SCIENTIFIC VERSES

Imaging the mind: Neurometabolism and functional activation

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Fundamental to many functional brain imaging methods is a remarkable feature of metabolism that tightly couples cerebral blood flow and energy metabolism to local neuronal activity, implying thereby that maps of local glucose and oxygen consumption and local blood flow provide information about ongoing neuronal activity. A major tool in basic and applied neuroscience that involves noninvasive imaging technology is the blood oxygenation level-dependent (BOLD) response, which forms the basis of functional magnetic resonance imaging (fMRI). FMRI uses hemodynamic responses as an indirect measure of neuronal function by measuring local fluctuations in magnetic susceptibility that depend on deoxyhemoglobin (dHb) concentration and hence relies primarily on hemoglobin-mediated tissue oxygenation, which in turn reflects metabolic activity linked to extracellular neuronal activity through local field potentials and multi-unit spiking activity [1]. However, though the general principles of brain metabolism and hemodynamic response to neuronal activity are known, we are only now beginning to understand the complex mechanisms that link energy metabolism to neuronal signaling.

A healthy human brain consumes a fifth of the total energy required by the body, and hence has to depend on a continuous supply of fuel for oxidative metabolism supplied by the blood to meet the demands of the active neuronal populations. There is a lack of fuel reserves in the brain and its high energy demand makes it highly vulnerable to interruptions of blood flow. However, several defensive strategies against interruptions of cerebral blood flow (CBF) give the brain an incredible operational resilience. Foremost among these defenses is the control the brain exercises over the distribution of blood through its humoral and neural manipulation of the cardiovascular system. Secondly, cerebrovascular autoregulation concentrates the cerebrovascular effects of normal fluctuations in arterial blood pressure that occur during normal brain function, thus maintaining a stable cerebral perfusion despite fluctuations in arterial pressure [2]. The third mechanism of neurovascular regulation, one that is most relevant for understanding the numerous studies of brain activation and connectivity using functional magnetic resonance imaging, is the oxidative metabolism and glucose utilization in response to information processing, which is still limited by an incomplete appreciation for the understanding of the BOLD signal and for defining the neurophysiological correlates of behavior and characterizing the networks for numerous cognitive processes.

Information transfer in the brain along the axons occurs by the process of neurotransmitter release at synapses and their subsequent interaction with specific receptors on target neurons. These neurotransmitter-receptor interactions then lead to changes in the membrane voltage distribution, initiated by changes in the membrane current flow, thereby changing the extracellular electric field and altering depolarizing frequency. These elements of the brain's physiology of information transfer and their role in the generation of action potentials, increased oxidative metabolism and glucose utilization, and enhanced local cerebral blood flow therefore would help us comprehend the basis of the hemodynamic BOLD signal, and hence of the relationship between action potentials, local field potentials and fMRI. This article is an attempt to understand the cellular energy production associated with neuronal activation, in particular the oxidative and non-oxidative energy metabolism and the mechanisms determining their balance in neurons and astrocytes, and their relationship with CBF.

The nature of metabolism in the brain

In order to understand the link between metabolic changes affecting the physiological parameters controlling neuronal activity, it is necessary to consider the release of neurotransmitters, their subsequent reuptake, and the propagation of membrane potential reversal. All three processes involve work coupled to ion transport (K^+, Na^+) across the membrane.

Ion transport

For neurons to actively maintain electrochemical membrane potential, specific ion channels in the neuronal membrane capable of exchanging free ionic species across the membrane between extra- and intracellular spaces [3]. To maintain the membrane potential at a stable point, the electrical conductance for each of the ionic species must be matched by the

active transport of the ions against its concentration gradient by pumping against an electrochemical gradient, which is an energy-requiring process. A key membrane enzyme in brain cells is, therefore, the phosphorylation-type-, Na^+,K^+-ATPase, which combines with the energy-rich molecule ATP, Mg^{2+}, K^+ and Na^+ to form an enzyme-substrate association during which ATP is hydrolyzed, thus releasing energy and inducing conformational changes in the enzyme to translocate K^+ and Na^+ in the appropriate directions [4, 5]. However, while about 40 percent of the energy produced by the neuron's mitochondria is utilized for maintaining the membrane potential, other factors like work done to move the Na^+ ions that enter to depolarize the membrane back into extracellular space again to restore the resting membrane potential, are also important. 50-60 percent of the average energy consumption has been found to remain when ion transport was completely blocked by the inactivation of Na^+,K^+-ATPase [6, 7].

Biochemical pathways of energy production in neurons

The brain maintains a constant concentration of the energy-rich compound ATP by a negative feedback process sensitive to the increase of ATP utilization. Both short-term and long-term regulatory mechanisms are used to maintain a constant concentration of ATP by hydrolysis of phosphocreatine (PCr), a small, phosphorylated molecule found in brain tissue. More prolonged increases in ATP utilization rate are balanced by increases in rates of glycolysis (enzyme-catalyzed reactions necessary to maintain the orderly, exothermic breakdown of glucose into pyruvate and lactate) and oxidative phosphorylation (breakdown of pyruvate to generate CO_2).

Hydrolysis of PCr

The 'low energy' compound ADP is produced when ATP is hydrolyzed by ATPases, releasing P*i* in the process. The enzyme creatine kinase (CK) catalyzes the interconversion of ADP and PCr with ATP and creatine, with one form of the enzyme in the cytosol where it is active enough to maintain the reaction at near equilibrium, i.e. [ADP][PCr] $\rightleftharpoons$ [ATP][creatine] at a constant pH [8]. Thus, as concentration of ADP (which is 1-2 orders of magnitude smaller than that of ATP) rises, the concentration of PCr must decrease to maintain ATP concentration at an almost constant level.

Except in cases of very high metabolic activity, PCr diffuses faster than ATP due to the rate-limiting nature of CK-transphosphorylation in the mitochondria [9]. This proves advantageous to the cell because cytosolic PCr quickly replenishes the transfer of high-energy PO_4^- bonds that occur in the cytosol through a mitochondrial ATP-linked CK form.

Glycolysis

The series of enzyme-catalyzed reactions in the cytosol responsible for the breakdown of glucose into pyruvate and lactate together with ATP release is known as glycolysis. Under normal conditions, pyruvate and lactate production is linked to their oxidative metabolism (aerobic glycolysis). Since glucose is the preferred energy substrate for the brain, understanding how glucose metabolism is controlled is key to the understanding of how energy production and utilization are linked. Under conditions of maximal neuronal activity, or sometimes in pathological conditions, glycolysis rates may be limited or enhanced by rising and falling levels of glucose. Normally, glucose metabolism is tightly controlled by the reactions catalyzed by the enzymes phosphofructokinase and hexokinase.

Pyruvate and lactate productions link glycolysis to oxidative metabolism through the lactate dehydrogenase (LDH) buffers, and by metabolic competition for pyruvate. Fluctuations in the concentration of pyruvate is thus a useful indicator of overall kinetics of bioenergetic changes in a cell, which include three pathways for pyruvate: export from cells, conversion to lactate and transport into the mitochondria [10], and two for lactate: conversion to pyruvate and export from cells. For pyruvate, its changes in tissue contents is given by:

$$\Delta M_{pyr}(t) = \frac{(2\Delta J_{glc})}{(1-\Lambda)k}(1-e^{-kt})$$

where

ΔJ_{glc} = rate of change of glycolysis,

$$k = \frac{(\Pi k_{pyr} + \Lambda k_{lact})}{1+\Delta},$$

k_{pyr} and k_{lact} are clearness of pyruvate import into the mitochondria, $k_{pyr} = \left(\frac{\sum T_{\max}}{k_t}\right)_{pyr}$ and lactate export across cell membranes, $k_{lact} = \left(\sum \frac{T_{\max}}{k_t}\right)_{lact}$,

Π = fraction of active mitochondria in the tissue, i.e. those that are stimulated to metabolize pyruvate in the cells, and Λ = ratio of Michaelis

constants (K_m) of LDH towards lactate and pyruvate, i.e.

$\Lambda = \left(\frac{K_m^{lact}}{K_m^{pyr}} \right)$. The ratio $\frac{\Pi k_{pyr}}{\Lambda k_{lact}}$ is known as the tissue oxidative capacity (ω) as influenced by the mitochondrial activity, i.e. Πk_{pyr}, the kinetic profile of LDH i.e. Λ and the blood-brain clearance of lactate, i.e. k_{lact}. For lactate, the change of its tissue content is a function of the effective ratio of LDH affinities towards its two substrates in terms of Λ:

$$\Delta M_{lact}(t) = \frac{(2\Lambda\Delta J_{glc})}{(1+\Lambda)k}(1-e^{-kt})$$

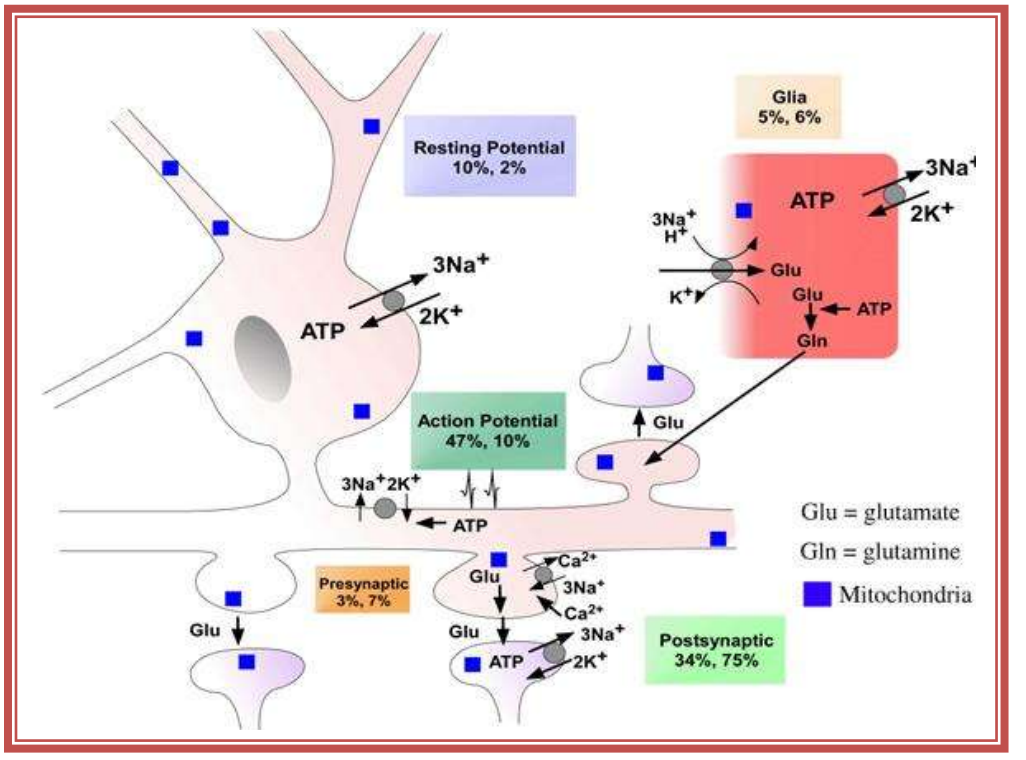
Since Λ varies with the isoenzyme subtype, which may range from lower for LD1 (adapted to functioning in an aerobic cell such as cardiac tissue) to highest for LD5 (adapted to functioning in an anaerobic cell such as muscle tissue), the higher the ratio between LDH's 'affinities' or effective binding, the lower is the ratio between the Michaelis constants of lactate and pyruvate, and consequently the more rapid is the approach to a new steady state, with a time constant of $1/k$.

This difference has important consequences for cellular physiology. Kinetic properties of LD1 subtype render this isoenzyme particularly effective for a tissue of high oxidative capacity since it allows rapid buildup of pyruvate. On the other hand, the LD5 subtype is more suitable for buffering the increase of pyruvate in a tissue with lower oxidative capacity. Thus LD1 and its messenger RNA (mRNA) subtype may be exclusively found in neurons, while both LD1 and LD5 and their respective mRNA subtypes may be present in astrocytes [11, 12]. However, other factors like different temperatures may also affect the chemical kinetics *in vitro*.

The tight coupling of regional neurometabolic activity with synaptic activity and regional cerebral blood perfusion constitutes a single functional unit, described generally as a neurovascular unit. This is central to any discussion of hemodynamic response linked to any neuronal activation. In normal as well as in injury conditions, neurons, astrocytes and endothelial cells of the vasculature interact to generate the complex activity-induced cerebral hemodynamic responses. Neurons and astrocytes have highly integrated signaling mechanisms, yet they form two separate networks. Bidirectional neuron-astrocyte interactions are crucial for the function and survival of the central nervous system. The primary purpose of such regulation is the homeostasis of the brain's microenvironment.

What kind of neuronal activity does the BOLD signal represent?

Neuronal activity that is most directly discernible from changes in rCBF is a range of processes from biochemical to neurotransmitter-based synaptic signaling of populations of neurons. As these neurons integrate information and fire at different spatial and temporal scales, activity in these neuronal populations generates local field potentials (LFPs), which can be recorded at the immediate vicinities of a microelectrode and reflect localized dendritic and synaptic signaling. Several studies have suggested that both LFPs and spiking



Schematics of an excitatory neuron and an astrocyte

The percentage of grey matter signaling energy predicted to be consumed is shown in shaded boxes (the first number for rodents and the latter for primates.) Almost all energy is spent in reversing Na^+ and K^+ fluxes across the neuronal membrane, or reversing Ca^{++} fluxes with Na^+/Ca^+ exchanger. **(Figure courtesy: Soumya Iyengar)**

activity are linked the BOLD signal in terms of increases of CBF. However, CBF increases may be observed even in the absence of spiking, e.g. CBF increase in cerebellum may reflect signal processing by inhibitory interneurons and other non-spiking electrophysiological events, suggesting that local neuronal integrative processes may cause CBF increase irrespective of whether those processes generate action potentials. Indeed, a recent report has demonstrated a strong coupling between LFPs and changes in tissue oxygenation, suggesting thereby that perisynaptic activity (which include classical events of synaptic transmission, excitatory/inhibitory postsynaptic potentials and several dendritic- and somatic spike-dependent integrative processes) places the maximum demand on brain metabolism [13].

Role of glutamate in the regulation of synaptic transmission

That active neurons release vasoactive agents into the extracellular space has been known for more than a century [14]. These vasoactive agents reach the endothelial cells (ECs) of the vascular tissue by diffusion and connects the rCBF consequences of neurotransmitter release, in particular glutamate and γ-amino butyric acid (GABA), to neuronal signaling. However, the mechanisms implicated for the linkages between these neurotransmitters and vasoactive species are not very well understood. It has been shown that excitatory activity in cerebellar Purkinje fibers, which receive major excitatory inputs from glutametergic parallel fibers and glutamate, aspartate or N-methyl-aspartyl-glutamate-utilizing (an NAA-Glu complex) climbing fibers, may lead to increase in rCBF that can be blocked by non-NMDA-type GluRs and is replicable using exogenous glutamate application. In neocortical and hippocampal tissue slices too, glutamate has been found to have a significant vasodilatory effect. In addition, exogenous glutamate application or selective GluR agonists dilate pial arterioles and precapillary microvessels. *In vivo*, these vasodilatory effects include the recruit-ment of NMDA-type GluRs as well [15].

In contrast to other neurotransmitters and neuromodulators like actylcholine, GABA and neuropeptides that are vasoactive by themselves, glutamate increases rCBF by an indirect mechanism of release of powerful vasoactive factors from other cells using several Ca^{2+}-dependent enzymatic mechanisms. This indirect vasodilatory effect of glutamate may be attenuated by blocking the NOS pathway and metabolites of cyclooxygenase-2 (Cox2). However, in the somatosensory cortex, NOS-related mechanisms are far less common.

In many of the model responses, CBF responses were studied by perturbing or attenuating the mechanisms by pharmacological or genetic approaches. However, it is observed that the inhibition of any of the mediators does not completely block the CBF response, partly because multiple vasoactive agents cooperate in a brain region-specific manner to increase CBF during neuronal activation, and partly because of differences in vascular microcircuitry that is involved.

What triggers the release of vasoactive factors?

The increase in the sudden demand for energy imposed on the neuronal tissue as a result of synaptic signaling is usually thought to increase CBF because of oxygen and glucose deficits. Curiously though, oxygen concentration at the site of activation falls during stimulation, and when present, are small and transient and cannot on their own account for flow changes. Indeed, CBF increases can persist even when the neuronal tissue is not under oxidative stress, as in hypoxia, which leads us to the conclusion that sudden changes in the energy budget is not the only factor that triggers the hemodynamic response (HDR). HDR is thus considered to be linked to the cellular and molecular processes underlying information transfer between brain cells. It would seem to suggest that CBF increases evoked by neuronal activity are synaptic neurotransmitter-based signaling in origin rather than being metabolic.

Conclusion

The close coupling observed between neuronal oxidative glucose consumption and glutamate recycling has been shown to occur during neuronal activation [16]. In the astrocytes, neurotransmitter glutamate recycling has been suggested to be the main factor in ATP required for glutamine amydation provided by aerobic glycolysis whose stimulation is triggered by glutamate uptake [17]. Based on reports of higher glucose phosphorylation (~50%) in astrocytes compared to its oxidative metabolism (~14%) [18], a coupling mechanism between neuronal activation and glucose utilization can be envisaged, one that suggests a cooperation-like behavior between the astrocytic and neuronal metabolisms to fulfill the energy needs of the active brain. This understanding is essential to interpret fMRI data correctly as well as to make better estimation of hemodynamic response functions.

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