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Depolarisation Phenomena in Traumatic and Ischaemic Brain Injury

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With 11 Figures

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Abbreviation List

ADC	Apparent diffusion coefficient
ATP	Adenosine triphosphate
Ca^{2+}	Calcium ion
CA1	The CA1 region of the hippocampus
Cl^-	Chloride ion
Cl^-_e	Extracellular chloride ion
CSD	Cortical spreading depression

CBF	Cerebral blood flow
DC	Direct current
ECoG	Electrocorticography
ECS	Extracellular space
Hb(O)	Haemoglobin (oxidised form)
HSP	Heat shock protein
HSD	Hypoxic spreading depression – like depolarisation
IEG	Immediate early gene
IL	Interleukin
IP3	Inositol trisphosphate
K^+	Potassium ion
K_e	Extracellular potassium ion
MCAO	Middle cerebral artery occlusion
mM	Millimoles per litre
mRNA	Messenger ribonucleic acid
mV	Millivolts
Na^+	Sodium ion
PID	Peri-infarct depolarisation
pO_2	The partial pressure of oxygen
NO	Nitric oxide
Na-K ATPase	Sodium-potassium ATPase
NIRS	Near infrared spectroscopy
nm	Nanometres
NAD(H)	Nicotinamide adenine dinucleotide (reduced form = NADH)
Vm	Neuronal membrane potential
BDNF	Brain derived neurotrophic factor
NF-κB	Nuclear factor kappa-B
NMDA	N-methyl-D-aspartate
TPA	Tissue plasminogen activator
TBI	Traumatic brain injury

History, Definitions and Introduction

In 1944 a young Brazilian physiologist, Aristides Leão, was studying for his doctorate in Harvard University. According to Somjen [1], he was attempting to study propagation of epileptic activity in the cerebral cortex, and he approached the problem by applying electrical stimulation to the frontal convexity cortex of anaesthetised rabbits, and recording from an array of corticography electrodes posterior to this (Fig. 1). Instead of seeing propagating epileptic activity, he observed a period of electrical silence, which was first seen adjacent to the stimulating electrodes, and did indeed propagate from the site of stimulation backwards along the cere-

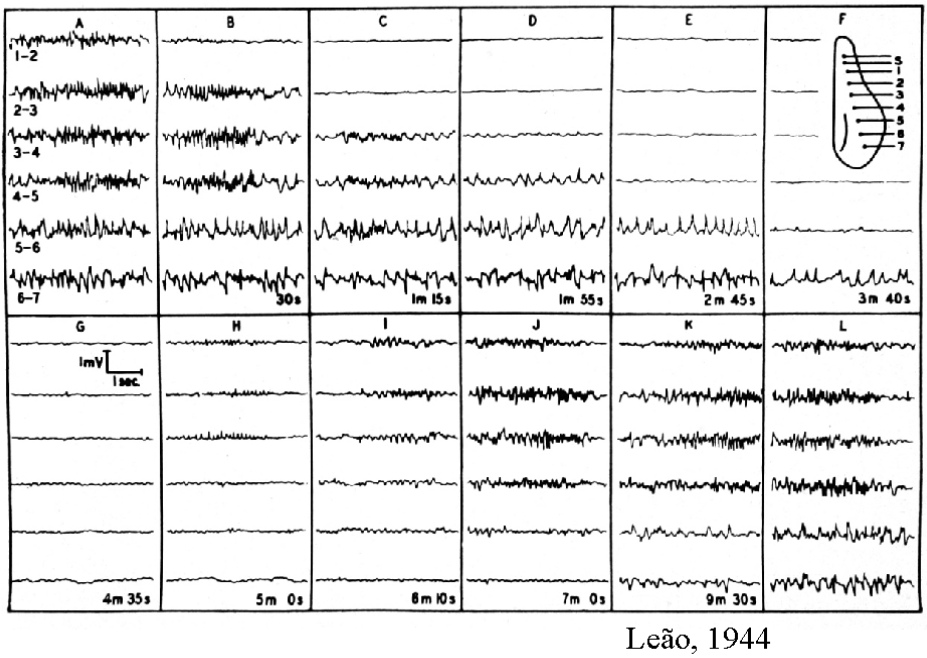


Fig. 1. Leão's original demonstration of cortical spreading depression, demonstrating a time sequence of twelve separate recordings spanning some 10–11 minutes, from a linear array of seven electro-corticographic (*ECoG*) electrodes extending antero-posteriorly over the right hemisphere of a rabbit anaesthetised with barbiturate. A pair of bipolar electrical stimulating electrodes are placed at the front of the hemisphere, and following stimulation, a wave of electrical silence is seen to propagate backwards from the site of stimulation, followed after approximately 7–9 minutes by spontaneous recovery at each site. (Reproduced with permission from Leão [2])

bral hemisphere – at a rate of some 3 millimetres per minute. The phenomenon resolved after 5–15 minutes, with – apparently – full resumption of cortical electrical activity. He reported his findings in a landmark paper entitled "Spreading depression of activity in the cerebral cortex" [2]. The event which he described became known as "spreading depression" or "cortical spreading depression" [of Leão] (CSD), and has remained a subject of intense interest to neurophysiologists. Although the electrophysiological and haemodynamic features have become very well characterised, with mass focal depolarisation of neurones and glia as the defining event, its most enigmatic challenges have remained its uncertain physiological role in grey matter, and its relevance – if any – to human disease states.

Since 1977–1978, stroke research laboratories have become aware of a feature of cerebral cortex in the ischaemic penumbra which shares certain

characteristics with CSD, but also differs from it in critical aspects. ''Peri-infarct depolarisations'' (PIDs) arise spontaneously in cortex at the edge of the core ischaemic territory and propagate in the penumbra, but unlike CSD, they are harmful in that they cause progressive recruitment of the penumbra into the core territory, thus enlarging the infarct [3]. Somjen refers to such events as *hypoxic spreading depression-like depolarisations (HSD)* [1]. The evolution of this concept, and increasing awareness among some clinicians of its existence, has prompted increasing speculation as to whether CSD or PIDs occur in the injured human brain. Demonstrations of CSD-like events in models of traumatic brain injury, the imaging in the laboratory of propagation of PIDs across the cerebral cortex in models of focal cerebral ischaemia, the knowledge that not only cerebral cortex but also deep nuclei and the hippocampus may be subject to CSD, and particularly the recent confirmation that such events do indeed occur in patients with serious head injury [4], seem likely to open a fresh chapter in clinical brain injury research. This is an area of research to which neurosurgeons are uniquely placed to contribute.

The features of cortical spreading depression as it is observed in the experimental laboratory have been the subject of a number of authoritative reviews extending over many years, and the reader seeking the most detailed information is directed to them [1, 5–7]. We have relied extensively on these reviews as well as on the original sources. In this review, we shall draw together the principal physiological, chemical and haemodynamic features of CSD and PIDs, and consider their possible functions and effects in the context of acute ischaemic and traumatic injuries to the human brain. We shall also explore methods for detection of depolarisations in the injured human brain, and the actual and potential impact of this information on our understanding of the pathophysiology of the injured human brain and on our clinical management of traumatic and ischaemic brain injury. The broader term ''depolarisation'' will be used where neither CSD nor PID is specifically under discussion.

Cortical Spreading Depression

The ''Onset'' Phase of CSD

Initiation of CSD

Leão's observations were made in rabbits under barbiturate anaesthesia, and the stimulus to the cortex was bipolar electrical current delivered from an induction coil, but several other stimuli are also effective. Dialysis through an implanted microcatheter or superfusion of the exposed cortex with potassium chloride (KCl) at 130 mM or more is effective in the rat brain [8], as is local application of KCl with a wick. Neurosurgeons should

also be aware that needling of the cortex is effective, and it seems inescapable that more complex surgical manipulations of similar, *susceptible* tissue are likely to be effective if, as seems clear from the recent findings in patients [4], CSD does indeed occur in the human brain. There is also experimental evidence that spreading depression occurs in the spinal cord [9]. What determines susceptibility, by which is meant the frequency of occurrence of CSD (rather than vulnerability to damage from depolarisations), is an important theme of this review. The factors which are currently believed to affect this are species differences, location in the brain, haemodynamic and metabolic conditions in the cortex, anaesthesia, and systemic metabolic variables (essentially – in the present state of knowledge – plasma glucose). All of these factors are best considered after we have first reviewed the basic electrophysiological, haemodynamic and metabolic properties of CSD.

The DC Potential Transient

For the purposes of a discussion focussed on brain injury, the CSD complex is best considered in its two phases, onset and recovery, since, as we shall see, it is probably deficiencies in the recovery process that underlie the differences between CSD and PIDs. When Leão measured the DC potential difference between a point on the cortex in the path of the propagating wave of depression and a remote reference point, he noted a transient negativity of some 10 to 15 mV. The observation has been repeated many times, and when sought, the DC potential transient is an invariable feature of both the CSD and PID patterns of depolarisation. The nature of the DC potential transient – presumably indicating a brief accumulation of negative charge in the cortex – is still unknown, although an increase in one or more anions, – lactate, amino-acids, or bicarbonate – has been suggested as a cause [10].

Mass Neuronal Activity: Grafstein – 1956

Studying areas of cerebral cortex isolated electrically by subpial transection but with perfusion intact, Grafstein recorded unit activity with an extracellular microelectrode, and found a short phase of intense firing at the onset of the DC potential change, followed by prolonged silence [11] (Fig. 2). There is no suggestion that this transient neuronal activity conveys any physiological information, and as we shall see it is initiated by local changes in the extracellular environment. The observation suggests that excitatory or depolarising influences on neurones – not necessarily synaptic – contribute to the initiation of the CSD event as it reaches a new locus. It is of interest for current researchers studying models of stroke that

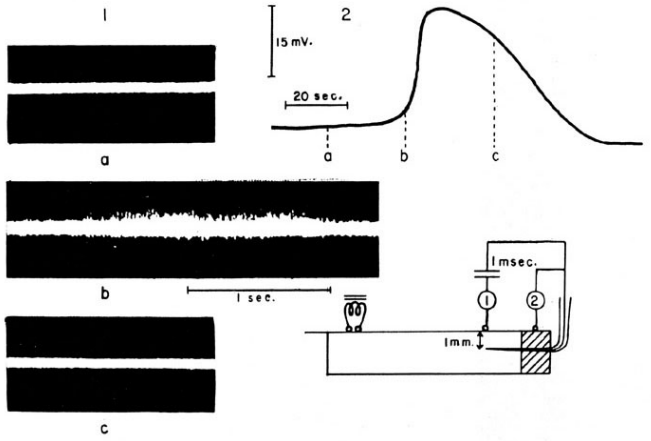


Fig. 2. Comparison of extracellular microelectrode recording (*Panel 1, a,b,c*) and simultaneous recording of DC potential (*Panel 2*) in a slab of cerebral cortex isolated electrically by subpial transections (Grafstein, 1956). The cortex was stimulated remote from the recording electrodes, initiating a wave of spreading depression. In 1a, no spontaneous neuronal firing is present prior to arrival of the wave, but as depolarisation commences there is a *brief* phase of intense neuronal firing (*b*) followed by silence (*c*) when depolarisation has begun to recover. (Reproduced with permission from Grafstein [11])

Grafstein was able to suspend and then restore resolution of the DC potential transient by first occluding and then releasing the middle cerebral artery (MCAO) in rabbits, showing that resolution of the DC potential is energy-dependent. Her proposal that potassium ion liberated by neuronal depolarisation caused subsequent depolarisation of adjacent neurons still forms the basis of current thinking on mechanisms of CSD propagation (see below).

Thus Grafstein's 1956 paper demonstrated or inferred three of the key features of CSD – mass neuronal depolarisation, mediation by potassium ion, at least in part, and the dependence of recovery on availability of perfusion and energy. The findings and inferences have remained substantially unchallenged, and form the foundation of our understanding of depolarisation events in the cerebral cortex; the paper is perhaps one of the key contributions to neuroscience in the past 50 years.

Changes in Extracellular Ion Concentrations $[K^+]_e$, $[Na^+]_e$, $[Cl^-]_e$, $[Ca^{2+}]_e$

A transient, marked increase in K_e from the normal 3 mM to 60 mM or more is a striking and regular feature of CSD, and lasts for approximately 30–40 seconds in total, often resolving with an undershoot below

the baseline [12]. There are accompanying decreases in $[Na_e]$ [13], in $[Cl^-]_e$ and in $[Ca^{2+}]_e$ [14].

Changes in Membrane Potential and Conductance During CSD

The first intracellular recordings from a neuron during passage of a wave of CSD were made by Collewijn and Van Harreveld, who concluded, after allowing for the simultaneous change in extracellular potential, that neuronal membrane potential (V_m) reached zero briefly [15]. This pattern of depolarisation to zero volts is different from that of the action potential, where V_m may reach +20 mV, and could imply simultaneous opening of several or all membrane conductances in CSD; this might represent mechanical opening of membrane pores with no ion-specific conductance properties, but according to Somjen it is not necessary to postulate such special channels in order to explain the membrane potential changes in CSD [1].

Redistribution of Water: Tissue Impedance

An increase in electrical impedance of tissue is largely a measure of cell swelling, and Leão and Martins-Ferreira demonstrated an increase in impedance during CSD in 1953 [16]. Measurements of extracellular space volume using indicators such as tetramethylammonium, together with morphological evidence, support this, and the basis most probably lies in the excess of the decrease in $[Na^+]_e$ over the increase in $[K^+]_e$ [13]. This would imply a net movement of ions into cells, accompanied by osmotically obliged water, in turn raising impedance to current flow in the ECS. However, Somjen points out [1] that there is evidence that when impedance is measured some current flow is through rather than around cell membranes, perhaps more especially glia, and that there is also evidence of a marked drop in neuronal membrane resistance during SD [17, 18]. Whatever their precise nature, these changes in CSD appear closely related to the transient reduction in apparent diffusion coefficient (ADC) that can be detected in the rat [19] and cat [20] brains during CSD using magnetic resonance diffusion-weighted imaging (see also page 33: Section on Occlusive Stroke).

Mode of Propagation of CSD

Early experimental studies of propagation of spreading depression were aided by the use, principally by Martins-Ferreira and Oliveira Castro [21], of the isolated chick retina, in which the presence and propagation of spreading depression is evident to the naked eye from a transient change in optical properties. They were able to establish a ''ring'' of retina in which

the phenomenon could be constrained to propagate in circular fashion, at a rate measured at 3.7 mm/minute – similar to that originally described by Leão, and they found that alkaline conditions, or increased K_e or Cl_e, all accelerated propagation, whereas acidification or an increase in Mg_e slowed it.

In one of her 1956 papers [11], and noting the likelihood of neuronal depolarisation (as the basis for the brief phase of spontaneous spike discharges), Grafstein suggested that the resulting liberation of *potassium* ion into the ECS could occur in sufficient concentration to cause adjacent neurones to depolarise, thus causing – or at least supporting – propagation. The simultaneous reduction in ECS volume (see above) would increase the effective $[K]_e$, thus facilitating depolarisation of neurones in the path of the wave.

The *separate* idea that potassium ion might diffuse slightly further in the ECS and cause depolarisation in *non*-contiguous neurones was explored in detail by Gardner-Medwin, who determined a rate for cortical extracellular diffusion of K^+, and showed that this was slower than that of CSD propagation [22]. A further argument against extracellular diffusion of K_e as the basis of propagation is that in CSD, no increase in K_e can be recorded in the cortex prior to the DC depolarisation (unlike PIDs, where a gradual, prior increase in K_e *does* occur [23, 24].

A second candidate agent explaining propagation is glutamate released into the extracellular space (ECS) by mass neuronal depolarisation, and in turn depolarising adjacent neurons. Van Harreveld induced CSD by application of compounds in brain extracts, one of which was glutamate [25], and he and Fifkova later demonstrated release of glutamate during CSD in the retina [26]. However, glutamate dialysed into the cortical ECS does not elicit CSD, nor does inhibition of glutamate reuptake [8, 27].

Propagation of CSD via Glial and/or Neuronal Gap Junctions

The possible roles of intercellular coupling either of neurones or of astrocytes in initiation and propagation of CSD have received much attention in the last few years. In the case of astrocytes, it is now abundantly clear that in cultures of astrocytes studied with intracellular calcium-sensitive dyes, waves of transient increase in intracellular calcium ion (Ca_i) can be initiated – by glutamate [28], nitric oxide (NO) [29] or mechanical stimulation [30] – and will then propagate across the culture at a rate very similar to that of CSD in the intact cortex [6]. Nedergaard has shown that in mixed glia-neuronal cultures, such glial waves are associated with elevations in neuronal calcium concentrations [31]. Transmission of calcium waves through glial cultures is believed to occur through glial gap junctions – specialised and specific membrane openings whose molecular

structure is now well-characterised and which are usually readily permeable to ions and compounds of smaller molecular weight; examples are inositol trisphosphate (IP_3) and potassium. IP_3 is thought to mediate propagation of Ca_i waves through its role as a ligand for IP_3-receptor-Ca-conductance complexes on the endoplasmic reticulum, and glial gap junctions are also thus a probable substrate for the mechanism of "spatial buffering" of increases in K_e, as envisaged by Somjen [32]. Propagation is also mediated by an extracellular agent, ATP [33].

At least in the cell culture preparations in which glial communication has been studied, the capacity to propagate Ca_i waves seems exceptionally well supported, by a range of agents that include ATP [33], nitric oxide [29], and inositol trisphosphate (IP_3), the latter via glial gap junctions [34]. The demonstration that an intracellular calcium wave *precedes* the arrival of spreading depression [35] also lends support to the idea that CSD propagation is mediated primarily by glia. A further argument for the concept is based on the fact that halothane, which blocks glial gap junctions [36], also reduces the frequency of CSD in the gyrencephalic brain [37], and reduces MCAO infarct volume and PID frequency by an effect either on perfusion or on intrinsic PID susceptibility [38].

Other findings argue against this hypothesis. First, CSD is more readily elicited in areas of grey matter with relatively *lower* glia : neuron ratio, such as the CA1 layer of the hippocampus (in experimental studies) [39], and the occipital cortex in humans [40, 41] (if it is accepted that migraine with visual aura is a manifestation of CSD, as discussed below). Secondly, the use of specific agents toxic to glia such as fluorocitrate or fluoroacetate fails to prevent CSD [42, 43]. Third, CSD can occur in the absence of Ca_i waves [44].

The Recovery Phase of CSD, and the Responses of Cerebral Metabolism and Blood Flow to CSD

Resolution of the cation transients might in theory be due either to restitution of normal, resting distributions by active transport, or in the case of the increased $[K]_e$, to diffusion through the extracellular space (which would necessarily be slower than the observed resolution rate [22]), to spatial buffering by the astrocytes through gap junctions [32], or to passive elution through cerebral perfusion (probable only under conditions of energy failure [45]). Grafstein's experiment with MCAO described above is perhaps the earliest evidence for a role for energy-dependent active transport in the recovery phase, and evidence for the concept has steadily accumulated. Demonstration of the cation transients that are an integral feature of CSD makes it almost inevitable that restoration of resting cation distributions should necessitate a considerable increase in ATP utilisation.

Indeed CSD, and epileptic seizures, are perhaps the most extreme forms of activation challenge to reactivity of cerebral metabolism and blood flow (CBF).

Detailed studies by Rosenthal & Somjen and their colleagues of CSD in the normally perfused brain indicated transient oxidation of the mitochondrial respiratory chain [46]. In the light of subsequent work demonstrating transient *increases* in perfusion [47] and in tissue pO_2 during CSD [48], one simple interpretation of Rosenthal's work is that the redox potentials of the respiratory chain coenzymes are in equilibrium, and are determined by the balance between the rate of ATP hydrolysis and availability to mitochondria of molecular oxygen from cerebral perfusion.

Glucose Utilisation During Recovery from CSD

Studies of normal, functional activation in the human brain using positron emission transverse tomography [49] indicated for the first time that the rate of glucose utilisation increased in greater proportion to oxygen utilisation, suggesting upregulation of glycolysis rather than of oxidative glucose utilisation. The finding of transient increases in brain lactate of some 30% in experimental studies of somatosensory activation [50] supported this interpretation, and suggested a degree of dependence on glycolytic generation of ATP during activation. The very large cation shifts that occur in CSD make it highly likely that similar and greater – but still transient – changes in glycolysis would occur during repolarisation after CSD. However, an extracellular lactate transient need not necessarily mean a shift to anaerobic metabolism, and Back and colleagues showed that in the normally perfused brain CSD is accompanied by an *in*crease in partial tissue pressure of oxygen [48]; this may be attributed to the hyperaemic response to CSD which is described below.

The model of the cerebral metabolic response to activation developed by Magistretti and colleagues [51] envisages that glycolytic activity is predominantly in the astrocytic compartment (where almost all glycogen in the brain is held [52, 53]), stimulated by an increase in extracellular glutamate during functional activation. It is further proposed that astrocytes deliver lactate to neurons, which, relying on lactate dehydrogenase activity in reverse, convert lactate to pyruvate. This pyruvate is then metabolised via the tricarboxylic acid cycle. Glucose transport across the blood brain barrier is highly efficient, to the extent that total unidirectional flux into the brain under non-activated conditions is approximately twice the rate of utilisation by glycolysis [54]. This, allied with the hyperaemic response to CSD discussed below, endows the cortex with its capacity to meet the challenges of activation. It is not appropriate to pursue further this important topic in this context, and the reader is referred to work by Magis-

tretti and colleagues [55], to a review questioning some aspects of this ''compartmented glial glycolysis'' model [56], and to the review by Chen and Swanson of astrocytic function and changes in brain injury [57]. Changes in glucose metabolism in focal ischaemia and during PIDs are described later in this review.

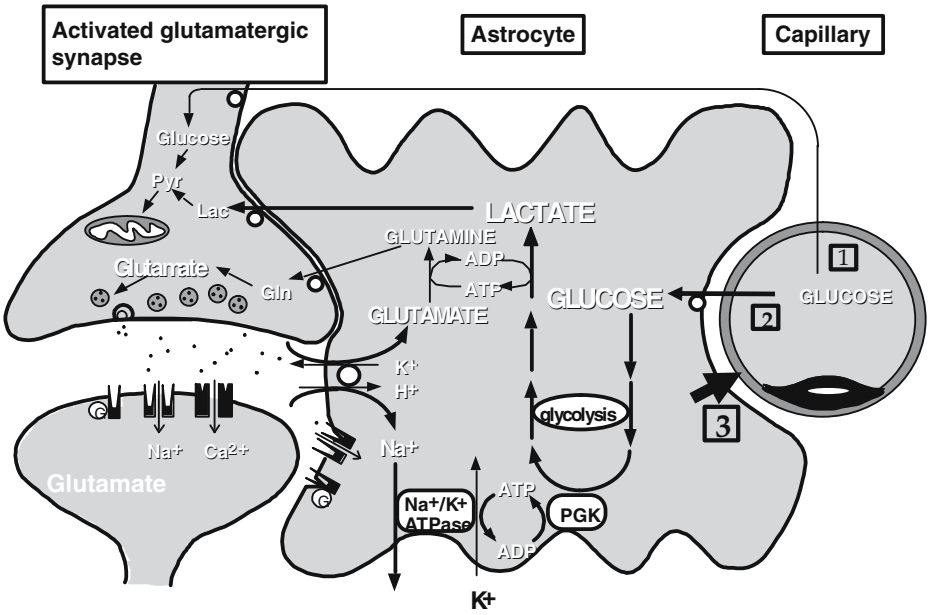


Fig. 3. Schematic diagram illustrating current concepts of the role of astrocytes in cerebral perfusion and metabolism (adapted with permission from Tsacopoulos and Magistretti [55]: Copyright 1996 by the Society for Neuroscience). Cerebral capillaries are extensively invested by astrocyte end feet, and extraction of glucose from blood to brain (probably the astrocyte compartment) is highly efficient (*arrow 2*). During activation, and especially in cortical spreading depression, the glycolytic pathway in astrocytes is upregulated, and the different kinetics of glial and neuronal lactate dehydrogenases favour net movement of lactate from astrocytes to neurons; the (limited) brain glycogen pool is located in astrocytes. Under resting conditions, glycolysis in neurons may be sufficient to meet energy demands (*arrow 1*). Neurotransmitter glutamate released into the synaptic cleft is re-accumulated into astrocytes by high-affinity cotransport with Na^+ ion, making use of the normal electrochemical gradient generated by Na^+/K^+ ATPase.

Several mechanisms regulate cerebral perfusion, with a prominent role proposed for astrocytes (*arrow 3*) [140]. First, their high membrane conductance for K^+ allows astrocytes to buffer the increased extracellular levels resulting from activation, with a direct vasodilator effect of K^+ on the microcirculation via the astrocyte cytosol and end feet. Adenosine- and nitric oxide-based mechanisms also contribute. Recent work by Zonta *et al.* now supports an additional mechanism of astrocyte-mediated vasodilation during activation [141]

Haemodynamic Response

Leão himself was the first to demonstrate hyperaemia in association with CSD; he observed a doubling in width of pial surface arterioles during CSD [58]. If CSD induced in the prefrontal region of the rat is assumed to propagate anteroposteriorly in the cerebral hemisphere at a constant rate, serial coronal sectioning of the hemisphere after it has been frozen at a single time point will provide in the section sequence a time series of the response of the brain to the propagation wave. Using autoradiography for CBF, and reasoning in this way, Lauritzen *et al.* showed that CSD is closely followed by an intense ($>200\%$) but brief (2 minutes) transient hyperaemia [47]. An extended phase of mild hypoperfusion (80–90% control) follows, lasting for some 60 minutes. This feature of CSD was later used by the same group to allow mapping with isotope scanning of a phase of hypoperfusion associated with migraine with aura that propagated forwards in the cerebral hemisphere at a rate in accordance with that of CSD – a finding that argues quite strongly for CSD as the basis of migraine with aura [59].

Histology of the Cortex Following CSD

A careful histological study by Nedergaard & Hansen [60] found no evidence of classical ischaemic pathological changes in the cortex following CSD *in the normally perfused cortex* of rats. As will be described later, the situation is very different in focal ischaemia.

Molecular Responses to CSD

Expression after induction of CSD of some of the immediate early genes (IEG) that respond to stress has been studied extensively, principally in rats, mice and transgenic mice. The IEG responses to MCAO have also been studied. In many such MCAO studies, increases in gene expression extend to the whole hemisphere rather than remaining within the core and penumbral regions. It is generally believed that such widespread upregulation represents a response to a depolarisation event that started as a PID in the ischaemic territory but then propagated throughout the rest of the hemisphere as CSD. According to Sharp *et al.* [61] this applies to c-fos and jun-B. Cyclooxygenase-2 is also induced by CSD [62]. In some cases, the association is relatively specific: for example, the degree of induction of the mRNAs encoding brain-derived neurotrophic factor and heat-shock protein-72 in response to CSD induced in the rat is dependent on the number of CSDs [63]. It needs to be stated that in MCAO other gene expression patterns may relate more to cell damage than to CSD. Thus HSP70, a heat shock protein, behaves as a protein chaperone, increasing in

the presence of denatured proteins [64], although expression in the infarct core may be limited by ATP depletion [65].

CSD as an Initiator of Inflammation

That cerebral ischaemia causes an increase in levels of interleukin-1β (IL-1β: an inflammatory cytokine) in the brain is well established [66–68]. CSD has a similar effect: Jander and colleagues recently showed that mRNA levels for IL-1β and tumour necrosis factor-α (TNF-α, also an inflammatory cytokine) are increased 24- and 60-fold respectively 4 hours after CSD induction with KCl [69]. Expression of the IL-1β protein was largely confined to microglia in the superficial cortical layers. These authors suggest that ''cytokine expression following CSD forms part of a physiological stress response that contributes to the development of ischaemic tolerance in this and other preconditioning paradigms'' (see below). That IL-1β can promote CNS repair has also been shown [70]. Another view of the effects of IL-1β comes from the work of Blamire *et al.*, who examined the effects of microinjection of recombinant IL-1β into the striatum of 3-week-old rats, and found significant reduction in apparent diffusion coefficient (ADC) and increases in cerebral blood volume and blood brain barrier permeability [71]. ADC reductions are usually attributed to a shift of water from extra- to intracellular compartments, but a reduction in water mobility in the intracellular compartment may occur [72]; both explanations are in keeping with an adverse effect of IL-1β.

Pre-Ischaemic Conditioning with CSD as Protection in Experimental Stroke

In experimental studies of stroke in rats, it is possible to confer a degree of protection from the effects of a period of ischaemia by prior induction of CSDs [73]. Levels of mRNAs for FOS, BDNF, and tPA, are increased by preischaemic conditioning with CSD [74]. TNF-α and IL1-β are believed to contribute to increased tolerance of ischaemia [75, 76], and an antagonist to nuclear factor κ-B (NF κ-B) blocked NF κ-B activity and reduced the pre-conditioning effect [77].

It seems very likely that one or more of the currently identified expression cascades – or other(s) still to be detected, underlie the protective effect of preischaemic conditioning with CSD, and increasing understanding of the molecular response to CSD may in time allow us to identify which of the several genes upregulated by CSD is/are responsible for the protective effects of preconditioning, and so perhaps lead to novel therapy for cerebral ischaemia, or at least better protection of the brain when some degree of prospective risk exists.

Factors Determining Ease of Induction of CSD

Species Differences and Cytoarchitecture

It has long been clear that CSD is more readily induced – and its repetition maintained – in rats than in larger experimental animals [5], with primates seen as the most "resistant" group of species. However, it is certainly possible to induce CSD in the primate brain [78]. A specific attempt to compare PID frequency in cats and squirrel monkeys after MCAO showed that PIDs do indeed occur spontaneously in a primate species, but failed to confirm a species difference in frequency of PIDs because of wide variability within both species [79]. However, the results revealed a clear dependence of PID frequency on plasma glucose level: this is discussed below in the context of PIDs.

One of the most widely canvassed explanations for species differences starts with the observation that lissencephaly is characteristic of the CSD-prone species, whereas the more resistant brains are gyrencephalic. There are however also regional differences in susceptibility within the brain of a given species, with the hippocampus particularly liable to CSD, together with – in migraineurs-with-aura – the occipital cortex. A clue to the puzzle comes from consideration of the cytoarchitecture and glial : neuronal ratios of different brain regions and in the brains of different species. Thus neurons are particularly tightly packed, glia relatively sparse, and CSD frequent, in the CA1 layer of the hippocampus. Migraine with aura typically commences with a visual aura on or near the fixation point (although auras apparently arising from the somatosensory cortex also occur), and the glia : neuron ratio in the occipital cortex is lower than elsewhere in neocortex [40, 41].

Tower and Young compared the glia : neuron ratio with brain size in a group of mammals ranging from mice to whales and elephants, and, using a log : log plot, demonstrated a convincing hierarchy in which the glia : neuron ratio increases in proportion with brain size [80]. Primates are distributed appropriately for their brain size within this hierarchy, rather than all of them possessing a high glia : neuron ratio independent of brain size, as might be predicted on "evolutionary" grounds. The issue is of interest in relation to the discussion above on mechanisms of CSD propagation, and the relationship of CSD propensity with Tower and Young's hierarchy is more in keeping with a *homœostatic* role for glia in the context of CSD than with one in which they *propagate* CSD.

Spreading depression has also been observed in experiments on the spinal cord [9, 81], and the possibility therefore arises that perilesion depolarisations might contribute to the evolution of spinal cord damage – at least in grey matter – not only in trauma but also in vascular lesions.

Drugs and Anaesthetic Agents

The reduction of CSD frequency by agents known to block glial gap junctions such as halothane and propofol has been referred to above. The role of increased K_e in initiating CSD has been referred to above, and glutamate and other excitatory amino acid agonists can also effect this [25, 47, 82]; conversely, it is widely recognised that CSD and/or PID frequencies can also be reduced by the action of some excitatory amino acid antagonists, notably antagonists of the n-methyl-D-aspartate (NMDA) class of glutamate receptors [83–86]. Such mechanisms may operate in ischaemic and traumatic brain injury, although with no proven definitive therapeutic benefit in humans to date, and are considered below in the context of PIDs.

Factors Precipitating Migraine with Aura

Evidence favouring CSD as the basis of the migraine aura has gradually accumulated since Leão and Morison first suggested this [87], and is reviewed in Migraine page 29. We can learn something of the mechanisms of CSD induction from descriptions from migraineurs (with aura) of the precipitating factors they implicate. Sometimes onset follows relaxation after a period of intense concentration or physical exercise. The onset is attributed to hunger by some migraineurs with aura, and we may speculate in the light of discussion below (page 25: Relationship of Cortical Glucose Availability with PID Frequency) that hypoglycaemia is responsible in these individuals. The various other precipitating factors do not at present appear relevant in this context.

Genotype

Familial patterns of migraine incidence and inheritance are well recognised, and there is evidence that familial hemiplegic migraine is due to a calcium channelopathy [88]. Migraine with (non-hemiplegic) aura is much more common, and appears sometimes to have a familial element. It seems likely that other gene/ion-channel abnormalities will emerge in due course. A patient with the appropriate genotype seems likely to be at increased risk of depolarisations occurring in association with stroke, subarachnoid haemorrhage or serious head injury.

Haemodynamic and Metabolic Conditions in the Cortex

The role of ischaemia, trauma, increased K_e and glucose availability to the cerebral cortex in stroke and head injury will be considered below in relation to PIDs. Disturbances of magnesium metabolism have also been invoked as an additional factor increasing migraine risk [89].

Peri-Infarct Depolarisations (PIDS)

Historical

In their 1977 paper Branston *et al.* [45] referred to spontaneous, transient increases in extracellular potassium ion concentration (K_e) which occurred in the ischaemic penumbra following experimental MCAO. Similar, spontaneous events were later reported in another MCAO preparation, also in a gyrencephalic species [90]. It was suggested then that such events, PIDs [3] or HSDs [1], were "not necessarily benign" [91], and specific studies have confirmed this page 24: Evolution of PID Patterns with Time Pathogenic Potential and Recruitment of Penumbra into Core Territory). The critical points of difference between CSD and PIDs are that CSD in completely healthy cortex requires an initiating stimulus and does not damage normally perfused and metabolising grey matter, whereas PIDs are *spontaneous* and do cause damage, and in the case of the ischaemic penumbra, appear to play a large part in recruiting this zone of tissue into the expanding core infarct until this reaches what appears to be a "predestined" size (assuming no treatment).

Detection with Electrodes, and Characteristics of PIDs in Experimental in Vivo Models

PIDs have usually been documented from recordings of the cortical DC potential, and traditionally this has been regarded as a reference detection method. Such electrodes need to be non-polarisable, and usually consist of a glass micropipette filled with physiologically neutral electrolyte and inserted into the cortex, or a chlorided silver ball placed on the cortical surface. Twin-barrelled surface contact or glass microelectrodes allow the signal from an ion-selective barrel (most often to K^+) to be compared with that from an adjacent electrode, both of them referenced to a remote ground electrode. The time course of K_e as recorded from such an electrode during a PID resembles that of CSD in respect of onset and peak amplitude, but may differ in that the recovery phase may be more prolonged. In baboons, a linear, direct relationship of K_e clearance half time with degree of ischaemia was shown, and interpreted as indicating that clearance was no longer by Na-K ATPase (energy-dependent), but relied instead on passive elution by residual perfusion [45]. Studying MCAO in rats, Gill and colleagues [85] distinguished "small" (duration ~1 minute) and "big" PIDs, both recorded with DC electrodes, the latter having much longer time courses. In the same study, this group showed that the time course of depletion of extracellular calcium mirrored that of the DC potential, indicating that "big" PIDs were associated with protracted increases in intracellular calcium, likely to be cytotoxic.

The Response of CBF to a Peri-Infarct Depolarisation

The hyperaemic response to a CSD wave is well recognised from observation of cortical vessels [58], serial section autoradiography in the rat brain (generating a time series as the event propagates along the hemisphere) [47], laser Doppler flowmetry [48], and, by inference, from monitoring of transient increases in tissue pO_2 [48]. Following MCAO, the CBF response is greatly attenuated, or even reversed; thus laser Doppler flowmetry in a deteriorating patient with an intracerebral haematoma at first revealed transient increases in perfusion coupled to probable CSD episodes, but the perfusion responses reversed to transient *hypo*perfusion as brain swelling progressed [92] (Fig. 8). Back *et al.* showed that the positive tissue hyperoxia of CSD becomes a transient *decrease* in tissue pO_2 in focal ischaemia [48].

Detection and Tracking of PIDs with Imaging

In open-skull animal models of stroke, it is usually necessary to leave electrodes at a fixed location rather than probing different cortical areas sequentially, and it is also not possible to determine the extent of propagation of a presumed PID wave with one or more electrodes in the cortex. The use of a method that acquires sequential images of the exposed core and penumbral areas offers a solution if the variable being imaged is affected by the pathophysiology. When illuminated with fluorescent light at 370 nm, the cortex will fluoresce blue, emitting light in the range 445–470 nm; the fluorochrome responsible is the reduced species of the nicotinamide adenine dinucleotide redox couple (NAD/NADH), the coenzyme for succinic dehydrogenase in the mitochondrial respiratory chain. Only NADH – the reduced species – fluoresces, so that oxidation of the couple leads to a fall in fluorescence, whereas reduction causes an increase. Interpretation of such images needs to take account of the capacity of haemoglobin, particularly when oxidised, to absorb or quench blue light (hence its colour!). This method was applied in non recovery MCAO studies in cats [93], and revealed spontaneous *increases* in 450 nm fluorescence that appeared almost always to originate near the core territory and propagate outwards into the penumbra at rates in the range 1–4 mm cortex per minute and hence very characteristic of CSD (Figs. 4–6). Propagation is invariably around the walls of a sulcus, with no evidence that the event can spread directly between gyri lying in contact at the surface. Time courses of the events could be classified into (1) fluorescence increases that did not reverse, (thus closely resembling the time course of terminal depolarisation as recorded with a K_e or with a DC-potential electrode), and which occurred on penumbral cortex close to the core, (2) more peripheral

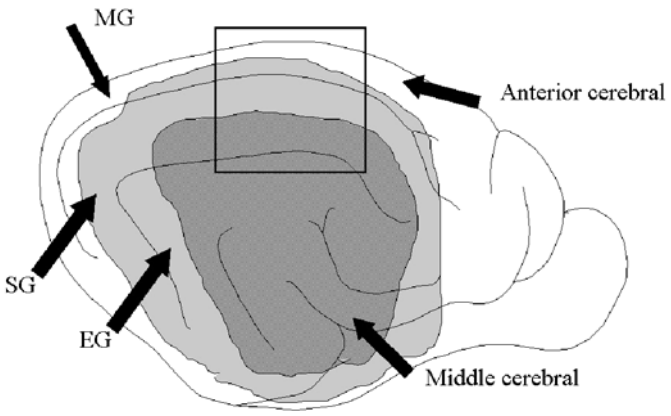


Fig. 4. Schematic diagram illustrating the concept of an ischaemic penumbra or boundary zone in experimental focal cerebral ischaemia in the cat brain, induced in this case by permanent occlusion of the right middle cerebral artery. The ectosylvian (*EG*), suprasylvian (*SG*) and marginal (*MG*) gyri lie at respectively increasing distances from the proximal Sylvian fissure. Directions of arterial inputs from the anterior and middle cerebral (*MCA*) arteries are indicated (posterior cerebral omitted for clarity). The heavily shaded area represents the *core* cortical territory associated with permanent MCA occlusion; terminal depolarisation has occurred within an hour or less of occlusion and is irreversible except by early reperfusion. The lighter shaded area (penumbra) is the site of recurrent peri-infarct depolarisations originating at the edge of the core and propagating outwards into the penumbra (see text and Fig. 5). The square area represents the field of view in each panel of Fig. 5

transient increases in fluorescence that had propagated centrifugally from cortex affected by PIDs with the first pattern, and (3) transient *decreases* in fluorescence, occurring in cortex close to the anterior cerebral artery input, and probably lying just outside penumbra (Figs. 4–6). In some cases, a single PID was seen to propagate from penumbra into anterior cerebral territory, changing its polarity from increase to decrease as an unseen interface was crossed (Fig. 5).

Increases in fluorescence may represent either reduction of the redox couple or a decrease in haemoglobin at the same locus, or a combination of the two, although also not excluding a small increase in haemoglobin outweighed by a larger NADH increase. Whichever the explanation, the observed increase in raw fluorescence indicates either vascular or metabolic compromise, and the method has been used largely to confirm propagation of the events, and to detect them. The depression in crude fluorescence grey level in normally perfused cortex *outside* the penumbra accords well with the depression of *compensated* fluorescence during CSD as shown by Rosenthal and Somjen [46].

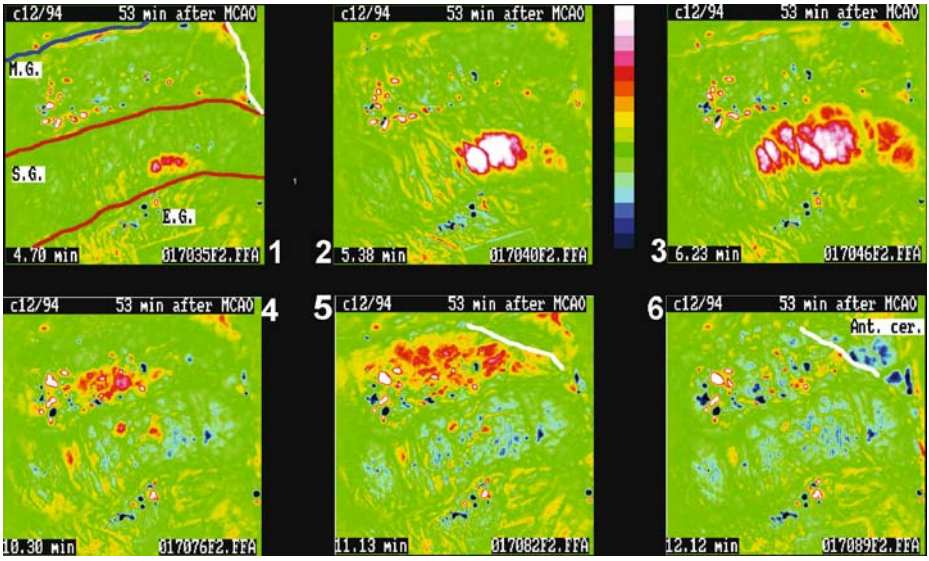


Fig. 5. *Sequence of digital images illustrating initiation and propagation of a peri-infarct depolarisation in the penumbra following experimental middle cerebral artery occlusion.* (For orientation of the image field in relation to the whole hemisphere please see Fig. 4) After exposure of the brain a sequence of grey scale fluorescence images was acquired 53 minutes after occlusion of the middle cerebral artery. The baseline image acquired at time zero was subtracted from each subsequent image and the difference image calculated and displayed in pseudocolour. Green background indicates no change in fluorescence while colours up through the rainbow spectrum to red, pink, white represent increases in fluorescence, and changes into blue, purple or black, decreases respectively. *Panel 1: EG* ectosylvian gyrus (ischaemic core). *SG* Suprasylvian gyrus (inner penumbra). *MG* Marginal gyrus (outer penumbra). Principal middle cerebral input is from lower right of the field, and anterior cerebral from upper right (*panel 6*) (see also Fig. 4). White line in panel 1 represents the anterior margin of the craniectomy exposing the cortex. Red lines represent sulci, and blue line, the line of the sagittal sinus medial to MG. Shortly before the image shown in panel 1, an area of increased fluorescence emerges from the lower sulcus and propagates outwards (from MCA input) throughout the SG (*panels 2–3*). After an interval between panels 3 and 4, the depolarisation (verified by potassium-selective electrode on posterior SG) emerges onto the MG and propagates forwards and medially (*panel 5*) but on reaching cortex perfused by anterior cerebral artery (ant. cer.), the event dissipates, represented only by a *decrease* in fluorescence in panel 6 (upper right of panel). Thus, the white line drawn on MG in panels 5 and 6 represents an apparent interface between middle and anterior cerebral territory. In this example, fluorescence has returned to baseline in the suprasylvian gyrus, but after one or more subsequent similar events, fluorescence increases on this gyrus often culminate in a permanent increase, probably indicating terminal depolarisation (Fig. 6). (Reproduced with permission from Strong *et al.* 1996 [93])

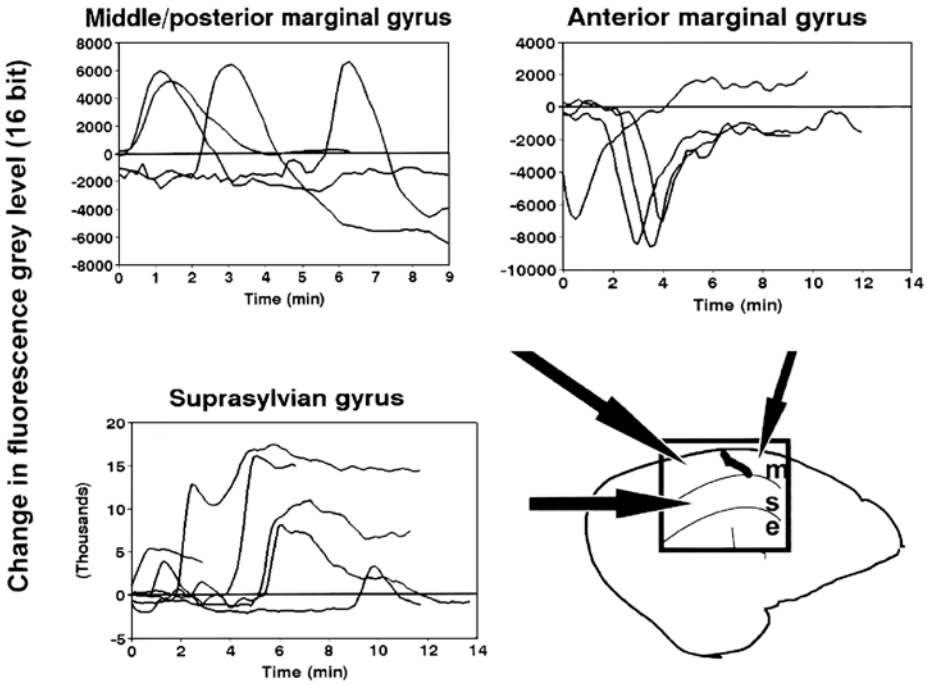


Fig. 6. Examples of time course of fluorescence events recorded from suprasylvian gyrus(s), middle and posterior marginal gyrus(m), and anterior marginal gyrus. (See also Fig. 5). On suprasylvian gyrus, the majority of fluorescence increases are sustained, probably indicating terminal depolarisation. On the middle and posterior MG, still within MCA territory but better collateralised, fluorescence increases are smaller than on SG, and not sustained. In the anterior MG, within anterior cerebral territory, fluorescence transients are all *de*creases, indicating either oxidation of the NAD/H couple, or an increase in total haemoglobin content in the parenchymal circulation, implying vasodilation. Please see also text (page 20: Detection and Tracking of PIDs with Imaging) (reproduced with permission from Strong *et al.* 1996 [93])

Initiation of PIDs

Experience with *in* vivo imaging suggests that the great majority of PIDs originate at the edge of core territory [93], and the high levels of K_e present in core areas are a probable cause [11, 27], but the same considerations apply as in CSD, and glutamate or other factors liberated from ischaemic tissue might contribute.

Terminal Depolarisation

In the core infarct territory established soon after experimental MCAO, the DC potential rapidly becomes negative, but, unlike a PID, does not

then resolve, instead becoming increasingly negative and reaching a plateau that is interpreted as indicating complete depolarisation of all cellular elements. Terminal depolarisation – effectively a failure to repolarise spontaneously (as does a PID) – is commonly taken to imply complete depletion of the ATP pool required for repolarisation, and will lead inevitably to infarction unless the ATP pool can be restored promptly by reperfusion.

Evolution of PID Patterns with Time, Pathogenic Potential, and Recruitment of Penumbra into Core Territory

PIDs – however detected – recur at irregular intervals during ischaemia, and observation over 10–12 hours reveals that, at least under chloralose anaesthesia, the pattern of recurrence eventually culminates in terminal depolarisation in outer areas of penumbra, similar to the sequence that occurs earlier in more central penumbra [38]. A feature of the progression, when K_e is monitored, is that resolution of each K_e PID transient towards the pre-transient baseline becomes steadily less complete with time, leading to a gradually increasing K_e baseline. Harris *et al.* showed that in the case of K_e, there is a striking acceleration of K_e increase when it reaches 13 mmol, suggesting a specific change in a membrane conductance [24]; terminal depolarisation follows, and the area of penumbra affected is thus recruited into the core infarct. This sequence of events suggests that number or frequency of PID events in the penumbra is a principal determinant of infarct size, and three pieces of evidence support this. First, Gill *et al.* showed that when the number of PIDs was restricted with the non-competitive NMDA antagonist dizocilpine in rats subjected to MCAO, infarct size was reduced [85]. Secondly, Mies and colleagues reported findings closely similar to those of Gill's group [94]. The association of a larger infarct with increasing PID number may simply reflect the operation of a different, underlying mechanism determining both infarct size and PID frequency. However, thirdly and conclusively, Busch *et al.* were able to increase infarct size in rats by inducing CSD events *outside* the penumbra which propagated into it and caused enlargement of the definitive core infarct [95].

Arising from the original demonstration that loss of evoked potential amplitude could be reversed upon reperfusion, the initial concept of the ischaemic penumbra was of a "sleeping beauty" – a zone of cortex whose function was *reversibly* suppressed in a stable fashion, so that function could be restored at a much later time point by the magical touch of a vascular neurosurgeon carrying out an extra-intracranial vascular bypass procedure [96]. The study of PIDs and manipulations of their frequency has demonstrated instead that – without early reperfusion – the ischaemic penumbra is a maturation phenomenon in which the core infarct gradually

expands into penumbra, thus "recruiting" it. The time course of this progression is probably shortest in rats – perhaps 3 hours, extending to 12 to 24 hours in cats, and is believed in humans to extend to perhaps 48 hours. The factors which might influence PID frequency and hence the rate of progression need to be considered.

Species Variations in PID Frequency

Tower and Young's observation of a relationship of cerebral cortical glia : neuron ratio with brain mass is relevant to brain injury since, as mentioned earlier, glial buffering of potassium ion concentration and uptake of neurotransmitters, especially glutamate, are important mechanisms for homeostasis of the extracellular space. It is therefore not a matter of surprise that the frequency of PIDs following MCAO in rats should be high [85], but much less so in cats [79]. CSD is also difficult to induce in monkeys [78]. Efforts to make a direct comparison of PID frequency between cats and primates were frustrated by considerable inter-experiment variability in frequency within a species, but variations in plasma glucose emerged from these experiments as a cause of this variability; this is discussed below (some page: Relationship of Cortical Glucose Availability with PID Frequency). The inference from such comparisons is that PIDs in humans might be rarer still – perhaps vanishingly so – and the relevant, new evidence is described later.

Effects of Drugs and Anaesthetic Agents on PID Frequency

The beneficial effects of NMDA-type glutamate receptor blockade on PID frequency and on infarct size have been reviewed above (page 18: Drugs and Anaesthetic Agents). The AMPA/kainate-type glutamate receptor antagonist NBQX has been shown to reduce PID frequency and volume of ATP depletion in rats subjected to MCAO [97], and this agent has also been shown to reduce ischaemic lesion volume [98]. It is of interest that, unlike MK-801, NBQX does not prevent induction of CSD in the normal brain [99]. The volatile anaesthetic agent halothane may achieve its experimental neuroprotective effect by reducing PID numbers [38], and can, like propofol, block CSD [37]. The fact that halothane also blocks gap junctions in cultures of astrocytes [36] supports the argument for a role of glial gap junctions in the propagation of CSD [100].

Relationship of Cortical Glucose Availability with PID Frequency

As CBF progressively falls in focal ischaemia, a shift to anaerobic glycolysis is inevitable once oxygen extraction is maximal. At that point, a dramatic loss in efficiency of glucose utilisation is equally inevitable, with a

fall in net ATP yield per mole glucose utilised from 38 to 2 moles. Glucose utilisation increases to compensate [101]; this is possible despite presence of ischaemia, due to the remarkable effectiveness of the capillary glucose uptake/transport mechanism. This concept is based on several lines of evidence. Hansen showed that following cardiac arrest in rats, delay before terminal ischaemic depolarisation was proportional to plasma glucose, indicating an inverse relationship between depolarisation rate (the dependent variable) and glucose availability in the brain [23]. In 1986, Nedergaard and Astrup showed in rats (MCAO) that hyperglycaemia reduced the frequency of PIDs (although a plasma level in excess of 30 mmol/L was needed to achieve this) [102]. They also showed an increase in phosphorylation of [^{14}C]2-deoxyglucose (an index of metabolic rate) that was related to frequency of PIDs, and predicted that with ischaemia accompanied by PIDs the brain free glucose pool would tend towards zero as delivery and extraction from plasma would quickly become inadequate, given the high, anaerobic utilisation rate. In cats (MCAO), dependence of homœostasis on plasma glucose is demonstrable at glucose levels that are frequently encountered in clinical practice: thus Strong *et al.* showed a striking increase in PID frequency in this situation when mean post-occlusion plasma glucose fell below 4.5 mmol/L (the lower limit of normal quoted for clinical plasma glucose assays in our institution is 3.3 mmol/L) [79]. Our subsequent, unpublished work suggests that the threshold may be nearer 6.5 to 7 mmol/L. This is of potential importance for clinical management since insulin is used to control hyperglycaemia in many intensive care units, with the target range varying in different units. At least one trial of glucose and insulin (to restrict ischaemic acidosis) in acute stroke is under way [103]. There is also striking (and influential) evidence favouring the use of insulin in the intensive care of systemic critical illness [104].

In summary, the initiation of a PID appears to be a random event in which an elevated K_e level at the edge of core infarct territory causes depolarisation of neighbouring tissue because membrane homœostasis there is partially impaired. The impairment is due to a combination of factors in which reduction of glucose availability (the multiple of perfusion (absolute, ml/100 g/min) and plasma glucose levels) as ischaemia deepens becomes particularly important. It seems that reduction of glucose availability increases the probability of initiation of a PID.

The Metabolic "Signature" of PIDs

The transient hypoperfusion or reduction in tissue pO_2 that occurs in association with a PID has been described above. Given the likelihood of transient tissue glycopœnia during recovery from a PID, and the critical dependence of the ATP pool on the balance between on the one hand,

ATP utilisation for restitution of cation gradients during PID recovery, and glucose availability on the other, it becomes valuable to measure the available tissue glucose pool with sufficient time resolution to detect the effects on it of a PID. This has recently been achieved with the use of cerebral microdialysis coupled with rapid sampling of dialysate by means of an online, automated flow–injection assay [105, 106]. The technology allows enzymatic assay of microlitre dialysate samples for glucose and lactate at intervals of 30 seconds each. When dialysate was sampled from penumbral tissue closely adjacent to the core area after MCAO in cats, a PID arriving at the microdialysis probe was associated with complete disappearance of glucose from the dialysate within approximately 3 minutes. In more peripheral penumbra, PIDs were accompanied by transient, stereotyped increases in lactate and decreases in glucose, superimposed in the case of recurrent PIDs on decreasing glucose and increasing lactate baselines (Fig. 7) [107]. This reproducible combination of transient metabolite changes may be taken as a typical metabolic "signature" for a PID, of potential value for the monitoring of patients with severe TBI or acute cerebral ischaemia.

The Role of Depolarisations in Pathophysiology of CNS Disorders in Humans

Speculation and then evidence have accumulated, at first gradually [59, 108, 109], but now more steadily [4, 92, 110], that depolarisations do indeed occur in the human brain – in the functional disorder of migraine with aura as well as in acute traumatic brain injury. It seems that it will only be a matter of time before evidence emerges that they also occur in acute ischaemic or haemorrhagic lesions affecting grey matter in the CNS.

Cortical spreading depression and peri-infarct depolarisations compared:
It is appropriate at this point to summarise the similarities and differences between cortical spreading depression (CSD) and peri-infarct depolarisations (PIDs). CSD is a general, *asynchronous*, neuronal and glial depolarisation that usually commences at a focus in the cerebral cortex, and usually in response to quite vigorous efforts to induce it. It propagates radially in the cortex at 2–5 mm/minute, is accompanied by intense but transient hyperaemia, and does not result in histologically demonstrable cell damage.

A PID is a general neuronal and glial depolarisation that occurs *spontaneously* in an ischaemic boundary zone, especially when plasma glucose is mildly reduced, and propagates into adjacent boundary zone territory at the same velocity as CSD. There is little or no recruitment of perfusion, and, probably as a result of this, ischaemic damage accumulates in the

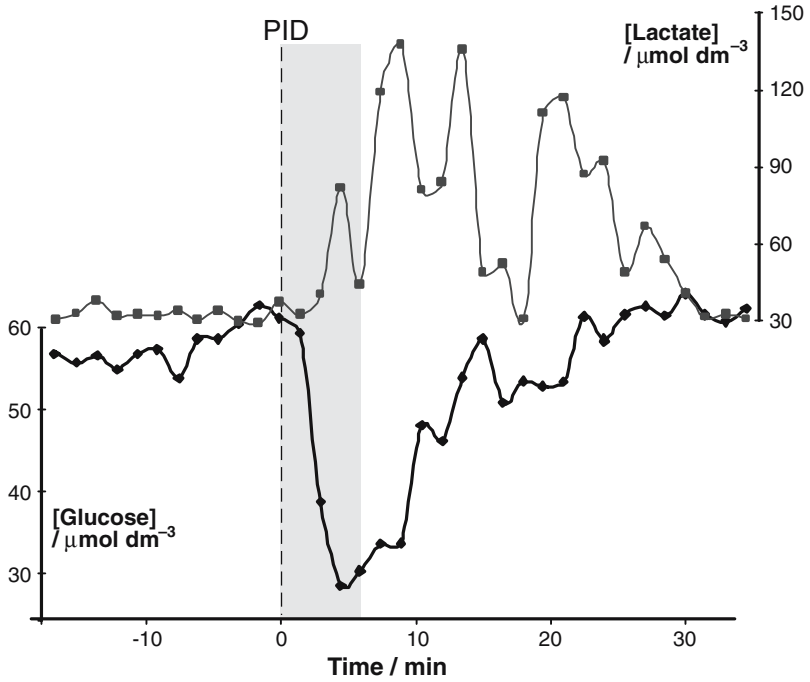


Fig. 7. Time courses of lactate (upper trace) and glucose (lower) concentrations in dialysate from a probe placed in the marginal gyrus (peripheral penumbra) of the cat brain (chloralose anaesthesia). Samples were analysed at 30-second intervals using an enzymatic flow-injection assay [106]. The data demonstrate the typical transient increase in dialysate lactate and decrease in glucose that accompany a PID; this was verified by fluorescence imaging [93]. (Reproduced with permission from Parkin *et al.* [142])

affected territory, culminating after varying periods, perhaps 24–48 hours in humans, in terminal depolarisation and complete infarction.

Spreading depolarisations and epileptic seizures compared:
The essential electrophysiology of an epileptic seizure affecting the cerebral cortex differs from that of CSD in that a degree of *synchronous* firing/depolarisation of neurons is required to generate the dipole whose presence is detected by EEG/ECoG electrodes during a seizure. The transient phase of *asynchronous* neuronal firing at onset of CSD results simply in silence at overlying electrodes. However, the apparent capacity of a Jacksonian fit (a rare event) to propagate across the cerebral cortex resembles the behaviour of CSD, and in the light of the new concepts of non-synaptic communication between different cells in grey matter, one may envisage that Jacksonian epilepsy and CSD might propagate through similar mechanisms. CSD and an epileptic fit both result in transient increases in the metabolic

load inherent in membrane repolarisation, and hyperaemia is a feature common to both. Provided the hyperaemic response is sufficient to permit prompt restoration of "resting" transmembrane cation gradients, neither CSD nor an epileptic fit should lead to any neuronal necrosis. A focal fit is a recognised complication of surgery to clip a ruptured middle cerebral artery aneurysm, and we, probably like most neurosurgeons, view such fits with concern as to their cytotoxic potential since vasospasm may attenuate the hyperaemic response that is required.

Migraine

Classical migraine, now designated migraine-with-aura, is characterised by the migraineur's experience of a visual, somatic motor or sensory symptom as the first component of a stereotyped sequence. The typical visual aura starts as a central scotoma and propagates outwards into the more peripheral visual field (usually a hemifield) as a scintillating, often multicoloured pattern. In 1941 Lashley published a description of his own visual aura, and suggested that it represented propagation of an unknown disturbance across the visual cortex at a rate which he calculated lay in the range of some 3 mm per minute [111], and Leão and Morison suggested that CSD was the basis of migraine with aura [87]. Milner [108] drew attention to the similarity of Lashley's figure for migraine aura propagation with Leão's for CSD propagation, and since then evidence has gradually accumulated that CSD is the basis of migraine with aura. For example, Lauritzen and colleagues [59] mapped CBF using the intra-arterial xenon method and reported propagation in serial images of a phase of reduced blood flow following migraine with aura – probably representing the oligaemic phase of the haemodynamic response to CSD. Woods, Iacoboni and Mazziotta achieved similar results with positron emission tomography [112], and Hadjikhani and colleagues recently described transient loss of normal magnetic resonance blood-oxygen level dependent (BOLD) responses to repetitive visual stimuli during migraine with visual aura. This inhibition propagated outwards from the occipital pole at a rate that was appropriate for CSD [110]. Gardner-Medwin and colleagues had earlier demonstrated propagation of a similar MRI change in experimental CSD [113].

Transient Global Amnesia

Transient global amnesia (TGA) is a neurological syndrome possibly arising in the hippocampus and characterised by sudden onset of complete memory loss; TGA is believed to have as its basis CSD in the hippocampus. The individual appears to be completely alert and can commu-

nicate, but enquires frequently about present events. Most episodes last around eight hours but can last for 24 hours and indeed an episode lasting seven days has been described. The onset of memory loss may occur during an emotional stimulus or physical exertion. A history of migraine is recognised in up to 25% of TGA patients. Cerebral blood flow studies using the 133-xenon inhalation method in TGA patients suggest temporary regional hypoperfusion [114]. Marked hypoperfusion in the region of the posterior cerebral arteries has been displayed with single photon emission computed tomography [115]. Diffusion-weighted magnetic resonance imaging during an episode of TGA indicated a decrease in the interstitial space and cellular oedema of the temporal lobe [116]. The induction of spreading depression by the injection of KCl in the hippocampus creates an irreversible retrograde amnesia in the rat [117, 118]. It is currently believed that the amnesic effect of CSD depends on the duration and density of the phenomenon, repetitive CSD causing a more sustained retrograde amnesia.

Trauma

Depolarisation and Concussion

The suggestion that neuronal depolarisation might account for disturbance of consciousness following head injury originates with a paper by A. Earl Walker and colleagues in 1944 [119], and there is ample, more recent experimental evidence – from use of DC potential or ion-selective electrodes in *in vivo* small-animal models of traumatic brain injury – that is compatible with this concept (some page: Recurrent Depolarisations following Experimental Traumatic Brain Injury (TBI)). However, other mechanisms may also contribute to or account for concussion. For example, there is evidence to implicate sublethal, reversible diffuse white matter shearing injury as a mechanism of concussion [120, 121], and it is beyond the scope of this review to explore this issue in detail.

Recurrent Depolarisations Following Experimental Traumatic Brain Injury (TBI)

The term ''peri-*infarct* depolarisation'' is not strictly applicable to a depolarisation occurring spontaneously in the periphery of a *traumatic* contusion or intracortical haematoma, but several reports (below) of depolarisations in experimental TBI raise two questions. First, do such depolarisations have the characteristics of CSD or of PID, and second, is there evidence for similar events in the injured human brain? Until it becomes clear whether or not depolarisations around a contusion have the characteristics of an (ischaemic) PID, it seems wiser not to assign the term ''PID'' or ''CSD'' to them. Although there is evidence for ischaemia surrounding

such lesions in humans [122, 123], it is by no means clear that the ischaemia is distributed as widely in TBI as it is in MCAO (unless intracranial pressure is markedly elevated). Notwithstanding these uncertainties about the extent or severity of ischaemia, there is ample experimental evidence from electrode studies for the occurrence of depolarisations in the rat brain following TBI [124–127] or in association with an intracerebral haematoma [128].

Kubota and colleagues [125] and Sunami and colleagues [126] showed a relationship between severity of contusion (from fluid percussion injury) and subsequent frequency of CSD-like events; they also found marked elevations in local cerebral glucose utilisation in those hemispheres in which CSD occurred. Similar observations, suggesting hyperglycolysis and later a *hypo*metabolic state, were reported by Hovda, Lee, and Katayama and their colleagues [129]. B. Nilsson and colleagues studied the effect of mild, non-lethal acceleration head injury on cerebral blood flow and metabolism in rats, and found marked but transient increases in CBF and in brain lactate : pyruvate ratio [130, 131]. Although DC potential was not recorded, the time course of the changes closely resembles the transient hyperaemia associated with CSD described above [47]. P. Nilsson *et al.* demonstrated a relationship between CSD and neuronal damage after a weight drop injury [132].

Direct Detection and Characterisation of Depolarisations in Humans, and Their Role in Human Traumatic Brain Injury

Is there any *direct* evidence for the occurrence of depolarisations in the human brain? In the course of stereotaxic neurosurgical procedures, Sramka and colleagues were able to demonstrate CSD in deep grey matter [109]. Mayevsky and colleagues used a multimodal monitoring system located on the right frontal convexity in 14 patients [92]. In only one did they find evidence for CSD, but the findings in this individual were striking. Recurrent ECoG suppressions were seen, associated with transient increases in CBF (laser Doppler), oxidation of NADH, and elevations of K_e, a combination of features closely compatible with CSD, although – despite the authors' claims – proper verification of propagation of the events was not possible at the single monitoring point used. As brain swelling progressed, the NAD/H transients became reduction rather than oxidation events, and the CBF transients became negative – the features now of PIDs (Fig. 8). Although this group saw CSD/PID events in only one patient, it is important to note that their regular use of a right frontal monitoring site irrespective of the site of any contusion or haematoma, although standard practice at the time of the study, will have precluded detection of events confined to the margins or "traumatic penumbra" of a lesion elsewhere

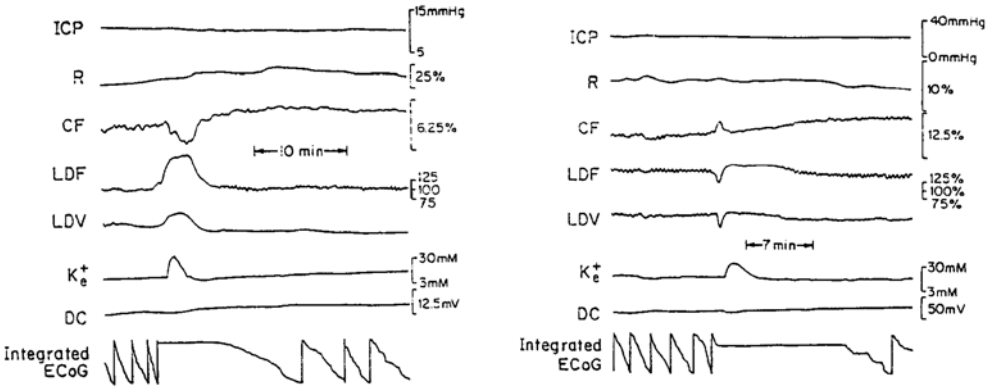


Fig. 8. Traces of intracranial pressure (*ICP*), cortical reflectance (*R*), compensated fluorescence (*CF*), laser Doppler flow (*LDF*), laser Doppler blood volume (*LDV*), extracellular potassium (*K*) DC potential (*DC*) and time-integrated cortical surface EEG activity recorded from a multiparametric probe assembly in the right frontal region of a patient with a severe left parietal contusion [92] (Reproduced with permission from: Mayevsky A et al (1995) J Cereb Blood Flow Metab 15, S1, p S34). The right panel was acquired several hours after the left, following deterioration and shortly before death. In the left-hand panel, a single event – characterised by an increase in perfusion, decrease in fluorescence, increase in extracellular potassium, and a period of electrical silence – has the characteristics of CSD. The characteristics of the event in the right panel have changed in that fluorescence now increases, but perfusion (*LDF*) shows a decrease. The characteristics now correspond more with features of PID rather than CSD

in the brain. There now appears to be wide recognition of the value of locating detection devices, at the very least for research purposes, near the edge of focal lesions.

Recently our group has undertaken a prospective, pilot study designed to detect – or exclude – CSD/PID in patients undergoing emergency craniotomy for traumatic and spontaneous intracranial haematoma [4]. Linear strips of 6 corticography electrodes were placed on the cortex *adjacent to the focal contusion*, lying over both marginal as well as healthy cortex. A working definition of CSD as "suppression of amplitude of the voltage envelope by 50% or more, occurring between one electrode pair and propagating to the next 2 adjacent sites" was adopted, and 14 patients were studied. During periods of observation that lasted up to 63 hours, 6 definite and 23 possible episodes that met this definition were seen in 14 patients (Fig. 9). A second pattern was seen 19 times in 8 patients, in which essentially synchronous suppression was seen in all channels. A proportion of these apparently synchronous events may have been due to arrival of a CSD wave propagating across the array rather than along it, but modelling the statistical distribution of a set of CSD waves reaching

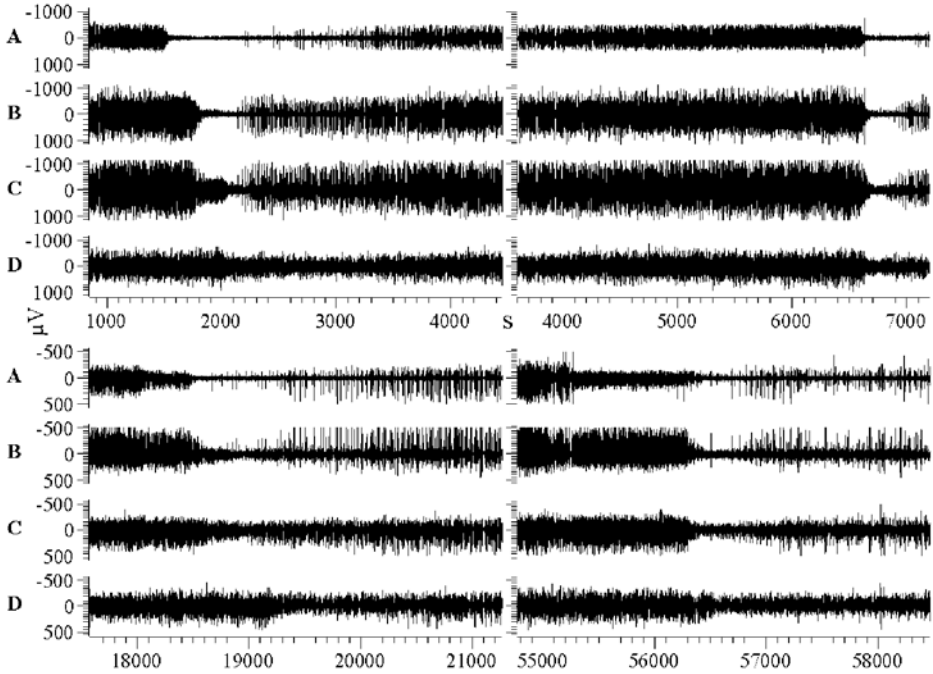


Fig. 9. Examples of two time-compressed electrocorticographic (*ECoG*) traces from two patients (Patient 1: upper, Patient 2, lower) (four bipolar traces per patient). In the first patient there is a sudden reduction of ECoG amplitude propagating sequentially to the next two adjacent channels and to a lesser extent to the third channel. Propagation rate was 2.4 and 2.3 mm per minute, corresponding closely with velocities characteristic of CSD. In the second trace from this patient, ECoG suppression occurs rapidly, recovering most slowly in the upper channel as in the left panel. Synchronous suppression suggests arrival of a wave from a site equidistant from all electrode pairs and to one side of the array, rather than, as in the first panel, propagating along the length of the electrode array. In the lower panel (Patient 2) a phase of ECoG amplitude suppression again propagates along the electrode array, with the respective time points indicating propagation rates of 1.4, 5.0 and 1.0 per minute. (Strong *et al.* 2002 [4]: reproduced with permission)

the strip from a range of angles between 0 and 90 degrees did not fully account for the frequency of synchronous events that we recorded, and the existence of a second pattern of truly synchronous event (possibly due to a partial seizure elsewhere in the cortex [133]) could not be excluded.

Cerebrovascular Disease

Occlusive Stroke

The extensive experimental evidence of PIDs in MCAO models has not as yet been mirrored by studies in patients with occlusive stroke. In 1995

Hasegawa *et al.* had shown that MR diffusion-weighted imaging of rats yielded clear evidence of a transient depression of the apparent diffusion coefficient (ADC) for water that propagated across the cortex at a rate appropriate for CSD in response to stimuli capable of inducing it; they also found evidence for propagating depolarisations in ischaemia [19]. However, Back *et al.* [134] used the same approach in patients with stroke and were unable to detect evidence of PIDs; there are significant practical problems posed by this approach when applied in patients whose condition may be unstable, and the time actually available for actual imaging in this study was relatively short. In the light of the intermittent occurrence of CSD-like episodes now reported from ECoG recordings in trauma [4], it is likely that extended periods of image acquisition will be needed to capture depolarisations in stroke or trauma with MR imaging methods.

Intracerebral Haemorrhage

Using a collagenase that generates a spontaneous intracerebral haematoma in swine and monitoring DC potential (as well as other variables) Mun-Bryce and her colleagues recorded recurrent, spontaneous CSDs originating in perilesion cortex [128].

Subarachnoid Haemorrhage (SAH)

Although there is at present no direct evidence for CSD or PIDs in patients with SAH, there is persuasive experimental evidence to suggest the likelihood of depolarisations occurring in these patients, perhaps restricted to those in intermediate or poor grade. For example, Dreier and colleagues showed that superfusion over the cortex of rats of a combination of increased K^+ and free haemoglobin (such as would arise from lysed erythrocytes in the subarachnoid space) could induce recurrent CSDs [135]. These were accompanied not by hyperaemia but by ischaemia, and thus meet the essential criteria for designation as PIDs. When haemoglobin (which scavenges nitric oxide) was replaced with the nitric oxide synthase inhibitor *N*-nitro-L-arginine, the same effect was observed. This group suggested that this mechanism might account for non-haemorrhagic deterioration in patients with SAH. The common clinical observation of fluctuations in clinical state of intermediate grade SAH patients over intervals often of less than an hour is compatible with the capricious behaviour of CSDs and PIDs in the laboratory; clearly, other explanations are possible and cannot be discounted, but, taken together, the demonstration of CSD-like events in TBI [4] and the work of Dreier and colleagues provide support for this hypothesis.

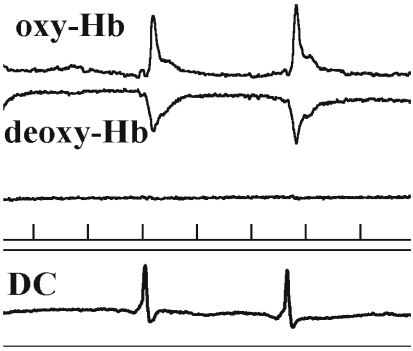
Non-Invasive Detection of Depolarisations in Ischaemic and Traumatic Brain Injury

Availability of a simple non-invasive method for detection of depolarisations would greatly aid studies of their frequency, properties and effects. Using near-infrared spectroscopy (NIRS), Wolf *et al.* characterised non-invasively the transient changes in oxidised and reduced haemoglobin (HbO, Hb) that accompany CSD in the rat brain [136]. A transient increase in HbO was accompanied by a reduction in Hb, a combination suggesting hyperaemia (Fig. 10, upper). There are clinical NIRS data from this department in one patient with TBI (hitherto unpublished: Fig. 10 lower) and from the Berlin group in two with ischaemic stroke (Fig. 11), in which the HbO and Hb transients closely resembled those seen with CSD in the laboratory. However, in neither case was it feasible to confirm depolarisation by ECoG or DC potential measurement. The potential use of serial measurements of the apparent diffusion coefficient for water using diffusion-weighted MRI has been discussed above.

Characterisation of Depolarisation Events in the Injured Human Brain

It will be clear from the distinction drawn between CSD and PIDs throughout this review that although it would seem that PIDs are invariably cytotoxic and therapy should aim at their control, this is much less certain in the case of CSD. The doubt arises from the evidence that experimental preconditioning with CSD confers protection against subsequent insults (page 16: Pre-Ischaemic Conditioning with CSD as Protection in Experimental Stroke). Since an episode of depolarisation detected by ECoG may represent either CSD or PID, it becomes important to distinguish which has occurred.

Several monitoring methods already well or partly established in clinical or research use are capable of making this distinction. The different responses of cerebral cortical tissue pO_2 to CSD and PIDs (transient increase and decrease respectively) have been well characterised by Back [48]. Mayevsky *et al.* showed that laser Doppler flow monitoring would provide similar information [92]. Dirnagl suggests that the NIRS profile of CSD-linked hyperaemia, transient increase in HbO and decrease in reduced Hb, is reversed in PID – a decrease in HbO with an increase in Hb [137]. Finally, our experimental work with rapid-sampling microdialysis in the MCAO stroke model shows that the occurrence of transient depletion of dialysate glucose and increase in lactate would indicate a PID [105] (Fig. 7). To date we have been unable to identify any comparable "signature" for CSD.



Change in chromophore concentration (μM) (patient with frontotemporal contusion):-

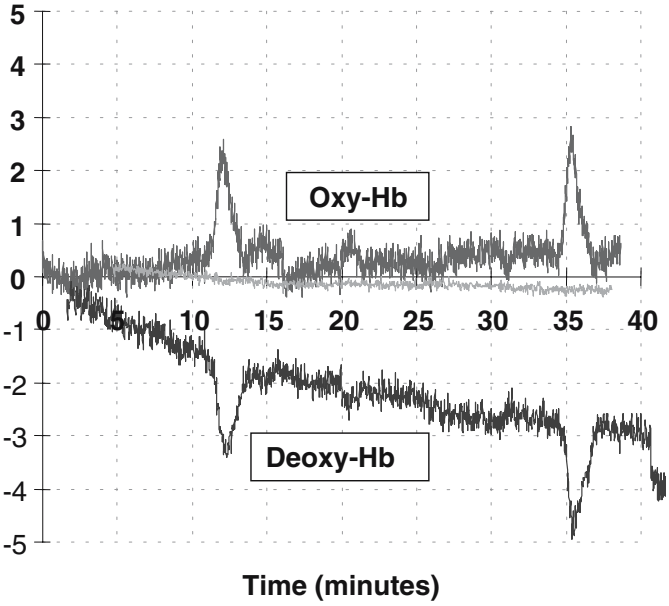


Fig. 10. (*Upper panel*) Time course of near infra-red spectroscopy (NIRS) data (oxy-haemoglobin and deoxy-haemoglobin) from the exposed rat cerebral cortex during an episode of induced CSD, verified by the changes in DC potential. Time bars are at 10 minute intervals. (Reproduced with permission from Kohl *et al.* [139]). (*Lower panel*) Time courses of changes in oxidized haemoglobin (upper trace), cytochrome oxidase (middle trace) and deoxyhaemoglobin (lower trace) in a ventilated patient following severe head injury, obtained non-invasively with NIRS (Cheng, Prowse and Strong, unpublished). The traces show stereotyped combinations of increased HbO and decreased Hb, separated by an interval of some 25 minutes, and suggesting increased oxygen availability characteristic of CSD; ECoG was not available to verify CSD (Please see (page 15: Haemodynamic Response and page 31: Direct Detection and Characterization of Depolarisations in Humans, and Their Role in Human Traumatic Brain Injury). The time course and patterns of the HbO and Hb transients recorded clinically (lower panel) correspond closely with those known to be linked to CSD as illustrated in the upper panel. (Vertical axis is change in chromophore concentration in micromolar)

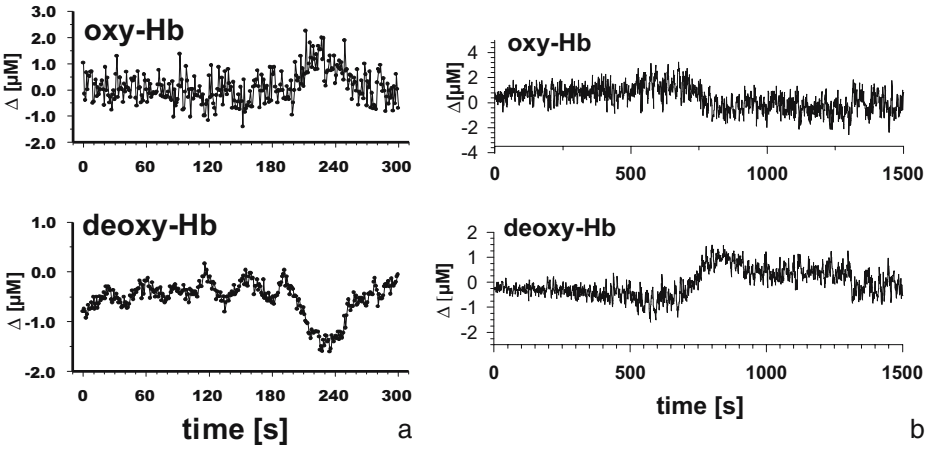


Fig. 11. NIRS traces from 2 patients during the acute phase of ischaemic stroke. (a) Transient increase in HbO with decrease in Hb, suggesting a hyperaemic response to a (unverified) CSD wave. (b) Sustained reduction in HbO signal and increase in Hb, suggesting PID, characterised by *de*crease in HbO signal, and *in*crease in the deoxy Hb signal (depolarisation or propagation not verified by electrophysiology). (Reproduced with permission from Dirnagl, 2001 [137])

The Biological Significance of CSD

Nearly 60 years after its first description by Leão, we remain uncertain of the biological role of a phenomenon that seems to be at times beneficial and a normal response of the brain, and at other times, in the case of PIDs, harmful, principally under conditions of ischaemia.

As one speculation, perhaps the apparent paradox can be explained if we see CSD as reflecting the operation of well-conserved intercellular communications in the brain – serving to protect the brain against inflammation and infection. We do well to bear in mind that our relative mastery of infection in the central nervous system – incomplete and perhaps temporary – is only very recent on the time scale on which evolution operates, and there has probably been more survival value for our own and other vertebrate species in effective responses to CNS infection before and during reproductive life than in avoidance of the cost of aberrant, deleterious operation of the same mechanism in the ageing or irretrievably injured brain.

A quite different speculative view of CSD emerges from the recent rapid growth in our knowledge of the physiological role of astrocytes in modulating synaptic function, to the extent that the synapse is now seen as a tripartite entity – pre- and post-synapse, and astrocyte [138]. Perhaps the probability of CSD (variable depending on glia : neuron ratio and other

factors) is an inevitable consequence of this arrangement and of the intimate communication between astrocytes through their gap junctions.

We may also speculate that the depolarisation events that we are able to observe propagating across the cortex with current methods and stimuli may occur much more frequently than we can presently detect, but restricted to microfoci of grey matter, not propagating widely, and below the limits of the available resolution and sensitivity. Results of imaging work with glial and organotypic cultures seem to support this possibility.

As the methods of molecular biology expand, so does the range of gene responses to CSD that have been documented, and it is by no means yet certain which are the most significant. However, it does seem likely that we shall learn as much about the biological significance of CSD from greater knowledge of the expression cascades that it initiates as from the longer-established neurophysiological approaches to CSD.

For neurosurgeons studying and caring for acute brain injury, the only certainties are that *PID*s, when identified, should be controlled, and that there is more to be learned about the effects of *CSD* on the human brain before we can reach a view on whether to attempt to control it.

Summary

1. *Cortical spreading depression* is a non-physiological global depolarisation of neurones and astrocytes that can be initiated with varying degrees of difficulty in the normally perfused cerebral cortex in the experimental laboratory. Induction is typically with electrical stimulation, needling of the cerebral cortex, or superfusion of isotonic or more concentrated potassium chloride solution. The phenomenon propagates across the cerebral cortex at a rate of 2–5 mm per minute, and is accompanied by marked but transient increases in cerebral blood flow, in local tissue oxygen tension, and most probably in metabolic rate.
2. *Peri-infarct depolarisation* is also a depolarisation event affecting neurones and glia, with an electrophysiological basis similar or identical to CSD, but occurring *spontaneously* in the ischaemic penumbra or boundary zone in focal cerebral cortical ischaemia. Most such events arise from the edge of the ischaemic core, and propagate throughout the penumbra, at a rate similar to that of cortical spreading depression.
3. Cortical spreading depression in the normally perfused cortex does *not* result in histological damage whereas peri-infarct depolarisations *augment* neuronal damage in the penumbra, and are believed by many authors to constitute an important, or the principal, mechanism by which electrophysiological penumbra progressively deteriorates, ultimately undergoing terminal depolarisation and thus recruitment into an expanded core lesion.
4. There is some experimental evidence to suggest that under some circumstances induction of episodes of cortical spreading depression can confer protection against subsequent ischaemic insults.

5. Although cortical spreading depression and peri-infarct depolarisations have been extensively studied in the experimental *in vivo* models, there is now clear evidence that depolarisations also occur and propagate in the human brain in areas surrounding a focus of traumatic contusion.
6. Whether such events in the injured human brain represent cortical spreading depression or peri-infarct depolarisation is unclear. However, invasive and probably non-invasive monitoring methods are available which may serve to distinguish which event has occurred.
7. Much further work will be needed to examine the relationship of depolarisation events in the injured brain with outcome from cerebral ischaemia or head injury, to examine the factors which influence the frequency of depolarisation events, and to determine which depolarisation events in the human brain augment the injury and should be prevented.

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