
Does glutamate image your thoughts?

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Functional imaging methods exploit the relationship between neuronal activity, energy demand and cerebral blood flow to functionally map the brain. Despite the increasing use of these imaging tools in basic and clinical neuroscience, the neurobiological processes underlying the imaging signals remain unclear. Recently, interest has been focused on uncovering the signals that trigger the metabolic and vascular changes accompanying variations in neuronal activity. Advances in this field have demonstrated that release of the major excitatory neurotransmitter glutamate initiates diverse signaling processes between neurons and astrocytes, and that this signaling could be crucial for the occurrence of brain imaging signals. In this article we review the hypothesis that glutamate represents a common trigger for both neurometabolic and neurovascular coupling.

At the forefront of cognitive and clinical neuroscience research in humans are the non-invasive functional imaging approaches of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). These techniques yield brain images that give insights into many brain functions, ranging from the processing of sensory information to complex cognitive tasks [1,2]. The application of brain imaging techniques to localize these neuronal processes is based on the assumption that local neuronal activity changes when a region is performing the task. Neuronal activity requires energy, and this is provided almost exclusively by oxidation of glucose[3]. Because the brain has very little energy in reserve, a continuous vascular supply of glucose and oxygen is mandatory to sustain neuronal activity. This supply is regulated locally and dynamically to meet the increased energetic demand of functional activation. Functional imaging utilizes the association between increased neuronal activity and energetic demand, by recording changes in blood flow, glucose use, or the metabolism and delivery of oxygen (Fig. 1). Despite the increasing use of these imaging tools in basic as well as in clinical applications, the neurobiological processes responsible for these imaging signals remain largely unknown. Recent advances in the field suggest that glutamate release triggers both vascular and metabolic responses, via different signaling processes [4]. As glutamate-mediated transmission is tightly regulated through neuron–glia interactions [5], these results suggest that brain-imaging signals are dominated by changes in energy usage and blood flow related to the functioning of both neuronal and glial parts of glutamatergic synapses [6].

Energetic cost of glutamate transmission

The majority of neuronal information is conveyed via the rapid, excitatory glutamate-mediated system:
Opinion

80–90% of cortical synapses are glutamergic [7]. Therefore, an important energy load is created by the operation of glutamatergic synapses. Moreover, a growing body of experimental data indicates that the activity of these synapses is tightly regulated by dynamic interactions between astrocytes and neurons (Fig. 2). The different steps in glutamate-mediated transmission include action potentials, glutamate release, glutamate uptake and recycling by astrocytes (glutamate–glutamine cycling), and excitatory pre- and postsynaptic activity. The prevailing hypothesis is still that the majority of the energy consumption is used to restore the ionic gradients and resting membrane potentials that are modified during the excitation phase. Recent analysis has shown that action potentials are responsible for much (47%) of the ATP consumption [8], as they are restored mainly by the Na⁺/K⁺-ATPase, which consumes ATP to re-establish Na⁺ and K⁺ gradients. ATP produced by glycolysis and oxidative phosphorylation fuels this pump [9]. An increased activity of the Na⁺/K⁺-ATPase is also required to reverse ion movements evoked by the activation of postsynaptic NMDA and non-NMDA ionotropic glutamate receptors: this activity could account for as much as 34% of the total ATP usage. Therefore, ~80% of the energetic cost of glutamate-mediated transmission is directly related to the activity of the neuronal Na⁺/K⁺-ATPase, a key enzyme in brain glucose metabolism [8] (Fig. 2).

Recent in vivo magnetic resonance spectroscopy (MRS) studies have suggested that the energy requirements of glutamatergic neurons might indeed explain a large fraction of the cortical glucose consumption, and that this demand is coupled to glutamate release and recycling (for a detailed review, see Ref. [10]). Briefly, ³¹C MRS studies in the rat, performed over a range of cortical activity, demonstrated that glutamate–glutamine cycling in the cerebral cortex is coupled in a 1:1 molar stoichiometry to neuronal glucose oxidation above isoelectricity (i.e. above basal energetic rate) [11]. These findings suggest that the energy requirements of pre- and postsynaptic events related to synaptic transmission could be met by the oxidative metabolism of one molecule of glucose per glutamate molecule released synthetically. Furthermore, the basal energetic rate unrelated to neuronal activity was only ~15% of the awake resting rate, indicating that some 85% of cortical energy consumption is coupled to neuronal activity. These experimental findings are in close agreement with the results of theoretical calculations by Attwell and Laughlin [8] (Fig. 2). It is of note that recent measurements of these rates in human cerebral cortex [12–15] are consistent with the relationship between glucose oxidation and glutamate–glutamine cycling obtained from rat cortex above the basal energetic rate. However, to date no analogous study of graded neuronal activity has been performed in humans.

The use of different isotopic precursors ([2-¹³C]glucose and [2-¹³C]acetate) has recently allowed direct measurement of the astrocytic contribution to brain energy metabolism in vivo by MRS, in both rats and humans [15,16]. Mathematical modeling of these data has shown that the glutamate–glutamine neurotransmitter cycle between astrocytes and neurons is the major pathway of neuronal glutamate repletion, thus demonstrating in vivo an important functional role for astrocytes in glutamate-mediated activity. In addition, these MRS studies have made it possible to assign fractions of total glucose oxidation in the brain to different cell populations. The recent study of Lebon et al. [15], in which [2-¹³C]acetate was used to target astrocytic metabolism specifically, demonstrated that astrocytic glucose oxidation accounts for ~14% of total brain consumption. This is slightly higher than theoretical calculations of astrocytic energy consumption as a percentage of total (7%) [8]. However, in these calculations, the only energy-requiring processes accounted for were maintenance of resting membrane potential and glutamate–glutamine cycling, even though activation of astrocytic NMDA and non-NMDA receptors by glutamate (see following discussion) is likely to impose additional energy requirements. In earlier ¹³C MRS studies, glucose oxidation by glutamatergic and GABAergic neurons was calculated to be 75–80% and ~10% of total cortical consumption, respectively [10]. For the first time, these results related one of the processes underlying functional imaging signals (energy metabolism) to specific neurotransmitter systems and cell populations.

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What triggers the increase in glucose uptake during activation?

Although the studies already mentioned demonstrate a quantitative relationship between cortical energy expenditure and glutamate-mediated neurotransmission, they do not relate glucose consumption to specific processes directly. In other words, we do not know which signaling pathway enables tight adjustment of glucose use (as seen by FDG-PET) to meet the increasing needs of the glutamatergic synapses. In addition to the possible dynamic regulation of glucose entry into neurons via the neuronal glucose transporter GLUT3, much attention has recently been paid to the role of astrocytes and, in particular, to the roles of glutamate transporters and the glial Na+/K+-ATPase. The uptake of glutamate is mainly performed by two subtypes of excitatory amino acid transporter (EAAT), GLAST (EAAT1) and GLT-1 (EAAT2), which are almost exclusively expressed in the astrocytes of adult animals [17, 18]. These transporters tightly regulate the level of extracellular glutamate within the synaptic cleft, to ensure a high fidelity of glutamate transmission and to prevent neuronal damage from excessive activation of glutamate receptors [19].

In vitro experiments have demonstrated that astrocytes respond to glutamate with increases in both glucose utilization and lactate production [20]. These effects were not receptor-mediated, but were dependent on glutamate transporters. As glutamate transport is coupled to the Na+ gradient with a precise stoichiometry of three Na+ ions for each transported glutamate molecule, glutamate uptake leads to a massive Na+ entry into astrocytes (as demonstrated by fluorescence microscopy) [21]. Further studies in cultured astrocytes have shown that glutamate enhances the activity of a specific glial Na+/K+-ATPase subunit [22], and that ouabain, a specific inhibitor of the Na+/K+-ATPase, prevents the enhancement of glucose utilization and lactate production by glutamate [20]. Thus, the activity of this glial enzyme has been directly implicated in the metabolic response. These findings are in accord with the MRS studies linking glucose utilization to glutamate–glutamine cycling, and provide an attractive mechanism to account for at least part of the FDG-PET signal (Fig. 3a). Recently, two studies have provided strong in vivo evidence that glial glutamate transporters play an active role in neurometabolic coupling (an idea that previously had been based entirely on in vitro data). These studies demonstrated that the metabolic response to somatosensory activation was significantly decreased both in GLAST and GLT-1 knockout mice [23] and following the downregulation of GLAST in rat brain by antisense oligonucleotides [24].

It is important to note that, although astrocytic glutamate uptake could trigger at least part of the observed changes in glucose metabolism, the energetic requirements of astrocytic glutamate uptake and recycling are small. Between two and five percent of the total energy consumption related to glutamate-mediated signaling is attributed to astrocytes [8] – a percentage consistent with the process of glutamate uptake and recycling by astrocytes being predominantly fuelled by glycolysis. One might hypothesize, therefore, that glycolytic...
energy supports the very rapid process of glutamate uptake by astrocytes, whereas oxidative metabolism supports aspects of synaptic transmission that occur over a slightly longer timeframe (e.g. restoration of pre- and postsynaptic ionic balance or recycling of vesicles). How this energy consumption is regulated, and whether glucose alone or other substrates (e.g. lactate) are oxidized by active neurons, remains to be determined.

**What triggers the increase in blood flow during activation?**

Despite a century of research, it is still unclear how the vascular supply of glucose and oxygen adapts to the changing needs of neurons (for a comprehensive review, see Ref. [25]). Roy and Sherrington’s ‘in series’ hypothesis suggested that increased neuronal activity leads to the accumulation of vasoactive catabolites, such as H+, K+ and adenosine, which decrease vascular resistance and thus increase blood flow until normal conditions are re-established [26]. However, this hypothesis could not account for all experimental increases in blood flow. To provide an alternative control system, which allows for rapid parallel changes in flow and neuronal activity, it was proposed that there is direct regulation of cerebral blood flow by neurotransmitters themselves [27]. This hypothesis has received strong anatomical support from the demonstration that cortical blood vessels are contacted by nerve terminals containing ACh, 5-HT, noradrenaline, dopamine and several other peptides [28–31]. The nerve terminals are sometimes in direct apposition with the blood vessel basal lamina, but more often are separated by specialized astrocytic processes (endfeet) that cover almost all brain capillaries. Immunohistochemical and molecular studies have identified different subtypes of receptors for these neurotransmitters on specific cellular components of the functional microvascular unit (i.e. endothelial and smooth muscle cells and their associated perivascular astrocytes) [32–34]. These findings might explain the vasoactive effect of neurotransmitters as a consequence of well-defined neurotransmitter–receptor interactions.

Although brain function relies mainly upon glutamate-mediated transmission, glutamate itself has received little attention in the context of neurovascular coupling. This has been mainly due to the facts that glutamate was shown to be devoid of vasoactive effects on isolated arteries in vitro and that there are no glutamate receptors on the vasculature [35]. However, when administered in situ, glutamate and glutamate-receptor agonists dilate arteries and...
increase blood flow. Furthermore, in accordance with previous observations that isolated neurons release the potent vasodilator agent NO in response to activation of NMDA receptors (and the associated increase in intracellular Ca\(^{2+}\)) [36], it has been shown that the vascular effect of glutamate in vivo is indeed mediated by NO [37]. Subsequently, it was reported that NO plays either an obligatory or a modulatory role in the hyperemic (increased blood flow) response to increased neuronal activity [38–41]. The involvement of NO in mediating the effect of glutamate is supported by the fact that the NO synthase (NOS) is physically anchored to the NMDA receptor by two postsynaptic density proteins, PSD-93 and PSD-95 [42,43].

NOS is not the only enzyme system that produces vasoactive molecules upon glutamate-induced changes in intracellular Ca\(^{2+}\). Phospholipase A\(_2\) releases arachidonic acid (AA) from membrane phospholipids in a Ca\(^{2+}\)-dependent fashion. AA is then metabolized via cyclooxygenases (COXs) to a number of vasoactive prostaglandins. Interestingly, inhibition of the COX-2 isoflorm of the enzyme, or targeted deletion of the gene encoding COX-2, attenuates the blood-flow response to somatosensory stimulation in mice [44]. COX-2 is localized in postsynaptic elements of excitatory neurons, where its constitutive expression is coupled to synaptic activity. Thus, via glutamate-induced increases in intracellular Ca\(^{2+}\) and activity of the phospholipase A\(_2\)–COX system, vasodilator prostaglandins could couple synaptic activity to cerebral blood flow.

A role for astrocytes in neurovascular coupling?

It has recently emerged that cell types other than neurons might also be involved in glutamate-mediated functional hyperemia. Indeed, the notion that astrocytes are active components of glutamate-mediated transmission has received strong experimental support over the last three years [45]. In addition to the fact that these glial cells are strategically positioned close to the vasculature, they can sense glutamate through functional NMDA [46] and non-NMDA [47] receptors, and can respond to glutamate activation with rhythmic elevations of intracellular Ca\(^{2+}\) levels that can spread to adjacent astrocytes as waves [5]. Astrocytes express different NOS isoforms [48,49] that are also regulated by Ca\(^{2+}\), and, consequently, these cells could release NO after activation by glutamate. Astrocytes contain not only COX, but also cytochrome P450 epoxygenase, which metabolizes AA to highly vasoactive epoxygenase products [50]. Recently, it was observed that both P450-epoxygenase and NO synthase inhibitors could block the cerebral blood flow response to in vivo administration of NMDA [51], indicating that astrocytes could participate in neurovascular coupling (Fig. 3b).

Concluding remarks

Although data do not provide yet definitive answers, they do suggest that glutamate could coordinate both the vascular and the metabolic responses to neuronal activity that underlie functional imaging signal changes. These effects of glutamate could involve its receptor-mediated action on neurons and/or astrocytes (metabolic and vascular responses), and its transport within the astrocyte (metabolic responses). These data support the notion that functional imaging signals are closely linked to the activity of glutamatergic synapses, so that any dysfunctioning of the dialog between glutamatergic neurons and astrocytes could lead to altered functional brain images, as has recently been observed [53].

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