Meeting Report

New Approaches to Functional Neuroenergetics

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INTRODUCTION

The coupling between brain energy metabolism and neuronal activity has, for more than a century, allowed researchers to monitor brain function (Roy & Sherrington, 1890; Siesjo, 1973; Sokoloff, 1981). A breakthrough in this effort, and one that made human studies routine, was the development 20 years ago of positron emission tomography, or PET (see Raichle, 1998). In combination with experimental paradigms and models developed in cognitive psychology, PET allowed the first high-resolution metabolic maps of functionally specialized regions of the human brain. A drawback of the PET technology was its reliance on cyclotron-generated short-lived radioisotopes. The subsequent development of functional magnetic resonance imaging (fMRI) made functional brain mapping widely available to scientists (Kwong et al., 1992; Ogawa, Menon, Kim, & Ugurbil, 1992). Almost weekly, new brain imaging results are highlighted in scientific journals and the popular media as providing new insights into the biological basis of human brain function and neurological and psychiatric disorders.

The application of PET and fMRI to localize cognitive processes is based on the assumption that functional neuronal activity increases when a region is involved in performing a cognitive task (Posner & Raichle, 1994). These functional neuronal activities are involved in the communication of information between neurons and include neurotransmitter release and action potentials. The energy required for these and other brain processes is provided almost exclusively by oxidative glucose metabolism (Siesjo, 1978). Functional imaging measures either glucose metabolism or neurophysiological parameters coupled to glucose metabolism (Sokoloff, 1981). Regions of increased functional neuroenergetic demand are identified by the corresponding increase in glucose metabolism. Depending on the form of the labeled tracer used, PET either directly measures the regional rate of glucose metabolism (CMRglc) or the coupled parameters of the cerebral metabolic rate for oxygen ($CMRO_2$), and the cerebral blood flow (CBF) rate. The fMRI blood oxygenation level dependent (BOLD) signal is sensitive to both $CMRO_2$ and CBF (Ogawa et al., 1998).

In a functional imaging study, a subject performs experimental tasks while the signal is acquired. Most cognitive neuroscience imaging studies rely on the PET CBF or the BOLD fMRI measurement. The acquired signal is analyzed to provide images of the spatial distribution and temporal dynamics of CBF or BOLD contrast. The *functional image* is an image of the increment in signal intensity during a task relative to a baseline state in which the subject rests in the scanner. Cognitive processes are localized by functional imaging using experimental paradigms and analyses based upon theories of cognitive neuroscience.

As an illustrative example, consider a study designed to assess whether the frontal lobe is involved in the general cognitive skill of verbal working memory. The subject would perform tasks requiring this cognitive skill, such as remembering lists of words, while being scanned. In one strategy the degree of involvement of verbal working memory in each task would be varied, but the requirements for other cognitive skills would be held constant. The relative intensity of the functional imaging signal in the frontal lobe during each task would be statistically correlated with the verbal working memory component. A positive correlation would support the involvement of frontal lobe neuronal activity in this skill (Posner & Raichle, 1994, provides an excellent summary of how imaging studies are designed).

Given the enthusiasm with which functional imaging is used to answer sophisticated questions about the functional architecture of the brain, it is easy to forget

that there are long-standing controversies concerning the neuroenergetic basis of the signal. To define some of the outstanding questions that remain in the field, and to encourage research to better understand the meaning of these measurements, the James S. McDonnell Foundation organized a workshop, Cerebral Metabolism and Human Cognition: A Workshop Discussing New Approaches to Functional Neuroenergetics.[1] The workshop took place over two days with focused talks and animated discussions on a variety of issues related to neuroenergetics and functional imaging. Some of the major questions identified and the results of these discussions are described in this report. Selected references are provided for key points raised by the participants.

What is the relationship between the imaging signal being acquired and the cellular localization of neuronal activity?

Louis Sokoloff (NIMH) reviewed studies on the cellular location of glucose metabolism in peripheral neurons. These studies showed that the uptake of radio-labeled deoxyglucose during electrical stimulation of peripheral neurons occurs mainly in the synaptic regions. The rate of glucose metabolism was close to linear with the stimulation frequency, which he interpreted as supporting a direct relationship between increases in CMRglc measured by PET and the total electrical activity of a region (Yarowsky, Kadekaro, & Sooloff, 1983). Gordon Shephard (Yale University) addressed the issue of the variations in neuroenergetic requirements as a function of cell type and brain region. He pointed out that the relationships measured in peripheral neurons do not hold for all classes of neurons. He presented high-resolution deoxyglucose autoradiography studies of the olfactory bulb that showed that unmyelinated axons have a high glucose metabolic rate (Greer, Stewart, Kauer, & Shepherd, 1981). Because unmyelinated axons are common in the cerebral cortex, they may contribute significantly to the neuroenergetic requirements measured by functional imaging. Shepherd, collaborating with Yale colleague Robert Shulman, is correlating metabolic and electrical maps of neuronal activity in the olfactory bulb with ultra-high-resolution fMRI (Yang et al., 1998). William Greenough (University of Illinois) observed that functional imaging of the developing or learning brain, in which the density of synapses and other neuronal structures changes with time, may help in the assignment of the neuroenergetics measured by functional imaging to specific cellular structures. Along these lines Kevin Behar (Yale University) discussed NMR measurements in the postnatal rat cortex that showed that $CMRO_2$ increased with age and correlated with synaptic development (Behar, Ariyan, Mason, Haddad, & Novotny, 1997). It was concluded that by further development of these experimental strategies and methods (e.g., higher-resolution autoradiography; more detailed correlation of metabolism with development) definitive answers would be obtained for these fundamental, long-standing questions.

What are the molecular mechanisms that couple neuroenergetic requirements to CMRglc and $CMRO_2$?

Although most functional neuroimaging studies assume that signal changes reflect neuronal activity, Pierre Magistretti (University of Lausanne) presented results identifying the astrocyte as the primary site of cerebral glucose consumption. Magistretti proposed that astrocytic glucose uptake is mechanistically coupled to neuronal energy requirements by the use of nonoxidative glycolysis to provide energy for removing glutamate, released by the nerve terminals, from the synaptic cleft. The lactate produced as a result of the incomplete oxidation of glucose is then transported from the astrocyte to the neuron as a substrate for oxidative energy metabolism (Pellerin et al., 1996). Robert Shulman's *in vivo* results from rat and human brain supported and extended the proposal by Magistretti. Shulman described ^{13}C NMR spectroscopic studies showing that the rate of astrocytic glutamate uptake increased with glucose metabolism with close to a 1:1 stoichiometry (Sibson et al., 1998). He proposed that 80% of cortical glucose oxidation is coupled to neuronal glutamate release and uptake by the astrocyte. Rolf Gruetter (University of Minnesota) discussed his own ^{13}C NMR spectroscopy data of human brain with enhanced sensitivity that is consistent with Shulman's findings (Gruetter, Seaquist, Kim, & Ugurbil, 1998). A lively discussion initiated by Albert Gjedde (University of Denmark) questioned the localization of glucose metabolism to the astrocyte. Among the objections were the presence of a high glucose transporter and glycolytic enzyme capacity in neurons as well as the lack of direct evidence *in vivo.* Although no consensus was reached, it was agreed that the results presented provided important new insights into the potential coupling of the functional imaging signal to neurotransmission.

Are functional neuroenergetic requirements temporally dependent?

Marcus Raichle (Washington University) and Louis Sokoloff each presented data suggesting, in the human and rat brain, respectively, that the fractional increase in CMRglc is greater than $CMRO_2$ during sensory stimulation. Mark Mintun (Washington University) discussed his recent PET results, showing that at longer stimulation times $CMRO_2$ increases, consistent with NMR and microdialysis data showing a transient increase in brain lactate during sensory stimulation (Prichard et al., 1991). Several discussants pointed out that even during the transient period the majority of the increase in cerebral energy requirements is supplied by glucose oxidation. Albert Gjedde described studies suggesting that the mismatch is highly stimulation and region dependent

(Marret et al., 1993). Robert Shulman proposed an explanation for the transient mismatch based upon the increased use of glycogen metabolism during the transition period between the resting and stimulated state. Other discussants noted that there are large variations in the reported degree of mismatch between CMRglc and $CMRO_2$. (Marret et al., 1993; Seitz & Roland, 1992). Results from BOLD fMRI studies find temporal variations between CBF and $CMRO_2$ only for the first few seconds of stimulation (Ogawa et al., 1998). It was concluded that with improvements in both fMRI and PET, as well as the more detailed chemical picture provided by MRS, a better understanding will emerge of the neuroenergetic basis and functional importance of this phenomenon.

Does blood flow spatially and temporally correlate with functional neuroenergetic requirements?

Workshop participants are using several different techniques and experimental models in an effort to address this question. Marcus Raichle's PET studies suggest that during sensory stimulation, the observed fractional increase in CBF is similar to that seen for CMRglc, with both exceeding the increase in $CMRO_2$. Kamil Ugurbil (University of Minnesota), acquiring BOLD functional images with a 4 Tesla magnet system from the activated human visual cortex, reported that a rise in CBF is not localized to the active optical columns (Hu, Le, & Ugurbil, 1997). The adjacent nonactive columns also experience an increase in blood flow. Superior localization of the active columns was achieved using the BOLD signal immediately after the start of the stimulation. This early BOLD signal is believed, based on optical data (Malonek et al., 1996), to reflect primarily oxygen consumption. Robert Turner (Wellcome Institute of Neurology) and others supported Ugurbil's proposal of a spatial uncoupling of CBF and local neuroenergetic requirements based on optical data (Malonek et al., 1996). This conclusion was debated as participants discussed the well-documented autoradiographic data from rat studies showing a tight spatial coupling of the changes in blood flow and oxidative metabolism, electrical measurements, and fMRI (Greenberg, Hand, Sylvestro, & Reivich, 1979; Ueki, Linn, & Hossmann, 1988). Jeff Dunn (Dartmouth College) supported a tight spatial coupling of $CMRO_2$ and CBF with his own data, showing that the oxygen tension in the brain remained low during activation. Thomas Woolsey (Washington University) suggested that CBF responds to local neuronal activity prior to CMRglc and $CMRO_2$, a view supported by his elegant studies of rat whisker barrels (Moskalenko et al., 1996). He also suggested an alternate explanation for the primate optical measurements based upon imprecision in the spatial correspondence of the vessels on the cortical surface with the columns that they serve. Louis Sokoloff criticized the standard interpretation of the BOLD fMRI measurement for not appropriately taking into account the physical constraints on blood volume. Ugurbil and others vigorously defended the BOLD measurement. Despite the controversies the discussants agreed that many of these issues could be resolved through appropriate functional imaging studies in animal models to allow correlation with invasive methods that allow measurements of blood flow and neuroanatomy at extremely high spatial resolution. Because BOLD fMRI provides the highest spatial and temporal resolution of the metabolic functional imaging methods, the resolution of these issues is critical for continuing progress in functional imaging.

What are the molecular mechanisms that regulate CBF?

Data supporting several candidates for the molecular regulation of CBF during functional activation were presented, including adenosine, nitric oxide, and adrenaline. A novel proposal for CBF regulation was put forth by Joseph Williams (Washington University). Working with Thomas Woolsey on the rat whisker barrel, he showed that the plasma lactate/pyruvate ratio, as well as other factors that alter the brain redox potential, effect CBF. Although the results from several speakers were promising, no single factor explained the full range of CBF autoregulation. The consensus among discussants was that these mechanisms warrant further investigation.

How will understanding of functional neuroenergetics aid in localizing cognitive processes?

If one goal of brain imaging studies is to map cognitive operations onto their neurobiological correlates, it is important to have a sense of how an understanding of neuroenergetics contributes to this goal. Randy McIntyre (University of Toronto) is tackling this issue by studying the nature of the electrophysiological activity underlying cognitive function in humans and primates. McIntyre's data suggests that both an increase in the total number and frequency of neurons firing and alterations in patterns of firing in activated tissue accompany increases in functional activity. Several discussants pointed out that it is not known whether functional imaging is more sensitive to changes in neuronal firing patterns or total electrical activity. The results presented by Shulman and Magistretti supporting a relationship of the functional imaging signal with neurotransmitter release (Pellerin et al., 1996; Sibson et al., 1998) were interpreted by some to support a tighter relationship with total electrical activity. Seiji Ogawa (Lucent Technologies) described studies in which BOLD fMRI correlated well with evoked potential recordings in sensory stimulation paradigms in animals (Ogawa et al., 1998). These studies were also interpreted as supporting a correlation with total electrical activity. It was generally agreed that more studies defining the electrophysiological basis of the electro-

encephalogram, EEG (and MEG) signal and the neuroenergetic basis of the functional imaging signal are needed to better understand the relationships between these mapping methodologies.

Several discussants gave examples of how a better understanding of the functional imaging signal would aid in their research. Kia Nobre (Oxford University) discussed how knowledge of the electrophysiological correlates of the functional imaging signal would assist in her studies using functional imaging to localize signal sources and EEG mapping to provide high temporal resolution information. Several possibilities for combining electrophysiological and metabolic imaging studies were discussed.

An animated discussion arose over new PET findings presented by Marcus Raichle that demonstrated in some regions decreases in CBF, compared with the "at rest" or control condition, during cognitive tasks (Raichle, 1998). These findings were considered paradoxical because it is generally assumed in functional imaging studies that functional energy demand is increased during tasks. Various explanations were offered by workshop participants for this finding, including increased inhibition of total neuronal activity of the region by projecting dopaminergic and serotinergic neurons, an increase in inhibitory GABAergic neuronal activity, and physiological constraints on CBF that lead to an uncoupling between CBF and glucose metabolism. Robert Shulman proposed, based on his findings of high neuroenergetic activity in the resting cortex, that the negative CBF signal did not require a novel neurobiological explanation. He proposed that in these regions internal mental processes during the resting baseline condition simply have a greater neuroenergetic demand than the processes induced by the externally directed task (Shulman & Rothman, 1998). Although consensus was not achieved, the participants felt that the discussion was valuable in providing a specific example of how understanding the underlying neuroenergetics would distinguish interpretations of the role of a region in supporting cognitive processes.

Several discussants supported the importance of developing functional imaging methods of specific neuroenergetic processes. Douglas Rothman (Yale University) suggested that a lack of proportionality between the functional imaging signal and specific neuroenergetic processes could lead to false negatives if proportionality is assumed in the statistical analysis. These pitfalls may be particularly severe for studying patient groups with altered coupling between cerebral blood flow and glucose metabolism. Steven Foote (NIMH) stated that improved neuroenergetic specificity in functional imaging would be valuable for the studies his institute is funding to apply functional imaging to study psychiatric disease. Along these lines there were several stimulating discussions among the participants about how to extend present methodologies to achieve higher specificity.

How will neuroenergetically "correct" functional imaging help us understand how the brain works?
Although most of the conference focused on issues of understanding the neuroenergetic basis of the functional imaging signal, there remains the question of what are the possibilities and limits of functional imaging in studying brain function. Several discussants offered suggestions of how understanding the neuroenergetic basis of functional imaging may lead to novel understanding of cognition. Stephen Hanson (Rutgers University) proposed that there has been a lack of new insight into cognition provided by functional imaging due to unnecessary assumptions of the present statistical paradigms. These assumptions result in regions being identified as active only if the time course of the imaging signal agrees with the expectations of the experimenter. He suggested that less restrictive assumptions, made possible by improvements in functional imaging methods, will reveal novel insights into cognitive function. Several participants commented that as our understanding of the neuroenergetic meaning of the imaging signal is improved, it is essential to develop a parallel understanding of the "neuronal code," in which electrical and synaptic activity leads to information processing. Once this understanding is developed, neuroenergetically specific functional imaging, particularly ultra-high-resolution fMRI obtained with the new high magnetic field systems, may provide a window on information processing in humans at a neuronal network level.

CONCLUSIONS

Although there were many vigorous scientific disagreements, the participants could agree that the conference played an important role in identifying key areas where our understanding of neuroenergetics and functional imaging needs to be strengthened. The final session of the workshop concentrated on the future studies needed to address the questions raised during the meeting. Of particular concern is the critical need for research spanning the many levels of complexity separating neuroenergetic processes at a synaptic level from complex human behavior deriving from the coordinated activity of vast networks of neurons. Given the experimental uncertainties at all levels of study, the ability to test for consistency across levels will greatly strengthen our understanding of cognitive processes.

It was generally agreed that the gaps in our knowledge remaining after decades of research on cerebral glucose metabolism should send a cautionary note to those newly interested in brain imaging. The common usage of the functional image as synonymous with a neuronal activity map should be avoided or, at minimum, be care-

fully qualified. However, there was optimism generated by the progress being made in closing these gaps. The achievement of a detailed understanding of the neuroenergetic basis of the functional imaging signal was agreed to be critical for understanding the neurobiological basis of human cognitive behavior.

Note

1. A listing of the participants can be found on the Meetings Section of the JSMF website www.jsmf.org.

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