

COMMENTARY

The Elusive Initial Dip

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We must suppose a very delicate adjustment whereby the circulation follows the needs of the cerebral activity. Blood very likely may rush to each region of the cortex according as it is most active, but of this we know nothing.

William James, The Principles of Psychology (1890)

Blood does indeed rush to each region of the brain in response to local neural activity, but we are still struggling to understand this phenomenon in a quantitative way. The fact that cerebral blood flow (CBF) increases in a focal way makes intuitive sense, because ionic gradients degraded by neural activity must be restored and neurotransmitter molecules repackaged. This requires energy metabolism, and CBF serves both to deliver glucose and oxygen, the metabolic substrates that fuel the brain, and to carry away carbon dioxide and heat, the waste products of metabolism. But what has been surprising, and is still puzzling, is why blood flow increases so much more than the metabolic rate of oxygen.

Since Fox and Raichle (1986) first pointed out this discrepancy, numerous studies with both positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have confirmed that with neural activation the fractional change in CBF is at least twice as large as the fractional change in oxygen metabolism. This was originally called an “uncoupling” of CBF and oxygen metabolism, because the CBF increase appeared to be excessive for the small increase of oxygen metabolism. However, the recent elegant work of Hoge *et al.* (1999) shows a simple graded relationship between CBF and oxygen metabolism in the human visual cortex during sustained visual stimulation. This suggests a close coupling of CBF and oxygen metabolism changes, although with a much larger fractional change in CBF.

Furthermore, in the past few years an alternative explanation for the mismatch of CBF and oxygen metabolism changes has been developed based on the idea that oxygen delivery is limited at rest (Gjedde *et al.*, 1991, 1999; Buxton and Frank, 1997; Hyder *et al.*, 1998). In the context of this oxygen limitation model the large increase of CBF is required to support the

smaller increase of oxygen metabolism because the oxygen extraction fraction E must drop with increased flow. The basic premise of the model is that tissue pO_2 is near zero and that the diffusion distance from capillary to mitochondria is fixed (no capillary recruitment). Then in order to increase the diffusion gradient for oxygen E must be reduced in order to raise the average capillary pO_2 . At steady state the product of E and CBF must match this increased flux down the oxygen gradient, so CBF must increase more than oxygen metabolism to overcome the decrease of E . The oxygen limitation model provides a possible explanation for why a large change in CBF may be necessary to sustain a small change in oxygen metabolism.

The question of whether and how CBF and oxygen metabolism are coupled during neural stimulation is critical for understanding the blood oxygenation level-dependent (BOLD) signal measured with fMRI. The BOLD signal results directly from the decreased oxygen extraction, because deoxyhemoglobin tends to reduce the MR signal through magnetic susceptibility effects. Functional MRI has blossomed in the past few years to become the premier technique for mapping patterns of activation in the human brain. Yet the fundamental physiological effect that underlies the technique, the mismatch of the changes in CBF and oxygen metabolism, is still poorly understood. While the results of Hoge *et al.* (1999) in humans and Mandeville *et al.* (1999a) in rats suggest coupling of flow and metabolism with sustained activity changes (3 min in the human experiments, 30 s in the rat experiments), the question of whether the two physiological processes are coupled during dynamic changes is still unresolved. With the advent of event-related paradigms in fMRI and the use of stimuli with subsecond

durations, this question has become even more important.

THE INITIAL DIP

In this issue of *NeuroImage*, three papers deal with the fundamental dynamic relationship between CBF and oxygen metabolism changes in response to brief stimuli (Jones *et al.*, 2001; Lindauer *et al.*, 2001; Mayhew *et al.*, 2001). These papers focus on the controversial phenomenon known as the initial dip (also called the fast response). The phenomenon was first reported by Grinvald and colleagues (Frostig and Grinvald, 1990; Malonek and Grinvald, 1996) using optical techniques to measure dynamic changes in oxyhemoglobin and deoxyhemoglobin in a cat model. After a brief stimulus they found an initial increase in deoxyhemoglobin content, followed by a later and more pronounced decrease in deoxyhemoglobin. The later decrease corresponds to the standard BOLD signal increase in fMRI, but the initial deoxyhemoglobin increase would correspond to an initial dip in the BOLD signal, and the BOLD terminology is generally used to describe the effect. Prompted by these animal experiments, several groups using fMRI found a brief, weaker signal decrease prior to the standard BOLD signal increase in the human visual cortex (Ernst and Hennig, 1994; Menon *et al.*, 1995; Hu *et al.*, 1997) and recently in the motor cortex (Yacoub *et al.*, 2001).

There are two reasons why the initial dip is an important phenomenon to understand: it may be more localized to the active region of tissue than the later BOLD signal increase used in most fMRI mapping experiments, and it may be evidence of a dynamic uncoupling of CBF and oxygen metabolism. Malonek and Grinvald (1996) found that the columnar structure of the cat visual cortex could be more clearly delineated by mapping the initial deoxyhemoglobin increase rather than the later, larger deoxyhemoglobin decrease. They hypothesized that the initial dip is due to an early focal increase in oxygen metabolism accomplished by an increase of oxygen extraction before the flow increase has started and that the flow increase when it does occur is controlled on a coarse spatial scale. This would lead to a phenomenon of "watering the garden for the sake of one thirsty flower," a poetic image that not only explains why the initial dip is better localized but also potentially provides another explanation for why the CBF increase is always larger than the oxygen metabolism increase. Oxygen metabolism and CBF changes could be matched within a column, but if the CBF increases over several adjacent columns due to coarse CBF control, the tissue average increase of CBF would be larger than the average oxygen metabolism increase. This hypothesis has significant implications both for the ultimate spatial res-

olution that can be achieved with fMRI and for the interpretation of BOLD signal changes.

However, the initial dip has been highly controversial in both the optical imaging and fMRI literature because it is not always found. This has led to heated discussions of the models on which the optical analysis techniques are based, possible effects of different anesthetics, and even species differences. To counter the criticism of the earlier optical studies, Vanzetta and Grinvald (1999) used a different technique, based on phosphorescence quenching by oxygen of an agent confined to the blood, to demonstrate an initial dip in the average pO_2 of blood. They found that this initial dip preceded the volume change derived from optical measurements of total hemoglobin content and added support to both the existence of the dip and the argument that the increased extraction precedes the flow change.

The two papers by Jones *et al.* (2001) and Lindauer *et al.* (2001) in this issue further sharpen the controversy. Both used optical measurements in a rat model with stimulation of the whisker pad, and both used a more sophisticated path length analysis than had been used in the earlier studies. Yet the two groups came to opposite conclusions: Jones *et al.* (2001) found a reproducible initial increase in deoxyhemoglobin, consistent with earlier work from this group (Mayhew *et al.*, 2000), while Lindauer *et al.* (2001) found no evidence for a fast response. Lindauer *et al.* (2001) also used the phosphorescence quenching technique used earlier by Vanzetta and Grinvald (1999) and found no evidence for an initial dip in blood pO_2 . In addition, Lindauer *et al.* (2001) provide a detailed discussion of how the optical pathlength analysis can lead to an artifactual initial increase of deoxyhemoglobin. Although this problem may have affected the earlier optical studies, the fact that Jones *et al.* (2001) used a similar analysis and found a fast response indicates that in general the fast response in optical studies cannot be explained away as a processing artifact. Lindauer *et al.* (2001) also raise a technical concern that needs to be resolved with the phosphorescence quenching technique. The calculation of pO_2 from the phosphorescence decay time assumes a single well-mixed blood compartment with a uniform pO_2 , and this is certainly not the case. The full implications of this assumption need to be clarified, and this may require a more detailed model for the dynamic changes in different blood compartments.

Despite a significant effort over the past few years, the controversy remains as strong as ever. Given the high quality of the labs involved, and the different techniques employed, we can only conclude that the different findings are not due to experimental errors but rather represent a true variability of the physiological effect. Faced with this stubborn variability, we must ask what the future of the initial dip will be. Will it become the signal of choice for precise mapping stud-

ies with fMRI? And what will it tell us about the coupling of CBF and oxygen metabolism?

SPATIALLY ACCURATE ACTIVATION MAPPING

Is the ultimate spatial resolution attainable by fMRI limited by the hardware and signal-to-noise ratio or is it limited by the physiology of the CBF response to activation? This is a critical question for the future of fMRI, but the answer is not known. Recently Kim *et al.* (2000) used the initial dip in the BOLD signal (corresponding to the initial deoxyhemoglobin increase) to map the columnar structure of the cat visual cortex with an fMRI experiment similar to the optical experiment of Malonek and Grinvald (1996). They also found that the maps produced from the later positive BOLD signal did not reveal the columnar structure as clearly. However, questions about the robustness and reproducibility of the initial dip and maps made from it still need to be answered (Logothetis, 2000). Furthermore, the intrinsic weakness of the fast response in fMRI makes it unlikely that mapping the initial dip will become the standard approach for BOLD-fMRI except perhaps at very high magnetic fields.

Nevertheless, such mapping studies based on the initial dip support the concept that when it is detectable it is better localized than the later positive BOLD response. However, this finding alone does not necessarily indicate that the arterial delivery of blood is poorly localized. Increased inflow produces changes in blood oxygenation which travel down the vascular tree. With time these oxygenation changes may move a substantial distance (many millimeters) from the site of activation. This draining vein effect has created a localization problem in fMRI studies from the beginning, and so it is not surprising that the later signal change is more poorly localized than the initial signal change, regardless of whether the change is positive or negative. Recently Menon *et al.* (1999) have used this idea to image ocular dominance columns in humans using the early rising part of the BOLD signal (i.e., corresponding to the beginning of the deoxyhemoglobin decrease, not the initial dip). Furthermore, Cheng *et al.* (2000) have recently shown that under the right conditions the ocular dominance columns in humans can be measured reproducibly using the sustained signal increase due to longer stimulations.

The problem of spatial spread due to venous drainage could occur even if the change in arterial delivery is quite focused and well-localized. In recent years a different approach to doing fMRI has been developed using arterial spin labeling (ASL) techniques [see Calamante *et al.* (1999) for a review]. The ASL signal depends on the delivery of magnetically tagged arterial blood and not on blood oxygenation. For this reason the ASL signal reflects the arterial side of the vasculature, while the BOLD signal reflects the venous side. In

addition, the ASL signal is reproducible and stronger than the initial dip. Recent initial work by Duong *et al.* (2000) showed that the columnar structure of the cortex can be mapped with ASL techniques, suggesting that ASL may provide more precise activation maps than the standard BOLD maps. Further work will be required to directly compare maps based on the initial dip with ASL maps and to test whether these techniques for mapping CBF provide similar spatial resolution with greater sensitivity and reliability for studies requiring high spatial specificity.

IMPLICATIONS FOR MODELS OF THE COUPLING OF CBF AND OXYGEN METABOLISM

Ultimately, the initial dip may prove to be more important in what it tells us about the dynamic coupling of CBF and oxygen metabolism changes. In particular, the hypothesis of Malonek and Grinvald (1996), that the initial dip is due to increased oxygen extraction prior to the increase of blood flow, suggests a dynamic uncoupling of flow and metabolism. Even if this uncoupling is only transient, it nevertheless provides a useful probe of how flow and oxygen metabolism changes are connected.

However, the central problem in unraveling these relationships is that changes in oxy- and deoxyhemoglobin concentrations (and the BOLD effect) depend on the combined changes in CBF, blood volume, and oxygen metabolism, all of which may be changing on different time scales. For example, the poststimulus undershoot of the BOLD signal that is often observed is likely due to blood volume returning to baseline more slowly than CBF (Buxton *et al.*, 1998; Mandeville *et al.*, 1999b). This potential for different time constants for different physiological processes makes the interpretation of dynamic optical and BOLD studies intrinsically ambiguous. These difficulties are made clear in the analyses in the Jones *et al.* (2001) and Mayhew *et al.* (2001) papers. A number of assumptions must be made in order to quantitatively interpret the optical signals in terms of oxygen metabolism changes, and thorough testing of these assumptions will need to be done. In particular, the interpretation of dynamic changes likely will require more detailed modeling of how arterial and venous blood volumes change and the time constants for each.

The paper by Mayhew *et al.* (2001) is a thoughtful initial attempt at evaluating the dynamic data in terms of the oxygen limitation model. The simplest form of the oxygen limitation model assumes that all extracted oxygen is metabolized and that the diffusivity of oxygen remains constant with increased flow (Buxton and Frank, 1997). This simple model shows why the flow increase must be much larger than the oxygen metabolism increase, but is probably too simple for a complete model. For example, there could be

small changes in diffusivity with activation due to capillary dilatation or a shift of the oxygen/hemoglobin saturation curve due to increased CO₂ in the blood. If the diffusivity does increase with flow, then the same oxygen metabolism change could be supported by a smaller CBF increase. Hyder *et al.* (1998) modified the mathematical framework of the model to include a variable diffusivity proportional to CBF, with a free parameter characterizing that proportionality. Mayhew *et al.* (2001) tested these two forms of the model against their dynamic measurements and concluded that neither gave a particularly good account of the data. This may be due in part to the fact that the oxygen limitation model is a steady-state model, and applying it to dynamic measurements will require a more complete dynamic model. However, the more likely, and perhaps more interesting, possibility is that flow and oxygen metabolism are uncoupled during the initial dip.

The fundamental difficulty for interpreting changes in total deoxyhemoglobin and oxyhemoglobin is that these measured quantities are affected by both changes in oxygen extraction fraction E and changes in blood volume V (Buxton *et al.*, 1998; Hathout *et al.*, 1999). Malonek and Grinvald (1996) hypothesized that the change in E precedes the change in V , and Vanzetta and Grinvald (1999) argued that the drop in average blood pO₂ measured by phosphorescence quenching occurred before the blood volume increase measured with optical techniques. However, the telling optical experimental result to support the interpretation that E increases before V would be a drop in oxyhemoglobin corresponding to the increase in deoxyhemoglobin, yet none of the published reports shows a decrease in oxyhemoglobin, including Jones *et al.* (2001). Instead, a typical finding is that oxyhemoglobin remains constant during the beginning of the rise in deoxyhemoglobin. This implies that the fast response must be a combination of a simultaneous increase of E and V . If it was purely a change in V , then both oxy- and deoxyhemoglobin signals should go up together, and if it was purely an increase of E , then the oxyhemoglobin content should fall when the deoxyhemoglobin content rises. The observed pattern of an increase in deoxyhemoglobin with roughly constant oxyhemoglobin (at least initially) could arise if there is a transient increase of E in combination with an increase of arterial volume. Then the oxyhemoglobin would be increased on the arterial side and decreased on the venous side, potentially with no net change, while the deoxyhemoglobin would show a pure increase. In addition, Jones *et al.* (2001) report that the flow change measured by laser Doppler flowmetry closely matches the blood volume curve derived from the optical data.

The finding that oxygen extraction and flow are changing together loosely supports the idea of coupled changes, but the observation that E increases rather

than decreases is a problem from the viewpoint of the oxygen limitation model in its current form. It is perhaps worth speculating about how such an observation could be reconciled with the idea of limited oxygen delivery. In fact, this question focuses on an assumption of the original oxygen limitation model that is undoubtedly too simple: the assumption of zero backflow of oxygen to the blood is equivalent to assuming that the pO₂ in the tissue is zero at all times. This is clearly not true, because observations consistently show an increase in tissue pO₂ with activation. Indeed, an elevation of tissue pO₂ with activation seems to argue against the oxygen limitation model, because it appears that excess oxygen is being delivered to tissue that is not being metabolized. Furthermore, studies of the oxidation state of cytochrome oxidase indicate that at rest oxygen concentration is not a limiting factor in determining the rate of oxidative metabolism in the mitochondria (Springett *et al.*, 2000), which we would certainly expect to be the case if the mitochondrial pO₂ is really zero. However, a revised form of the oxygen limitation model, along the lines sketched by Mayhew *et al.* (2001), could reconcile all of these observations with the central idea of oxygen limitation and may provide an explanation for the variability of the initial dip.

The necessary addition to the model is the inclusion of a buffer of available oxygen, which might operate in the following way. *In vitro* studies indicate that oxygen concentration does not become limiting for cytochrome oxidase activity until the pO₂ is reduced to below 1 mmHg. If the mitochondrial pO₂ is maintained at a higher level, say 5 mmHg, then oxygen metabolism can be controlled based on the local need for energy metabolism and would be independent of the actual pO₂ in the mitochondria. However, the mitochondrial pO₂ is still much less than the average capillary pO₂, so the arguments of the oxygen limitation model still apply. By regulating CBF to maintain the mitochondrial pO₂ at a fixed level, there would always be sufficient oxygen available for oxidative metabolism, yet oxygen delivery nevertheless would be fundamentally limited. That is, if CBF suddenly stopped, the delivery of oxygen from the capillary would drop, and the mitochondrial pO₂ would quickly decrease until the oxygen concentration became the limiting factor in oxidative metabolism.

Then the activation scenario is that there is a buffer of oxygen availability, and CBF is regulated to maintain this buffer at a constant level. To increase the diffusion gradient with a fixed mitochondrial pO₂, the capillary pO₂ must be increased. To do this the extraction fraction E must decrease, and so the flow must increase substantially more than the oxygen metabolic rate in order to increase the product of E and CBF. As a result the average tissue pO₂ also will increase, consistent with microelectrode recordings (the mean tissue pO₂ measured with an electrode must lie some-

where between the capillary pO_2 that increases and the mitochondrial pO_2 that stays constant). The apparent overabundance of oxygen in tissue during activation would thus be required to support the increased oxygen metabolism. This concept of a buffer of oxygen that is maintained during activation, rather than initially consumed, would also reconcile the oxygen limitation model with the sophisticated mathematical model of oxygen delivery developed by Hudetz (1999; Hudetz, personal communication). That model predicted that only small flow changes would initially be required to support an oxygen metabolism increase, because the buffer could be consumed, and that large increases in CBF would be required only for large increases in oxygen metabolism. To maintain the buffer (i.e., prevent the mitochondrial pO_2 from dropping), large CBF increases would be required for all levels of oxygen metabolism change.

It is important to keep in mind in this discussion of the regulation of CBF to maintain oxygen metabolism that we are really talking about the hypothesized functional role of CBF regulation, not the actual mechanisms of CBF regulation. For example, it may well be that the regulation of CBF is not directly controlled by oxygen content at all (Wolf *et al.*, 1997), even if the ultimate function of the CBF increase is to make possible an increase in oxygen metabolism. For example, the hypercapnia studies of Jones *et al.* (2001) (and earlier fMRI studies) show that despite the fact that oxygen delivery has already been increased in excess of what is needed due to the CO_2 inhalation, CBF nevertheless increases further with activation. It may be that the chemical signals that mediate increased blood flow simply have additive effects. For example, the arteriolar dilatation due to chemical agents released through elevated neural activity may be tuned to provide the necessary flow increase to support the increase in oxidative metabolism associated with the increased neural activity. Then the vasodilatory effects induced by changes in extravascular pH due to the elevated CO_2 levels may add to produce an additional increase in CBF.

Finally, the concept of a buffer of oxygen availability may provide an explanation for the variability of the initial dip, as suggested by Mayhew *et al.* (2001). The initial dip could arise if oxygen metabolism is increasing faster than CBF, so that both capillary and mitochondrial pO_2 drop, with the mitochondrial pO_2 dropping more in order to increase the oxygen concentration gradient. In this scenario a part of the oxygen for metabolism comes from the increased flux from the capillary, and a part comes from drawing down the buffer reserve of oxygen by lowering mitochondrial pO_2 . However, if the buffer of available oxygen is increased, by inhaling a gas mixture richer in oxygen, then more of the initial oxygen needed could come from the buffer and less from the capillary. With

no initial change in the oxygen extraction there would be no initial dip. Mayhew *et al.* (2001) report that hyperoxygenation reduces or eliminates the initial dip, consistent with this view, and the experiments of Lindauer *et al.* (2001) used a higher concentration of inspired O_2 than the experiments of Jones *et al.* (2001). Further studies of how the initial dip changes with physiological manipulations will be critical for testing these ideas. Such studies might even provide a bonus for high-resolution mapping studies if physiological manipulations can either enhance the initial dip or make it more robust.

The initial dip may seem to be such an elusive phenomenon that so much experimental work and theoretical speculation focused on it cannot possibly be worth the effort. Yet the initial dip is a thread that connects to many fundamental unanswered questions in the critical area of neurovascular coupling. A better understanding of why the initial dip occurs, and what it signifies when it does occur, will lay a more solid foundation for fMRI studies of brain function. Although fMRI has not yet had a large impact on clinical diagnosis, the possibility of probing the connections between neural activity, blood flow, and oxygen metabolism noninvasively has enormous potential for studies of the developing and aging brain and neurodegenerative disease. To realize that potential, we must first understand the basic physiology of how neuronal activity is coupled to energy metabolism and blood flow.

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