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Cortical spreading depression and migraine: new insights from imaging?

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The possibility that spreading depression (SD) of cortical activity, a phenomenon observed in all vertebrates, causes the aura of migraine remains an open question in spite of nearly half a century of investigation. SD is also thought to be associated with the progressive neuronal injury observed during cerebral ischaemia. Thus, the ability to detect and investigate SD in humans might prove clinically significant. Animal studies of cortical spreading depression (CSD) have benefited greatly from the advent of relatively non-invasive imaging techniques. The use of these new imaging techniques for clinical studies will accelerate progress in this area of neurobiology.

Migraine is a debilitating condition affecting 10–15% of the population[1,2], and is characterized by episodic attacks of severe headache that are often unilateral, pulsating and aggravated by movement. It is associated with nausea, vomiting, photophobia or phonophobia. Accordingly, migraine has a large economic cost[3].

The cerebral cortex of migraineurs is thought to be abnormally responsive to repeated stimuli[4], so that a wide variety of stimuli (flickering light[5], noise, even the smell of strong perfume[6]) might act as triggers for an attack. Anecdotal evidence indicates that many migraineurs can predict the onset of their attacks. Premonitory symptoms (e.g. repetitive yawning,

> 'Forty years ago the progression of visual symptoms during aura was noticed to have remarkable similarities with the spreading depression of cortical electro-encephalographic (EEG) activity…'

fatigue, stiff neck, photophobia) might precede a migraine attack by many hours. A possibly related phenomenon called migraine 'aura' occurs in about one-third of sufferers. Aura precedes the headache phase, and commonly includes visual disturbances, but other sensory, motor or speech disturbances can be involved. Aura has a slow, progressive onset and can be associated with movements of scintillations or fortification illusions, followed by a 'scotoma' or blind spot, across the visual field. In a classic reference,

Box 1. Ionic and cellular components of cortical spreading depression

Cortical spreading depression (CSD) involves a temporary, but major, localized redistribution of ions between intracellular and extracellular compartments. This ion redistribution is energy dependent[a], becoming clinically significant in ischaemia when brain metabolism is impaired. During CSD initiation the concentration of extracellular K^+, $[K^+]_o$, rapidly rises, causing brief neuronal excitation then depolarization and a period of electrical silence during which the direct current (DC) potential at the brain surface falls. In tandem, $[Na^+]_o$ and $[Cl^-]_o$ levels decrease as these ions enter cells. Consequently, water enters cells, the extracellular space is reduced, and cells swell[b]. Ca^{2+} ions also move inwards, but slightly later than the outward movement of K^+, suggesting that Ca^{2+} movements follow K^+ fluxes. Additional negative ion species move outwards to maintain electrical balance, the excitatory neurotransmitter glutamate probably being the most important[c].

In the isolated chick retina[d], human neocortical tissue[e] and cat brain[f], NMDA receptor antagonists block SD completely. By contrast, in rat hippocampus, glutamate (and Ca^{2+}) facilitates SD initiation, whereas NMDA antagonists (and low $[Ca^{2+}]_o$) delay its onset but fail to block SD completely[g–i].

Both volume-activated ion channels[j,k] and glial cells probably play important roles in the restoration of normal cellular homeostasis. The former are stimulated during cell swelling, and the latter provide spatial buffering that prevents increased levels of $[K^+]_o$ and $[Glu^-]_o$ during normal neuronal activity. However, they might also prolong CSD: volume-activated ion channels release glutamate during CSD (Ref. l); and although gliotoxins prolong CSD (Ref. m), they also reduce glutamate efflux from glial cells[n]. CSD and PID appear more difficult to evoke in brains of larger animals in which the ratio of glia to neurones tends to be higher, suggesting that glial cells are important for limiting CSD activity[o]. Such limiting forces might be greater in the more complexly folded human brain, and could explain the paucity of literature accounts of CSD during neurosurgery.

Cooperation between glia and neurones maintains normal ion and transmitter levels during neuronal activity. During CSD the magnitude of the ion movements and the synchrony of these fluxes might be abnormal. A possible mechanism for such a greatly enhanced, but transient, synchrony might involve changes in the permeability of gap junctions[p,q], because the gap junction blockers octanol and halothane prevent CSD propagation, especially in gyrencephalic species[f,q,r].

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Lashley[7] mapped the progression of his own visual aura, calculating that cortical function was affected at a rate of ~3 mm.min^{-1}.

Forty years ago the progression of visual symptoms during aura was noticed[8] to have remarkable similarities with the spreading depression of cortical electroencephalographic (EEG) activity described by Leão[9]. Although CSD is relatively easy to observe in animal brains, technical challenges probably account for the dearth of human CSD studies[10]. Nevertheless, some reports do indicate the occurrence of CSD in humans[11,12].

Here we consider the evidence linking CSD with migraine and its aura, and show how modern *in vivo*

Box 2. Cortical spreading depression, electrophysiology and migraine

Cortical spreading depression (CSD) in animal models (mainly rabbit and rat) affects single neurone spike activity, synchronization of synaptic potentials and steady membrane potentials produced by neurones or glia. Together these are recorded as a flattening of the EEG and a reduction in the cortical direct current (DC) potential. Although depression of the EEG is rarely complete, CSD changes its amplitude, shape and frequency when recorded from the exposed pia-arachnoid surface, with frequency changes sometimes occurring in advance of the main changes. Reduction or abolition of somatosensory evoked potentials also occurs.

The characteristic surface-negative, DC slow-potential change of CSD advances via all cortical layers, arriving earliest via the most superficial ones in which ion movements are greatest. Single neurones discharge briefly at the onset of the EEG depression and are then suppressed, followed by a slow recovery of spontaneous activity. Average cell membrane potentials are reduced during the negative phase of CSD, during which the passive electrical properties of depressed neural tissue are reflected by changes in overall resistive and capacitive components of cortical impedance. Currents that are tangential to the skull arising from CSD alter the magnetic fields external to the head. Such changes are detected by magnetoencephalography (MEG), but poor spatial resolution requires the locations of the current sources to be interpreted using computer modelling.

Most migraine studies have been largely interictal, with changes noted in evoked potentials and on transcranial magnetic stimulation (TMS) (Refs a,b). These changes are thought to reflect an abnormal excitability of cortical neurones. This altered excitability might enhance interictal visual acuity in migraineurs[c], but perhaps leads to a situation in which cortical activity is insufficiently damped and therefore CSD can 'break through' when the level of afferent input to the brain breaches some threshold.

TMS studies, comparing migraineurs with and without aura and controls, showed that cortical responsiveness is altered in at least two respects[d]. (1) TMS of the occipital cortex produces brief scintillations (phosphenes) in the visual field: in migraineurs with aura a lower phosphene threshold was detected compared with other subjects. (2) TMS of the motor cortex evokes a muscle contraction response: in migraineurs, an increase in the excitability threshold for muscle contraction has been reported. Such studies, however, produce mixed results from different protocols and study groups. Nevertheless these results imply that ictal studies of migraine should be investigated by a combination of MRI and simultaneous region-specific TMS, or by TMS assessments before and after ictal MEG. Acquisition of EEG or MEG during MRI is also likely to be fruitful. These approaches would also be compatible with an acute experimental pharmacotherapeutic intervention.

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imaging will probably advance this field of neurobiology.

Cortical spreading depression

CSD probably starts with a cellular efflux of K^+, leading to depolarization and a period of relative electrical silence (Box 1). The subsequent energy-dependent restitution of ion gradients eventually restores normal neuronal activity. The ionic activity, however, results in a wave of neuronal depolarization propagating away from the initiation site at a velocity of ~3 mm.min^{-1}. Because the depolarization–restoration process takes ~1.5 min, the wave is only ~5 mm deep.

A phenomenon similar to CSD, peri-infarct depolarization[13] (PID), has been described in models of cerebral ischaemia[14], subarachnoid haemorrhage[15] and cortical trauma[16], and has also been observed in humans[17]. PID has been implicated in the progressive development of brain injury following ischaemia[18]. It is possible, therefore, that an inhibitor of CSD and/or PID could be valuable in treating migraine and neuronal injury in stroke or brain trauma.

CSD, migraine and the cerebrovasculature

Leão observed that CSD also leads to transient dilatation of pial arteries, increasing venous flow and 'arterialization' of the blood[19]. Following this CSD-associated transient hyperperfusion, cortical hypoperfusion ensues that persists long after CSD waves have passed[20–24]. The hypoperfused area might reflect the cortical surface traversed by CSD events, as opposed to the small region involved in individual CSD wavefronts.

The discovery of prolonged hypoperfusion during migraine strengthened its hypothetical link with CSD (for reviews see Refs 10,25,26). Imaging studies of patients experiencing migraine with aura describe the development of unilateral regions of occipital hypoperfusion that tend to spread rostrally from the
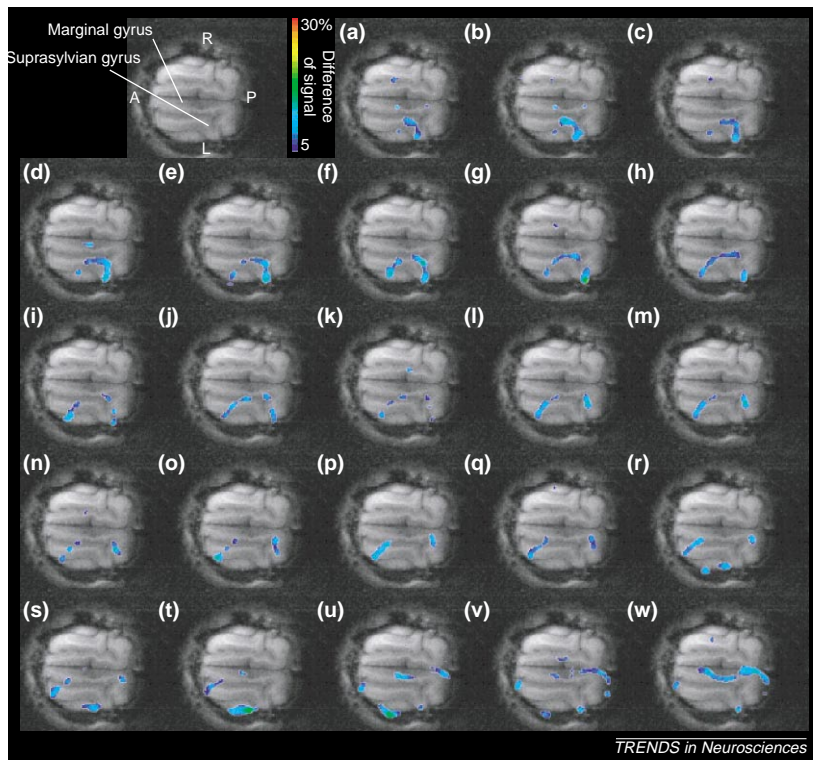
Fig. 1. Cortical spreading depression (CSD) propagating across the surface of the cat brain *in vivo*. Images were obtained at 10 s intervals starting ~50 s after KCl application; the coloured overlays represent decreased cerebral water diffusion caused by water movement into cells during the passage of the CSD; overlays are superimposed on an anatomical image of the brain surface. In frames (a–k), the elliptical wave front travels both anteriorly and posteriorly along the suprasylvian gyrus from its centre, where the event was initiated. In frame (n), the wave begins to pass into the marginal gyrus posteriorly. The wave also moves laterally from the suprasylvian gyrus across the ectosylvian and suprasylvian sulci, out of the image plane, to reappear on the ectosylvian gyrus (frames r–v), and then on the marginal gyrus (frames t–w). Abbreviations: A, anterior; P, posterior; L, left; R, right. The event propagated with velocity 3.2 ± 0.1 mm.min⁻¹; the field of view is 5 cm. Modified, with permission, from Ref. 34.

subcortical regions). This idea could explain some of the spreading physical symptoms that occur in some migraineurs. These perfusion changes spread at a rate that is consistent with the propagation of CSD (Refs 25,27,28).

CSD, migraine and EEG changes
In animals CSD 'flattens' the EEG response and decreases the direct current (DC) potential on the brain surface[29]. This suggests that EEG and similar non-invasive electrophysiological techniques, such as magnetoencephalography (MEG), might be valuable for investigating CSD in humans[4] (Box 2). The rapidly fluctuating voltages of EEG waveforms do not lend themselves to the detection of CSD because of the high-pass filtering normally used to process EEG signals. Changes in DC potential have been difficult to detect during neurosurgery, but have been shown

occipital cortex and to persist into the headache phase. However, spreading cerebral perfusion changes have also been reported in migraine without aura, perhaps because SD occurs in brain regions subserving functions other than vision (including
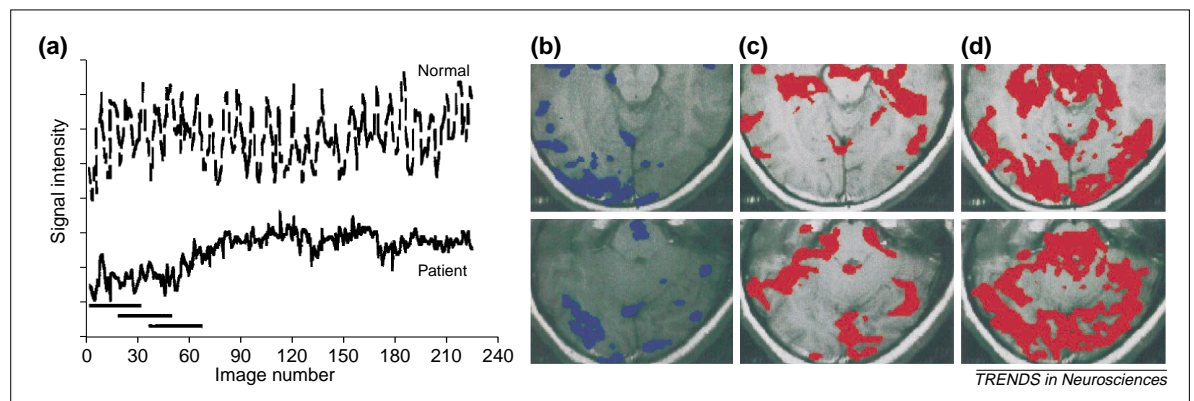

Fig. 2. Blood oxygen level-dependent (BOLD)-fMRI of the occipital cortex during a migraine attack. A migraine attack was provoked using an alternating red–green checkerboard pattern projected to the patient in the magnet. The pattern alternated at 9 Hz and was on or off every 14 s. The patient experienced visual symptoms that appeared at the onset of a severe headache 6.1 min after the start of the visual stimulus (~image 104). The visual changes reported were different to the patient's usual aura. (a) (Upper trace): in normal subjects, no migraine attack was provoked and the visual cortex responded to the on-off visual stimulus with corresponding changes in BOLD signal for 12.4 min (224 images). (Lower trace): in the patient, cortical responsiveness was inhibited during the migraine attack and the BOLD signal intensity was also increased from baseline (horizontal bars = 1.75 min each). (b)–(d) The spatial extent of baseline BOLD signal changes in the patient. Two adjacent axial images (upper, lower) through the occipital cortex are shown; BOLD intensities were compared with the mean signal intensity of the first 56 images and significant changes were superimposed on these anatomical images. The field of view is 24 cm. (b) In the right occipital lobe (i.e. left of image), during images 83–112, significantly decreased (blue) BOLD signal changes occurred before symptom onset; these changes were observed only in patients who reported visual symptoms. (c) Significant increases in BOLD signal (red) that coincided with the suppression of cortical responsiveness were also observed during this time period, especially in the left visual cortex (i.e. right of the image) but also in the association cortex. (d) During symptoms (images 113–168), increased BOLD signal had spread by 9.8 min (image 168) into the left hemisphere (i.e. right of the image). The authors suggest that the BOLD intensity increase that coincided with the suppression of visual activation might reflect vasodilation similar to that observed during the earliest phase of cortical spreading depression (CSD). They also indicate that the waves of suppression were of variable duration, and were more widespread anatomically than might be expected from the localized nature of the signal intensity increase of experimental spreading depression, or of the spreading suppression of visual activation or neurologic symptoms of their patients. Modified, with permission, from Ref. 28. See also Ref. 38 which amplifies these findings and provides further strong evidence that CSD underlies migraine aura.

with intracranial electrocorticography[17] after human head injury. Focal DC potential shifts propagating across the cortex should give rise to changes in the magnetic field outside the head. Thus, MEG has been used in animals to detect CSD (Refs 30,31). Furthermore, DC potential shifts have been detected with MEG during migraine aura and headache[32]. Nevertheless, reliable detection of small signal changes, perhaps as a result of CSD, during migraine remains a difficult task. These techniques will probably be combined with other non-invasive techniques, such as transcranial magnetic stimulation and *in vivo* imaging.

CSD, migraine and imaging

Both CSD and PID have been imaged in animal brains using MRI. Transient, localized changes in cerebral water diffusibility, arising from altered tissue water compartmentalization (Box 1), can be detected during CSD with diffusion-weighted MRI. Both CSD and PID, in normal and ischaemic brain, respectively, have been detected in animals in this way[15,33–35] (Fig. 1). CSD has also been detected with MRI techniques that are sensitive to transient hyperaemic changes[36,37]. There are, however, some technical impediments to imaging CSD in humans.

- Migraine attacks usually occur unpredictably, often without prior warning, and volunteers are rarely available for imaging at the start of an attack.
- In animals, CSD wavefronts occupy very small brain areas (Fig. 1); if this is also true in humans, such small regions might not be easily detectable.
- Cortical folding could limit the spread of CSD in the human brain[31,35], further increasing the difficulty of detection.
- The clinical timing of CSD or PID events is uncertain, so that continuous imaging would be necessary to maximize their detection.

Repetitive imaging in migraineurs has used functional MRI (fMRI) to detect blood-oxygenation level-dependent (BOLD) activations during aura indicative of the transient, propagating cerebral hyperperfusion of CSD. Because aura often involves visual disturbances, investigations have concentrated on the occipital cortex. Recently[28,38] subjects were imaged soon after the onset of a migraine attack, and the responsiveness of their occipital cortices was monitored while they observed a reversing checkerboard pattern. Transient increases in the baseline BOLD signal coincident with visual aura were detected, whereas the BOLD response to the on–off visual stimulus was suppressed (see Fig. 2). By comparing signals from different regions, it was shown that the period of suppression travelled across the occipital cortex[28,38]. A case of spontaneous migraine with aura, involving no visual stimuli, showed a rather widespread BOLD activation that propagated faster than expected for CSD (Ref. 39). A similar study[27], using $H_2^{15}O$-positron emission tomography, recorded a spreading hypoperfusion in a spontaneous migraine attack. Perhaps hypoperfusion can rapidly follow a transient BOLD activation: further studies with careful temporal resolution should clarify this point.

Conclusions and future studies

Evidence is accumulating that CSD and PID might be associated with migraine and other neurological disorders. New imaging techniques will provide a greater understanding of these phenomena and could lead to new methods of treatment. Although repetitive BOLD fMRI already represents a step forward in migraine and CSD research, future studies using repetitive diffusion-weighted and other types of MRI, in combination with other non-invasive techniques such as EEG and MEG, will lead to new insights into these important medical problems.

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The endoplasmic reticulum: one continuous or several separate Ca²⁺ stores?

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The Ca²⁺ store and sink in the endoplasmic reticulum (ER) is important for Ca²⁺ signal integration and for conveyance of information in spatial and temporal domains. Textbooks regard the ER as one continuous network, but biochemical and biophysical studies revealed apparently discrete ER Ca²⁺ stores. Recent direct studies of ER lumenal Ca²⁺ movements show that this organelle system is one continuous Ca²⁺ store, which can function as a Ca²⁺ tunnel. The concept of a fully connected ER network is entirely compatible with evidence indicating that the distribution of Ca²⁺-release channels in the ER membrane is discontinuous with clustering in certain localities.

The ER is crucial for Ca^{2+} signalling. The ER can act as a sink for Ca^{2+} that enters the cell through channels in the plasma membrane, but can also be a source for Ca^{2+} release into the cytosol in response to intracellular messengers, such as inositol 1,4,5-trisphosphate [$Ins(1,4,5)P_3$] (Refs 1,2); cyclic ADP-ribose or nicotinic acid adenine dinucleotide phosphate[3], generated following activation by

neurotransmitters. Because Ca^{2+} can interact with many different molecular targets, signal specificity requires subcellular localization of the cytosolic Ca^{2+} signal[4,5] and/or special oscillation and spiking patterns[5,6]. The ER is essential in this respect, because clustering and mixing of various Ca^{2+}-release channel types in specific regions can focus Ca^{2+} signals in space and time[7–9]. Failure of these processes can lead to serious pathological conditions[6]. Changes in ER Ca^{2+} storage and release might also be important in neuronal ageing[10].

Here we discuss whether the ER consists of one continuous vesiculo-tubular system or of separate isolated compartments. This issue is separate from, but relevant to, the discussion concerning ER heterogeneity. The question about the continuity and discontinuity of the ER lumen has consequences for cytosolic Ca^{2+} signal generation and is related to the discussion about the quantal Ca^{2+} release phenomenon: a sustained elevation of the cytosolic $Ins(1,4,5)P_3$ concentration to a submaximal level

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'More recently, the question of continuity or discontinuity has been directly addressed by monitoring movements of Ca²⁺ within the ER lumen.'

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causes only a transient and partial liberation of Ca^{2+} from the ER and further increments in the $Ins(1,4,5)P_3$ concentration elicit additional pulses of Ca^{2+} release[11–13]. This could be explained by subdivision of the ER into separate compartments,