1 mm from the edge of the cup (●) in a normal preparation (continuous lines) and in the same preparation after death (dashed line).

at 0·5 mm depth intervals. The voltage changes were proportional to the current, up to ±0·5 mA, and a standard 0·1 mA (5 μA per mm^2 of cup area) was passed at each depth (Fig. 5). The steepest gradient, close to the tissue surface, was 13·1 mV mm^{-1} (±2·2 mV mm^{-1} S.D., $n = 4$). Similar gradients were observed near the edge of the cup (Fig. 5). When the animal was killed the voltage gradients increased markedly, by as much as 2·5 times (Fig. 5, dashed line). This increase has previously been seen and studied by Van Harreveld & Ochs (1956). At depths of 2–4 mm the voltage gradient was about half as great, presumably because here the current was spread over a larger area. The current density close to the surface will have been equal to the total current divided by the cup area. With the observed superficial voltage

gradient this implies a tissue resistivity of 260 Ω cm (cf. 356 Ω cm measured at low frequencies in rabbit cortex by Ranck (1963)).

Measurements using blocks of agar jelly

In two experiments measurements of K^+ transport number were made on blocks of agar jelly (1 % agar by weight) made with each of the standard saline solutions (3 mM-KCl + 150 mM-NaCl, with and without Ca^{2+} and buffers: see Methods). Currents of 0·4 mA were passed in each direction for 400 sec at room temperature (20–22 °C). The total change of $[K^+]$ seen after 400 sec was 0·15–0·16 mM, corresponding to a total K^+ efflux or influx of 20–21 n-mole, and was equal and opposite for currents in the two directions. No lag was detectable between the switching on and off of the current and the attainment of steady rates of change of $[K^+]$ in the cup. These results correspond to a K^+ transport number of 0·012–0·013, compared with values calculated for aqueous solutions with the same compositions: 0·0115–0·012 (using figures for mobilities from Conway, 1952).

Potential differences induced by superfusion with altered $[K^+]$

The K^+ concentration within saline flowing in a cup over the surface of the brain was altered rapidly, within less than 4 sec (see Methods) and observations were made of the size and the time course of the potential difference that developed between the fluid with altered composition in the cup and a pool with unaltered composition outside. Typical results are shown in Fig. 6*A–C* for three areas of the rat brain (neocortex, cerebellum and hippocampus). With an increase in $[K^+]$ from 3 to 12 mM a potential difference of 1·5–2·5 mV (cup negative) developed, rising fast initially and then more slowly to its full level after 2–3 min. Similar experiments were carried out with cups positioned on the surface of the isolated brain of the frog *Rana temporaria*, maintained *in vitro* (Gardner-Medwin *et al.* 1979). The potential changes in this preparation were similar in amplitude but substantially faster (Fig. 6*D*).

In the experiments on rats there were no statistically significant differences between records from different brain areas nor between cups with diameters 2·1 and 3·2 mm. A cup with 1·03 mm diameter, tested on the neocortex, gave approximately 30 % smaller responses. The records from the three areas, with the two larger cup sizes, were pooled and averaged (Fig. 6*E*) for changes in $[K^+]$ from 3 to 12, 6 and 0 mM. The times for rise to half maximum for the averaged responses were approximately 20 sec (12 mM), 11 sec (6 mM) and 25 sec (0 mM). Curves in Fig. 6*E* show the results expected from the simple tissue model without linear approximations (continuous lines) and with linear approximations (dashed lines), using the same parameters as for Figs. 3 and 4. The linear approximations differ considerably here, especially for $[K^+] = 12$ mM, because the changes of $[K^+]$ represent substantial deviations from resting conditions. The agreement between the results and the predictions is within 15 % for the $[K^+]$ increases, except in the first few seconds. For the K^+-free fluid (▼) the voltage changes were only about half as great as predicted (see Discussion).

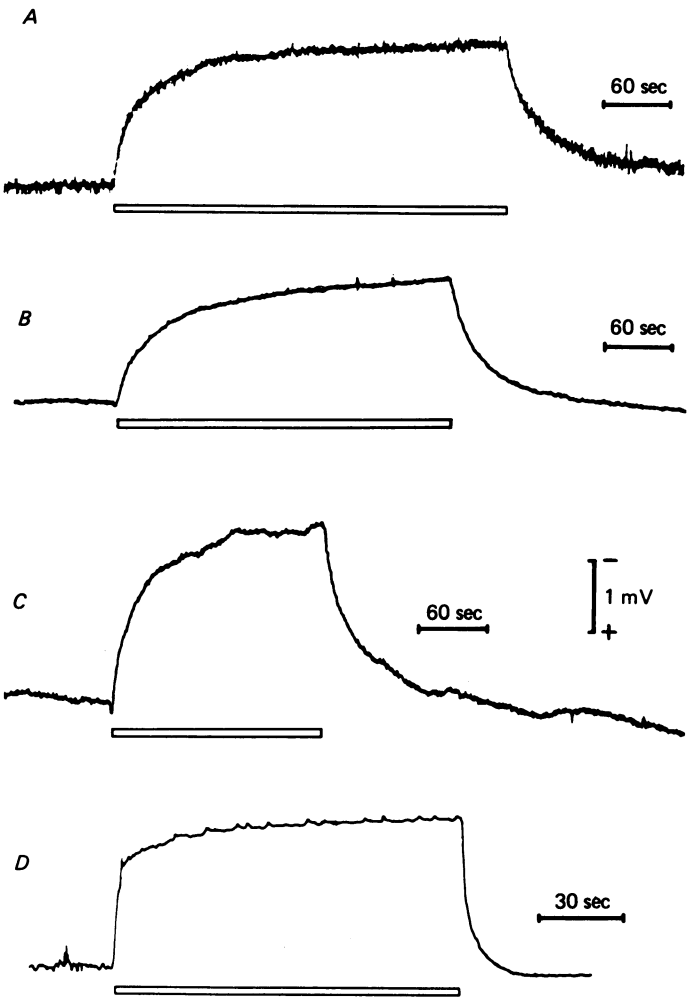

Fig. 6. *A–D*, potential changes recorded in a cup (2·1 mm diameter) on different types of brain surface when the cup fluid was switched from normal saline (with 3 mM-K^+ in *A–C* and 2·5 mM-K^+ in *D*) to saline with four times greater [K^+] during the periods indicated by bars. Records are from the pia-arachnoid surfaces of the neocortex (*A*) and cerebellum (*B*), from the dorsal alvear surface of the hippocampus (*C*), all in anaesthetized rats, and from the pia-arachnoid surface of the optic lobe of the frog brain isolated *in vitro* at 15 °C (*D*). Calibration: 1 mV (all records). Note the faster time scale in (*D*). *E*, average potential changes (means ± S.E. of means) as a function of time after the solution change, in experiments on anaesthetized rats. Data from five animals for the three brain areas illustrated in *A–C* are pooled. Solutions changed from 3–12 mM-K^+ (●, $n = 17$), 6 mM (■, $n = 5$) and 0 mM (▼ $n = 6$) with composition otherwise as stated in Methods. The curves show the results expected from calculations based on the simple tissue model (continuous lines) and on the linear approximations (dashed lines): see Fig. 3 and Methods. Data obtained partly in collaboration with J. Gibson.

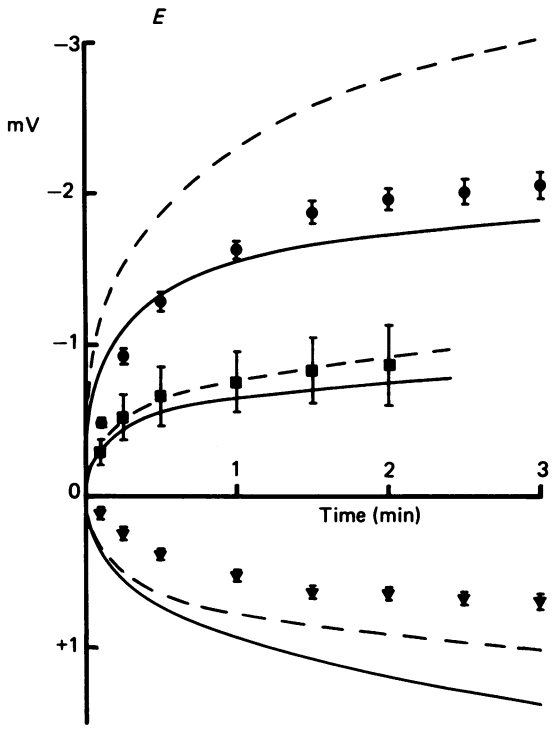

Fig. 6*E*. For legend see opposite.

DISCUSSION

The transport number for K⁺ in brain tissue

The estimated potassium transfer across the surface of the neocortex during current flow corresponds to a K^+ transport number *ca.* 0·06 for outward current densities up to about 20 μA mm^{-2} and for inward current densities up to about 10 μA mm^{-2}. This figure, based on the changes of $[K^+]$ in a surface cup, may be expected to underestimate the K^+ transport number within the bulk of the brain tissue for two reasons. First, it is shown in a separate paper (Gardner-Medwin & Nicholson, 1983) that outward current across the brain surface produces a build-up of K^+ (measured with ion-selective micro-electrodes) in the layers of tissue close to the surface. Conversely, inward current produces K^+ depletion. Therefore in both cases the K^+ flux across the tissue surface must be less than that deeper in the tissue to account for the superficial changes in tissue K^+ content. The data suggest that about 15% of the deep K^+ flux was not exchanged with the cup (Gardner-Medwin & Nicholson, 1983) and the transport number in the bulk of the brain tissue would therefore be underestimated by this amount. Secondly, the estimation of the transport number ignores possible changes in the volume of cup fluid due to current-induced water movement. Water movement was not measured, though it probably led to small underestimates of the K^+ flux (see below). A change in fluid

volume more than *ca.* 5% is unlikely, since it would have produced a conspicuous (0·3 mm) change in fluid level.

Some water movement in the direction of current flow can be expected, due to the following two factors.

(i) Electro-osmosis due to fixed negative charges lining the aqueous clefts of the tissue. The amount of electro-osmotic flow is uncertain in many of the membranes and epithelia where attempts have been made to study it (House, 1974). A flux of 40 molecules/electronic charge (7·5 μl./Coulomb) represents a high electro-osmotic efficiency and would have led to a change of fluid volume in the cup no greater than 0·6% in any of the present experiments.

(ii) Water flux due to changes in the osmotic contents of the cup. The reaction occurring at the Ag/AgCl electrode used for passing current is as follows:

$$e + AgCl \rightleftharpoons Ag + Cl^-.$$

With outward current (cup electrode negative), Cl^- ions are released from the electrode while electroneutrality is maintained partly by a loss of Cl^- to the tissue (*ca.* 60%) and partly by a gain of Na^+ and K^+ (*ca.* 40%). With outward current of 400 μA for 400 sec the osmotic contents of the cup will have increased by *ca.* 3·3%. If enough water were to accompany this osmotic gain to maintain the cup contents at normal osmolarity the fluid volume would have increased correspondingly, by 3·3%. The inferred K^+ flux, which accounted for a 25% increase of $[K^+]$ in these circumstances, would have been underestimated by about 15%. However, calculations based on unrestricted diffusion of water and solutes through the tissue, employing solutions of the diffusion equations for a steadily increasing osmolarity at the surface (Carslaw & Jaeger, 1959: eqn. 2·5 (5)) suggest that equilibration would have been very far from complete after 400 sec and that the underestimate from this cause would have been less than 0·1%.

The expected transport number for K^+ in extracellular space of brain tissue

The extracellular contribution to the K^+ flux induced by current flow through the tissue will be determined by the proportion of the current flowing in the extracellular space and by the extracellular transport number for K^+ (t_K). The transport number depends on the concentrations (c_i), mobilities (u_i) and moduli of the charge numbers ($|z_i|$) for all the ions in solution (MacInnes, 1961) according to the following equation:

$$t_K = \frac{u_K c_K}{\Sigma(|z_i| u_i c_i)}.$$

In order to calculate t_K for the extracellular space we need to consider both the composition and the relative mobility of ions within this space.

Small molecules and ions diffuse relatively freely between cerebrospinal fluid and the extracellular space in mammalian brain (Davson, 1970). Nevertheless, only small changes in composition occur when cerebrospinal fluid flows over the brain surface. From this it has been inferred (see Davson, 1970) that the extracellular fluid is approximately in equilibrium, at least in respect of the principal ions (Na^+, Cl^- and K^+), with cerebrospinal fluid. This conclusion is supported by the fact that ion-selective electrodes register little or no difference of electrochemical potential between external fluid and extracellular sites under normal resting conditions (Nicholson, 1980).

Equilibrium between extracellular and external fluid does not necessarily imply equality of ionic concentrations or activities in the two fluids. A Donnan equilibrium could exist between the two compartments resulting, for example, from the existence of fixed negative charges on molecules within the extracellular clefts. Evidence for such a Donnan equilibrium has been cited by Treherne & Schofield (1981) for the

nervous system of the cockroach, where the ratio of exchangeable Na^+ to exchangeable Cl^- in the extracellular space exceeds the ratio of these ions in the bath with which they are equilibrated by a factor of about 3·0. If these results were to apply to the mammalian central nervous system, then monovalent cations and anions would have concentrations in the extracellular space respectively 1·7 and 0·58 times their concentrations in cerebrospinal fluid. The corresponding Donnan potential in the extracellular space would be −15 mV. Measurements of the distribution space for extracellular markers suggest that such large effects probably do not occur in the vertebrate central nervous system since the measured space (15–25 %) does not appear to depend on the charge on the marker molecules, either from experiments with radioactive markers (see Bradbury, 1979: Table 3·3) or from measurements with ion-selective electrodes close to a site where marker ions are released ionophoretically (Nicholson & Phillips, 1981). The fact that micro-electrodes in extracellular sites do not register the postulated Donnan potential of −15 mV is probably less good evidence against the existence of a Donnan equilibrium, since this negative result might be due simply to the disruption of a charged matrix in the neighbourhood of the electrode tip.

The diffusion of molecules within the extracellular space appears to be relatively free and little affected by either molecular size (Patlak & Fenstermacher, 1975) or charge (Nicholson & Phillips, 1981). The diffusivity is approximately 0·4–0·5 times that in free aqueous solution, corresponding to a tortuosity factor (λ) for the extracellular clefts of 1·4–1·6. This suggests that the relative mobility of different ions in extracellular space is likely to be the same as in free aqueous solution. Relative mobilities are little affected by temperature (Conway, 1952) and tables given by Conway for 25 °C are used here.

The cation concentrations in rat cerebrospinal fluid were measured by Manthei, Wright & Kenny (1973): Na^+ 152, K^+ 3·4, Ca^{2+} 2·2, Mg^{2+} 1·8 mM. Assuming Cl^- to be the principal anion, this gives a transport number of 0·012. This is close to the value (0·011) calculated for human cerebrospinal fluid from an analysis given by Bradbury (1979) and to values for the saline solutions used in these experiments (0·0114–0·0132 for $[K^+] = 3·0-3·5$ mM). If, as discussed above, the extracellular composition is the same as for cerebrospinal fluid, then the expected K^+ transport number in extracellular space is *ca.* 0·012. If one were to assume a Donnan equilibrium with fixed negative charges giving the parameters inferred for the cockroach nervous system (Treherne & Schofield, 1981: see above), the K^+ transport number would be 0·020, or 66 % higher than that in cerebrospinal fluid. The figure of 0·012 is used in these papers as the basis for comparison with the data and Donnan effects are assumed to be negligible.

The expected extracellular transport number would be altered if the conditions of an experiment affect significantly the extracellular composition. Thus the high K^+ transport numbers observed in these experiments (up to *ca.* 5 times the value for cerebrospinal fluid) might be explained on the basis of extracellular K^+ transport alone if the experimental conditions led to substantial increases of $[K^+]_o$ to approximately 15 mM. If this were the case, then the smaller currents used for some of the measurements, which would presumably cause less disturbance than larger currents, should have yielded smaller transport numbers. No such effect was observed

(Fig. 3), nor was there any increase of the transport number measured with alternating currents that would presumably have caused more effective activation of neurones in the tissue. Measurements with K^+-selective micro-electrodes (Gardner-Medwin & Nicholson, 1983) confirm that the increases of $[K^+]_o$ with outward current were too small to account for the results in this way; with inward currents $[K^+]_o$ actually decreased.

The contribution of transcellular flux to the K^+ transport number

If, as discussed above, the K^+ transport number for current through brain tissue exceeds substantially the transport number in extracellular space, then the majority of the K^+ flux must pass through cells. Only a small fraction of the tissue current need pass through the cells, however, to raise the K^+ transport number from 0·012 to 0·06. If the cellular current is carried across cell membranes almost exclusively by K^+, as is probably the case for current through glial cells (Somjen, 1979; Gibson, 1980), then this cellular fraction could be as little as 5 %. Cellular transport accounting for most of the K^+ flux would therefore be compatible with the conclusion drawn from quite different evidence (Van Harreveld, 1972) that extracellular space provides the principal route for current flow through the tissue.

Since the transport number for K^+ is inferred here from measurements of flux only across the tissue surface, not within the bulk of the tissue, it might reflect simply a property of the specialized structures (pia-arachnoid membranes and glial specializations) at the tissue surface. Quantitative consideration of the fluxes makes it unlikely that this could be the case, though the most direct evidence against this possibility comes from $[K^+]_o$ measurements beneath the tissue surface (Gardner-Medwin & Nicholson, 1983).

The outward K^+ flux across the surface with a current of 400 μA was *ca.* 13 p-mole mm^{-2} sec^{-1}, approximately five times bigger than the flux expected for extracellular transport alone. If this high flux were due to a selective permeability of cells at the surface, with K^+ transport in the bulk of the tissue provided only by extracellular flux, then the K^+ content of the tissue beneath the surface would have been depleted at a rate of about 10 p-mole mm^{-2} sec^{-1}. The high surface flux could be maintained in such a situation by diffusion of extracellular K^+ towards the K^+-depleted surface; but calculations employing parameters for diffusion in extracellular space measured by Nicholson & Phillips (1981) show that a K^+ gradient of approximately 50 mM mm^{-1} would be required and that this could be maintained for no longer than *ca.* 0·9 sec before $[K^+]_o$ had fallen to zero underneath the postulated K^+-selective structures. Thus though a high surface flux could result *initially* as a result of selective surface permeability alone, this flux could not be maintained unless it were matched by flux through the bulk of the tissue. The flux measured in these experiments shows no decrease with currents maintained for 400 sec and must therefore be presumed to reflect a high flux per unit current within the bulk of the tissue. Measurements of $[K^+]_o$ (Gardner-Medwin & Nicholson, 1983) indicate that the flux is actually lower at the surface, as can be explained if the transcellular flux occurs through a network of cells with an electrical space constant *ca.* 0·2 mm. This depth dependence of the transcellular flux provides a satisfactory explanation for the time course of the observed surface flux (Fig. 4).

Potassium movement in a concentration gradient

The question whether extracellular K^+ flux is augmented by a K^+ flux through the membranes and cytoplasm of cells is probably more important for fluxes caused by a gradient of $[K^+]_o$ than for fluxes associated with net current flow through the tissue,

since K^+ flux is a mechanism by which local increases of $[K^+]_o$ may be diminished by dispersal. Theoretical arguments can be put forward, based on specific assumptions about the transport mechanisms, that the ratio of transcellular and extracellular fluxes might be expected to be the same (for gradients maintained over long distances) in the two situations; but the question is best approached from an empirical point of view.

As explained in the Introduction, a specific transcellular K^+ flux not accompanied by fluxes of other ions leads to a negativity in extracellular space associated with regions of high $[K^+]_o$. This negativity has been described for many situations in which raised $[K^+]_o$ results both from efflux from cells and from exogenous application (see Heinemann, Lux, Marciani & Hofmeier, 1979; Somjen, 1981 for reviews). The potential changes are larger than could be expected from diffusion potentials due to the different mobilities of K^+ and Na^+ in aqueous solution and it has been argued that they are due primarily to currents associated with K^+ flux through glial cells. The potential shifts observed in the present experiments, averaging 2·0 mV at 2 min after a change from 3 to 12 mM in the superfusing fluid (Fig. 6*E*) are comparable with those reported in earlier studies with superfusion by Zuckermann & Glaser (1970: 1·5–2·0 mV for changes to 9–18 mM) and by Heinemann & Lux (1977: 10 mV for a change to 30 mM). Aqueous diffusion potentials calculated with the Henderson equation (MacInnes, 1961) for K/Na substitutions are *ca.* 0·3 mV for a 9 mM change in $[K^+]_o$.

The local relationship between K^+ transfer mediated via transcellular current loops and that due to extracellular diffusion can be inferred fairly simply by application of electrochemical principles to the extracellular space. If the current density associated with transcellular K^+ flux through a given portion of tissue is I, then the extracellular voltage gradient associated with the return current path is I/σ, where σ is the extracellular conductance of the tissue. If the same flux per unit area of tissue were to occur by diffusion in extracellular space it would require a $[K^+]_o$ gradient of $\lambda^2 I/(\alpha DF)$, where $\alpha D\lambda^{-2}$ is the flux diffusion coefficient for K^+ in extracellular space (Gardner-Medwin, 1980). We can use the Einstein relation between diffusion and mobility coefficients (Bockris & Reddy, 1970), and the standard electrochemical equations relating mobility and conductance, to show that this expression $(\lambda^2 I/(\alpha DF))$ is equal to $c_B(n_B RT/F)^{-1}$ time I/σ, where c_B and n_B are the extracellular K^+ concentration and the K^+ transport number in extracellular space under base-line conditions. The ratio of extracellular voltage gradient to $[K^+]_o$ gradient is therefore $n_B(RT/F)/c_B$ (i.e. *ca.* 0·10 mV mM^{-1}) for equal transcellular and extracellular diffusion fluxes and it would be proportionately larger for a greater ratio of the two fluxes. The observed shifts of surface potential 2 min after superfusion with small changes of $[K^+]$ were *ca.* 0·3 mV mM^{-1} (Fig. 6*E*), suggesting a ratio transcellular : extracellular flux of 3 : 1. Since this is based on the surface displacements of voltage and concentration rather than on the gradients through the tissue, it represents a weighted average of the ratio at all the depths where there is a gradient. The transcellular flux during superfusion can be expected to vary from zero at the surface (where the cells terminate) to a maximum deep within the tissue; hence the ratio of transcellular to extracellular flux within the bulk of the tissue must be greater than 3 : 1. The slow development of the surface potential change can be seen, on this

argument, to be due to the fact that initially the gradients are restricted to the surface, where transport is principally by diffusion.

A more complete theoretical analysis based on a model of the situation with combined extracellular and transcellular K^+ flux (Gardner-Medwin, 1983) allows both the size and the time course of the potential shifts to be predicted. The data for increases of $[K^+]_o$ in fluid superfused over the surface of the rat brain (Fig. 6E) is fitted, within *ca.* 15%, using parameters $\beta = 5$, $\Lambda = 0{\cdot}2$ mm in the model. This corresponds to a ratio transcellular:extracellular flux of 5:1 for the low spatial frequency components of K^+ flux (Gardner-Medwin, 1983). The electrotonic space constant for the cells involved in transfer in the model, which affects the time course of the potential shifts, is 0·2 mm. These parameters are the same as those that fit the data for flux measurements (see above) and $[K^+]_o$ measurements (Gardner-Medwin & Nicholson, 1983) during current passage. When superfusion was with K^+-free solutions (Fig. 6*E*) the potential shifts were *ca.* 50% smaller than predicted. This discrepancy may be due to efflux of cytoplasmic K^+ caused by reduced Na–K pumping at low $[K^+]_o$ levels (Gardner-Medwin, 1983): this could lead to a smaller change of $[K^+]_o$ in the superficial tissue with a lowering of $[K^+]$ in the superfusate than with a rise of $[K^+]$. The effects of a fall in $[K^+]$ are probably of less physiological importance than those due to a rise, since substantial increases of $[K^+]_o$ and only fairly small reductions are normally observed (Heinemann *et al.* 1979).

Measurements by G. E. Cordingley (quoted by Somjen (1978): Fig. 3) have revealed substantially larger extracellular voltage shifts per unit of $[K^+]_o$ change than those observed here, in situations with electrical stimulation of neurones: *ca.* 1·8 mV mM^{-1} in cerebral cortex and *ca.* 2·2 mV mM^{-1} in spinal cord in the cat. The distribution of voltage changes in these situations with neuronal activation is very similar to the distribution of changes of $[K^+]_o$ (Cordingley & Somjen, 1978). Since the analysis of the relation between extracellular voltage and concentration gradients given above should apply also to these results they suggest ratios of transcellular to extracellular flux of 18:1 and 22:1, compared to the ratio 5:1 indicated in the present studies on rat brain. It is only possible at present to make suggestions to account for these differences: a difference between species, a possible underestimate of the $[K^+]_o$ changes measured with micro-electrodes, changes of extracellular space fraction, or an effect of other substances released with neuronal activation. The data are nevertheless clearly consistent with a predominantly transcellular K^+ flux contributing to K^+ dispersal.

The circumstances in which the potentially dominant transcellular route for K^+ movement is likely to be important for neuronal fuction are discussed elsewhere (Gardner-Medwin, 1981*b*, 1983). The identity of the cells involved is discussed by Gardner-Medwin & Nicholson (1983).

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