J. exp. Biol. (1981), **95**, 111–127
With 5 figures
Printed in Great Britain

POSSIBLE ROLES OF VERTEBRATE NEUROGLIA IN POTASSIUM DYNAMICS, SPREADING DEPRESSION AND MIGRAINE

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SUMMARY

The membranes of glial cells are highly selectively permeable to potassium. The implications of this and the possible reasons for it are discussed. Glial cells may contribute to buffering the extracellular K^+ concentration of brain tissue through several mechanisms. However, the only one that benefits from the K^+ selective permeability is the so-called 'spatial' buffer mechanism, which acts more effectively than extracellular diffusion in many situations to speed the dispersal of local accumulations of potassium.

The role of glial cells in buffering the extracellular K^+ concentration may help to prevent the occurrence of a phenomenon called Leão's spreading depression (SD). A K^+-induced K^+ efflux from neurones, occurring when the EC K^+ concentration rises above critical levels, is probably crucial in causing SD. The models that have been proposed to describe this process are discussed and related.

Spreading depression is not known definitely to occur in man. It seems probable, however, that it occurs during attacks of 'classical' migraine, associated with neurological symptoms. These neurological symptoms have often been attributed to vasoconstriction rather than to SD since certain vasodilators can relieve the symptoms. Experiments with SD in anaesthetized rats show that at least one of these vasodilator interventions (administration of a CO_2/O_2 mixture) stops also the propagation of a wave of SD. This strengthens the evidence for a possible relationship between migraine and SD. The involvement of SD in migraine probably deserves more critical attention than has hitherto been devoted to it.

INTRODUCTION

In these days of library computers it is possible to search the literature for papers linking two or more keywords. If one were to pick out the following associations:

neuroglia – potassium,
potassium – spreading depression,
spreading depression – migraine,

one would make quite an impressive collection. Try to link *neuroglia* with *migraine*, however, and there would be little to show. The aim of this paper is to explore the

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three associations set out above, to consider whether the associations have physiological significance, and to see whether it is appropriate to set them side by side.

Some of the individual topics referred to here are the subject of excellent recent reviews. Since there is no value in attempting to repeat the content of these reviews, I frequently refer the reader to them for background information, rather than to the original literature.

NEUROGLIA AND POTASSIUM

The association of neuroglia and potassium goes back nearly as far as the electrophysiological study of glial cells themselves (Kuffler & Nicholls, 1966). The membrane potentials of glial cells in many preparations, including the vertebrate central nervous system, follow the Nernst equilibrium potential for potassium quite closely (see Somjen 1975, 1979; Orkand, 1981). This could indicate either that the membranes are permeable almost exclusively to potassium or that other ions (e.g. Cl^-) move across the membranes so quickly in a new environment, to establish a new equilibrium, that they have only a transient effect on the membrane potential (cf. Hodgkin & Horowicz, 1960). This second possibility has usually been considered unlikely because of the small size of detectable transient potential changes (Bracho, Orkand & Orkand, 1975). Small transient potential changes with Cl^- replacements could be explained, however, by rapid glial equilibration keeping pace with the diffusion of ions into or out of the extracellular clefts, as well as by a relatively low Cl^- permeability. In the leech nervous system Gibson (1980), in my laboratory, has shown recently that the input resistance of glial cells does not increase detectably when external Cl^- ions are substituted. This appears to confirm, at least for this preparation, that Cl^- ions do not move easily across glial membranes. We are faced with the conclusion that glial membranes are highly selectively permeable to potassium and sensitive to external potassium levels – possibly more so than any other biological cells that have been studied (see Williams, 1970, for a review of many non-excitable cells). Why is this?

At least two types of answer to this question have been proposed, both of them originating from the pioneering work by Kuffler and his colleagues. Firstly, the membrane potential changes may act on the glial cells as *signals*, triggering off some metabolic or other response that is appropriate when there is elevated $[K^+]_o$ due to local neuronal activity. Such mechanisms are discussed elsewhere in this volume. The second type of explanation offerred for the K^+ selectivity, to be discussed here, is that it permits glial cells to act in one or more ways to buffer the changes of $[K^+]_o$ that occur around active neurones. This would mean that the glial cells are not only affected by the changes of $[K^+]_o$, but play a role in the K^+ dynamics of the tissue.

The first way in which glial cells might act to buffer a rise of $[K^+]_o$ is through the shift of K^+ across their membranes that is required to depolarize them. Enough K^+ must enter the glial cells to discharge their membrane capacitance by the amount of the observed depolarization. Calculations (Gardner-Medwin, 1980) suggest that the loss of K^+ from the extracellular (EC) space through this *capacitative shift* of ions would only diminish a $[K^+]_o$ rise by at most about 2% in vertebrate brain tissue. It is unlikely to represent a significant buffering action.

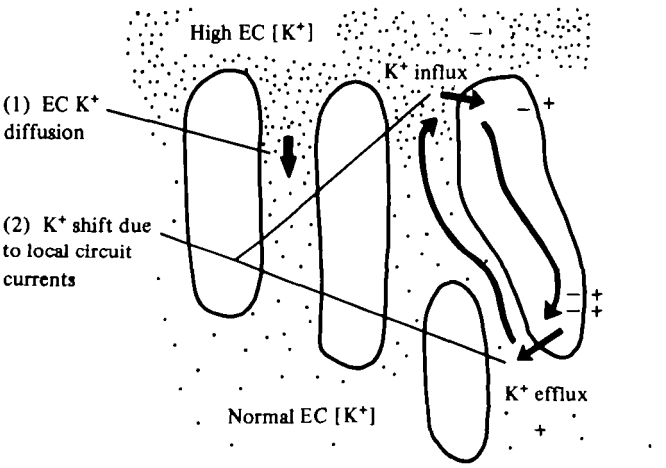

Fig. 1. Mechanisms for movement of K^+ in an extracellular concentration gradient. (1) Diffusion in EC space. (2) K^+ influx and efflux across cell membranes, associated with local circuit currents and a gradient of depolarization in cells with K^+-selective membranes: the *spatial buffer* mechanism (Orkand *et al.* 1966). Note that the spatial buffer currents are carried principally by K^+ across the cell membranes and principally by Na^+ and Cl^- in EC space. This produces an effective transfer of K^+ from regions of high to low EC concentration: partly through K^+/Na^+ exchange and partly KCl transfer.

If more K^+ is to enter across glial membranes than the amount associated with the capacitative shift, it represents an inward K^+ current that must be balanced by a separate outward current across the cell membranes. This outward current must either be a K^+ current leaving the cells through some other region of their membranes where the conditions are different, or it must be due to a flux of ions other than K^+ across the membranes. The possible involvement of other ions is discussed later. The first possibility, that K^+ enters in one place and leaves elsewhere, is the so-called 'spatial' buffer mechanism (Orkand, Nicholls & Kuffler, 1966; Trachtenberg & Pollen, 1969), that effectively transfers K^+ from a region with high $[K^+]_o$ to a region where $[K^+]_o$ is lower (Fig. 1).

The spatial buffer mechanism is a purely passive mechanism, a form of facilitated diffusion, that does not require that the membranes should be permeable to anything other than K^+. It can be expected to operate to some extent through neurons as well as glial cells; but certain properties of glial cells suggest that they may be particularly effective – their greater depolarization in high $[K^+]_o$ and their electrical coupling, known to occur in some preparations and presumed to occur in mammals from the existence of glial gap junctions (Kuffler & Nicholls, 1976). An essential feature of the spatial buffer mechanism is the development of a current loop through intracellular and extracellular space when there is a gradient of $[K^+]_o$. This is a direct consequence of the gradient of depolarisation (Fig. 1). The fraction of the current carried by K^+ (the K^+ *transport number*) is different in different arms of the loop, being close to 1·0 for current across the membranes and very small in EC space (*ca.* 0·012 in mammalian brain: Gardner-Medwin, 1977); it is this difference that leads to a net removal of K^+

from EC space in one place and addition to it in another. The expected changes of cytoplasmic K^+ concentration depend on the cytoplasmic K^+ transport number (Gardner-Medwin, Coles & Tsacopoulos, 1981; Coles & Tsacopoulos, 1981); but they are not directly important for the buffering of $[K^+]_o$.

My own recent work has suggested that the spatial buffer mechanism in mammalian brain is in some situations very much the most important mechanism responsible for K^+ flux: up to five times more important than EC diffusion. The evidence for this is based on three types of experiment. These involved firstly the determination of the K^+ transport number for bulk mammalian cortex by measuring the changes of $[K^+]$ in a cup on the cortical surface when current was passed through the brain tissue (Gardner-Medwin, 1977). This gave a value *ca.* 0·06, about five times higher than could be accounted for by purely EC movement. This showed that some types of cells (referred to as *transfer cells*) must account for at least 80% of the K^+ flux in an electrical gradient. Secondly, the changes in $[K^+]_o$ beneath the surface of the brain were measured during current passage (Gardner-Medwin & Nicholson, 1978), showing that the transcellular flux occurs throughout the bulk of the tissue rather than just within a layer at the surface. Thirdly, the potential changes induced by alterations of $[K^+]_o$ at the surface of brain tissue were measured (Gardner-Medwin, Gibson & Willshaw, 1979) and shown to agree with those predicted from the transport number measurements. The conclusion suggested by this work is that there are, in mammalian brain, transfer cells (either neurones or glia) responsible for a much larger flux than EC transport processes when these fluxes are caused by either electrical or concentration gradients maintained over distances greater than about 0·1 mm. In concentration gradients, the operation of these cells is precisely the spatial buffer mechanism. The implications of these results are discussed more fully in the report of a work session of the neurosciences research program (Nicholson, 1980). The experiments do not demonstrate that neuroglia rather than neurones are responsible for the measurements: but experiments reported by Dietzel & Heinemann reported at the same work session, in which similar results to those of Gardner-Medwin & Nicholson (1978) were obtained in gliotic scar tissue, suggest that it may be predominantly non-neural cells that are involved. The situations in which the inferred characteristics of the spatial buffer mechanism should most affect K^+ accumulation are discussed by Gardner-Medwin (1981*a*) and in papers in preparation. In addition to these substantial effects on $[K^+]_o$, it has been suggested by Dietzel *et al.* (1980) that the spatial buffer mechanism may act indirectly to stabilize Na^+ and Ca^{2+} concentrations in EC space around active neurons as well: this is because the ion movements associated with the spatial buffer currents produce water movement and a shrinkage of EC space around regions (particularly epileptic regions) with substantial neuronal activity. Thus the direct consequences of glial depolarization in high K^+ environments may be quite far-reaching and important.

As described above, a K^+ buffering action of glial cells might involve fluxes of other ions than K^+ to balance the K^+ influx. There are many possibilities for such mechanisms (Gardner-Medwin, 1980). Some of them may be of substantial importance, but at present the discussion of them is unfortunately largely speculative. In the present article I shall simply ask whether the high K^+ selectivity of glial membranes and their

close adherence to the K^+ equilibrium potential has any bearing on the possible existence of these buffering mechanisms. If these mechanisms operate, with either passive K^+ entry or active transport, these seem to be good reasons to expect that the membrane depolarisation in high $[K^+]_o$ should be *less* than the change of the K^+ Nernst potential. These are discussed below.

Net uptake of K^+ into glial cells by *passive* means will occur only if the glial cell depolarization is less than the change in the Nernst equilibrium potential for potassium. This follows from the definition of the equilibrium potential, as that at which there is no net passive flux. The fact that glial cells appear to depolarize to about the full extent of the Nernst potential when their membranes are subject to a uniform alteration of extracellular K^+ suggests, therefore, that passive net uptake into glial cells is *not* a role for which they are optimized. For a given K^+ permeability of their membranes, glial cells would do a better job at buffering changes of EC K^+ concentration by passive net uptake and release if (like twitch fibres in vertebrate muscle) they had evolved to have a substantial Cl^- permeability as well. As things are, a substantial passive K^+ entry in regions with high EC K^+ levels will only occur if the K^+ build-up is restricted in extent so that the local glial depolarisation is held to a lower value than the Nernst depolarization by electrotonic coupling to regions with less K^+ build-up: this is the spatial buffer mechanism again, which acts to disperse a local build-up of K^+ but not to clear K^+ from the EC space if the build-up is widespread (Gardner-Medwin, 1980).

If K^+ uptake involves *active* transport, with stimulated Na/K exchange, one should expect the electrogenic nature of the exchange pump (Tang, Cohen & Orkand, 1980; Orkand, Orkand & Tang, 1981) to result in a depolarization less than the full change of the Nernst potential, or even a hyperpolarization, in raised $[K^+]_o$. The fact that glial cells depolarize to about the extent of the change in the Nernst potential in high $[K^+]_o$ is evidence that electrogenic pumping of this sort is not stimulated to a large extent. Of course if the passive permeability of the membranes is high, there might be only a tiny electrogenic potential to be seen: but this means that any pumped fluxes would be small compared to the passive flux cocurring by the spatial buffer mechanism with only a few mV of driving potential. There might perhaps be a *neutral* Na/K exchange pump that is stimulated significantly in normal glial cells under conditions with raised $[K^+]_o$, with the electrogenic pump of Tang *et al.* (1980) only evident under conditions with Na^+ loading of the cells. Such a conclusion would be very interesting, but has no clearcut evidence to support it. The evidence for stimulated Na/K exchange in glial membranes from other types of experiments does not unfortunately lead to clear quantitative predictions in relation to the tissue K^+ dynamics. Henn, Haljamäe & Hamburger (1972) showed that Na/K ATPase is more sensitive in glial cells than in neurones to raised $[K^+]_o$; but since the resting pump rate in glial cells is presumably low as a result of the low Na^+ permeability, this does not necessarily imply any substantial involvement in K^+ dynamics. Hertz (1978) has observed an increased (and apparently saturable) $^{42}K^+$ uptake into cultured astrocytes in raised $[K^+]_o$; but over the range of concentrations of physiological interest there seems to be no indication how much of this uptake might have been attributable to Na/K exchange rather than to simple exchange flux with a non-linear concentration dependence due to the

depolarization in high $[K^+]_o$. Further evidence would seem to be required to establish Na/K exchange firmly as a significant factor in glial buffering of $[K^+]_o$.

To summarize this section, it is possible that glial cells might play several roles in buffering the changes of $[K^+]_o$ caused by neuronal activity. They could do this to some extent by a passive or active net uptake of K^+ into their cytoplasm when $[K^+]_o$ is raised, but in neither case is it well established that this occurs to a significant extent; the high selectivity of glial membranes for K^+ is not a feature that would assist these processes or that provides any evidence for their occurrence. The existence of a K^+ permeability much higher than that for other ions does, on the other hand, render glial cells more effective in transferring EC K^+ from regions of high concentration to regions of low concentration, thereby tending to diminish local rises of concentration. It is this 'spatial' buffer mechanism that may have provided the evolutionary pressure that has led to glial cells possessing such selective permeability.

POTASSIUM AND SPREADING DEPRESSION

The phenomenon of Leão's spreading depression (Leão, 1944) is the one clearcut syndrome that we know can be set off by high K^+ levels in EC space. There are many other effective triggering stimuli (e.g. glutamate, trauma, anoxia, electrical, mechanical and other chemical stimuli). Potassium is of particular interest because, once spreading depression (SD) is initiated, it rises to extremely high EC levels (30–80 mM) and because the disperasl of EC K^+ into hitherto unaffected regions of tissue is a strong contender for the means by which the disorder spreads (Grafstein, 1956; Nicholson & Kraig, 1980). It is the link between spreading depression and potassium, and hence neuroglia, that I shall discuss here.

The properties of spreading depression (see Bures, Buresová & Krivánek, 1974) include a very profound, probably complete, depression of neuronal activity preceded often by a period of increased activity. It can occur in many types of neural tissue, including the grey matter of the cerebral and cerebellar cortex, certain subcortical brain structures and the retina, but not apparently the spinal cord (Somjen, 1978). The phenomenon lasts in any one region of tissue for a minute or so, depending on the criterion, and is followed by complete recovery. It propagates through the tissue at about 3 mm/min, with the band of affected tissue being typically a few millimetres wide at any one time. Repeated waves can be elicited with continuous severe stimuli though there is a refractory period of the order of a minute after each wave. Several observable phenomena are associated with SD that are not currently believed to be involved in its propagation, such as circulatory changes, increased O_2 consumption and changes of tissue volume, transparency and electrical impedance.

The severity of the neuronal disturbance is easily understood in the light of measurements made with ion-selective microelectrodes during SD (Nicholson & Kraig, 1980). Rises of EC K^+ activity and falls of Ca^{2+} activity (each by factors as large as 10 times) have been observed, with also substantial falls of Na^+ and Cl^- activities. The approximate distribution of K^+ increases within a wave of SD is shown in Fig. 2(*a*).

A complex pattern of negative and positive extracellular potential changes has been observed in spreading depression (Fig. 2*b*; Nicholson & Kraig, 1980), but has no

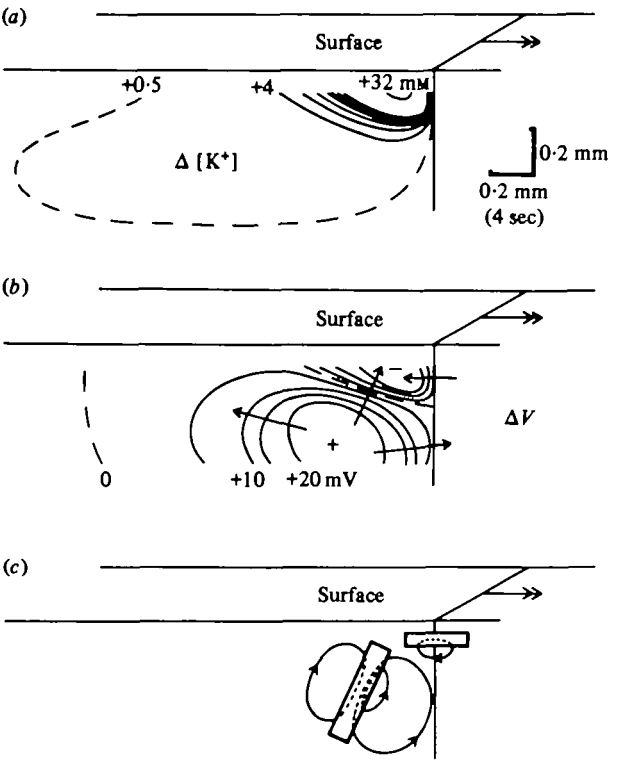

Fig. 2. Extracellular K^+ concentration and voltage changes during spreading depression. (*a*) An approximate contour plot showing the pattern of K^+ concentration increases beneath the surface of the rat cerebellar cortex during a wave of SD propagating towards the right. The instantaneous pattern is inferred from data of Nicholson & Kraig (1980) giving the time course of $[K^+]$ increases observed at different depths. Assumed velocity of propagation: 3 mm/min; contours: multiples of 4 mM increase above baseline (*ca.* 3 mM), except dashed line: 0·5 mM increase. (*b*). Contour plot showing changes of EC voltage during SD (contour steps 5 mV; otherwise as in (*a*)). Arrows indicate the directions of EC current flow inferred from the voltage gradients. (*c*). Postulated contributions of glial cell elements to dispersal of the K^+ build-up in (*a*), both ahead of the SD wave and to deeper tissue, through spatial buffer action. Note that the pattern of expected EC current flow is similar to that observed, in (*b*). Data for the most superficial tissue is not presented in the source material.

been analysed fully in terms of current sources and sinks. It invariably seems to include a sharp negative going wave at about the time of onset of the ionic changes in the regions most affected. This implies that as a wave approaches a region there is first a period with current leaving cells, followed by a period with current entering cells, much as with a propagating action potential. These local circuit currents may well be flowing principally through the membranes of glial cells, since these have been observed to become depolarized earlier than neurones in the onset of spreading depression (Sugaya, Takato & Noda, 1975). If this is the case it would make the explanation for the negative EC potential in spreading depression the same as for other sustained negative EC potential shifts, often associated with glial depolarization and raised EC K^+ (Somjen, 1973). The local circuit currents at the onset of spreading depression are, this hypothesis, spatial buffer currents (Fig. 1). The earliest detected extracellular

concentration change during the onset of SD is a gradual rise of K^+ concentration (Kraig & Nicholson, 1978). It seems quite likely that this may be largely due to an efflux of K^+ from glial cell membranes ahead of the region with the full disturbance (Fig. 2*c*). It has long been thought possible that diffusion of EC K^+ ahead of a region with spreading depression might trigger off the disturbance in the new region (Grafstein, 1956). The contribution of spatial buffer current through glial cells, which according to the conclusions of Gardner-Medwin (1980, 1981*a*) may act much faster than EC diffusion in altering the EC K^+ concentration around a local disturbance, would not alter the principle of this hypothesis at all. It might, however, increase the expected propagation velocity.

The various hypotheses for the mechanism of spreading depression have been much discussed elsewhere (e.g. Bures, Buresová & Krivánek, 1974; Nicholson & Kraig, 1980). Common to them all is the idea that neural tissue can be intrinsically non-stable through the existence of positive feedback mechanisms, such as K^+-induced K^+ release or glutamate-induced glutamate release. It seems possible that more than one such mechanism exists (Van Harreveld, 1978), and that they may be inextricably inter-related once instability has set in, in the manner put forward by Nicholson & Kraig (1980). The idea that the complex sequence of events is triggered off by a spread of K^+ from the previously affected region is not really proven, but gains the most general acceptance in the light of evidence showing the early rise of K^+ concentration, the speeding of propagation by imposed electric fields in the direction that would hasten the spread of a positive ion (Grafstein, 1956) and the agreement (within a factor of 2 or so) of the propagation velocity with that calculated on the basis of simple models of spread by the influence of potassium (Grafstein, 1963; Tuckwell & Miura, 1978).

The roles of neuroglia in K^+ dynamics could be relevant in several ways to the initiation and propagation of spreading depression. I omit here any discussion of the processes going on in the recovery phase and the possible glial involvement in transmitter uptake and release (Varon & Somjen, 1979). If we assume that spread of K^+ is responsible for the propagation (or is at least a facilitating influence), then we have three ways in which the *spatial buffer* action of glial cells may affect spreading depression. It may hasten the process of propagation, as mentioned above: this is not of any obvious benefit. It may raise the threshold for initiation in situations where a local build-up of K^+ around intensely active neurones is a contributing factor, by dispersing the K^+ more effectively (Gardner-Medwin, 1981*a*). Lastly, it might in some situations help to cause failure of propagation or to prevent the spreading depression process occurring at all, as perhaps in the spinal cord. A net uptake of K^+ into glia by active or passive processes would also help to raise thresholds and to prevent propagation.

The conditions that determine whether spreading depression can occur or can propagate are not at present amenable to a full theoretical analysis: there are too many unknowns. A simple analysis originating from an unpublished manuscript by A. L. Hodgkin has been set out by Grafstein (1963) and by Bures *et al.* (1974), for the purpose of an order-of-magnitude calculation of the expected propagation velocity. This supposes that the rate of influx of K^+ into cells (or more strictly, the rate of decline of EC K^+ concentration taking account of possible EC volume changes) is a direct function (f) of the K^+ concentration rise ($\triangle c$): Fig. 3(*a*). This is an approximation

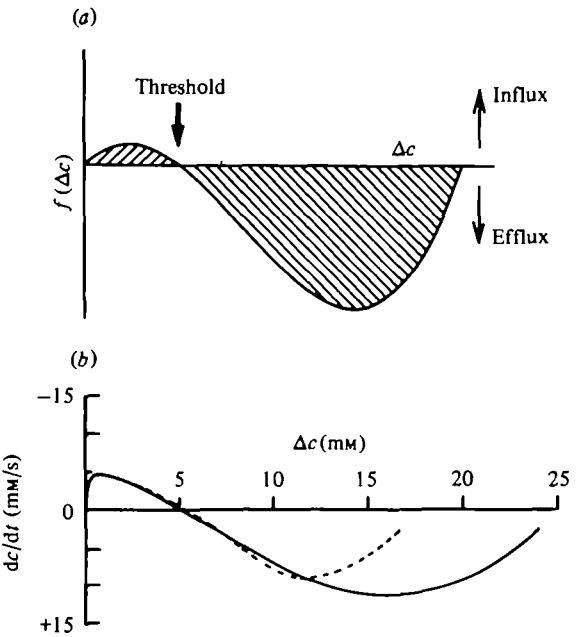

Fig. 3. The K^+ influx function: a postulated basis for the unstable EC composition observed in spreading depression. If K^+ were suddenly added uniformly to the tissue EC space, increasing the concentration (c) by an amount Δc, f is the rate at which c would decline due to K^+ influx into cells. (*a*) General features of $f(\Delta c)$, following the analysis by Grafstein (1963) and Hodgkin (unpublished). The negative zone of f (postulated for values of Δc above a 'threshold') represents K^+-induced K^+ efflux, producing a precipitous rise in K^+ concentration. (*b*) The particular function implied in equations used by Tuckwell & Miura (1978) to model the propagation of SD. Full line: calculated directly from the equations, assuming $[K^+]_o$ alters with no change in the other concentration variables that enter into the equations. Broken line: calculated assuming that other parameters change as $[K^+]_o$ rises according to the relation found in Tuckwell & Miura's explicit numerical solution for the onset of propagating SD (their Fig. 3). Note that the changes of other parameters (principally $[Ca^{2+}]_o$) do not much affect the K^+ flux until $[K^+]_o$ is well above threshold. This suggests that they are not an essential feature of Tuckwell & Miura's model in the *initial* phase of a propagating SD wave, and that a simpler analysis in terms of a fixed K^+ influx function would correctly predict the $[K^+]$ changes in this phase.

since K^+ influx may also depend significantly on other factors that differ for any particular K^+ concentration, depending on the recent history of the tissue (e.g. Ca^{2+} concentration, transmitter pools and internal concentrations). These other factors probably need not be taken into account in considering the initial phase of SD with rapidly rising EC K^+ concentration, though they become important in determining the time course and recovery phase of the SD wave (Tuckwell & Miura, 1978).

It is by no means straightforward to measure the influx function $f(\Delta c)$ with the tissue intact; but it is possible to attempt to construct it with a combination of data from various sources (Tuckwell & Miura, 1978). Its essential features are a positive region for small values of Δc (since without this the resting tissue would be unstable) and a negative region for larger values of Δc (that represents K^+-induced K^+ efflux due to transmitter action and neural activity, leading to the instability of spreading depression). The crossover point between the two regions is a 'threshold', since a higher [illegible]form K^+ level than this will lead to an explosive increase of K^+ concentration.

Hodgkin in his analysis assumed, largely for simplicity, that the function $f(\triangle$ might have the form of a cubic equation; he then proceeded to calculate conditions for propagation and the expected propagation velocity based on K^+ diffusion. The influx function calculated by me from the assumptions of Tuckwell & Miura (1978) is shown in Fig. 3(*b*)(full line). Tuckwell & Miura's relatively complex analysis does not assume that parameters other than EC K^+ concentration stay constant during the critical early phase of SD that determines the speed of propagation. If the rate of change of K^+ concentration is plotted as a function of the EC K^+ concentration for the early phase of the calculated wave in their model (dotted line, Fig. 3*b*), the discrepancy from the influx function obtained by ignoring the changes of the other parameters (full line) is not great. Of course the particular assumptions made by Tuckwell & Miura may be open to question; but it seems reasonable to conclude from their work that the simple approach that considers only the dependence of K^+ influx and efflux on EC K^+ concentration may be adequate for describing the early phase of SD.

It is possible to show in general (without assuming a cubic equation) that a condition for propagation of a spreading depression wave by diffusion of EC K^+ ahead of the wave is that the positive hatched area to the left of the threshold in Fig. 3(*a*) (representing the influx function) must be smaller than the hatched negative area to the right of threshold (my own unpublished mathematical analysis). This is clearly true for the particular assumptions of Tuckwell and Miura (1978): Fig. 3(*b*). When this condition is satisfied, a region of tissue with K^+ concentration above 'threshold', and an extensive flat boundary on to normal tissue, will propagate forwards rather than collapse backwards. Propagation may nevertheless fail if the region of affected tissue is not extensive, or if dispersal of K^+ can occur into tissue that is not susceptible, such as white matter (with presumably a smaller, or non-existent, negative region in $f(\triangle c)$). Thus propagation depends on geometrical factors as well as on the K^+ influx function. In most regions of the nervous system the synaptic neuropil is sandwiched in layers, up to a few millimetres thick, between white matter and/or fluid spaces. The geometrical factors and the dispersal of K^+ sideways into the adjacent regions, aided by the spatial buffer mechanism, may play an important role in limiting the prevalence of spreading depression. With a typical build-up of K^+ concentration in SD, taking 10 sec or so over a zone several hundred micrometres thick, sideways diffusion alone would have little effect in reducing the build-up; but this is just the kind of situation where the spatial buffer mechanism makes a substantially greater contribution to dispersal than diffusion (Gardner-Medwin, 1981*a* and in preparation).

Glial cells also contribute directly to the K^+ influx function $f(\triangle c)$. The total influx is the sum of the influx into all the cells facing the EC space. The negative zone of $f(\triangle c)$, which is assumed to be the cause of the problem of spreading depression, is due to the tendency of neurones to release more K^+ when sufficiently depolarised in high K^+ levels: both through increased excitability and firing rates and through induced transmitter release and permeability changes. Glial cells do not behave in this way. When the K^+ concentration rises, they can be expected to contribute a net influx at all levels. Though the extent to which they do this is uncertain, it will add a purely positive function to $f(\triangle c)$ and will increase the ratio of the positive to the negative areas in Fig 3(*a*). In this way the glial cells may be serving to counteract an intrinsic ins[illegible] bility of nervous tissue due to the neuropil (Gardner-Medwin, 1980).

In summary, the increase of EC potassium levels associated with spreading depression is probably a major factor causing the propagation of this phenomenon. Neuroglia may play several roles in relation to this: They may account for the observed extracellular potential changes; they may raise the threshold for any stimuli that initiate spreading depression; they may hasten the rate of propagation and in some situations they may exert sufficient stabilizing influence on the K^+ dynamics of the tissue that they render it not susceptible to spreading depression at all.

SPREADING DEPRESSION AND MIGRAINE

Spreading depression (SD) has been observed experimentally in many vertebrate species (see Bures *et al.* 1974). A comparison between species suggests that in the cerebral cortex it is more easily elicited in lower mammals without convoluted cortex. Damage to the tissue renders it particularly susceptible, but such damage is probably not necessary for the spread of SD, at least in some species (Marshall, 1959). Spreading depression might occur in man, but the evidence is not clear. Bures (1959) refers to two observations of indicative phenomena, made by himself and by H. H. Jasper; but he gives few details. Goldring (1963), on the other hand, has failed to observe SD in human cerebral cortex in conditions that might have been expected to elicit it. In man there are only a few justifiable techniques and procedures that could be used in attempting to characterize SD if it occurs.

Milner (1958) pointed out that there are similarities between the syndrome that might be expected if SD were to occur in the primary visual cortex of man and the visual disturbance that commonly occurs as an early symptom of classical migraine. It is the suggestion that spreading depression and migraine are related that I shall mainly consider here: it seems to have received only occasional passing mention in the literature on both spreading depression and migraine.

In no species has it been established that SD occurs in a normal healthy animal without artificial procedures to initiate it. This does not of course mean that it never occurs. It is interesting to speculate on the consequences if it did or does occur. Spreading depression in the spinal cord of vertebrate species might be expected to induce temporary partial paralysis; in the respiratory control centres it might cause death through asphyxia. It is likely that there would be a strong biological advantage in protecting the nervous system against such occurrences and it is therefore interesting that Somjen (1978) has noted that SD cannot be elicited in the spinal cord with normal techniques. The consequences of SD in the cerebral cortex might depend on the region affected and on the species. With the increasing encephalization of function in higher mammals and the increasingly severe effects of decortication on behaviour, it may be increasingly important to protect against widespread SD. Only a small region of tissue (a band a few millimetres across) is affected by a single wave at a time, however, so in some regions of the brain the incapacity resulting from a single wave might be only slight.

Migraine is a common disorder in man. It takes many forms, with various overlapping combinations of symptoms. The symptoms causing most distress are headache and nausea, which may range from being almost absent to extreme severity. Symptoms of abnormal function of neurones, associated with so-called 'classical' migraine, are common: these vary between subjects and are normally evident well before the

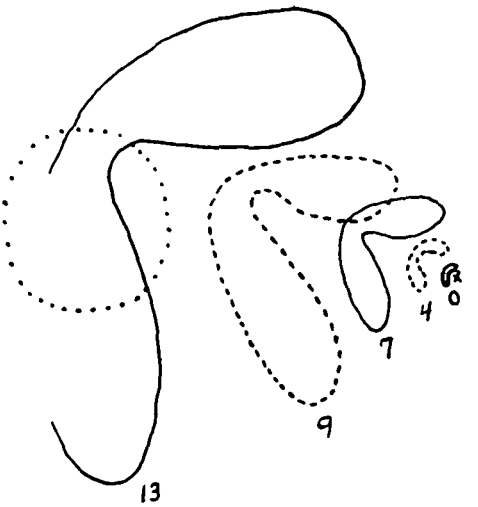

Fig. 4. Successive maps of a scotoma in classical migraine. Charts of the blind region were plotted with both eyes open, at intervals indicated in minutes. The fixation point is marked (×) and the blind spot of the homolateral eye (approx. 15° from ×) is indicated by the circle at the left. Reproduced from Fig. 3 of Lashley (1941).

headache phase. The most clearly characterized initial symptom is a visual disturbance involving a scotoma (blind region) and abnormal flickering sensations, both of which move around through one half of the visual field. The scotoma is profound and can be easily plotted with conventional techniques.

There is little direct evidence linking migraine with spreading depression. The principal case can be made forcefully by quoting a paper by Lashley (1941), who made a careful quantitative study of his own migraine. Note that Lashley had no knowledge of spreading depression and that his paper was published 3 years before Leão's first description of SD (Leão, 1944).

'Maps of the scotomas of opthalmic migraine* sketched at brief intervals during an attack suggest that a wave of intense excitation is propagated at a rate of about 3 mm per minute across the visual cortex. This wave is followed by complete inhibition of activity, with recovery progressing at the same rate. Sometimes the inhibition spreads without the preceding excitatory wave' (Lashley, 1941).

One of Lashley's scotoma maps is reproduced in Fig. 4. With hindsight, the similarity between Lashley's inferences and the properties of spreading depression is almost uncanny, as recognized by Milner (1958). Milner suggested also that other manifestations of migraine might be due to SD in other regions of the cortex and that, for anatomical reasons, these might be more amenable to study with non-invasive techniques than disorders of the visual cortex. Curiously, the literature on migraine and SD very nearly seems to stop there. Perhaps this is because of difficulties (referred to above) in demonstrating SD in human cerebral cortex, though Lashley had actually made a big point of how his inferred cortical disturbance seemed to be restricted to one

* This term, used by Lashley, clearly refers to a case that would be described nowadays as *classical migraine with binocular visual aura.*

particular region (the primary visual cortex) and to be incapable of spreading to adjacent regions. Perhaps it is also because at the time of Milner's (1958) suggestion the principal theories of migraine were already established and appeared to be inconsistent with conclusions that have been drawn about SD. We need therefore to look at the migraine literature to assess the case against a relationship to spreading depression.

If SD occurs during migraine it is probably during the initial phase, before headache occurs. We are thus principally concerned with the underlying mechanisms of the initial phase. The early symptoms are usually attributed to vasoconstriction and ischaemia. This theory originated in the last century and has gained particular support from experiments of Wolff in the 1940s and from recent direct evidence for reduced cerebral blood flow early in migraine (see, for example, Wolff, 1980). The idea that vasoconstriction could adequately account on its own for the complexity and discrete nature of the neurological symptoms in migraine has been repeatedly challenged however, for example by Sacks (1970). These puzzling discrete symptoms of migraine are just the kinds of things one might expect from spreading depression – notably in the case of the visual disturbances. In this context it is particularly surprising to find Sacks (1970, p. 196) referring to Milner's (1958) suggestion of an involvement of SD in migraine as 'ingenious if absurd'. It would of course be absurd to suggest that migraine was nothing more nor less than SD: if SD occurs, there must be something abnormal in the first place to initiate it. One of the interesting possibilities is that the consequences of SD might spread far beyond the direct effects of local neuronal depression through the release of substances into the cerebrospinal fluid or plasma.

What might be the relationship between SD, if it occurs in migraine, and vasoconstriction? There is some evidence that vasoconstriction occurs early in SD, followed by vasodilatation (Van Harreveld & Stamm, 1952), though others with different techniques have found evidence only for vasodilatation (see Bures *et al.* 1974). Vasoconstriction might also be the factor (or one of the factors) precipitating SD in migraine. This would be consistent with the various theories of the aetiology of migraine implicating the autonomic system or platelets as primary causes of reduced blood flow (Johnson, 1978; Hanington, 1978).

The principal evidence that vasoconstriction might directly cause symptoms in classical migraine comes from the effects of vasodilators (amyl nitrite; CO_2/O_2 mixtures) given during the pre-headache phase. These were shown by Wolff and his colleagues to relieve transiently the early symptoms and (in the case of CO_2/O_2 mixtures) to be capable of aborting an attack, including the expected headache. Since the propagation of SD probably does not involve the circulation, it would be surprising if relief from symptoms caused by SD could be obtained by purely vasodilator interventions. It seemed to me worthwhile therefore to examine the effects of amyl nitrite and CO_2/O_2 mixtures administered during SD in anaesthetized rats, in situations analogous to Wolff's tests on migraine patients. There was in fact already evidence that apnoea induced in rats after they had been breathing pure O_2 could prevent the initiation of SD waves from the site of a KCl crystal (Lehmenkühler, Speckmann & Casper, 1976). My experiments (Gardner-Medwin, 1981*b*) have shown that CO_2/O_2 mixtures (10–15% CO_2), given after a single SD wave has been initiated, can stop the

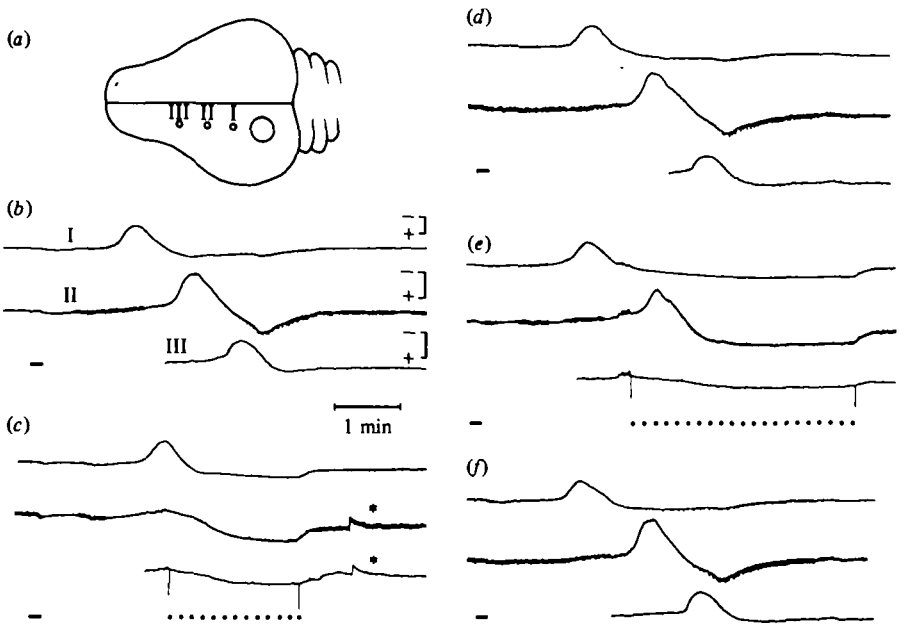

Fig. 5. The effect of CO_2-O_2 mixtures on waves of spreading depression. (*a*) Diagram of the brain of an anaesthetized rat (urethane I.P., 1·4 g/kg) showing the sites of a 3 mm diam. cup on the exposed pial surface (large circle) and of subdural Ag/AgCl electrodes at approximately 3 mm spacing (I–III). (*b–f*) Voltages recorded at the three electrode sites relative to a reference electrode in the mouth, during spreading cortical depression induced by switching perfusion of the cortical cup from 147 mM-NaCl + 3 mM-KCl (37 °C) to 150 mM-KCl (37 °C) for 10 sec (bars at left). (*c*, *e*) During the period indicated by dots, the rat's tracheal cannula was connected to a chamber (5 ml) continuously supplied with O_2 (86 %) + CO_2 (14 %) instead of to room air. The surface-negative wave characteristic of SD failed to reach electrodes II or III in (*c*) and electrode III in (*e*), when the gas was administered *ca.* 30 sec later than in (*c*). Trace III starts late because the pen was used also to record the initial potential changes in the cup (not shown). *An artefact produced on two channels by power transients. Calibration bars: 5 mV.

propagation of this wave within a minute or so (Fig. 5). It remains uncertain whether this action is due to vasodilator or some other effects of the gas mixture; but the similarity with the results on migraine (Marcussen & Wolff, 1950) weakens the argument that the neural symptoms in the two phenomena must be caused by different mechanisms. My experiments with amyl nitrite vapour administered during SD in rats have been less clearcut. The drug easily elicits a widespread non-propagating dysfunction in rat cortex, possibly due to a drop in blood pressure. This is consistent with the effect of large doses administered during migraine in man. Schumacher & Wolff (1941) found that they needed carefully controlled doses in their migraine subject to alleviate his scotoma: larger doses produced only a few seconds of improved vision before a more widespread scotoma set in. I have not at present succeeded in stopping the propagation of a wave of SD with amyl nitrite. This may be because of a true difference between SD and migraine, because of species differences, or because of inadequate dose control. Wolff's writings suggest that, in migraine also, the effects of CO_2/O_2 mixtures were more reproducible and more consistently in the direction of relieving the syndrome than amyl nitrite, when applied early enough in an attack.

The headache phase in migraine is normally attributed to vasodilatation (Wo[illegible]

1980). If SD does occur in migraine, it is an open question whether it is simply an incidental phenomenon or whether (for example, by the release of active substances) it is a causal link in the chain of events that leads to vasodilatation and to headache. Some forms of migraine ('common' migraine) have a phase with headache and nausea not preceded by clearcut neurological symptoms. These may indicate that the headache can occur wholly in the absence of SD, or they may perhaps be indicative of SD occurring in a region of the brain where it fails to give rise to overt symptoms. These issues are hard to investigate at present, especially since it is by no means easy to study headache in animals, nor the microenvironment of the brain in man. But there are observations in the migraine literature, for example extracranial vasodilatation and changes of serotonin levels in the blood, that so far as I know, have not even been studied in relation to SD.

The case for a link between SD and migraine, though not strong, is probably stronger than the case against such a link. Spreading depression may turn out to be either crucial or irrelevant in the disease process; in the meantime, it is surely worth investigation in this context. If and when a relationship may be clearly established, this may be the time to return along the chain of associations in this review and to ask whether one of the roles of neuroglia in the vertebrate nervous system may be to prevent or to minimise the syndrome of migraine.

I should like to thank C. D. Marsden, C. Nicholson, J. A. Coles and J. F. Ashmore particularly for helpful comments on this manuscript and A. L. Hodgkin for providing a copy of his unpublished mathematical treatment relating to spreading depression.

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