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REVIEW
The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional neuroimaging signals

Chase R. Figley[1] and Patrick W. Stroman[1,2,3]
[1]Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada
[2]Department of Diagnostic Radiology, Queen's University, Kingston, ON, Canada
[3]Department of Physics, Queen's University, Kingston, ON, Canada

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Abstract

Data acquired with functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) are often interpreted in terms of the underlying neuronal activity, despite mounting evidence that these signals do not always correlate with electrophysiological recordings. Therefore, considering the increasing popularity of functional neuroimaging, it is clear that a more comprehensive theory is needed to reconcile these apparent disparities and more accurately explain the mechanisms through which various PET and fMRI signals arise. In the present article, we have turned our attention to astrocytes, which vastly outnumber neurons and are known to serve a number of functions throughout the central nervous system (CNS). For example, astrocytes are known to be critically involved in neurotransmitter uptake and recycling, and empirical data suggests that brain activation increases both oxidative and glycolytic astrocyte metabolism. Furthermore, a number of recent studies imply that astrocytes are likely to play a key role in regulating cerebral blood delivery. Therefore, we propose that, by mediating neurometabolic and neurovascular processes throughout the CNS, astrocytes could provide a common physiological basis for fMRI and PET signals. Such a theory has significant implications for the interpretation of functional neuroimaging signals, because astrocytic changes reflect subthreshold neuronal activity, simultaneous excitatory/inhibitory synaptic inputs, and other transient metabolic demands that may not elicit electrophysiological changes. It also suggests that fMRI and PET signals may have inherently less sensitivity to decreases in synaptic input (i.e. 'negative activity') and/or inhibitory (GABAergic) neurotransmission.

Introduction

Despite its immense complexity, the human nervous system, in its most fundamental form, is composed of only two general cell types – neurons and neuroglia – along with an intricate circulatory network to deliver essential metabolites and carry away metabolic byproducts. Whereas neurons were traditionally thought of as the workhorses of this system, providing the computational backbone that underlies our autonomic (i.e. breathing, heart rate, etc.), sensory and motor functions, as well as our 'higher-level' cognitive abilities, there is now a large body of evidence indicating that astrocytes, a particular type of neuroglia, are anything but passive bystanders in neural signaling. Moreover, these data suggest that astrocytes, and the physiological processes occurring therein, are responsible for the metabolic and hemodynamic changes that are detected with functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) techniques.

Correspondence: Patrick W. Stroman, Centre for Neuroscience Studies, Room 228 Botterell Hall, Queen's University, Kingston, ON, Canada K7L 3N6.
E-mail: stromanp@queensu.ca

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Inferring 'brain function' from functional neuroimaging data

Because of their non-invasive nature, as well as their ability to bridge the gap between neuroanatomical structures and their functional involvement in sensory, motor and cognitive processing, PET and, particularly, fMRI have become widely available and commonly used techniques throughout the biological and psychological sciences. Moreover, fMRI is increasingly finding its way into clinical assessments for characterizing neurological and psychiatric diseases, evaluating the efficacy of pharmacological interventions, and even neurosurgical planning (Detre, 2006; Jezzard & Buxton, 2006; Matthews *et al.*, 2006). However, given the incredible advances in both instrumentation and methodology, the interpretation of the resultant data is, increasingly, less limited by technical obstacles – such as spatial and temporal resolution, and signal-to-noise and contrast-to-noise ratios – and increasingly more limited by what can be inferred from the signal changes themselves (Logothetis, 2008; Leopold, 2009; Narasimhan *et al.*, 2009; Smith, 2009; Welberg, 2009).

The application of functional neuroimaging techniques to the study of task-related changes in 'brain activity' relies on a number of implicit assumptions, not least of which is that the relatively macroscopic measurements made with PET or fMRI accurately reflect changes in neuronal activity and cognitive state. The central dogma

here is that: (i) a particular task/stimulus will invoke specific changes in neuronal activity, (ii) that this will elicit local metabolic and hemodynamic responses, and (iii) that these will produce spatiotemporal intensity changes in PET and fMRI signals. It is obvious, however, that the real contribution of functional neuroimaging to cognitive and systems neuroscience lies in the potential to infer changes in cognitive state from the imaging data *a posteriori* (i.e. inductively), through so-called 'reverse inference' (Poldrack, 2006, 2008). For these inferences to be accurately interpreted, and to establish levels of confidence in their interpretations, it is imperative to have a well-founded understanding of what the signals themselves mean in terms of underlying changes in neurophysiology – particularly what physiological processes are involved and how these interact with each other.

In this respect, it has been pointed out that PET and fMRI techniques alike are inherently limited because they reflect only surrogate physiological changes based on mass neuronal activity (Arthurs & Boniface, 2002; Logothetis, 2008) and, as previously discussed (Raichle, 1998) but often ignored, mass astrocyte activity (Fig. 1). In light of recently reported disparities between hemodynamic and neurophysiological changes (Schummers *et al.*, 2008; Sirotin & Das, 2009), it seems that a modified interpretation of functional neuroimaging signals is needed, and that this may require going beyond the traditional view that these signals are based exclusively on neuronal processes (Narasimhan *et al.*, 2009; Welberg, 2009). Thus, in the present article, we will review how astrocytes affect task-related neurometabolic and neurovascular changes, and discuss the implications for interpreting functional neuroimaging data on the basis of both neuronal and astrocytic activity.

Because neuroscientists and other experts in the field occasionally confuse their jargon, using 'fMRI signal' synonymously with the 'blood oxygen level-dependent signal' (i.e. the 'BOLD signal'), it is sometimes assumed that 'fMRI' means 'BOLD', and vice versa, when in fact these have two very distinct meanings. Although the BOLD signal is certainly the most widely used fMRI contrast mechanism, there are in fact a number of distinct fMRI methods (selected by using different imaging parameters) that are sensitive to physiological changes other than blood oxygen levels. So far, these have not been widely employed beyond methodological developments and a small number of specialized applications, but this is an important technical differentiation nonetheless. We therefore begin with a brief overview of fMRI and the different fMRI contrast mechanisms, so that these principles can be kept in mind throughout subsequent sections.

A brief introduction to fMRI – BOLD and beyond

Typically, fMRI experiments involve a series of interleaved tasks or conditions designed to elicit (and hopefully measure) task-dependent changes in neuronal activity. This is normally achieved by acquiring a series of magnetic resonance images throughout the experiment, so that the signal-intensity time-course in each voxel can be compared with the experimental paradigm, thereby allowing determination of which, if any, regions show significant correlations between the experimental conditions and functional neuroimaging signals. By convention, these regional correlations are statistically thresholded and displayed as activity maps on top of higher-resolution anatomical images (Friston *et al.*, 2006). However, when attempts are made to draw conclusions from these maps or infer cognitive changes, the questions still remain – what do these signal changes actually mean, and how can these macroscopic observations be reconciled with the complex microscopic processes (i.e. changes in neuronal firing rate, neuroenergetics, and cerebrovasculature) that occur during action, perception, and/or cognitive processing?

As alluded to previously, the answers to these questions are complicated by the fact that there are actually a variety of techniques (all broadly classified as fMRI) that use magnetic resonance imaging (MRI) methods to infer 'function' from different physiological changes. The BOLD fMRI signal results from increased blood flow (or 'functional hyperemia') and an overabundant supply of oxygen, relative to oxygen utilization, proximal to regions of neural activity. Therefore, because of the magnetic characteristics of oxyhemoglobin (diamagnetic) and deoxyhemoglobin (paramagnetic), changes in blood

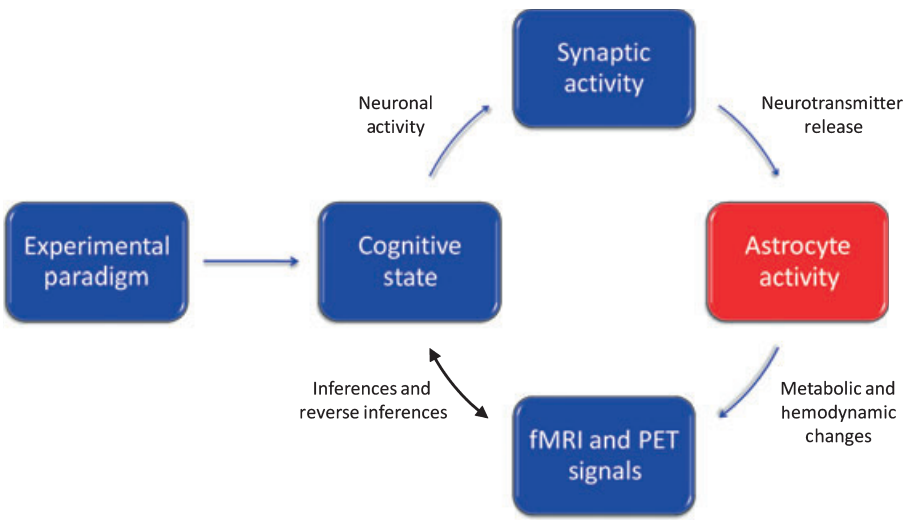

FIG. 1. In this article, our goal is to discuss the important (although often overlooked) roles of astrocyte activity (red) and how these processes fit into the commonly inferred mechanism(s) of functional neuroimaging signals (blue). A preponderance of evidence now shows that task-induced neurometabolic and neurovascular processes, and thus PET and fMRI signals, may reflect astrocyte activity more directly than neuronal activity *per se*. Because many functional neuroimaging experiments are now using these techniques to explore task-related changes in the underlying sensory, motor and/or cognitive states through 'reverse inference', it is important to understand exactly what these measurements mean. The fact that functional neuroimaging signals are more closely linked to astrocyte activity than to neuronal activity has a number of implications regarding the interpretation of functional neuroimaging data in terms of the underlying microscopic neurophysiological changes (see text for details).

oxygenation lead to measurable changes in magnetic-susceptibility-weighted MRI signals, making them 'blood oxygen level-dependent' (Ogawa *et al.*, 1990a,b). Two other hemodynamic-based fMRI methods, which more directly measure changes in either microvascular dilation/constriction or changes in blood flow (as opposed to blood oxygenation), are known as vascular space occupancy (VASO) and arterial spin labeling (ASL) perfusion, respectively (Williams *et al.*, 1992; Wong *et al.*, 1997; Lu *et al.*, 2003). On the other hand, there are also non-hemodynamic fMRI methods, such as signal enhancement by extravascular water protons (SEEP) (Stroman *et al.*, 2002, 2008; Figley *et al.*, 2010) and high *b*-value diffusion-weighted fMRI (Le Bihan *et al.*, 2006; Le Bihan, 2007), that have been reported to detect changes in activity-related cellular swelling.

The selection of imaging parameters, and therefore the type of contrast (i.e. BOLD, VASO, ASL perfusion, SEEP, or diffusion-weighted fMRI), depend on the experimental objectives and the anatomical regions being investigated. An important similarity is that all of these techniques represent, at best, an indirect measure of the local neural processing. What they actually measure are the average neurometabolic and/or neurovascular changes that occur over the experimental time-course.

Electrophysiological comparisons have shown that magnetic-susceptibility-weighted (i.e. BOLD) fMRI signal changes most accurately reflect the neural input and local processing of neuronal populations (Logothetis *et al.*, 2001; Logothetis, 2003, 2007; Logothetis & Pfeuffer, 2004; Viswanathan & Freeman, 2007). However, a recent study has shown that acute cerebrovascular responses – such as those detected with BOLD, VASO and ASL perfusion fMRI methods – can occur in the absence of stimulation or changes in neuronal firing rate (Sirotin & Das, 2009): a finding that is causing quite a stir throughout the functional neuroimaging community (Leopold, 2009; Narasimhan *et al.*, 2009; Smith, 2009; Welberg, 2009). Thus, given the rapidly growing number of fMRI and PET papers appearing in scientific journals, it seems that more than ever we need a better understanding of what, precisely, these and other functional neuroimaging techniques are measuring. Fortunately, a number of recent studies have shed some much needed light, literally in some cases (by using optical fluorescence and transmittance methods), on the cellular/molecular processes regulating the neurometabolic and neurovascular events that ultimately give rise to these functional neuroimaging signals.

Box 1 – A brief overview of astrocyte physiology during glutamate uptake and recycling

Upon neuronal 'activation', presynaptic neurons release neurotransmitters such as glutamate or GABA into the synaptic cleft, where they are able to activate specific receptors on the postsynaptic membrane. Because astrocytes and their end-processes surround virtually every neuron and synapse in the CNS, they are able to respond quickly and efficiently to neuronal and synaptic changes. One of the major roles of astrocytes is to clear synaptic glutamate, which resets the synapse, prevents neurotoxicity and molecular 'spill-over', and potentially provides molecular feedback to the presynaptic neuron (Agulhon *et al.*, 2008). For each glutamate molecule, this requires a concomitant uptake of three sodium ions in order to maintain a neutral electrochemical balance (Zerangue & Kavanaugh, 1996) and the subsequent activation of Na^+/K^+-pumps in order to restore the osmotic gradient to baseline levels. Once glutamate has entered the astrocyte, it is then converted into glutamine and passed back to the adjacent neurons.

Whereas Na^+ uptake is a passive process, the activation of Na^+/K^+-pumps and the conversion of glutamate to glutamine are both energy-intensive processes (each consuming one molecule of ATP), requiring the astrocyte to use a total of two ATP molecules per molecule of glutamate taken up (Pellerin & Magistretti, 2004). It has been suggested that astrocytic glycolysis (i.e. where glucose is non-oxidatively converted to lactate) may be invoked to quickly satisfy these increased metabolic demands (Sibson *et al.*, 1998), and that the lactate from astrocytes may be used as a metabolic substrate to sustain oxidative phosphorylation in adjacent neurons (Pellerin & Magistretti, 1994, 2004; Bouzier-Sore *et al.*, 2003, 2006; Kasischke *et al.*, 2004) – a process that has come to be known as the astrocyte–neuron lactate shuttle hypothesis (ANLSH). Therefore, because increased glutamate uptake is thought to increase astrocytic activity (measured by intracellular Ca^{2+} fluxes) and extracellular lactate concentration, both of which are known to regulate the polarity of the cerebrovascular microcirculation (Gordon *et al.*, 2008), it follows that astrocyte activity and metabolism regulate activity-dependent increases in cerebral blood flow. This further implies that functional neuroimaging techniques such as PET and fMRI are linked to astrocyte activity, which (all else being equal) is quantitatively related to synaptic glutamate release.

Neuroenergetic and metabolic changes in astrocytes during neural signaling

One of the important functions of astrocytes is to surround neuronal synapses and take up neurotransmitters such as glutamate and GABA after they have been released from presynaptic neuron terminals. This serves to quickly and efficiently clear the synapse before the next cascade of neurotransmitter release, while simultaneously preventing molecular 'spill-over' to adjacent synapses (Kullmann & Asztely, 1998). In addition, astrocytic clearance of neurotransmitters has been shown to play a vital role in preventing glutamate-induced neurotoxicity (Rothstein *et al.*, 1996). Nonetheless, the uptake and subsequent cycling of neurotransmitters does not come without a cost to the astrocyte, and these processes are accompanied by a series of energy-intensive processes (Box 1). However, despite keen interest and considerable research efforts to determine the precise metabolic pathway(s) invoked by astrocytes, and the complex interplay with neuronal metabolism, these issues have proven to be both exceedingly complex and hotly debated. Although there is now some general agreement within the research community, certain questions (such as where, on the cellular/molecular level, these task-related neurometabolic changes take place) have yet to be fully resolved.

Until relatively recently, neurometabolism was assumed to be a strictly oxidative (i.e. oxygen-requiring) process – an assumption based on the superior efficiency of oxidatively converting glucose into more useful forms of energy, such as that stored in adenosine triphosphate (ATP). However, in the middle to late 1980s, an important series of PET studies challenged this assumption by showing that cerebral glucose consumption exceeds what can be explained by oxygen utilization in regions of brain activity (Fox & Raichle, 1986; Fox *et al.*, 1988), demonstrating that the metabolic needs of active neural tissue are met in a partially non-oxidative manner. Initially, this conclusion was quite controversial [as reviewed by Barinaga (1997)], but it was later supported by the finding of activity-related increases in cerebral lactate, the metabolic end-product of glycolysis (Prichard *et al.*, 1991). Thus, following transient changes in neural activity, it is now generally agreed that: (i) blood delivery increases with metabolic demand; (ii) blood flow and glucose consumption increase more than oxygen utilization; and (iii) both

oxidative and non-oxidative processes are initiated to meet the increased metabolic requirements. Nevertheless, differing opinions exist concerning how, when and in what cell types these non-oxidative metabolic processes normally occur.

Synaptic terminals represent the most energy-demanding loci in nervous tissue (Schwartz *et al.*, 1979; Mata *et al.*, 1980), suggesting that synaptic activity requires significant baseline neurometabolism to balance excitatory and inhibitory inputs to neurons (even in the absence of postsynaptic spiking activity) and implying that neurometabolic changes following transient neural activity are likely to reflect deviations in net synaptic input (Raichle & Gusnard, 2002). Therefore, because of their cytoarchitectural arrangement surrounding both synapses and capillaries (i.e. the sources of oxygen and glucose delivery), and their roles in mediating synaptic activity [see Agulhon *et al.* (2008) for a recent review], a number of studies have attempted to identify the metabolic processes occurring in astrocytes during neural signaling.

Early *in vitro* experiments demonstrated that during aerobic conditions: (i) extracellular lactate production increases with glucose availability in cultured astrocytes, but not in cultured neurons; and (ii) that blocking electron transport, as would happen under anaerobic conditions, dramatically increased the lactate efflux from both neurons and astrocytes (Walz & Mukerji, 1988). These data were among the first to suggest that cultured astrocytes may rely on 'aerobic glycolysis' to meet some of their energy demands (that is, they use glycolysis even when they could use more efficient oxidative processes), but that glycolysis and lactate production can further increase (at least as much as threefold) upon anaerobic challenge. The subsequent discovery that neurons possess an endogenous lactate transport system (Dringen *et al.*, 1993), combined with the observation that increased neurotransmitter (glutamate) uptake stimulates the conversion of glucose to lactate in cultured astrocytes (Pellerin & Magistretti, 1994), then led to the proposal of the so-called 'astrocyte–neuron lactate shuttle hypothesis' (ANLSH). The essence of this hypothesis is that lactate from astrocytic glycolysis is used as an energy substrate to fuel neuronal oxidative metabolism during the increased activity of glutamatergic neurons (Pellerin & Magistretti, 1994). By coupling glucose utilization (via astrocyte glycolysis) to neuronal activity and oxidative phosphorylation, this mechanism is consistent with previous studies that have implicated a non-oxidative metabolic component during focal brain activation. Therefore, in addition to oxidative metabolism, non-oxidative processes have gained significant attention for their potential role in supporting active central nervous system (CNS) tissue.

Although some studies have not found significant lactate changes *in vivo* (Kauppinen *et al.*, 1997; Boucard *et al.*, 2005; Xu *et al.*, 2005), there have also been several magnetic resonance spectroscopy studies that have shown activity-dependent lactate increases in different brain areas (Prichard *et al.*, 1991; Sappey-Marinier *et al.*, 1992; Kuwabara *et al.*, 1995; Frahm *et al.*, 1996; Richards *et al.*, 1997; Yanai *et al.*, 1997; Urrila *et al.*, 2003; Mangia *et al.*, 2007a,b; Maddock *et al.*, 2009). As a result, there is now a large body of empirical evidence suggesting that glycolytic metabolism increases with corresponding elevations in brain activity. Similar results have also been found using *in vivo* on-line microdialysis, which has shown that cerebellar lactate concentrations increase immediately and are sustained in response to climbing fiber stimulation (Caesar *et al.*, 2008; Kasischke, 2008). In their study, Caesar *et al.* demonstrated that these lactate transients correlated with increased blood flow and oxygen consumption under normal conditions, and further established that selective blocking of postsynaptic AMPA receptors all but abolished the postsynaptic currents, as well as the metabolic and hemodynamic changes. This confirms that lactate production is mediated by postsynaptic events, and suggests that lactate, glucose and oxygen metabolism are tightly coupled to the activation of Ca^{2+}-permeable AMPA receptors on the perisynaptic glia (Caesar *et al.*, 2008). How these lactate concentrations return to baseline is, however, still somewhat of an open and contentious issue.

Immunostaining of brain slices for lactate dehydrogenase (LDH), which regulates reduction/oxidation between pyruvate and lactate, has shown that LDH-1 subunits are prevalent in both astrocytes and neurons, whereas LDH-5 is present exclusively in astrocytes – a finding that has been inferred to corroborate the existence of a lactate flux between astrocytes and neurons (Bittar *et al.*, 1996). Furthermore, by measuring NAD(P)H fluorescence changes, it has been suggested that *in situ* neuronal activity immediately triggers oxidative metabolism in postsynaptic dendrites, followed by a slightly delayed onset of astrocytic glycolysis (Kasischke *et al.*, 2004). The immediate lactate decrease proposed in this study has been taken as strong support for the ANLSH (Pellerin & Magistretti, 2004), because it implies that rapid lactate metabolism by oxidative phosphorylation in neurons could be supported by increased, albeit slightly delayed, astrocyte glycolysis (which would serve to restore the availability of extracellular lactate).

As it is beyond the scope of this article to provide a full review of the topic, however, it should be noted that the validity of the ANLSH and the role of lactate as a metabolic substrate for neurons has been enthusiastically debated (Chih *et al.*, 2001; Chih & Roberts, 2003; Bonvento *et al.*, 2005; Korf, 2006; Schurr, 2006; Simpson *et al.*, 2007; Mangia *et al.*, 2009b). For example, more recent work suggests that: (i) NAD(P)H transients, as reported by Kasischke *et al.*, may reflect mitochondrial dynamics rather than increased glycolytic metabolism (Brennan *et al.*, 2006); (ii) these measurements can be influenced by pre-stimulus NAD(P)H levels resulting from prior electrical stimuli (Brennan *et al.*, 2007); and (iii) various laboratory (*in vitro* and *in situ*) conditions could result in different metabolic processes than those occurring under normal physiological conditions *in vivo* (Dienel & Hertz, 2001; Dienel & Cruz, 2008). For these reasons, the source(s) of *in vivo* lactate production are, as yet, not completely agreed upon.

Furthermore, there is also controversy regarding the fate of this lactate; specifically, whether or not the subsequent decreases in lactate concentration result from local metabolic breakdown by the adjacent neurons. Lactate uptake has been measured in cultured neurons with ^{13}C-NMR spectroscopy, where the accumulation of enriched glutamate and aspartate confirm that ^{13}C-lactate is readily taken up and oxidatively metabolized through the tricarboxylic acid cycle (Schousboe *et al.*, 1997; Waagepetersen *et al.*, 1998); and the ANLSH has been further supported by the finding that cultured neurons, when exposed to both lactate and glucose, oxidized lactate in a 4 : 1 ratio as their primary metabolic substrate (Bouzier-Sore *et al.*, 2003, 2006). However, the finding that astrocytes have a high capacity to take up and disperse lactate in brain slices (Gandhi *et al.*, 2009) suggests that extracellular lactate could also be cleared by astrocytes and transferred through astrocytic syncytia to either blood vessels or other brain regions. For recent in-depth reviews of neuron–glia interactions and metabolic coupling, see Shulman *et al.* (2004), Magistretti (2006), Pellerin *et al.* (2007), and Mangia *et al.* (2009a).

To briefly recap, astrocytes are known to utilize oxidative processes in order to meet many of their energy demands (Hertz *et al.*, 2007), but cell cultures have also been shown to rely heavily on glycolysis (as discussed). While it was once thought that astrocyte extensions, such as those surrounding synapses, were too narrow to contain mitochondria (Hertz *et al.*, 2007) – implying that increased energy demands in these regions would have to be met though glycolysis – more recent studies have shown that mitochondria can inhabit astrocyte processes

(Lovatt *et al.*, 2007; Pardo *et al.*, 2011). Nevertheless, the fact that both glucose and glutamate have been found to stimulate lactate production in cultured astrocytes, despite the apparent presence of mitochondria and sufficient oxygen availability, suggests that these cells may, through some physiological endowment, be preferentially glycolytic. It is also easy to see how these results, along with the observation of activity-related lactate increases *in vivo*, can be taken to fit with *in vitro* evidence that lactate can, and may preferentially, act as a metabolic substrate for neurons. Therefore, although controversial, the ANLSH has gained significant popularity because of supporting empirical data, and the appeal of a logical and parsimonious explanation for the interaction between astrocytic and neuronal metabolic processes. However, regardless of its potential role in the ANLSH, astrocyte glycolysis and lactate production – in response to neurotransmitter uptake (i.e. synaptic activity) – may also have significant implications in terms of neurovascular coupling, as will be discussed in the next section.

Astrocyte-mediated neurovascular changes during neural signaling

The notion that neural processes are accompanied by local vascular changes dates back to well over 100 years ago (Roy & Sherrington, 1890), but the regulatory processes governing this cerebrovascular ebb and flow have, until quite recently, remained a mystery. It has been known for some time that astrocytes circumscribe most (if not all) neuronal synapses and more than 99% of the total cerebrovascular surface area (Agulhon *et al.*, 2008), but only recently have multiple laboratories independently substantiated that astrocytes control local cerebrovascular microcirculation (Zonta *et al.*, 2003; Mulligan & MacVicar, 2004; Gordon *et al.*, 2008; Petzold *et al.*, 2008; Schummers *et al.*, 2008). A complete review of astrocyte physiology is beyond the scope of this article [for comprehensive reviews of astrocytic regulation of neuronal activity and blood flow, see Agulhon *et al.* (2008), Koehler *et al.* (2009), Carmignoto & Gomez-Gonzalo (2010)]. Therefore, we will discuss only a small number of recent papers describing how astrocytes couple neuronal activity to cerebrovascular changes, and therefore hemodynamic-based functional neuroimaging signals.

For astrocytes to cause functional hyperemia, it stands to reason that astrocyte activity – typically measured by intracellular Ca^{2+} fluxes – must be initiated before vascular responses (Carmignoto & Gomez-Gonzalo, 2010). Fortunately, a number of studies have measured the latency of Ca^{2+} responses in astrocytes (Table 1), confirming that stimulus-induced astrocyte activity is rapidly invoked *in vivo* (i.e. within one or a few seconds). Of the studies summarized in Table 1, some also measured the time-course of vascular changes. Winship *et al.* (2007) reported hemodynamic changes beginning approximately 1 s following stimulus onset, with a peak response beyond the duration of their measurements (probably several seconds); Schummers *et al.* (2008) measured an initial hemodynamic response at approximately 2 s, and a peak response after approximately 9 s; and Petzold *et al.* (2008) reported dilation of penetrating arterioles approximately 2 s following stimulation. In general, these findings, along with the relatively slow onset of the BOLD fMRI signal (Friston *et al.*, 1994, 1998a,b; Boynton *et al.*, 1996; Buckner *et al.*, 1996; Aguirre *et al.*, 1998), fit with the notion that astrocyte activity precedes functional hyperimia in response to physiological stimuli.

Blocking of neuron–astrocyte signaling and astrocyte function has also provided valuable insights into the normal role of astrocytes in controlling cerebral microcirculation. In a study reported by Zonta *et al.*, *in vivo* administration of metabotropic glutamate receptor (mGluR) antagonists resulted in markedly decreased, although still detectable, changes in cerebral blood flow approximately 1 s following somatosensory stimulation (Zonta *et al.*, 2003). Because mGluR antagonists are known to reduce astrocyte Ca^{2+} fluxes in response to synaptic glutamate, these data imply that decreased vascular responses to the stimuli resulted from decreased astrocyte activity. It is perhaps also worth noting that, in this study, the use of mGluR antagonists had no effect on baseline blood flow, blood pressure, arterial P_{CO_2}, or arterial pH, suggesting that increased astrocyte activity is the primary cause of functional hyperemia. Likewise, a more recent study by Schummers *et al.* revealed that activity-related hemodynamic increases were reduced by more than 80% as a result of selectively blocking glutamate transport into astrocytes (Schummers *et al.*, 2008), despite a concomitant increase in neuronal activity (probably reflecting the decreased glutamate clearance that is normally performed by the astrocyte end-processes – see below). Therefore, because functional hyperemia is blocked as a result of astrocytic inhibition, and in spite of increased neuronal activity, this strongly suggests that astrocyte activity is more closely coupled to neurovascular tone and microcirculation than neuronal activity *per se*.

Finally, in an *in vivo* study by Petzold *et al.*, the authors demonstrated that: (i) local cerebral blood flow was correlated with presynaptic glutamate release; (ii) blocking postsynaptic neuronal (but not astrocytic) receptors had no effect on functional hyperemia; (iii) astrocytes displayed robust functional responses (Ca^{2+} fluxes) in response to stimuli; and (iv) these astrocytic responses correlated with vascular tone and microcirculation (Petzold *et al.*, 2008). In addition to decreased functional hyperemia, this study also reported increased extracellular concentrations of glutamate and slower glutamate clearance as a result of inhibition of astrocytic glutamate uptake, thereby offering a potential explanation for the elevated postsynaptic neuronal activity reported by Schummers *et al.* (see above).

By uncoupling neuronal and astrocytic processes, these studies have demonstrated that astrocyte activity, more directly than neuronal activity, is reflected by blood flow, blood volume and blood oxygenation, further implying that astrocyte activity likely represents

TABLE 1. Time-course of *in vivo* astrocyte activity following various physiological stimuli

Reference	Stimulus (Region of interest)	Latency of initial astrocyte Ca^{2+} response (s)	Latency of peak astrocyte Ca^{2+} response (s)
Wang *et al.* (2006)	Whisker stimulation (mouse barrel cortex)	Approximately 3 (cell body) 3–6 (end-processes)	3–12 (cell body)
Winship *et al.* (2007)	Mechanical limb stimulation (mouse somatosensory cortex)	< 0.5	Approximately 0.5 (cell body) 0.5–4.0 (end-processes)
Schummers *et al.* (2008)	Visual stimulation (ferret visual cortex)	1–3*	4–6*
Petzold *et al.* (2008)	Olfactory stimulation (mouse olfactory glomeruli)	< 1	Approximately 1

Post-stimulus Ca^{2+} signals have been differentiated between astrocytic cell bodies (soma) and end processes (endfeet) when possible. *Refers to responses at low (i.e. 0.6 and 0.9%) isoflurane levels.

a common neurophysiological basis for all hemodynamic neuroimaging methods (Wolf & Kirchhoff, 2008). In addition to this type of empirical evidence, a recent paper by Gordon *et al.* has added a mechanistic explanation for how astrocytes are involved in regulating local cerebrovasculature in step with the local metabolic requirements of active neural tissue (Gordon *et al.*, 2008). They found that *in situ* neuronal activation under low-oxygen conditions led to arteriole dilation, while activation of the same neurons during high-oxygen conditions led to arteriole constriction. In their report, the authors demonstrated that increased astrocyte activity – indicated by increased astrocytic Ca^{2+} concentrations – led to increased glycolysis and extracellular lactate concentration, particularly during lower-oxygen conditions. However, even during high-oxygen conditions, the addition of lactate was found to cause some neurovascular dilation, implicating lactate (possibly via astrocytes – see previous section) as the link between neurometabolic and neurovascular regulation.

These data, combined with *in vitro* data (Walz & Mukerji, 1988), suggest that during lower-oxygen conditions – such as those that might occur following rapid increases in oxidative phosphorylation in postsynaptic neurons (Kasischke *et al.*, 2004) – astrocytes may increase their dependence on glycolytic metabolism. This type of oxygen-dependent metabolic response in astrocytes, where the demands of increased neurotransmitter cycling lead to an increased extracellular lactate concentration, provides a conceptual framework for activity-dependent vasodilation. Conversely, when oxygen levels increase and/or neurotransmitter cycling decreases, the dependence of astrocytes on glycolytic metabolism (i.e. lactate production) is thought to be decreased, thereby coupling the local neurovascular supply to neurometabolic demand. This mechanism is also supported by new work *in vivo*, which has shown that increased blood flow to focally active brain regions is highly correlated with co-localized elevations in lactate concentration, but negatively correlated with the cerebral metabolic rate of oxygen ($CMRO_2$) (Lin *et al.*, 2010), further suggesting that increased cerebral blood flow is driven by glycolytic, as opposed to oxidative, metabolism under normal physiological conditions. Therefore, at least within the context of transient increases in astrocytic glycolysis (regardless of whether lactate is metabolized locally by adjacent neurons or not, thereby avoiding many of the primary concerns surrounding the ANLSH), these findings firmly support the notion that astrocytes and astrocytic processes form the basis of functional neuroimaging signals in relation to synaptic activity (i.e. neurotransmitter release).

Implications for a unified theory of functional neuroimaging

The revelation that astrocytes mediate the neurometabolic and neurovascular changes associated with neural signaling demonstrates that they are intimately involved in generating the signal changes measured with functional neuroimaging methods such as fMRI and PET. These cellular/molecular mechanisms are summarized in Fig. 2, and discussed below in terms of a number of specific functional neuroimaging (PET and fMRI) modalities.

2-[^{18}F]fluoro-2-deoxy-d-glucose (FDG)-PET

As its name implies, this technique is sensitive to the accumulation of FDG-6-phosphate that occurs within tissues metabolizing increased amounts of glucose. Therefore, if the ANLSH is correct and glucose is preferentially metabolized by astrocytes, as opposed to neurons, during elevated brain activity (Pellerin & Magistretti, 1994, 2004; Kasischke *et al.*, 2004), images generated with this technique are likely to reflect increased astrocytic activity more directly than increased neuronal activity (Pellerin & Magistretti, 2004). Moreover, there is now a preponderance of evidence suggesting that astrocytes not only use the glucose, but also control local glucose and oxygen delivery via their control over vasodilation, vasoconstriction, and regional blood supply (Zonta *et al.*, 2003; Mulligan & MacVicar, 2004; Gordon *et al.*, 2008; Schummers *et al.*, 2008).

^{15}O-PET

With the positron-emitting O_2 inhalation technique (Mintun *et al.*, 1984; Ohta *et al.*, 1992), the local $CMRO_2$ can be estimated by measuring the conversion of $^{15}O_2$ to ^{15}O-labeled water *in vivo*. Although baseline $CMRO_2$ levels reflect both neuronal and astrocytic metabolism, it is uncertain at this point which of the two is responsible for activity-related $CMRO_2$ increases; however, the ANLSH (outlined above) predicts that increased $CMRO_2$ during focal brain activation can most likely be attributed to the large and fast metabolic demands of neuronal processes (Kasischke *et al.*, 2004; Pellerin & Magistretti, 2004). However, ^{15}O-PET methods have also been used to study cerebral blood flow and blood volume; this can be achieved either through inhalation of trace concentrations of ^{15}O-labeled carbon monoxide or carbon dioxide, or through direct injection of radiolabeled water (Raichle, 1983). Therefore, given that the latter measurements are determined by the delivery and clearance of labeled radionuclide (carried in the bloodstream) and that astrocytes control functional hyperemia, it follows that these signals are mediated by astrocyte activity.

BOLD fMRI

In areas of increased neural activity, oxygen delivery exceeds the rate of oxygen utilization, thereby reducing the concentration of deoxyhemoglobin and increasing the magnetic-susceptibility-weighted MRI signal (Ogawa *et al.*, 1990a,b). Therefore, because the BOLD fMRI signal arises from changes in both $CMRO_2$ and blood flow, the resultant signal changes depend on many interacting factors. As previously explained, astrocytes play an important role in neurometabolic processes, and particularly during elevated brain activity and/or lowered oxygen conditions it is likely that they rely increasingly on glycolysis (using glucose, but not oxygen, and producing lactate) to meet their metabolic needs. It is thought that by increasing the local production of lactate, which acts as a vasodilator (by blocking transporter-mediated prostaglandin E_2 uptake), increased blood flow is directed towards metabolically active regions. A recent report by Gordon *et al.* (2008) found that this type of neurometabolism can have profound effects on cerebral blood flow in arterioles, leading to astrocyte-mediated vasoconstriction when oxygen levels are abundant and vasodilation when more oxygen is required, suggesting that localized hemodynamic changes and the physiological basis of BOLD fMRI are directly controlled by astrocytes. One exception, however, could be the brief 'initial dip' that is sometimes observed in the BOLD fMRI signal (Buxton, 2001). Because oxidative phosphorylation within the neurons is thought to occur before glycolysis in astrocytes – and therefore the production of vasoactive lactate (Kasischke *et al.*, 2004) – the brief initial dip in blood oxygenation can most likely be attributed to the early component of neuronal oxygen consumption that precedes increased blood delivery.

SEEP fMRI

Unlike magnetic-susceptibility-weighted fMRI methods, SEEP contrast is based on predominantly proton-density-weighted imaging

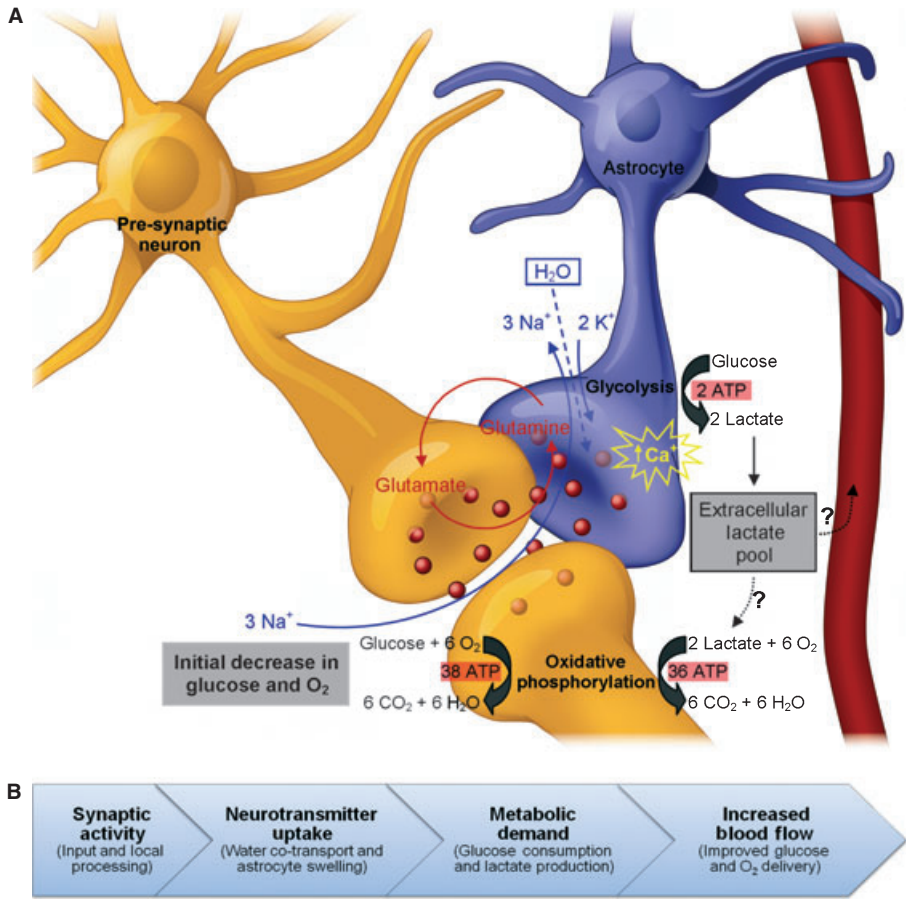

FIG. 2. As stimulus-driven lactate production and intracellular Ca^{2+} fluctuations in astrocyte end-feet elicit cellular/molecular and hemodynamic changes, astrocytes and their metabolic processes are likely to form the physiological bases of functional neuroimaging signals. (A) Increased synaptic input leads to astrocyte activity (e.g. activation of Ca^{2+}-permeable AMPA receptors), increased metabolic demand (e.g. uptake and recycling of neurotransmitters), and increased astrocyte glycolysis (i.e. lactate production). Although lowered oxygen availability (i.e. as a result of rapid oxidative metabolism) is not a prerequisite for sustained hemodynamic increases, as shown by responses to prolonged stimulation, the initial decrease may serve to boost lactate production at the onset of increased synaptic activity. The higher concentration of extracellular lactate resulting from glycolysis then acts as a vasodilator to increase the delivery of blood rich in glucose and oxygen. The fate of lactate is somewhat less clear (indicated by question marks). According to the ANLSH, it may be used locally as a metabolic substrate to support ongoing neuronal oxidative phosphorylation; however, another possibility is that it is transported to the blood. Note that the production of 38 ATP molecules from one molecule of glucose (as shown) is the theoretical maximum, and may be lower *in vivo*, for several reasons (Salway, 2004). (B) Although driven by synaptic activity, and thus tightly coupled to the cumulative neuronal input, these changes demonstrate that astrocytes are critically involved in the metabolic and hemodynamic processes leading to ^{15}O-PET, FDG-PET, BOLD, VASO, ASL perfusion, SEEP and diffusion fMRI signals (perhaps with the exception of the initial 'negative dip' that is sometimes, but not always, observed in hemodynamic measurements). As the input to a region determines the amount of astrocyte activity and the amount of neurotransmitter uptake/recycling that must be performed, this theory also explains why functional neuroimaging data correlate better with the neuronal input and local processing (i.e. LFPs) than with neuronal output (i.e. single-unit or multi-unit recordings).

parameters, where signal changes occur as a result of cellular swelling and increased tissue water content (i.e. more water protons) proximal to regions of neural activity (Stroman *et al.*, 2002, 2005; Figley *et al.*, 2010). It has recently been shown in live cortical tissue slices that SEEP fMRI signals are highly correlated with changes in light transmittance (Stroman *et al.*, 2008), substantiating an activity-dependent influx of water into cells as a result of increased neuronal depolarization and synaptic activity. Evidence that neurons lack aquaporins (Nielsen *et al.*, 1997; Amiry-Moghaddam & Ottersen, 2003) – that is, the porous membrane structures that allow water to passively diffuse between the intracellular and extracellular space – suggests that cell swelling is exclusive to astrocytes under normal physiological conditions. Conversely, neurons have been shown to swell irreversibly, and only under pathological conditions (Andrew *et al.*, 2007; Risher *et al.*, 2009). Thus, it is far more likely that activity-dependent cell swelling, and therefore the SEEP fMRI contrast, results from neurotransmitter uptake and concomitant water transport into astrocytes than from neuronal swelling.

Diffusion-weighted fMRI

High *b*-value diffusion-weighted fMRI is sensitive to changes in tissue water diffusivity (i.e. the ability of water to freely diffuse), which is also believed to change as a result of cellular swelling (Darquie *et al.*, 2001; Le Bihan *et al.*, 2006; Le Bihan, 2007). In this case, it has been proposed that the increased cell volume may serve to expand the membrane surface area, thereby enhancing glutamate clearance and other astrocyte–neuron interactions as the astrocytic processes swell around the synaptic cleft (Nedergaard *et al.*, 2002). Overall, these activity-related changes are thought to increase the proportion of water molecules within the boundary layer (i.e. a region of restricted diffusion) along the cell membranes of swollen tissues. Therefore, for the same reasons as outlined for SEEP fMRI (discussed above),

diffusion-weighted fMRI signals at high *b*-values are more likely to be attributable to astrocyte swelling than neuronal swelling under normal circumstances.

VASO and ASL perfusion fMRI

VASO measures changes in microvascular dilation and/or constriction (i.e. the volume of vascular space) (Lu *et al.*, 2003), whereas ASL perfusion is sensitive to changes in cerebral blood flow, accounting for both vessel diameter and flow velocity (Williams *et al.*, 1992; Wong *et al.*, 1997). Again, given that astrocytes directly control vascular tone (i.e. vasoconstriction/vasodilation), and therefore cerebral microcirculation, the basis for these neuroimaging techniques is more closely related to astrocytic than to neuronal activity.

The fact that all of the aforementioned PET and fMRI techniques are based on function-related changes in astrocyte activity suggests that functional neuroimaging results obtained with different contrasts – for example, BOLD vs. SEEP fMRI, as previously reported (Stroman *et al.*, 2003, 2005) – can be directly compared, taking into account that cell swelling methods may be more localized to active regions of neurotransmitter cycling than hemodynamic methods, where the signal may originate in downstream venules and draining veins (Figley *et al.*, 2010). However, because astrocyte activity does not produce electrochemical changes such as the depolarization of neurons, this has a number of important implications for comparing functional neuroimaging data with electrophysiological data, including: single-unit and multi-unit recordings, local field potentials (LFPs), electroencephalography, and other techniques such as magnetoencephalography.

Implications for detecting subthreshold synaptic activity

The notion that neurometabolism, blood flow and cell swelling are more closely related to astrocytic changes than neuronal activity *per se* supports and explains previous hypotheses that functional neuroimaging signals may also reflect subthreshold neuronal activity, simultaneous excitation and inhibition, and modulatory processes (such as feedback from other brain or brainstem regions) that might not necessarily elicit electrophysiological changes (Heeger & Ress, 2002). Thus, astrocytes and astrocyte activity are likely to account for previously reported occurrences of subthreshold neuronal activity observed via hemodynamic, but not electrophysiological, techniques (Toth *et al.*, 1996), thereby reconciling the apparent hemodynamic–electrophysiological disparities more recently reported by Sirotin & Das (2009) (Narasimhan *et al.*, 2009; Smith, 2009; Welberg, 2009).

This astrocyte-based interpretation of functional neuroimaging also explains why hemodynamic changes correlate better with LFPs than with either single-unit or multi-unit recordings (Logothetis *et al.*, 2001; Logothetis, 2003, 2007, 2008; Logothetis & Pfeuffer, 2004; Viswanathan & Freeman, 2007). Because LFPs represent the input to a given region, as well as the local processing, the aforementioned studies have established that hemodynamic measurements reflect coordinated synaptic activity more than the spiking output of a region [based on the net sum of excitatory and inhibitory inputs, where increased gluatamatergic (excitatory) input serves to increase the postsynaptic firing output, and increased GABAergic (inhibitory) input serves to decrease the firing output]. Therefore, balanced or subthreshold changes in glutamatergic and GABAergic input can exist – with potentially large increases in synaptic activity and metabolic demand – without eliciting corresponding changes in neuronal output. In this regard, temporal correlations between the synaptic inputs are also important. For example, increased but asynchronous synaptic inputs which are metabolically and astrocytically demanding, would be expected to produce functional neuroimaging signals without eliciting a corresponding increase in the firing rates of postsynaptic neurons (Salinas & Sejnowski, 2000, 2001, 2002).

The work of Viswanathan and Freeman has clearly demonstrated strong coupling between LFPs and changes in tissue oxygen concentration in the absence of spiking output (i.e. decoupling of postsynaptic neuronal activity from changes in blood flow), suggesting that hemodynamic functional neuroimaging signals reflect synaptic more than spiking activity (Logothetis, 2007; Viswanathan & Freeman, 2007). The fact that increased neurotransmitter uptake and cycling occurs in astrocytes surrounding the presynaptic terminals – thereby causing increased astrocyte activity, metabolism, and functional hyperemia – could explain why LFPs (reflecting the synaptic input to a region and the local processing, but not necessarily the neuronal output) are the closest electrophysiological correlates of functional neuroimaging signals.

Although LFPs serve as the strongest electrophysiological–hemodynamic (i.e. astrocytic) correlate, it is important to recall that, in electrophysiology, 'not measured' does not mean 'not activated', even in the neuronal sense. It is important to consider that structures with concentric, spherical or random orientations can produce so-called 'closed-field' configurations. In such cases, individual electromagnetic dipoles may interfere with one another (Nunez & Silberstein, 2000), allowing a region with significant metabolic requirements, and indeed increased neuronal firing, to go unnoticed by even LFP, electroencephalography or magnetoencephalography measurements.

Implications for detecting inhibitory (GABAergic) activity

With this new way of interpreting functional neuroimaging methods – namely, that they reflect astrocyte function and astrocyte metabolism – what can be said of their ability to shed light on glutamatergic vs. GABAergic synaptic activity? Because astrocyte metabolism and activity are closely linked to excitatory presynaptic neuronal activity (Box 1), we now know that functional neuroimaging data are sensitive to glutamatergic neurotransmitter uptake and cycling. But what intracellular Ca^{2+} fluxes and metabolic changes occur in astrocytes, and what functional neuroimaging changes might be associated with presynaptic inhibitory (e.g. GABAergic) neurotransmitter release?

Whereas synaptic glutamate markedly increases glucose utilization, *in vitro* studies of astrocyte cell cultures have shown that GABA does not, suggesting that inhibitory neuronal activity may not be directly coupled with astrocytic metabolism (Chatton *et al.*, 2003). However, more recent evidence indicates that GABAergic activity increases metabolic demand in both neurons and astrocytes *in vivo*, but to a lesser extent than excitatory activity (Patel *et al.*, 2005), supporting previous observations that synaptic excitation is more metabolically demanding than synaptic inhibition (Waldvogel *et al.*, 2000) [see Hyder *et al.* (2006) for a review of neurometabolic coupling of glutamatergic and GABAergic processes]. But why is inhibitory neurotransmission less metabolically demanding? Why is GABAergic activity not as tightly coupled to astrocyte activity? And, most importantly, what does this mean for interpreting functional neuroimaging data?

Previous data have shown that neurons actually have a higher density of GABA transporters than astrocytes (Schousboe, 2003), and experimental evidence has recently confirmed a relatively large contribution of neuronal GABA uptake (Yang *et al.*, 2007). Therefore, because astrocytes take up all of the synaptic glutamate, but only a fraction of the synaptic GABA (i.e. a lower astrocytic uptake of

GABA than of glutamate), these findings suggest that the astrocytic GABA-to-glutamine cycling pathway is largely bypassed, a finding that implies an inherent excitatory (i.e. glutamatergic) bias towards astrocyte activity and metabolism, and one that has important implications for interpreting PET and fMRI data in terms of excitatory and inhibitory contributions (Fig. 3). Within the framework of the previous discussion, these results suggest that larger functional neuroimaging signal changes are elicited from excitatory than from inhibitory (i.e. glutamatergic vs. GABAergic) synaptic activity. For example, a decrease in excitatory input (i.e. decreased presynaptic glutamate release) with an equally large increase in inhibitory input (i.e. increased presynaptic GABA release) should yield a net decrease in functional neuroimaging signal (Fig. 3; lower left-hand panel). Conversely, an increase in either PET or fMRI signal intensity probably means increased excitatory input (Fig. 3; top row, all panels) and/or increased inhibitory input without decreased excitatory input (Fig. 3; middle left-hand panel). Given that the magnitude of this bias is uncertain, and because of the potential implications for interpreting functional neuroimaging signals, this phenomenon warrants further investigation.

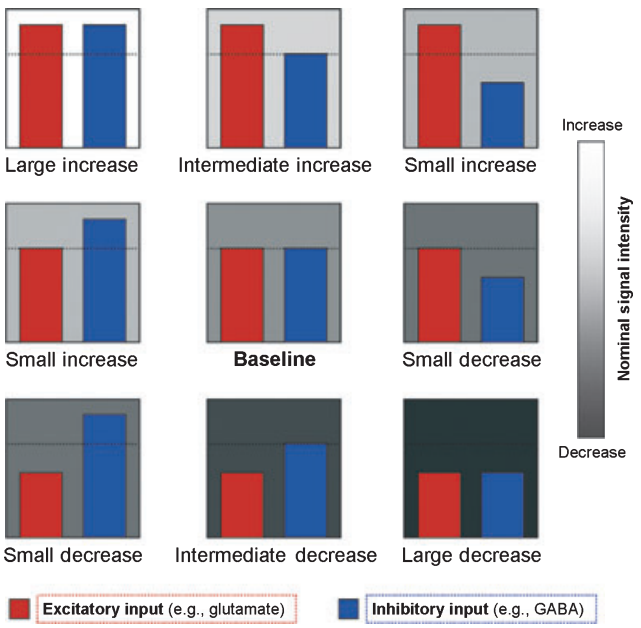

FIG. 3. A unified astrocyte-based interpretation of functional neuroimaging signals in terms of excitatory and inhibitory synaptic activity. Because glycolysis and intracellular Ca^{2+} fluctuations within astrocytes are elicited in response to neurotransmitter uptake and recycling, these processes and the subsequent cellular and hemodynamic changes are closely (if not inextricably) linked to presynaptic neuronal activity. Even subthreshold changes or simultaneous excitation and inhibition, which may have no bearing on postsynaptic action potentials, can cause changes in astrocyte activity and metabolism. Note, however, that there is probably some inherent excitatory bias – that is, greater signal intensity changes for a given amount of glutamatergic neurotransmission than for a similar release of GABA – because glutamate is preferentially taken up by astrocytes, whereas GABA is absorbed, to a large degree, by presynaptic neurons (altogether bypassing the astrocyte uptake and recycling pathway). The shading (light–dark) and text below each panel represent the expected direction and magnitude of functional neuroimaging signal change, given the different contributions of excitatory and inhibitory neurotransmitter release shown. These predictions, based on synaptic activity and the corresponding astrocytic changes, explain why imaging data reflect neuronal input and local processing, and illustrate how balanced excitatory and inhibitory input can elicit both increased (top left panel) and decreased (bottom right panel) functional imaging signals without corresponding electrophysiological changes.

A quick note about 'negative activity'

Because the majority of functional neuroimaging data are analyzed in a model-driven manner, such as a general linear model analysis, 'negative activity' is somewhat of an elusive concept [for a historical overview of PET and fMRI data analysis techniques, including the general linear model, see Friston *et al.* (2007)]. In the most general sense, regions of negative activity are those where the measured signal changes are inversely correlated with the predicted model time-course (that is, the actual signal decreases when the predicted signal increases, or vice versa). Traditionally, these modeled response functions have been measured via task-related increases in blood flow and/or cellular swelling (Friston *et al.*, 1994, 1998a,b; Boynton *et al.*, 1996; Buckner *et al.*, 1996; Aguirre *et al.*, 1998; Stroman *et al.*, 2005). Therefore, measuring and displaying negative activity involves the implicit assumption that reduced neuronal activity should produce an equal but opposite (i.e. inverted) signal response. However, given the likelihood that functional hyperemia and cellular swelling are controlled by astrocyte activity and metabolism, this assumption is fraught with potential errors and warrants cautious interpretation.

Given the complexity of the physiological mechanisms leading from ensemble neuronal (synaptic) activity to macroscopic imaging signals (Fig. 2), it does not follow that decreased astrocyte metabolism/activity, blood flow, blood oxygen and cell swelling will remit as fast as they begin, especially given that most of the measured response functions are not temporally symmetrical in terms of their onset and subsequent decay. The fact that these signals tend to rise quickly (after a brief lag) and then slowly return to baseline levels (either with or without a post-stimulus undershoot) provides a strong indication that the 'negative' processes – that is, when the signal returns to baseline during the latter half of the response function – do not decrease on the same timescale as that by which they increase during the onset of activity. Overall, this suggests that negative activity, although still detected in many studies, may be prevalent but largely underestimated, owing to the relatively poor correlations in model-driven analysis methods. Therefore, given the inherent negative bias (i.e. reduced sensitivity) of most functional neuroimaging methods towards negative activity, conclusions based on decreased activation probably merit judicious interpretation, taking into account the various inputs to the region, where these originate from, and whether they are likely to be excitatory or inhibitory (see previous section). Recall that the signal measured at any given location represents the net synaptic activity (i.e. the input and local processing).

Concluding remarks

Owing to recent advances in our understanding of astrocyte function, the once highly neurocentric view of the CNS is rapidly evolving, and it is becoming clear that astrocytes serve a number of important functions that either directly or indirectly control: (i) neurotransmitter uptake and recycling; (ii) neurometabolism; and (iii) neurovascular dilation/constriction and blood flow. Moreover, it appears that astrocytes, in contrast to previous notions, may even have their own unique forms of signaling. There is now evidence that, under certain laboratory conditions, astrocytes are able to release signaling molecules called 'gliotransmitters' (Araque *et al.*, 1999; Halassa *et al.*, 2007; Agulhon *et al.*, 2008; Allen & Barres, 2009; Perea *et al.*, 2009), and recent work has shown that single astrocytes are able to undergo highly tuned intracellular Ca^{2+} fluxes (Schummers *et al.*, 2008). Since both of these signaling characteristics (i.e. tuned responses and communication via signalling molecules) were

previously thought to be exclusive to neurons, these reports suggest that astrocytes are probably more actively involved in sensory, motor and cognitive processing than was traditionally believed. Furthermore, as we have tried to highlight in this article, it is now established that astrocytes play a number of important neurometabolic and neurovascular roles that couple synaptic activity to the physiological changes underlying functional neuroimaging signals – a nuance that is often overlooked, but has a number of significant implications for interpreting PET and fMRI data alike. We have tried to show how these astrocytic changes support previous hypotheses that functional imaging data, unlike electrophysiological methods, are capable of detecting subthreshold activity, changes in simultaneous excitation and inhibition, and/or other neuromodulatory activity that might affect astrocytes independently of net electrochemical changes. Moreover, this astrocyte-based interpretation suggests that (all else being equal) the magnitudes of functional neuroimaging signals are positively biased towards increased excitatory synaptic activity, with decreased sensitivity to both inhibitory (i.e. GABAergic) synaptic changes and decreased neurotransmitter cycling (i.e. 'negative activity').

Just over a decade ago, Magistretti *et al.* published a seminal paper summarizing the cellular and molecular mechanisms that accompany neural signaling, noting that 'functional brain imaging techniques bring us part way to understanding how neuronal processes such as action potentials and neurotransmitter release lead to a given brain activity and its resulting behavioral state' (Magistretti *et al.*, 1999). Therefore, if the purpose of functional neuroimaging is ultimately to shed light on the cellular/molecular correlates of neural activity, it is perhaps ironic that it has taken this long for studies of neurotransmitter release and the corresponding neurometabolic changes, neurovascular regulation and electrophysiology to bring us closer to an understanding of functional neuroimaging methods. Perhaps even more surprising still are the conclusions that these studies have led to, revealing that most functional neuroimaging techniques (including those based on ^{15}O-PET and FDG-PET, as well as BOLD, VASO, ASL perfusion, SEEP, and diffusion fMRI contrasts) are, in fact, more closely tied to the underlying functioning and metabolic activity of astrocytes than of neurons. This interpretation, drawn from and supported by a large body of empirical data, reconciles many of the apparent disparities between functional neuroimaging data and electrophysiological recordings, and puts forward a heuristic for interpreting many different kinds of functional neuroimaging signals.

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Abbreviations

ANLSH, astroctyte–neuron lactate shuttle hypothesis; ASL, arterial spin labeling; ATP, adenosine triphosphate; BOLD, blood oxygen level-dependent; $CMRO_2$, cerebral metabolic rate of oxygen; CNS, central nervous system; FDG, 2-[^{18}F]fluoro-2-deoxy-D-glucose; fMRI, functional magnetic resonance imaging; LDH, lactate dehydrogenase; LFP, local field potential; mGluR, metabotropic glutamate receptor; MRI, magnetic resonance imaging; PET, positron emission tomography; SEEP, signal enhancement by extravascular water protons; VASO, vascular space occupancy.

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