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REVIEW

The physiological and biochemical bases of functional brain imaging

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Abstract Functional brain imaging is based on the display of computer-derived images of changes in physiological and/or biochemical functions altered by activation or depression of local functional activities in the brain. This article reviews the physiological and biochemical mechanisms involved.

Keywords Cerebral blood flow · Cerebral glucose utilization · Cerebral metabolism · Astrocytes · Glycolysis · ATPase · fMRI · Lactate shuttle

Functional brain imaging techniques are now widely used to identify regions of the brain involved in cognitive and various other neurological functions. These methods are all based on the display of the local activities of physiological and/or biochemical processes related to functional activity within images of the brain.

Tissues that do physical work, as, for example, mechanical work by heart and skeletal muscles and chemiosmotic work by kidney, derive the energy needed to support that work from the metabolism of substrates supplied to them by the blood. The greater the work load, the greater is the demand for energy and the higher the tissue's rate of energy metabolism. Although the physical nature of the work done by brain is less obvious, the pathways of its energy metabolism are similar to those of other tissues. In man, the normal adult male's brain comprises only about 2% of total body mass, yet it consumes approximately 20% of the body's total basal oxygen consumption. This probably indicates that even at rest the brain is functionally very active. The brain's oxygen consumption ($CMRO_2$) is almost entirely for the oxidative metabolism of glucose which in normal physiological conditions is the almost exclusive substrate for the brain's energy metabolism (Clarke and Sokoloff 1999). As far back as 1892, Roy and Sherrington (Roy and Sherrington 1890) had hypothesized that mechanisms exist in brain that adjust its rate of blood flow (CBF) to the nutritional demands of its energy metabolism. If so, then CBF should vary with the rate of energy metabolism and reflect the Level of functional activity in the brain. In comparison with other organs, the brain is extraordinarily heterogeneous with regard to both structure and function with different neurological functions localized to specific regions of the brain. Probably for that reason methods that measured average $CMRO_2$ and CBF in the brain as a whole failed to show a clear relationship between normal physiological functional activities and rates of blood flow and metabolism. What was needed were methods for measuring rates of blood flow and/or energy metabolism in the brain locally within the various structures of the brain.

The first step in the evolution of functional brain imaging was the development of the [^{131}I]trifluoroiodomethane ($CF_3^{131}I$) method for measuring local rates of blood flow within the various structures of the brain (lCBF) (Landau et al. 1955). This method utilized a unique quantitative autoradiographic technique in which the optical densities in autoradiographs of sections of the brain were quantitatively related to the local tissue concentrations of the radioactive tracer, and these concentrations were in turn determined by the rate of blood flow to the tissue. Essentially, the method provided pictorial maps of the local rates of blood flow throughout the brain exactly

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within each of its anatomic structures. This method was used to show that retinal stimulation with photoflashes markedly increased local CBF in all the structures of the visual pathways of the conscious cat, and these local increases were clearly visible in the autoradiographs (Sokoloff 1961), This study confirmed the validity of the Roy and Sherrington hypothesis and was the first true example of functional brain imaging.

The mechanisms by which CBF adjusts to changes in functional activity are still largely undefined, probably because there are so many of them. It was long believed that CBF is adjusted to the level of energy metabolism because increased CO_2, and reduced oxygen tensions, decreased pH, and increased adenosine concentration in the tissue, all consequences of increased energy metabolism, dilate cerebral blood vessels, and when these are changed in the opposite direction during reduced energy metabolism, the vessels constrict. Neurogenic control of the cerebral circulation is always a possibility but never proven. Vascular effects of neurotransmitters, such as glutamate, dopamine, GABA, etc., and nitric oxide, a very potent vasodilator, have all been implicated. It may well be that all contribute to some extent and to varying degrees in different parts of the brain so that none can be ruled out. Regardless of the mechanism, however, measurement of local CBF can be used to localize local functional activity.

Compared with blood flow, it should be expected that local energy metabolism would be more closely, more directly, and more specifically related to local functional activity because both processes are localized to the same individual cells. No individual cells, however, have their own private blood flows, and, therefore, blood flow should increase more diffusely in areas surrounding the activated cells. Furthermore, energy metabolism is influenced only by events within the cells, but blood flow is also influenced by chemical factors in the blood perfusing the tissue. All in all, it is to be expected that measurement of local energy metabolism should provide a more specific marker and better spatial localization of functional activity in the brain.

The development of the [^{14}C]deoxyglucose) ([^{14}C]DG) method made it possible to measure local rates of glucose utilization ($lCMR_{glc}$) throughout the brain (Sokoloff et al. 1977). This method employed a quantitative autoradiographic technique like that of the ($CF_3^{131}I$) method but, subsequently, added computerized processing techniques that scanned the autoradiographs and redisplayed them in color with the actual rates of $lCMR_{glc}$ encoded in a calibrated color scale. It measured $lCMR_{glc}$ quantitatively accurately with a spatial resolution (Full-Width-Half-Max, FWHM) of about 200 μm (Goochee et al. 1980), but it could qualitatively visualize and display altered $lCMR_{glc}$ in areas as small as 25 μm. Numerous applications of this method in both conscious and anesthetized animals clearly established that increased functional activity stimulates and decreased functional activity diminishes $lCMR_{glc}$ in anatomical components of the activated pathways (Sokoloff 1981). A surprising finding was that the increases in glucose consumption evoked by functional activation are confined to synapse-rich regions, i.e., neuropil which contains axonal terminals, dendritic processes, and also the astrocytic processes that envelop the synapses (Sokoloff 1999; Kadekaro et al. 1985). It was also shown that the magnitudes of these increase are directly and linearly related to the frequency of action potentials in the afferent pathways and not to the activity of the target neurons in their projection zones (Sokoloff 1999; Kadekaro et al. 1985; Yarowsky et al. 1983). $lCMR_{glc}$ is, therefore, increased in the projection zone of an activated pathway regardless of whether the pathway is excitatory or inhibitory. To determine which requires examining $lCMR_{glc}$ in the next synaptic station of the pathway. Regions rich in neuronal cell bodies do consume glucose, but their rates are essentially unaffected by neuronal functional activation (Sokoloff 1999; Kadekaro et al. 1985). Presumably, the glucose metabolized in neuronal cell bodies is mainly to support cellular vegetative and house-keeping processes, e.g., axonal transport, biosynthesis of nucleic acids, proteins, phospholipids and other lipids, as well as other energy-consuming processes not related directly to action potentials.

Studies with the [^{14}C]DG method in neural tissue slices showed that the activation of energy metabolism by functional activation is due mainly, if not entirely, to stimulation of Na^+,K^+-ATPase activity (Mata et al. 1980). Action potentials reflect the uptake of Na^+ into and extrusion of K^+ from the cells, thus depolarizing the cell membranes. These ion shifts stimulate the activity of Na^+,K^+-ATPase, the enzyme that pumps the Na^+ back out and the K^+ back into the cells, a process dependent on the energy derived from the breakdown of ATP. The breakdown of ATP in turn stimulates glucose metabolism to restore the ATP that had been consumed. In short, it appears then that the energy-requiring work of the brain supported by the increased $lCMR_{glc}$, during neuronal functional activation is due mainly to activation of Na^+,K^+-ATPase activity to restore the ionic gradients across the cell membrane and the membrane potentials that were degraded by the spike activity.

The [^{14}C]DG method lacks the cellular and subcellular resolution needed to identify the elements in neuropil, e.g., axonal, dendritic, and astrocytic processes, that contribute to the functional activation of $lCMR_{glc}$. This issue has been approached indirectly by studies in vitro with cultured neurons and astroglia in which the incubation medium is manipulated to simulate changes expected to occur in vivo during neuronal functional activation, e.g., increased

extracellular K^+ concentration ($[K^+]_o$), opening Na^+ channels and raising intracellular Na^+ concentration ($[Na^+]_i$), and raising extracellular neurotransmitter concentrations (Takahashi et al. 1995; Pellerin and Magistretti 1994). Membrane depolarization by raising $[K^+]_o$ stimulated glucose utilization in cultured neurons but not in astroglia (Takahashi et al. 1995), and opening Na^+ channels by addition of veratridine or monensin markedly stimulated glucose utilization in both types of cells. These stimulations were all blocked by addition of ouabain, an inhibitor of Na^+,K^+-ATPase, confirming a role for this enzyme in the mechanism of the increased glucose utilization. Of particular interest was the finding that addition of L-glutamate to the medium markedly stimulated glucose utilization in the cultured astroglial cells (Takahashi et al. 1995; Pellerin and Magistretti 1994). This stimulation was unaffected by inhibitors of NMDA or non-NMDA receptors but blocked by ouabain and absent in Na^+-free medium, excluding glutamate receptors but implicating Na^+,K^+-ATPase in the mechanism of this stimulation as well (Takahashi et al. 1995; Pellerin and Magistretti 1994).

L-glutamate is the most prevalent excitatory neurotransmitter in brain and is released by the axonal terminals in the synapses in the projection zones of glutamatergic pathways. Extracellular glutamate is extraordinarily toxic to neurons, but they are normally protected from toxic extracellular glutamate concentrations by the avid uptake of glutamate by the astrocytes. This uptake is mediated by a Na^+/glutamate co-transporter that transports the glutamate together with Na^+ into the astrocytes (Flott and Seifert 1991) where the glutamate is converted to glutamine by glutamine synthetase, another ATP-consuming enzymatic process. On the basis of these observations made in cultured neurons and astroglia and anatomical evidence that capillaries in brain are largely surrounded by astrocytic end-feet, Magistretti and Pellerin (1996) have hypothesized that oxidative metabolism of glucose in brain is compartmentalized between astrocytes and neurons, first uptake of glucose by astrocytes where it is converted by glycolysis to lactate and then the export of this lactate to the neurons where it is oxidized to CO_2 and water by the tricarboxylic acid cycle. This hypothesis, combined with what is already known about cerebral biochemistry, leads to the following scenario. Functional activation of a pathway is associated with increased spike activity and the release of neurotransmitters in its terminal projection zone. This neurotransmitter is mainly glutamate in most excitatory pathways. The released glutamate is cleared from the extracellular space by the co-transport of the glutamate and 2–3 Na^+ ions into the astrocytes. The glutamate taken up by the astroglia is converted by glutamine synthetase to glutamine which can then be released and recycled for use by neurons. The rise in intracellular Na^+ content in the astroglia stimulates Na^+,K^+-ATPase activity to pump out the Na^+ ions, and one molecule of ATP is consumed in the pumping out of 3 Na^+ ions. The conversion of one molecule of glutamate to glutamine also consumes one molecule of ATP. Therefore, each glutamate molecule that is released by neuronal functional activation, then taken up along with Na^+ into the astroglia, and converted there into glutamine results in the consumption of two molecules of ATP, the net amount of ATP produced by the glycolytic conversion of one molecule of glucose to two molecules of lactate. The lactate molecules thus produced in the astrocytes are exported to the neurons where they are oxidized by the tricarboxylic acid cycle to produce an additional 36 molecules of ATP. These ATP molecules are used by Na^+,K^+-ATPase to restore the ionic gradients in the axonal terminals and dendritic processes that were partially degraded by the spike activity. The energy metabolism supporting functional activity in brain is then shared by neurons and astroglia in a symbiotic relationship. Glucose is the essential substrate for the brain's energy metabolism, but different segments of the overall pathway in its metabolism are to some extent segregated in the two cell types, glycolysis in astrocytes and oxidation in neurons.

This lactate-shuttle hypothesis has raised some controversy (Dienel and Hertz 2001). Its relevance has been questioned because it is based mainly on studies of cells in culture, and also as originally presented, appeared to propose exclusive assignments of glycolysis to astrocytes and oxidative metabolism of the glycolytic products, lactate and pyruvate, to neurons. It is true that in astroglia in culture the rate of glycolytic metabolism of glucose far exceeds that of oxidation so that excessive lactate is formed and released into the medium. Neurons in culture, however, are also able to metabolize glucose in the medium to pyruvate and lactate and then to oxidize these glycolytic products to CO_2 and water, and astroglia are able to some extent to oxidize glucose and lactate to CO_2 and water (Itoh et al. 2003). The neurons, however, do show a kinetic preference to oxidize lactate than glucose to CO_2. For example, progressive increases in lactate concentration in the medium produce correspondingly progressive inhibition of glucose oxidation by neurons whereas glucose in the medium does not inhibit the oxidation of lactate (Itoh et al. 2003). This would indicate that the direct oxidation of glucose by neurons depends on the lactate concentration in the medium. The lactate-shuttle hypothesis is probably valid mainly in regions of the brain with major glutamatergic inputs where spike activity in the afferent pathway releases glutamate in the synapses. The glutamate is then taken up along with Na^+ by the astrocytes which stimulates glucose utilization to pyruvate and lactate. The ATP formed by glycolysis is consumed in the astrocytes to pump the Na^+ out of the cells and convert the glutamate to

glutamine, and the lactate is released into the extracellular space. The rise in extracellular lactate concentration leads to preferential uptake and oxidation of lactate and inhibition of glucose utilization by the neurons. The ATP generated by the oxidation of the lactate provides the energy needed to restore the ionic gradients and membrane potentials degraded by the spike activity in the neuronal synaptic membranes. This compartmentalization is probably of lesser magnitude and importance in regions with input pathways that use transmitters other than glutamate, and in no case is the compartmentalization of glycolytic and oxidative metabolism of glucose between astrocytes and neurons complete and fully obligatory.

Because of their use of autoradiography, the $CF_3^{131}I$ and [^{14}C]DG methods for measuring local CBF and glucose utilization were limited to studies in animals. Both methods were, however, subsequently adapted for use in humans by substituting positron-emitting tracers and positron emission tomography (PET) in place of autoradiography, i.e., $H_2^{15}O$ instead of $CF_3^{131}I$ to measure blood flow (Herscovitch et al. 1983) and [^{18}F]fluorodeoxyglucose (^{18}FDG)in place of [^{14}C]DG to measure glucose utilization (Reivich et al. 1979; Phelps et al. 1979). These PET methods, however, have far lesser spatial resolution than the autoradiographic methods, e.g., FWHM in the mm instead of μm range. Although these PET methods contributed very little to define the mechanisms that relate blood flow and energy metabolism to functional activity in the brain, their applications did initiate and establish the field of functional brain imaging in humans.

The advent of nuclear magnetic resonance imaging (MRI) introduced a new and now the most convenient and commonly used method for functional brain imaging (fMRI) in both animals and humans. It is based on the physical property of atomic nuclei that, when first oriented in a magnetic field and then temporarily reoriented by radiofrequency pulses, emit during their return to their previous orientation in the magnetic field radio signals at resonance frequencies and intensities characteristic of their chemical species, concentrations, and environment. The strongest signals are those obtained from hydrogen nuclei because they are in water and thus most prevalent. This physical phenomenon has been exploited for fMRI by taking advantage of the so-called Blood-Oxygen-Level-Dependent (BOLD) effect (Ogawa et al. 1990, 1992). The physical basis of this effect is as follows. Reduced hemoglobin, mainly in venous blood, is paramagnetic which causes it to alter and attenuate the MRI signal. Oxyhemoglobin, most prevalent in the arterial blood is diamagnetic and has no such effect. Blood flow is increased by dilatation of the blood vessels which augments the inflow of arterial blood and its oxyhemoglobin content into the tissue. If this increased blood flow and oxygen input is not matched by a proportionate increase in oxygen consumption and extraction of oxygen from the blood by the tissue, the venous blood draining the tissue within the field of view contains more oxyhemoglobin and less deoxyhemoglobin. This leads to a small increase in the MRI signal, i.e., the BOLD effect. Functional activation, at least initially, increases inflow of arterial blood more than the oxygen extraction which results in lower reduced hemoglobin content within the field of view and produces the signal exploited by fMRI to localize functional activity in the brain (Ogawa et al. 1992, Kwong et al. 1992).

The physiological basis of the discrepancy between the magnitudes of the changes in blood flow and oxygen extraction that lead to the BOLD effect evoked by functional activation is still unclear. It has been attributed to increases in blood flow without, despite comparable increases in glucose utilization, proportionate rises in oxygen consumption that would extract the oxygen from the blood (Fox and Raichle 1986; Fox et al. 1988). This is highly unlikely because the same intracellular changes produced by increased functional activity that stimulate glucose utilization also stimulate oxidative phosphorylation and the oxidation of the pyruvate and lactate produced by glycolysis. A more likely explanation is that there is a temporal dissociation between the increased rates of glycolysis and the oxidation of its products. The time course of increased oxygen consumption is likely to be delayed, acutely less intense, and more spread out over a longer time period than the increase in blood flow. The lactate and pyruvate produced by the initial stimulation of glycolysis may be oxidized more slowly but over a longer time span that extends beyond the end of the functional activation (Madsen et al. 1999). Another likely contributor to the BOLD effect is the different spatial distributions of the changes in blood flow and oxygen consumption within the field of view. Functional activation stimulates energy metabolism only in functionally activated cells. No cells, however, have their own private blood flows. When cells are activated, blood flow is increased not only to those cells but also to surrounding unaffected cells. The venous blood draining the mixture of activated and unaffected cells in the field of view then contains higher oxyhemoglobin and lower reduced hemoglobin contents giving the impression that functional activation increases blood flow more than oxygen consumption. The blood flow is disproportionately higher because it includes the increased blood flow in the surrounding tissues within the field of view that were not functionally activated. In addition, one must consider the potential role of the Munro-Kellie doctrine (Wolff 1936) which addresses the fact that because the brain is enclosed in a rigid box, the cranium, any increase in any one fluid compartment must be at the expense of one or more of the

other fluid compartments in the brain. Because the brain is incompressible, this leaves only arterial and venous blood and cerebrospinal fluid susceptible. During functional activation the arterial blood vessels are dilated bringing in more oxygenated blood which must displace venous blood and/or CSF. The exit channels for CSF have much higher resistance than those for the venous blood, and so the input of arterial blood mechanically displaces mainly venous blood which would give rise to a BOLD effect.

Even though the physiological basis of the BOLD effect during functional activation in brain is still undefined, it is clear that fMRI provides a potent method for localizing functional activities in the brain. It is non-invasive, uses no ionizing radiations, is very rapid so that numerous repeated scans can be done within one session and control and stimulated conditions compared, and provides better spatial resolution than PET. Its main disadvantage is that baseline conditions without functional activation between subjects cannot be compared.

References

Clarke DD, Sokoloff L (1999) Circulation and energy metabolism of the brain. In: Siegel G, Agranoff B, Albers RW, Fisher S (eds) Basic neurochemistry:molecular, cellular, and medical aspects, 6th edn. Lippincott-Raven, Philadelphia, pp 637–669

Dienel GA, Hertz L (2001) Glucose and lactate metabolism during brain activation. J Neurosci Res 66:824–838

Flott B, Seifert W (1991) Characterization of glutamate uptake in astrocyte primary cultures from rat brain. Glia 4:293–304

Fox PT Raichle ME, Mintun MA, Dence C (1988) Nonoxidative glucose utilization during focal physiologic neural activity. Science 241:462–464

Fox PT, Raichle ME (1986) Focal physiological uncouplingof cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc Natl Acad Sci USA 83:1140–1144

Goochee C, Rasband W, Sokoloff L (1980) Computerized densitometry and color coding of [14C]deoxyglucose autoradiographs. Ann Neurol 7:359–370

Herscovitch P, Markham J, Raichle M (1983) Brain blood flow measured with intravenous $H_2^{15}O$. I. Theory and error analysis. J Nucl Med 24:782–789

Itoh Y, Esaki T , Shimoji K, Cook M, Law MJ, Kaufman E, Sokoloff L (2003) Dichloroacetate effects on glucose and lactate oxidation by neurons and astroglia in vitro and on glucose utilization by brain in vivo. Proc Natl Acad Sci USA 100:4879–4884

Kadekaro M, Crane AM, Sokoloff L (1985) Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. Proc Natl Acad Sci USA 82:6010–6013

Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, Kennedy DN, Hoppel BE, Cohen MS, Turner R, Cheng HM, Brady TJ, Rosen BR (1992) Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc Natl Acad Sci USA 89:5675–4679

Landau WM, Freygang WH, Rowland LP, Sokoloff L, Kety SS (1955) The local circulation of the living brain: values in the unanesthetized and anesthetized cat. Trans Am Neurol Assoc 80:125–129

Madsen PL, Cruz NF, Sokoloff L, Dienel GA (1999) Cerebral oxygen/glucose ratio is low during sensory stimulation and rises above normal during recovery: Excess glucose consumption during stimulation is not accounted for by lactate efflux from or accumulation in brain tissue. J Cereb Blood Flow Metab 19: 393–400

Magistretti PJ, Pellerin L (1996) Cellular bases of brain energy metabolism and their relevance to functional brain imaging: evidence for a prominent role of astrocytes. Cereb Cortex 6: 50–61

Mata M, Fink DJ, Gainer H, Smith CB, Davidsen L, Savaki H, Schwartz WJ, Sokoloff L (1980) Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. J Neurochem 34:213–215

Ogawa S, Lee TM Kay AR, Tank TW (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci USA 87:9868–9872

Ogawa S, Tank DW, Menon R, Ellerman JM, Kim SG, Merkle H, Ugurbil K (1992) Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. Proc Natl Acad Sci USA 89:5951–5955

Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci USA 91:10625–10629

Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE (1979) Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)2-fluoro-2-deoxy-d-glucose: validation of method. Ann Neurol 6:371–388

Reivich M, Kuhl D, Wolf A, Greenberg J, Phelps M, Ido T, Cassella V, Fowler J, Hoffman E, Alavi A, Som P, Sokoloff L (1979) The [^{18}F]fluoro-deoxyglucose method for the measurement of local cerebral glucose utilization in man. Circ Res 44:127–137

Roy CS, Sherrington CS (1890) On the regulation of the blood supply of the brain. J Physiol 11:85–108

Sokoloff L (1961) Local cerebral circulation at rest and during altered cerebral activity induced by anesthesia or visual stimulation. In: Kety SS, Elkes J (eds) The regional chemistry, physiology and pharmacology of the nervous system. Pergamon Press, Oxford, pp 107–117

Sokoloff L (1981) Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. J Cereb Blood Flow Metab 1:7–36

Sokoloff L (1999) Energetics of functional activation in neural tissues. Neurochem Res 24:321–329

Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897–916

Takahashi S, Driscoll BF, Law MJ, Sokoloff L (1995) Role of sodium and potassium ions in regulation of glucose metabolism in cultured astroglia. Proc Natl Acad Sci USA 92:4616–4620

Wolff HG (1936) The cerebral circulation. Physiol Rev 16:545–596

Yarowsky P, Kadekaro M, Sokoloff L (1983) Frequency-dependent activation of glucose utilization in the superior cervical ganglion by electrical stimulation of cervical sympathetic trunk. Proc Natl Acad Sci USA 80:4179–4183