Neurovascular Coupling During Cortical Spreading Depolarization and –Depression

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Cortical spreading depolarizations (CSDs) are self-propagating waves of transient loss of neuronal transmembrane ion gradients, followed by prolonged suppression of neuronal activity (spreading depression). CSDs emerge spontaneously in animal models of traumatic brain injury,1 subarachnoid hemorrhage,2 and in focal ischemia3 where they are associated with infarct growth.1 In humans, CSDs have been demonstrated in traumatic brain injury,1 subarachnoid hemorrhage,6,7 and malignant hemispheric stroke,9 and they are believed to be the brain mechanism underlying migraine aura.9

CSDs are associated with dramatic changes in cerebral blood flow (CBF). During the depolarization phase of CSDs induced in healthy and well-perfused brain tissue in animal models, an early hyperemic response is observed, typically followed by prolonged oligemia after the neuronal repolarization. Despite the initial CBF increase, tissue hypoxia may develop in more distant territories of capillary supply.10,11 Similar CBF changes have been observed in patients with migraine aura.12–14

In the injured brain, CSDs can be accompanied by severe initial CBF reduction instead of a CBF increase during the depolarization phase, termed spreading ischemia.15 When this inverse hemodynamic response is observed, the energy-dependent recovery from CSD is delayed in a characteristic fashion, indicating a severe mismatch between oxygen supply and demand3 and a high risk of tissue damage.16 In rat and cat models of focal ischemia, CSD-related CBF transients range from monoclinic, positive CBF responses in peri-ischemic tissue, over biphasic transients in mildly ischemic tissue, to negative CBF transients in more severe ischemia.17–19

During CSD in animal models, capillary flow patterns become severely disturbed.10,20,21 The passage of a CSD causes erythrocytes in some capillaries to reduce their speed, whereas other capillaries reveal 4-fold increases in flow or higher.21 Flow cessations during CSD have been observed to affect those capillaries, where pre-CSD erythrocyte velocity and flux were already low, more than those with high pre-CSD velocity and flux,20 again suggesting severe maldistribution of blood flow among capillaries during CSD.

We recently showed that capillary transit time heterogeneity (CTH) reduces oxygen extraction efficacy, and thereby limits the oxygen utilization that can be supported for a given CBF and tissue oxygen tension (PO2).22 Therefore, flow–metabolism coupling mechanisms would be expected to adjust CBF and CBF responses to compensate for the reduced oxygen extraction efficacy downstream, see Figure 1A. Below, we describe this capillary dysfunction and its implications for net oxygen extraction and ATP production. We then analyze how CBF would be expected to change to maintain flow–metabolism coupling if CTH increases in relation to a CSD and then remains high during the subsequent cortical depression. Although changes in capillary patency/resistance are likely to affect capillary flow patterns in the injured brain (see Figure 1B), data regarding CTH during CSD are sparse. The purpose of this report is therefore to present predictions that can be tested in future experiments and clinical studies to support or rule out CTH-related effects in the coupling of CBF to tissue metabolism during CSD in the normal and injured brain.

Predicted Changes in CBF During Episodes of Increased CTH in Normal Brain

In the following, we assume that flow–metabolism coupling adjusts CBF to meet the metabolic needs of tissue whenever possible. We therefore examine which compensatory CBF changes are required to support various types of tissue metabolism if some erythrocytes pass through the capillary bed with flow velocities that are too high to permit complete extraction of their oxygen load. Direct observations of cortical capillaries in normal brain show that capillary flow velocities are heterogeneous during rest, but homogenize during functional activation and the accompanying hyperemia.22 Such
Figure 1. **A**, The importance of capillary flow patterns (yellow arrows) for the efficacy of oxygen extraction. Intravascular colors indicate blood saturation (red, fully oxygenated; darker blue, more deoxygenated) and surrounding darker blue colors indicate lower tissue oxygen tension—adapted from Østergaard et al.\(^2\) Copyright ©2013, The Authors (see: http://creativecommons.org/licenses/by-nc-sa/3.0/). In the resting, normal brain (top), erythrocyte velocities vary greatly among capillaries, with little oxygen being extracted from fast-flowing blood.\(^2\) As cerebral blood flow (CBF) increases (right), capillary flow patterns homogenize in parallel.\(^2\) This phenomenon reduces the functional shunting that otherwise occurs when erythrocytes pass through capillaries at short transit times. Although the mean transit time (MTT) of blood through the capillary bed is related to CBF through the central volume theorem (MTT=CBV/CBF, where CBV is the capillary blood volume), capillary transit time heterogeneity (CTH) indicates the distribution of capillary transit times relative to this mean (e.g., in terms of their standard deviation). Both MTT and CTH are measured in seconds, and the degree of functional shunting generally increases as CTH approaches MTT.\(^2\) The accompanying reduction in net oxygen supply reduces tissue oxygen tension as cells continue to use oxygen, increasing blood-tissue concentration gradients and net oxygen extraction.\(^2\) With flow responses attenuated, oxygen extraction can be increased from the 30% of normal brain up to near unity, and normal brain function maintained although capillary dysfunction becomes more severe.\(^2\) It is important to note that current state-of-the-art algorithms to generate maps of transit time-related metrics based on perfusion-weighted magnetic resonance imaging or computed tomography cannot distinguish changes in tracer retention caused by prolonged MTT (reduced CBF) from changes caused by capillary flow disturbances (capillary dysfunction).\(^2\) The effects of CTH must therefore be separately modeled to ascertain whether clinical signs of ischemia are indeed caused by limited blood supply or by capillary dysfunction.\(^2\) **B**, The acute changes in capillary morphology that accompany conditions in which CSD are common. In traumatic brain injury (TBI; i), massive swelling of the perivascular astrocytic end feet (AE) and flattening or compression of the capillary lumen (L, indicated by red arrows) is observed.\(^2\) Ischemia (iii) can cause pericytes to constrict and compress the capillary lumen. Top, A horseradish peroxidase (HRP)–filled capillary segment interrupted by a constriction, merged with an image that highlights pericyte α-smooth muscle actin in green (bar indicates 10 µm). Bottom, Multiple disruptions of a HRP-filled capillary, highlighting that pericyte constrictions may prevent reperfusion (no-reflow) or disturb capillary flow patterns after recanalization (bar indicates 20 µm).\(^2\) Ischemia data were obtained in mice. Reprinted from Yemisci et al\(^2\) with the permission of Macmillan Publishers Ltd. Copyright ©2009, Nature America, Inc.
reductions of CTH would be expected to secure efficient oxygen extraction during hyperemia as illustrated in Figure 1A (top right panel). A recent study suggests that both CBF and CTH are indeed controlled by capillary pericytes during functional activation.  

Figure 2A illustrates how CBF must be adjusted to compensate for an increase in CTH to support a constant rate of oxygen metabolism. The figure also indicates the parallel change in oxygen extraction fraction (OEF). We first consider a mild increase in CTH, defined here as a CTH increase for which the resulting OEF reduction can be compensated by an increase in CBF. 

A compensatory CBF increase shortens capillary transit times, and thereby increases CTH-related functional shunting of oxygenated blood. Hyperemia can therefore only compensate for mild increases in CTH. For higher CTH, attenuation of CBF becomes mandatory to meet the metabolic demands of the tissue. Note that an initial, transient hyperemia is expected as CTH increases beyond the threshold at which CBF starts to be attenuated.

**Predicted Changes in CBF to Support Increased Metabolic Demands When CTH Is Elevated**

Above, we considered how neurovascular coupling supports resting brain metabolism as CTH increases. We now consider how CBF must be adjusted to meet the additional metabolic demands of functional activation or repolarization after CSD. Figure 2C and 2D illustrates this for CTH increases to levels below and above the threshold of flow suppression, respectively (compare Figure 2A and 2B).

Figure 2C shows how functional hyperemia is exaggerated during a mild increase in CTH; by definition, metabolic needs can still be met by hyperemia alone, and larger CBF responses hence compensate for the lower OEF. In Figure 2D, CBF responses are illustrated both in the normal state and after a moderate increase in CTH. Note how both baseline CBF and CBF responses to certain metabolic challenges are reduced after such a CTH increase. Note that flow suppression is expected as a result of flow-metabolism coupling and therefore should depend on the rate of oxygen utilization in the tissue. Accordingly, the suppression of activation-related CBF is expected to occur at a lower CTH level than the CTH level at which resting CBF must be suppressed. We review evidence of this phenomenon in migraine below.

**Observed Changes in CBF, Metabolism, and Blood Oxygenation During CSD in Normal Mouse and Rat Brain: Migraine Aura**

Figure 3A shows typical CBF responses during the passage of a CSD in mouse brain. Depolarization is accompanied by a small increase, followed by a drop, in CBF. If we assume that the long-lasting capillary flow disturbances caused by a CSD give rise to elevated CTH during the course of the initial depolarization, then the observed course of CBF changes (a slight hyperperfusion followed by hypoperfusion) is consistent with the prediction in Figure 2D. The subsequent repolarization is energetically demanding, and the transient increase in
CBF in the mouse model of CSD indeed coincides with the restoration of membrane potentials, after which CBF returns to a level consistent with high CTH, that is, continued capillary flow disturbances. The prediction that CSD-related CBF-changes are coupled—through CTH—to the metabolic needs of the tissue may help explain the distinct appearance of CBF response to subsequent CSDs,11 elicited while CTH remains high; see Figure 3B. Note that the shapes of the first and second CBF response (here in mice) are nearly identical except for the CBF drop during the first CSD, which we ascribe to neurovascular coupling during a gradual increase in CTH.

The extent of CBF suppression required to maintain flow metabolism during and after CSD is predicted to depend on both CTH and oxygen utilization. The prolonged 60% CBF suppression after repolarization in mouse (Figure 3C) is therefore predicted to be the result of a pronounced increase in CTH after CSD, high oxygen utilization in the anesthetized mouse brain, or both. The 25% reduction in CBF before and after repolarization in rat (Figure 3D),31 in turn, is predicted to reflect a more modest CSD-related CTH increase, a lower oxygen utilization in the anesthetized rat brain, or both. During repolarization, the extent of CBF suppression is expected to depend mainly on oxygen extraction efficacy, and thereby the degree of capillary flow disturbance. In normal brain, an inverse relationship is therefore expected between the amplitude of the CBF response during repolarization and the CBF reduction before and after repolarization, as indeed observed in Figure 3C and 3D.31

The duration of post-CSD oligemia varies. This CBF reduction may reflect reduced cortical activity (see below), but may also reflect adjustments of CBF and CBF responses to account for prolonged capillary flow disturbances. This prediction is consistent with findings that responses to increased CO₂ levels, basal forebrain stimulation, and direct arteriolar application of vasoactive substances are reduced or disappear12 during post-CSD oligemia and that evoked P₉₁ changes are inversely correlated with the degree of baseline hypoxia.32 As
capillary flow patterns normalize, metabolic needs can be met at a higher CBF for a given \( \text{P}_{\text{O}_2} \). Such indirect evidence of improved capillary flow patterns was observed by Fordman et al who reported that blockage of 20-hydroxyeicosa- tetraenoic acid synthesis ameliorated post-CSD hypoperfusion, although having modest effects on the degree of hypoxia.32 These results are consistent with a role of 20-hydroxyeicosatetraenoic acid in the capillary flow disturbances and neurovascular coupling after CSD, possibly through its action as a powerful pericyte constrictor.30

Migraine Aura
In patients with migraine aura, CBF responses to vasodilatory stimuli, such as \( \text{CO}_2 \), are attenuated during the spreading oligemia,13 consistent with neurovascular coupling during a state in which CTH is elevated (Figure 2D). Hypoperfusion typically persists as patients develop headache, until patchy areas of hyperperfusion appear in previously hypoperfused areas after 2 to 6 hours.33 The late hyperperfusion is consistent with the CBF change predicted to occur as a large increase in CTH resolves.

Blood oxygen level–dependent (BOLD) functional magnetic resonance imaging localizes brain activity via the accompanying reductions in blood deoxyhemoglobin concentration [dHgb]. During functional activation, this effect gives rise to increased signal intensity in activated brain areas compared with resting conditions. Both increases in CBF and reductions of OEF tend to reduce [dHgb], and small CTH increases (Figure 2C) are therefore predicted to elevate BOLD signal amplitudes for a given tissue metabolism. Conversely, reductions in CBF and elevated OEF (Figure 2D) both attenuate BOLD signal amplitudes for a given tissue oxygen metabolism. Small increases in CTH would therefore be predicted to increase both baseline BOLD levels and the amplitude of task-related BOLD signal changes, relative to a condition of normal CTH. As CTH increases further, flow suppression is expected to affect task-related hyperemia (where metabolic demands are higher) first, then the baseline BOLD amplitude. Figure 4 illustrates how task-related BOLD changes observed during the onset and early phases of migraine aura44 are remarkably consistent with these predictions.

**ATP Production at Elevated CTH: Differential Effects of Capillary Dysfunction on Oxygen and Glucose Extraction**

After the initial, near-complete breakdown of ion gradients during a CSD, large amounts of ATP are needed to reestablish normal ion distributions.33 Figure 5 shows the course of CBF, and the tissue levels of glucose and ATP, during the passage of CSDs in rat brain.33 Autoradiographic measurements during CSD have revealed 100% increases in the uptake of glucose analogs into the affected tissue.36 These data are widely interpreted as indicating a 100% increase in neuronal energy turnover during the restoration of ion gradients after CSD.

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**Figure 4.** A functional magnetic resonance imaging (fMRI) slice oriented perpendicular to the calcarine fissure in a subject with inducible migraine aura that affects the right hemisphere.44 B and C, Blood oxygen level–dependent (BOLD) signal changes to a visual stimulus (alternating, 32 second epochs of radial flickering checkerboard vs black screen with a fixation point) recorded in single image voxels (brown arrows) within homologous areas of the right (B) and left (C) occipital lobe during the onset of migraine aura (onset time indicated by white arrow). In the right hemisphere voxel, note the initial increase in activation-induced BOLD signal amplitude (time period indicated by green line) followed by an increase in baseline BOLD signal level (indicated by the left-most yellow line). These changes are consistent with a mild increase in CTH, in that both elevated cerebral blood flow (CBF) and reduced oxygen extraction fraction (OEF; cf. Figure 2A) cause tissue deoxyhemoglobin levels to fall and, hence, BOLD fMRI signal amplitude to increase. C, Simultaneous signal changes in the unaffected hemisphere: these illustrate typical signal fluctuations over time. Next, coinciding with the onset of aura symptoms,14 stimulus-induced BOLD changes become attenuated (indicated by blue line)—and even disappear during the time period assumed to mark the passing of a cortical spreading depolarization and -repolarization.14 In B, note that BOLD signal intensities during stimulus-off periods (indicated by the right-most yellow line) return to the baseline recorded at the onset of the imaging session. Although stimulus-induced BOLD responses remain suppressed, consistent with either suppressed neuronal activation or moderately elevated CTH, these findings do not support that resting CBF is suppressed to compensate for a moderate increase in CTH as defined in Figure 2B. D, The stimulus-off BOLD signal intensities fell below preaura levels in areas V3a and V3 in the same subject, consistent with a larger (moderate) CTH increase and flow-suppression. Signal source analysis revealed that the spreading BOLD changes had in fact originated in V3a in this subject,14 and our analysis thus suggests that the aura phenomenon arises under conditions with a more severe oxygen supply–demand imbalance than observed as the wave subsequently spreads across the visual cortex. It should be noted that neuronal responses to the visual stimulus are likely to be affected by the spreading depression of activity. It is therefore difficult to ascertain to which degree stimulus-induced BOLD responses are diminished as a result of changes in neuronal or in capillary function, respectively. Adapted from Hadjikhani et al14 with permission of the authors. Copyright ©2001, National Academy of Science, USA. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.
ATP production during the repolarization phase is, however, heavily dependent on oxygen availability, keeping in mind that a 100% CBF increase does not ensure a 100% increase in tissue oxygen availability. Under aerobic conditions, one glucose molecule generates 29 to 30 ATP molecules by oxidative phosphorylation, but if oxygen availability is limited, lactate production yields only 2.

The extraction of glucose and glucose analogs in the brain is limited by CTH, and efficient glucose extraction during hyperemia depends on homogenization of capillary transit times, similar to the properties of oxygen extraction. Elevated CTH seemingly favors the extraction of glucose over oxygen, and increases in CTH would therefore be expected to reduce ATP yields from glucose metabolism, causing (1) conversion of larger amounts of glucose into lactate, (2) relative ATP depletion, and (3) lower glucose and oxygen levels in tissue, consistent with the observations in Figure 5. Indeed, simultaneous glucose and lactate measurements during CSD in normal brain suggest a sharp increase in anaerobic glycolysis during and immediately after the passage of a CSD.

**Elevated CTH in the Injured Brain: The Effects of Preexisting Capillary Dysfunction**

In the injured brain, CTH may be elevated in the affected tissue because of elevated intracranial pressure, astrocytic end-foot swelling, pericyte constrictions, and so forth—see recent reviews for evidence of capillary dysfunction in stroke, aneurismal subarachnoid hemorrhage, and traumatic brain injury. Flow suppression can only compensate for elevated CTH to a certain extent. We have demonstrated that a CTH threshold exists for which the metabolic needs of resting human brain are met at negligible tissue oxygen tension. Notably, the CBF value which optimizes oxygen extraction at this CTH threshold is approximately the classical ischemic threshold of 20 mL/100 mL/min. For even higher values of CTH, and in cases where CBF is critically restricted after a thromboembolic event, oxygen availability may therefore fall below the metabolic demands of repolarization (see below).

Figure 6A indicates how the metabolic needs of brain tissue can be met at high CTH: resting CBF is suppressed and OEF elevated. The left panel illustrates how repolarization can still occur at the speed observed in less affected tissue. If CTH is more severe (right panel), net oxygen extraction can still be improved by reducing CBF to the threshold indicated in the left panel, but it now limits the speed of repolarization. Because we assume CBF to be coupled to the metabolic needs of repolarization, the duration of the required CBF suppression is predicted to correlate with the duration of repolarization. In Figure 6A, we assumed that cortical electric activity remains depressed (dark gray area) for some time after CSD (light gray area). Note that the parallel reduction of metabolic demands is predicted to cause a transient increase in CBF as flow-metabolism coupling in this case entails less suppression of CBF.

Figure 6B shows slow potential changes consistent with CSDs in 2 aneurismal subarachnoid hemorrhage patients. The upper traces show dynamic changes in CBF (as relative values), whereas the lower traces show the CSD-induced depression of spontaneous activity. The left panel illustrates a CSD with normal hemodynamic response, whereas the inverse response (spreading ischemia) is demonstrated in the right panel. Human recordings suggest that PO, preceding spreading ischemia can in fact be normal, low, or even high. PO, inevitably falls
dramatically during spreading ischemia, whereas it seems to increase during the normal hemodynamic response.16

We speculate that the increase in CBF (indicated with an arrow) after repolarization reflects reduced metabolism as a result of the suppressed spontaneous electric activity.

Figure 6C shows sequential laser speckle contrast images (left) obtained in a rat model of focal ischemia, demonstrating the CBF responses to a CSD propagating from severely ischemic tissue outwards into less affected tissue.16 The time courses (D, K, Q and V) illustrate the characteristic transition from positive to negative and prolonged CBF responses in relation to CSD passages, according to the postocclusion CBF reduction.16,17 Note that CBF remains low after the CSD in the most severely ischemic tissue (labeled V and highlighted by a red circle), unlike the pattern in the right panel of Figure 6B. The corresponding tissue (within the dashed
line) was recruited into the infarct core after the CSD passage. Studies of focal ischemia in both rats and cats confirm that such persisting hyperperfusion after CSD is associated with high risk of tissue injury. Recent studies have demonstrated that ischemia and oxidative stress are sources of prolonged pericyte constrictions (Figure 1B). By this mechanism, waves of spreading ischemia might hinder (no reflow) or disturb (increase CTH further) the subsequent recovery of tissue perfusion and thereby add to the metabolic derangement of penumbral tissue. Dynamic imaging of CBF and capillary flow patterns during spontaneous CSDs in peri-infarct tissue may prove useful for studying this phenomenon in animals. Meanwhile, microvascular flow disturbances can be imaged in acute human stroke and related to subsequent infarct growth.

Discussion

CSD-related changes in CTH would change oxygen extraction efficacy, and our analysis suggest that flow-metabolism coupling would alter CBF in ways that mimic the apparent supply–demand imbalance observed during CSD and subsequent repolarization. Simultaneous recordings of CBF, CTH, and tissue oxygen tension during CSD and repolarization are needed to examine whether these predictions are true.

Our analysis of CBF and BOLD recordings during migraine aura suggest that this phenomenon is associated with dysfunctional capillary flow control. Such dysfunctions may exist interictally, affecting CTH and brain oxygenation between attacks. Indeed, migraineurs with visual aura show elevated interictal BOLD responses to visual stimulation, but normal resting CBF, comparing to both migraineurs without aura and controls. This could indicate that migraineurs with aura have normal CTH during rest, but fail to homogenize their capillary flows fully during functional activation where metabolic demands increase. Our analysis shows that the ability to homogenize capillary flow patterns may prove critical for tissue survival in relation to CSDs in injured tissue. If this ability is impaired in migraine with aura, patients would indeed be vulnerable to CSDs during ischemic events as proposed by recent studies in animal models with migraine mutations. Given the recent demonstration that pericytes actively control capillary diameter during functional activation and that pericyte function is impaired by ischemia and oxidative stress, we speculate that further studies of this cell type may improve our understanding, not only of migraine with aura, but also of the elevated stroke risk associated with these conditions.

It is important to note that existing studies of spreading ischemia can be interpreted in 2 radically different ways. The current view is that spreading ischemia represents a dysregulatory process in which the normal hyperemic response is disturbed by elevated potassium levels and reduced levels of NO. This view is supported by observations that red blood cell products—which include potassium and oxyhemoglobin, a powerful NO scavenger—induce vasoconstriction in isolated arteries and cause spreading ischemia with deleterious tissue outcome after topical application in tissue with normal or slightly reduced CBF. Indeed, administration of vasodilators cause spreading ischemia to revert to a more physiological CBF response, shortening the energy-dependent recovery from CSD. The view presented here proposes that abnormal flow responses partly reflect compensatory changes to optimize oxygen extraction in tissue affected by severe capillary dysfunction. Molecules the size of hemoglobin undergo rapid perivascular clearance, during which they must pass through the narrow capillary basement membrane where pericytes are located. Here, CTH would be expected to change as pericytes react to potassium, NO, and a range of vasodilators in much the same way as vascular smooth muscle cells. Because blood flow changes are closely coordinated across the microvasculature and can be initiated at the level of capillary pericytes, the 2 scenarios are therefore difficult to disentangle with current experimental evidence. Tissue injury is clearly imminent when spreading ischemia is observed, but in conditions where this devastating phenomenon occurs, it is unclear whether therapies that restore large vessel patency and normal CBF also improve clinical outcome if they fail to restore capillary flow patterns in parallel. Therefore, we propose that both the proximal (resistance) and capillary vascular segments must be considered to understand the metabolic derangement in cortical spreading ischemia, for example, by parallel CTH, CBF, $P_{O_2}$, and CSD recordings.

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