INTRODUCTION TO FUNCTIONAL MAGNETIC RESONANCE IMAGING

Principles and Techniques

RICHARD B. BUXTON
University of California at San Diego

CAMBRIDGE
UNIVERSITY PRESS

PUBLISHED BY THE PRESS SYNDICATE OF THE UNIVERSITY OF CAMBRIDGE
The Pitt Building, Trumpington Street, Cambridge, United Kingdom

CAMBRIDGE UNIVERSITY PRESS
The Edinburgh Building, Cambridge CB2 2RU, UK
40 West 20th Street, New York, NY 10011-4211, USA
10 Stamford Road, Oakleigh, VIC 3166, Australia
Ruiz de Alarcón 13, 28014 Madrid, Spain
Dock House, The Waterfront, Cape Town 8001, South Africa

http://www.cambridge.org

First published 2002

Printed in the United States of America

Typefaces Times Ten 10/13 pt. and Kabel *System* QuarkXPress™ [HT]

A catalog record for this book is available from the British Library.

Library of Congress Cataloging in Publication Data

Buxton, Richard B., 1954 –
Introduction to functional magnetic resonance imaging: principles
and techniques/Richard B. Buxton.
p. cm.
ISBN 0-521-58113-3 (hardback), ISBN 0-521-00275-3 (supplementary CD-ROM),
ISBN 0-521-00274-5 (hardback plus supplementary CD-ROM)
1. Brain – Magnetic resonance imaging. 2. Magnetic resonance imaging. I. Title.
RC386.6.M34 B895 2001
616′.047548–dc21 00-050235

ISBN 0 521 58113 3 hardback
ISBN 0 521 00275 3 supplementary CD-ROM
ISBN 0 521 00274 5 hardback plus supplementary CD-ROM

Contents

1

Energy Metabolism in the Brain

METABOLIC ACTIVITY ACCOMPANIES NEURAL ACTIVITY

Mapping Brain Activity

The goal of understanding the functional organization of the human brain has motivated neuroscientists for well over 100 years, but the experimental tools to measure and map brain activity have been slow to develop. Neural activity is difficult to localize without placing electrodes directly in the brain. Fluctuating electric and magnetic fields measured at the scalp provide information on electrical events within the brain. From these data the location of a few sources of activity can be estimated, but the information is not sufficient to produce a detailed map of the pattern of activation. However, precise localization of the metabolic activity that follows neural activity is much more feasible and forms the basis for most of the functional neuroimaging techniques is use today, including positron emission tomography (PET) and functional

magnetic resonance imaging (fMRI). Although comparatively new, fMRI techniques are now a primary tool for basic studies of the organization of the working human brain, and clinical applications are growing rapidly.

In 1890, William James published *The Principles of Psychology,* a landmark in the development of psychology as a science grounded in physiology (James, 1890). The possibility of measuring changes in brain blood flow associated with mental activity clearly lay behind the experiment performed by Angelo Mosso and recounted by James in the quotation at the beginning of Part IA. By current standards of blood flow measurement, this experiment is quaintly crude, but it indicates that the idea of inferring neural activity in the brain from a measurement of changes in local blood flow long preceded the ability to do such measurements (Raichle, 1998).

In fact, this experiment is unlikely to have worked reliably for an important reason. The motivation for this experiment may have been an analogy with muscle activity. Vigorous exercise produces a substantial muscle swelling due to increased blood volume, and thus a redistribution of weight. But the brain is surrounded by fluid and encased in a hard shell, so the overall fluid volume within the cranium must remain nearly constant. Blood volume changes do occur in the brain, and the brain does move with cardiac pulsations, but these changes most likely involve shifts of cerebrospinal fluid as well. As a result, the weight of the head should remain approximately constant.

Furthermore, this experiment depends on a change in blood volume, rather than blood flow, and blood flow and blood volume are distinct quantities. Blood *flow* refers to the volume per minute moving through the vessels, whereas blood *volume* is the volume occupied by the vessels. In principle, there need be no fixed relation between blood flow and blood volume. Flow through a set of pipes can be increased by increasing the driving pressure without changing the volume of the plumbing. Physiologically, however, experiments typically show a correlation between cerebral blood flow (CBF) and cerebral blood volume (CBV), and functional neuroimaging techniques are now available for measuring both quantities.

The working brain requires a continuous supply of glucose and oxygen, which must be supplied by CBF. The human brain receives 15% of the total cardiac output of blood, about 700 ml/min and yet accounts for only 2% of the total body weight. Within the brain the distribution of blood flow is heterogeneous, with gray matter receiving several times more flow per gram of tissue than white matter. Indeed, the flow per gram of tissue to gray matter is comparable to that in the heart muscle, the most energetic organ in the body. The activity of the brain generates about 11 W/kg of heat, and glucose and oxygen provide the fuel for this energy generation. Yet the brain has virtually no reserve store of oxygen, and thus depends on continuous delivery by cerebral blood flow. If the supply of oxygen to the brain is cut off, unconsciousness results within a few minutes.

The Energy Cost of Neural Activity

As is true for all organs, energy metabolism in the brain is necessary for the basic processes of cellular work, such as chemical synthesis and chemical transport. But the particular work done by the brain, which requires the high level of energy

metabolism, is the generation of electrical activity required for neuronal signaling. The connection between neural activity and energy metabolism is the foundation of functional neuroimaging, yet the physiological basis of this connection is still incompletely understood. To explore this connection, we begin by reviewing the basic processes involved in neural activity from the perspective of thermodynamics, in order to emphasize the essential role of energy metabolism. A more complete description can be found in Nicholls, Martin, and Wallace (1992).

The primary example of neural activity is the generation of an action potential and the release of neurotransmitter at a synapse. In the neuron there is an electric potential difference across the cell membrane, with the potential more negative inside. An *action potential* is a transient disturbance of that potential, a rapid depolarization of the membrane. The action potential propagates down the axon until it reaches a junction with another neuron at a *synapse,* and the arrival of the action potential then influences the firing of the second neuron by creating a local fluctuation in the postsynaptic potential. With an *excitatory postsynaptic potential* (EPSP) the potential inside is raised, moving the second neuron closer to firing its own action potential, and for an *inhibitory postsynaptic potential* (IPSP) the potential inside is decreased. Each neuron thus has the capacity to integrate the inputs from many other neurons through their cumulative effect on the postsynaptic potential. From an electrical viewpoint, the working neuron is an intricate pattern of continuously fluctuating membrane potentials punctuated by occasional sharp action potentials.

The resting potential, the action potential, and the fluctuating postsynaptic potentials all depend on maintaining the intracellular and extracellular concentrations of several ions in a state far from chemical equilibrium (Figure 1.1). For example, at rest there is an excess concentration of sodium (Na^+) ions and calcium (Ca^{++}) ions in the extracellular space and an excess concentration of potassium (K^+) in the intracellular space. In the absence of a potential difference across the cell membrane, the natural tendency of this system would be for a net diffusion of each ion species from higher to lower concentration. But because the electric potential inside the cell is negative compared to the outside, an electrical force that favors the motion of positive charges into the cell and negative charges out comes into play. The K^+ distribution is near equilibrium, in the sense that the tendency for the K^+ to diffuse down its concentration gradient and equalize the concentrations is balanced by the opposite tendency for the positive charges to accumulate on the negative potential side of the membrane. But the Na^+ distribution is far from equilibrium, and both the concentration gradient and the potential difference (the electrochemical gradient) across the membrane would tend to drive sodium into the cell. This is prevented at rest because the permeability of the membrane to sodium is very low.

However, the membrane permeability to sodium is sensitive to the voltage across the membrane so that, when the potential difference decreases, the permeability increases. The sodium permeability is a weak function of the potential inside the cell until the protential is raised to a critical threshold. Once this threshold is passed, the permeability increases sharply as the potential increases. The increased sodium flux into the cell raises the potential even more, further increasing the sodium flux. The result is a rapid depolarization of the membrane as the potential

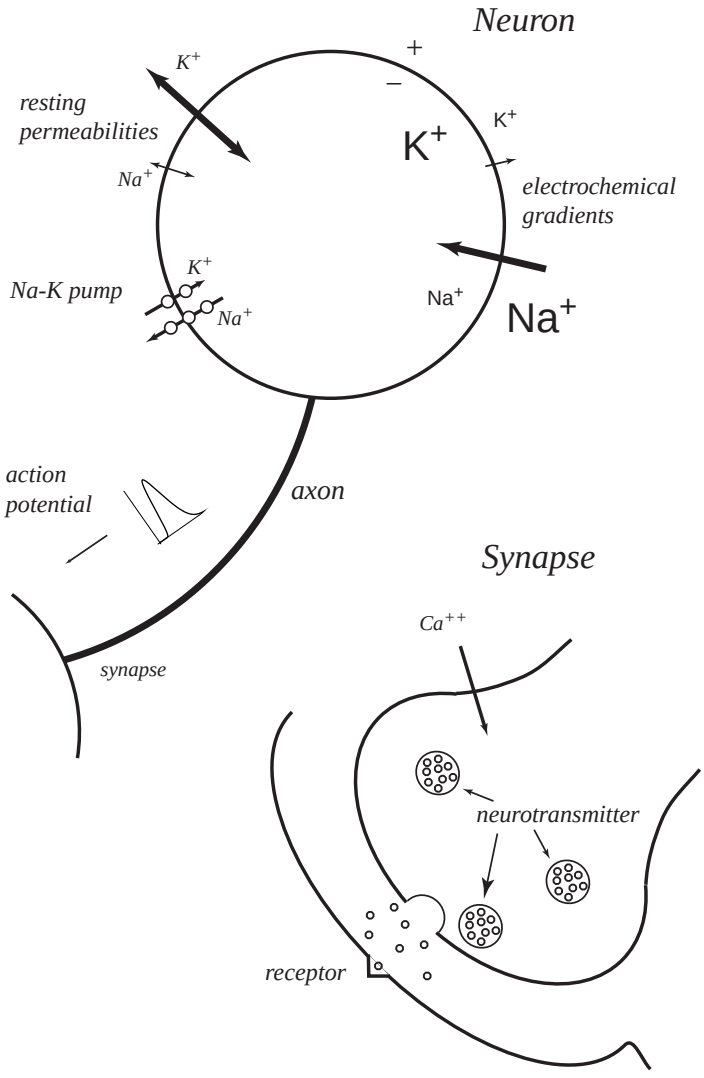


Figure 1.1. Neural activity. The schematic diagram shows the distribution and transport of the key ions, sodium, potassium, and calcium in the brain and the events following the arrival of an action potential at the synapse. The sodium distribution is maintained far from equilibrium by its low resting membrane permeability and by the action of Na-K-ATPase (the Na/K pump), which actively transports potassium into and sodium out of the cell. A transient increase in sodium permeability leads to a sharp depolarization of the membrane (an action potential), which travels down the axon and reaches the synapses with other neurons. The arrival of the action potential triggers an influx of calcium, which causes the prepackaged vesicles containing neurotransmitter to fuse with the membrane and spill into the synaptic cleft. Binding of the neurotransmitter to receptors on the postsynaptic neuron produces a postsynaptic fluctuation in the membrane potential. Recovery from neuronal signaling requires uptake and repackaging of neurotransmitter and restoration of ionic gradients, all uphill reactions that consume ATP.

inside the cell increases toward zero and even briefly becomes positive. The flux of sodium ions across the membrane in generating an action potential is then a passive approach to equilibrium as sodium flows down its electrochemical gradient and requires no driving energy. After a short time, the sodium permeability returns to

normal, and the potassium permeability increases to return the potential to its resting value. The result of this process is that there is a net flux of Na^+ into the cell and K^+ out of the cell during the depolarization.

The action potential travels down the axon as the small current through a patch of the membrane triggers a change in the sodium permeability of the next patch. The propagation of the action potential over a long distance would then require a small leak of ions down the entire length of the axon. In longer nerve fibers this leakage is minimized by the myelin sheath that surrounds the axon. Myelin is a poor conductor, so ion currents are small. But for the action potential to be able to propagate, the myelin sheath is periodically interrupted by bare patches called the nodes of Ranvier. At these nodes the sodium and potassium fluxes occur, effectively allowing the action potential to jump from node to node. Although the redistribution of ions in the creation of an action potential is small, it is nevertheless a degradation of the original distribution. Over time the neuron will run down as the ionic concentrations move toward equilibration.

At a synapse with another neuron, the arrival of the action potential triggers an increase of the membrane permeability to calcium, allowing Ca^{++} entry into the presynaptic terminal. Within the presynaptic terminal, neurotransmitter is concentrated in small packages called vesicles. Through mechanisms that are not completely understood, the influx of calcium triggers these vesicles to merge with the cell membrane and spill their contents into the synaptic gap. The neurotransmitter molecules drift across the gap and bind to receptor sites on the postsynaptic terminal. Glutamate is a common excitatory neurotransmitter (Erecinska and Silver, 1990). When the glutamate binds to the receptors, the postsynaptic potential is slightly depolarized, moving the neuron closer to producing an action potential of its own. In contrast, at an inhibitory synapse, the binding of the neurotransmitter causes a change in the postsynaptic potential that opposes depolarization, acting to inhibit the neuron from generating a new action potential. A common inhibitory neurotransmitter is gamma-aminobutyric acid (GABA), which is biochemically derived from glutamate. In each case, the action of the neurotransmitter is to alter the local membrane permeability, and a slight shift in the ionic concentrations then alters the local membrane potential. The effect on the second cell may be excitatory or inhibitory, depending on the type of synapse, but either way a signal has been sent from one neuron to another in the form of a slight shift in the postsynaptic potential.

From a thermodynamic point of view, each of these steps in neuronal signaling is a downhill reaction in which a system held far from equilibrium is allowed to approach closer to equilibrium. The high extracellular sodium concentration leads to a spontaneous inward ion flow after the trigger of a permeability increase occurs. Similarly, the calcium influx occurs spontaneously after its membrane permeability is increased, and the neurotransmitter is already tightly bundled in a small package waiting to disperse freely once the package is opened. We can think of neuronal signaling as a spontaneous, but controlled, process. Nature's trick in each case is to maintain a system away from equilibrium, waiting for the right trigger to allow it to naturally move toward equilibrium.

The production of EPSPs, IPSPs, and action potentials illustrates that the brain, like any physical system, is constrained by thermodynamics. We can think of the set of intracellular and extracellular ionic concentrations as a thermodynamic system whose equilibrium state would be one of zero potential difference across the cell membrane, with equal ionic concentrations on either side. Any chemical system that is removed from equilibrium has the capacity to do useful work, and this capacity is called the free energy of the system. The neuronal system, with its unbalanced ionic concentrations, has the potential to do work in the form of neuronal signaling. But with each action potential and release of neurotransmitter at a synapse, the free energy is reduced. Returning the neurons to their prior state, with the original ion gradients and neurotransmitter distributions, requires energy metabolism.

ATP Is the Common Energy Currency in the Body

Restoring the sodium and potassium gradients requires active transport of each ion against its natural drift direction and is thermodynamically an uphill process increasing the free energy of the system. For such a change to occur, the Na^+/K^+ transport must be coupled to another system whose free energy decreases sufficiently in the process so that the total free energy decreases. The reestablishment of ionic gradients thus requires a source of free energy, and in biological systems free energy is primarily stored in the relative proportions of the three phosphorylated forms of adenosine: adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) (Siesjo, 1978). Inorganic phosphate can combine with ADP to form ATP, but thermal equilibrium of this system at body temperature strongly favors the ADP form. Yet in the body, the ATP/ADP ratio is maintained at a far higher value, about 10 : 1 in the mammalian brain (Erecinska and Silver, 1994). The conversion of ATP to ADP thus involves a large release of free energy, enough to drive other uphill reactions. Despite the large free energy change associated with the reaction ATP to ADP, the ATP form is relatively stable against a spontaneous reaction. To make use of this stored free energy, the conversion of ATP to ADP is coupled to other uphill reactions through the action of an enzyme, generically referred to as an ATPase. The ATP/ADP system is used throughout the body as a common free energy storage system.

The transport of sodium and potassium against their existing gradients is accomplished by coupling the transport of ions to the breakdown of ATP to ADP. The enzyme Na-K-ATPase, also known as the Na/K pump, performs this task by transporting three sodium ions out of the cell and two potassium ions into the cell for each ATP molecule consumed. The Na/K pump is critical not just for energetic recovery from an action potential or a fluctuating postsynaptic potential, but also simply to maintain the cell's resting potential. The resting permeability to sodium is small, but not zero, so there is a constant leak of sodium into the cell. This excess sodium must be pumped out continuously by the Na/K pump, requiring a constant source of ATP. In addition, ATP is the indirect source of free energy for other processes that do not explicitly require ATP. For example, a mechanism exists to move bicarbonate ions into the cell in exchange for movement of chloride ions out of the cell (Thomas, 1977). The process is involved in the control of intracellular pH,

and for both ions the direction of transport is against the concentration gradient and so is a thermodynamically uphill process. The free energy for this transport comes from the sodium gradient itself, by coupling the transport to an influx of sodium down its electrochemical gradient. Ultimately, the sodium gradient must be restored by the action of the Na/K pump and the consumption of ATP.

The recovery from neural activity at the synapse similarly requires a number of uphill processes. The excess intracellular calcium is pumped out of the presynaptic terminal by two transport systems (Blaustein, 1988). One mechanism directly involves ATP, transporting one calcium ion out of the cell for each ATP consumed. The second system is driven by the sodium gradient, transporting one calcium ion out in exchange for an inward flux of three sodium ions. Note that by either transport system, one ATP is required to move one calcium ion out of the cell because in the second system the Na/K pump will ultimately be required to consume one ATP to transport the three sodium ions back out of the cell.

At the synapse, the neurotransmitter must be taken up by the presynaptic terminal and repackaged into vesicles. For glutamate the process of reuptake involves a shuttle between the astrocytes and the neurons (Erecinska and Silver, 1990). Astrocytes are one of the most common glial cells in the brain, frequently located in areas of high synaptic density. The glutamate from the synapse is transported into the astrocytes by coupling the passage of one glutamate with the movement of three sodium ions down the sodium gradient. The transport of the sodium back out of the cell requires the action of the Na/K pump and consumption of one ATP. In the astrocyte, the glutamate is converted to glutamine, which requires an additional ATP, and the glutamine is then released back into the synaptic gap. Glutamine does not bind to the glutamate receptors and so is inert as far as neuronal signaling is concerned.

The glutamine is passively taken up by the presynaptic terminal, where it is converted back to glutamate. Repackaging the glutamate into the vesicles then requires transporting the neurotransmitter against a strong concentration gradient, a process that requires more ATP. One proposed mechanism for accomplishing this is first to create a strong concentration gradient of H^+ ions, with the H^+ concentration high inside the vesicle (Erecinska and Silver, 1990). The inward transport of neurotransmitter is then coupled to a degradation of this gradient. The H^+ gradient itself is created by an ATP-powered pump.

In brief, a source of free energy is not required for the production of a neuronal signal but rather for the reestablishment of chemical gradients reduced by the action potential and the release of neurotransmitter at the synapse. Without this replenishment, the system eventually runs down like an old battery in need of charging. The restoration of chemical gradients is driven either directly or indirectly by the conversion of ATP to ADP. To maintain their activity, the cells must restore their supply of ATP by reversing this reaction and converting ADP back to ATP. This requires that the strongly uphill conversion of ADP to ATP must be coupled to an even more strongly downhill reaction. In the brain, virtually all the ATP used to fuel cellular work is derived from the metabolism of glucose and oxygen (Siesjo, 1978). Both oxygen and glucose are in short supply in the brain, and continued brain function requires continuous delivery of these metabolic substrates by CBF.

CEREBRAL GLUCOSE METABOLISM

In the preceding discussion, neural activity was discussed in terms of a thermodynamic framework in which uphill chemical processes are coupled to other, downhill processes. For virtually all cellular processes, this chain of thermodynamic coupling leads to the ATP/ADP system within the body. But the next step in the chain, the restoration of the ATP/ADP ratio, requires coupling the body to the outside world through intake of glucose and oxygen. Despite the fact that a bowl of sugar on the dining room table surrounded by air appears to be quite stable, glucose and oxygen together are far removed from equilibrium. When burned, glucose and oxygen are converted into water and carbon dioxide, releasing a substantial amount of heat. If a more controlled conversion is performed, much of the free energy can be used to drive the conversion of ATP to ADP, with metabolism of one glucose molecule generating enough of a free energy change to convert 38 ADP to ATP. As far as maintaining neural activity is concerned, the chain of thermodynamically coupled systems ends with glucose and oxygen. As long as we eat and breathe, we can continue to think.

But before considering how glucose metabolism works, we can consider how this chain of coupled thermodynamic systems extends to the rest of the world. The supply of glucose and oxygen is maintained by plants, which convert carbon dioxide and water into oxygen and organic compounds including glucose. The source of free energy for this strongly uphill process is sunlight, and the degradation of sunlight is coupled to these chemical reactions in photosynthesis. The source of the free energy of sunlight is that the photons, which started off in thermodynamic equilibrium when they left the sun, are far from equilibrium when they reach the earth. The energy density and the spectrum of photons in thermal equilibrium are determined by temperature, with higher energy photons at higher temperatures. The distribution of photon energies in the light leaving the sun is set by the sun's surface temperature (about 5700 K). As these photons travel away from the sun, they spread out so that the density of photons at the surface of the earth is much reduced. As a result, the photons arriving at earth have a spectrum characteristic of a 5,700 K source but an energy density equivalent to thermodynamic equilibrium at a temperature of only about 300 K. In other words, the photons arriving at the surface of the earth can be thought of as a system far from equilibrium, with the energy concentrated in high energy photons, whereas thermodynamic equilibrium favors more photons with lower energy. The degradation of these high-energy photons to low-energy photons thus releases a tremendous amount of free energy, which plants couple to chemical processes through photosynthesis. Life on earth thus depends on sunlight to drive chemical synthesis. It is interesting to note that it is not primarily the *heat* of the sunlight that is critical but rather the *spectrum* of the photons. Just as glucose and oxygen can combine when burned to produce heat, the free energy of the photons warms the surface of the earth. The same amount of heating could in principle be supplied by a lower temperature source of photons, but these photons would be inadequate to drive photosynthesis. So the existence of life on earth ultimately depends on the fact that the sun is hot enough to produce

high-energy photons, but far enough away so that the equilibrium temperature on earth is much lower.

Glycolysis and the TCA Cycle

We now turn to the question of how the combination of glucose and oxygen can be harnessed to produce ATP. The metabolism occurs in two stages: *glycolysis* and the *trans-carboxylic acid* (TCA) cycle (Figure 1.2). Glycolysis does not require oxygen but produces only a small amount of ATP. The further metabolism of glucose through the TCA cycle requires oxygen and produces much more ATP. Oxidative glucose metabolism involves many steps, and the following is a sketch of only a few key features. A more complete discussion can be found in Siesjo (1978).

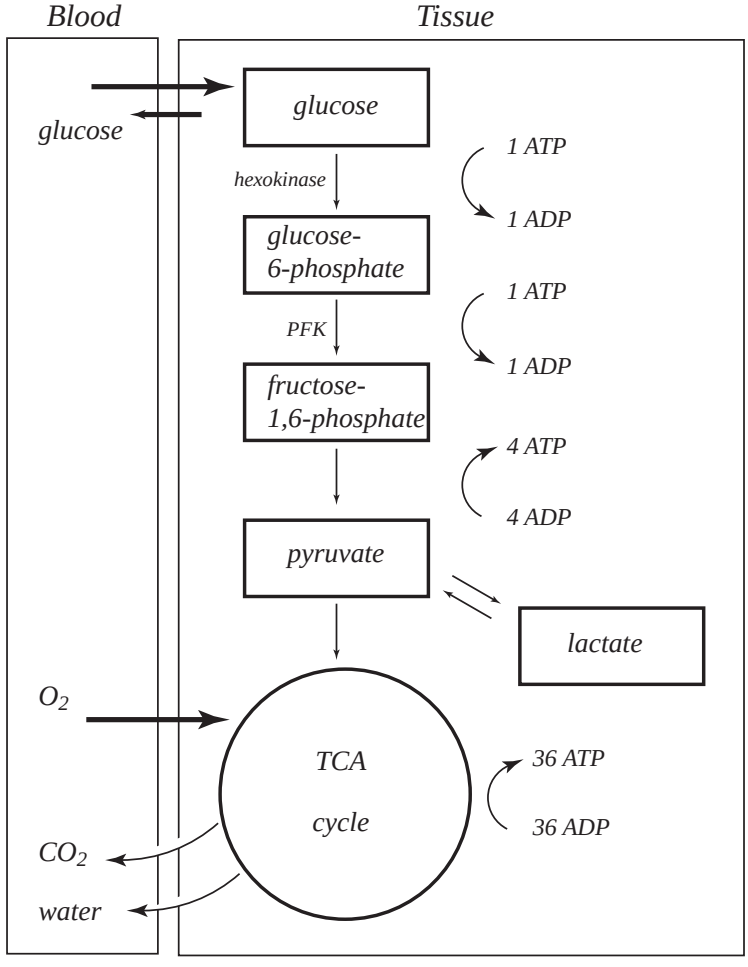


Figure 1.2. Schematic diagram of the major steps of cerebral energy metabolism. Glucose is taken up from blood and first undergoes glycolysis (the steps in boxes) to produce pyruvate, for a net conversion of 2 ADP to ATP. The pyruvate from glycolysis and oxygen extracted from the blood enter the TCA cycle and produce an additional 36 ATP. The waste products carbon dioxide and water are cleared from the tissue by blood flow.

In glycolysis, the breakdown of a glucose molecule into two molecules of pyruvate is coupled to the net conversion of two molecules of ADP to ATP. The process involves several steps, with each step catalyzed by a particular enzyme. The first step in this process is the addition of a phosphate group to the glucose, catalyzed by the enzyme hexokinase. The phosphate group is made available by the conversion of ATP to ADP, so in this stage of glycolysis one ATP is consumed and fructose-6-phosphate is produced. A second phosphorylation stage, catalyzed by phosphofructokinase (PFK), consumes one more ATP molecule. Up to this point two ATP molecules have been consumed, but in the remaining steps the complex is broken down into two pyruvate molecules accompanied by the conversion of four ADP to ATP. The net production of ATP is then two ATP for each glucose molecule undergoing glycolysis.

The possibilities for local control of glycolysis can be appreciated by noting that the activities of the key enzymes are sensitive to the local environment. Hexokinase is inhibited by its own product, so unless the fructose-6-phosphate continues down the metabolic path, the activity of hexokinase is curtailed. The step catalyzed by PFK is the major control point in glycolysis (Bradford, 1986). The enzyme PFK is stimulated by the presence of ADP and inhibited by the presence of ATP. In this way, there is a natural mechanism for increasing glycolysis when the stores of ATP need to be replenished. A number of other factors also influence the activity of PFK, including inhibition when the pH decreases, so it is likely that the cerebral metabolic rate of glucose (CMRGlc) can be adjusted to meet a variety of demands.

If the pyruvate is not further metabolized, it is reversibly converted to lactate through the action of the enzyme lactate dehydrogenase. The end point of glycolysis is then the production of two ATP molecules and two lactate molecules from each glucose molecule. But glycolysis alone taps only a small fraction of the available free energy in the glucose, and utilization of this additional energy requires further metabolism of pyruvate in the TCA cycle. In the healthy brain, nearly all the pyruvate produced by glycolysis is destined for the TCA cycle. The TCA cycle involves many steps, each catalyzed by a different enzyme, and the machinery of the process is housed in the mitochondria. Pyruvate (or lactate) and oxygen (O_2) must enter the mitochondria to become available for metabolism. At the end of the process, carbon dioxide and water are produced, and an additional 36 ATP molecules are created. The full oxidative metabolism of glucose thus produces about 18 times as much ATP as glycolysis alone. The overall metabolism of glucose is then

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O\ (+\ 38\ ATP)$$

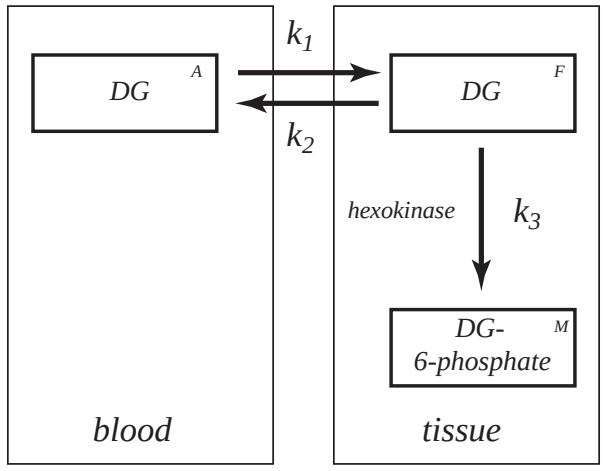
Blood flow delivers glucose to the brain, but only about 30% or less of the glucose that enters the capillary is extracted from the blood (Oldendorf, 1971). Glucose does not easily cross the blood brain barrier, and a transporter sysem is required (Robinson and Rapoport, 1986). This type of transport is called *facilitated diffusion,* rather than active transport, because no energy metabolism is required to move the glucose out of the blood. Glucose simply diffuses down its gradient from a higher concentration in blood to a lower concentration in tissue through particular chan-

nels (transporters) in the capillary wall. The channels have no preference for which way the glucose is transported; consequently, they also transport unmetabolized glucose out of the tissue and back into the blood. Once across the capillary wall, the glucose must diffuse through the interstitial space separating the blood vessels and the cells and enter the intracellular environment. There the glucose enters into the first steps of glycolysis. But not all the glucose that leaves the blood is metabolized. About half of the extracted glucose diffuses back out into the blood and is carried away by venous flow (Gjedde, 1987). That is, glucose is delivered in excess of what is required at rest. The net extraction of glucose, the fraction of glucose delivered to the capillary bed that is actually metabolized, is only about 15%. Carbon dioxide, the end product of glucose metabolism, diffuses out of the cell and into the blood and is carried off to the lungs to be cleared from the body.

The Deoxyglucose Technique for Measuring Glucose Metabolism

The development of the deoxyglucose (DG) technique was a landmark in the evolution of functional neuroimaging techniques (Sokoloff, 1977; Sokoloff et al., 1977) (Figure 1.3 and Box 1). With this method it became possible to map the pattern of glucose utilization in the brain with a radioactive tracer, whose distribution in an animal brain can be measured by a process called autoradiography. In *autoradiography,* a radioactive nucleus is attached to a molecule of interest and injected in an animal. After waiting for a time to allow the tracer to distribute, the animal is sacrificed, and the brain is cut into thin sections. Each section is laid on photographic film to allow the photons produced in the decay of the radioactive nucleus to expose the film. The result is a picture of the distribution of the agent at the time of sacrifice.

However, autoradiography cannot be used with labeled glucose itself because the brain concentration of the tracer at any single time point is never a good reflection of the glucose metabolic rate. Suppose that glucose is labeled with a radioactive isotope of carbon (e.g., ^{14}C). At early times the amount of tracer in the tissue does not reflect the local metabolic rate because some of that tracer will diffuse back out into the blood and will not be metabolized. If we wait a longer time, the unmetabolized tracer may have cleared, but some of the ^{14}C tracer that was attached to the glucose that *was* metabolized has also cleared as carbon dioxide. In short, to measure the glucose metabolic rate with labeled glucose, measurements at multiple time points are required, and this cannot be done with autoradiography. It is this central problem that was solved with the deoxyglucose method. Deoxyglucose differs from glucose only in the removal of one of the oxygen atoms. This analog of glucose is similar enough to glucose that it binds with the enzyme hexokinase catalyzing the first step of glycolysis. But because of the difference between DG and glucose, the DG cannot proceed down the glycolysis pathway, and the process halts after the DG has been converted to fructose-6-phosphate. The result is that the radioactive label on DG essentially sticks in the tissue. It cannot proceed down the metabolic path, and the clearance of the compound from the tissue is very slow. After a sufficient waiting period to allow clearance of the unmetabolized fraction, the tissue concentration of the label is a direct, quantitative reflection of local glucose metabolism (Figure 1.3).



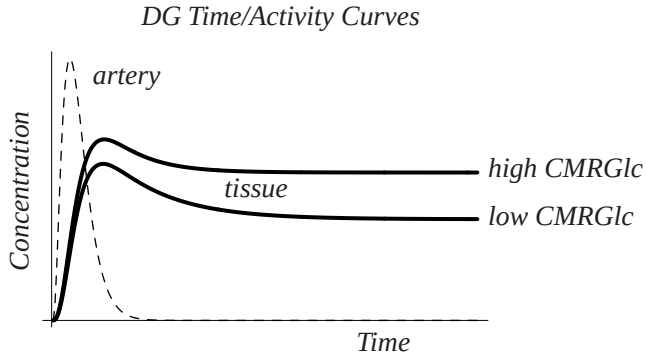


Figure 1.3. The deoxyglucose method for measuring the cerebral metabolic rate of glucose. The DG is metabolized similarly to glucose through the essentially irreversible phosphorylation catalyzed by hexokinase, but it cannot proceed farther and remains trapped in the tissue. The tissue concentration of the DG over time then shows an initial peak because more DG is taken up from the blood than will ultimately be metabolized. After a sufficient time for clearance of this unmetabolized fraction of the tracer, the tissue concentration directly reflects the metabolic rate.

With the adaptation of the DG method to positron emission tomography, studies of glucose metabolism were extended to the working human brain. Carbon-14, the radioactive tracer used in the DG autoradiographic method, cannot be used in humans because the electron emitted in the decay of the nucleus has a very short range in tissue, producing a large radiation dose in the subject but virtually no detectable external signal. In PET the radioactive tracers used are nuclei with an excess ratio of protons to neutrons, and the decay produces a positron. A positron is the antiparticle of an electron, with all the same properties as an electron except for an opposite sign of its charge. Normal matter contains only electrons, so a positron is an exotic particle. Positrons are emitted with substantial kinetic energy, which is dissipated within a few millimeters of travel through the tissue. When the positron has

BOX 1. DEOXYGLUCOSE TRACER KINETICS

In determining metabolic rates or CBF with radioactive tracers, the dynamic quantities that potentially can be measured are the arterial concentration and the tissue concentration curve over time. These time/activity curves are interpreted in terms of underlying physiological processes with a kinetic model, and we can illustrate the general approach with the deoxyglucose method. The uptake and metabolism of DG is modeled as shown in the upper part of Figure 1.3, with three compartments representing arterial blood *(A),* free unmetabolized tissue DG *(F),* and metabolized tissue DG *(M),* with the assumption that the metabolized form remains trapped in the tissue during the experiment. In compartmental modeling such as this, each compartment is assumed to be well mixed and described by an instantaneous uniform concentration *C.* The kinetics of the tracer are then described by

$$\frac{dC_F}{dt} = k_1 C_A(t) - k_2 C_F(t) - k_3 C_F(t)$$

$$\frac{dC_M}{dt} = k_3 C_F(t)$$

The parameters k_1, k_2, and k_3 are first-order rate constants. In the first equation, the three terms on the right describe, respectively, the delivery of DG by arterial flow, clearance of unmetabolized DG passed back to the venous blood, and metabolism of DG. The arterial concentration curve $C_A(t)$ drives the system, and the resulting total tissue concentration $C_T(t) = C_F(t) + C_M(t)$ then depends on the values of k_1, k_2, and k_3.

Ideally, the values of the k's for glucose and DG would be the same (i.e., transport and metabolism of the two molecules would be identical up to the point at which DG stops). Unfortunately, this is not the case, so a correction must be applied. But for now we can assume that glucose and DG behave identically to show how the tracer kinetic curve of DG is quantitatively related to the cerebral metabolic rate of glucose. If glucose metabolism is in a steady state with arterial glucose concentration C_0, then the rate at which glucose is delivered to the tissue is $k_1 C_0$. The fraction of this extracted glucose that continues down the metabolic path, rather than exiting into the blood, is $k_3/(k_2 + k_3)$. The metabolic rate (moles/g-min) is then

$$\text{CMRGlc} = C_0 \frac{k_1 k_3}{k_2 + k_3}$$

Turning now to the dynamic DG curves illustrated in Figure 1.3, there is an initial peak in the concentration, but over time C_T plateaus to a constant level. The peak occurs because more DG enters the tissue than will ultimately be metabolized, and the plateau occurs when the concentration in the first tissue compartment *(F)* has fallen to zero. In other words, by this time all the extracted tracer has either proceeded down the metabolic path or cleared from the tissue by venous flow. The important question is: How is the plateau DG concentration related to the k's? We can answer this question with reasoning similar to that used earlier for glucose, taking into account the dynamic nature of the arterial DG concentration $C_A(t)$. The amount of DG entering the tissue in a short interval dt is $k_1\, C_A(t)dt$, and so the total amount delivered during the experiment is the

integral of this term. But only a fraction $k_3/(k_2 + k_3)$ of this extracted DG is metabolized and trapped, so the plateau tissue concentration is

$$C_T(\infty) = \frac{k_1 k_3}{k_2 + k_3} \int_0^\infty C_A(t)\, dt$$

This is the same combination of k's needed to measure CMRGlc, so the final expression is

$$CMRGlc = \frac{C_0}{LC} \frac{C_T(\infty)}{\int C_A\, dt}$$

where we have also included the lumped constant LC, which accounts for the fact that the k's are not the same for glucose and DG. The lumped constant is determined empirically by comparing DG measurements with CMRGlc estimates derived with another method (Reivich et al., 1985).

Although one could analyze the entire time/activity curve of DG to make separate estimates of each of the k's, the power of this technique is that the plateau concentration alone directly reflects CMRGlc. (In practice, corrections can be made for residual DG in blood at the time of measurement, and some loss of the metabolized DG, but these are usually small corrections.) The integrated arterial curve and the lumped constant essentially define a global scaling factor that converts measured DG concentrations into units of CMRGlc. For studies of absolute CMRGlc such scaling is necessary, but for comparisons within a study (e.g., comparing CMRGlc in two different brain regions) a map of DG concentration alone is sufficient.

slowed sufficiently, it will annihilate with an electron. In this process the positron and the electron cease to exist, and two high-energy photons are created. In this annihilation process, energy and momentum are conserved, with the energy of each photon equal to the rest mass energy of an electron (511 KeV), and the photons are emitted in two directions close to 180° apart.

The emitted positron thus annihilates within a few millimeters of its origin, but the two photons travel through the tissue and can be measured with external detectors. Furthermore, because two photons traveling in opposite directions are produced, the detectors can be coordinated to count only *coincidence* detections, the arrival of a photon in each of two detectors within a very narrow window of time. The detection of such a coincidence then determines the origin of the photons, the site of the radioactive nucleus, to lie on a line between the two detectors. The total count of photons along a ray is proportional to the sum of all the activity concentrations along the ray. By measuring many of these projections of the radioactivity distribution, an image of that distribution can be reconstructed in an analogous way to x-ray computed tomography (CT) images.

Positron emitting nuclei are particularly useful for human metabolic imaging because the nuclei are biologically interesting (e.g., ^{11}C, ^{15}O), the radioactive half-lives are short, and the decay photons readily pass through the body and so can be detected. A short half-life is important because it reduces the radiation dose to the

subject, but this also requires that the isotope be prepared shortly before it is used, typically requiring an on-site cyclotron.

The PET version of the DG techniques uses ^{18}F-fluoro-deoxyglucose (FDG) as the tracer (Phelps and Mazziotta, 1985; Reivich et al., 1979). Fluorine-18 decays by positron emission with a half-life of about 2 hr. The tracer is injected in a subject, and after a waiting period of about 45 min to allow unmetabolized tracer to clear from the tissue, a PET image of the distribution of the label is made. In fact, PET images can be acquired throughout this period to measure the local kinetics of the FDG. Such time/activity curves can be analyzed with a kinetic model to extract estimates of individual rate constants for uptake of glucose from the blood and for the first stage of glycolysis (see Box 1). But the power of the technique is that the distribution of the tracer at a late time point directly reflects the local glucose metabolism.

To derive a quantitative measure of glucose metabolism with either the DG or FDG technique, two other quantities are required (see Box 1 for details). The first is a record of the concentration of the tracer in arterial blood from injection up to the time of the PET image (or the time of sacrifice of the animal in an autoradiographic study). The integrated arterial time/activity curve describes how much of the agent the brain was exposed to and essentially provides a calibration factor for converting the amount of activity measured in the brain into a measure of the local metabolic rate. The second quantity that is needed is a correction factor to account for the fact that it is really the metabolic rate of DG, rather than glucose, that is measured. This correction factor is called the *lumped constant* because it incorporates all the factors that make the uptake and phosphorylation rate of DG differ from glucose. An important question for the interpretation of FDG-PET studies in disease states is whether the lumped constant remains the same, and this question is still being investigated (Reivich et al., 1985).

The Association of Glucose Metabolism with Functional Activity

Over the last two decades numerous animal studies have clearly demonstrated a close link between local functional activity in the brain and local glucose metabolism (Kennedy et al., 1976; Schwartz et al., 1979; Sokoloff, 1981). An early monkey study examining the effects of visual occlusion showed a clear demonstration that the striate cortex is organized in alternating ocular dominance columns. This organizational pattern was known from previous, painstaking recordings from many cells, but the autoradiogram showed the full pattern in one experiment. These experiments also demonstrated that glucose metabolism decreases in association with a decrease of functional activity. When only one eye was patched, the ocular dominance columns associated with the patched eye appeared lighter (less exposed) on the autoradiogram than the columns corresponding to the open eye. With reduced visual input from the patched eye, glucose metabolism was also reduced.

Activation studies, in turn, showed an increase of glucose metabolic rate in the functionally active regions (Schwartz et al., 1979). Furthermore, with functional activity of different degrees, the change in glucose metabolism also showed a graded response (Kadekaro, Crane, and Sokoloff, 1985). The connection between functional

activity and glucose metabolism through ATP-dependent processes was demonstrated by an experiment in which the activity of the Na/K pump was blocked by a specific inhibitor, with the result that the increase of glucose metabolism with electrical stimulation was suppressed (Mata et al., 1980). In short, animal studies with DG and autoradiography, and human studies with FDG and PET (Phelps and Mazziotta, 1985), have found a close correspondence between local neural activity and local glucose metabolism.

The Location of Glucose Metabolism in the Brain

In the brain, the consumption of glucose is heterogeneous. The metabolic rate in gray matter is three to four times higher than that in white matter. The low metabolic rate in white matter suggests that the energy cost of sending an action potential down an axon is small, most likely because of the efficient propagation along myelinated fibers. Instead, the energy metabolism is more closely associated with the synapses. Within the layers that make up cortical gray matter, the glucose metabolic rate is highest in layer IV, an area rich in synaptic connections. This area also shows the largest changes in CMRGlc with activation. High-resolution studies of the precise location of the increased glucose metabolism suggest that it is not the cell body of the neuron, but rather these areas of dense synaptic connections that show the largest increase in metabolic rate (Sokoloff, 1991).

The regions exhibiting high glucose metabolic rates also contain high concentrations of astrocytes, one of the nonneuronal cell types that make up about half of the brain. A recent theory proposes that glycolysis occurs preferentially in the astrocytes, and the resulting lactate is shuttled to the neurons for further metabolism by the TCA cycle in the mitochondria. Based primarily on work with cultures of astrocytes and neurons from mouse cerebral cortex and the retina of the honeybee drone (Magistretti and Pellerin, 1996; Tsacopoulos and Magistretti, 1996), the theory presents an appealing picture (Figure 1.4). Astrocytes are closely connected to the blood supply, with projecting endfeet that surround the capillary, so they are well positioned for uptake of glucose from the blood. Furthermore, glycolysis in astrocytes is stimulated by glutamate, a common neurotransmitter, and astrocytes are intimately involved in the uptake and reprocessing of this neurotransmitter. Glutamate released at the synapse is taken up by the astrocyte, converted to glutamine, and returned to the extracellular space where it is taken up by the neurons and converted back to glutamate, as described earlier. This suggests a possible mechanism for coupling neural activity to energy metabolism: the release of glutamate at the synapse stimulates glycolysis and lactate production, and the lactate is then transported to the neurons for oxidative metabolism and the further generation of ATP. In addition, the energy cost to the astrocyte of taking up one glutamate and converting it to glutamine is two ATP, which can be precisely met with the glycolysis of one glucose molecule. Additional support for this hypothesis was recently presented based on NMR studies of the metabolism of glucose labeled with ^{13}C (Sibson et al., 1998). With NMR it is possible to follow the chemical fate of the labeled carbon as it enters the brain as glucose and enters the glutamate pool through the TCA cycle. These studies in the rat cerebral cortex in vivo found that

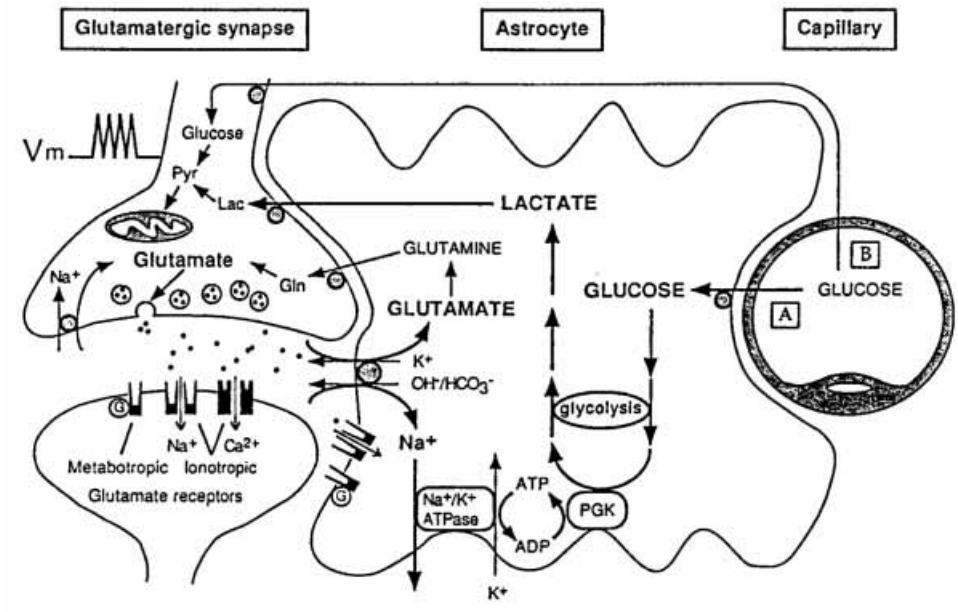


Figure 1.4. The role of astrocytes in energy metabolism. The astrocytes are closely involved in the uptake of the neurotransmitter glutamate from the synaptic cleft, and the conversion of it to glutamine, which then is taken up by the neuron and converted back to glutamate. By a current theory of energy metabolism in the brain, glycolysis occurs primarily in the astrocytes, and the lactate produced is then shuttled to the neurons for further oxidative metabolism. (Reprinted with permission from Magistretti and Pellerin, *Cerebral Cortex* **6:**50–61, 1996; copyright 1996 by Oxford University Press.)

the rate of glutamate-neurotransmitter cycling was closely matched to the rate of oxidative glucose metabolism. This model directly illustrates the close integration of neural activity and energy metabolism.

REFERENCES

Blaustein, M. P. (1988) Calcium transport and buffering in neurons. *Trends Neurosci.* **11,** 438–43.

Bradford, H. F. (1986) *Chemical Neurobiology.* W. H. Freeman and Co.: New York.

Erecinska, M., and Silver, I. A. (1990) Metabolism and role of glutamate in mammalian brain. *Prog. Neurobiol.* **35,** 245–96.

Erecinska, M., and Silver, I. A. (1994) Ions and energy in mammalian brain. *Prog. Neurobiol.* **43,** 37–71.

Gjedde, A. (1987) Does deoxyglucose uptake in the brain reflect energy metabolism? *Biochem. Pharmacol.* **36,** 1853–61.

James, W. (1890) *The Principles of Psychology.* Harvard: Cambridge, MA.

Kadekaro, M., Crane, A. M., and 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,** 6010–13.

Kennedy, C., Rosiers, M. H. D., Sakurada, O., Shinohara, M., Reivich, M., Jehle, J. W., and Sokoloff, L. (1976) Metabolic mapping of the primary visual system of the

monkey by means of the autoradiographic [14C]deoxyhemoglobin technique. *Proc. Natl. Acad. Sci. USA* **73,** 4230–4.

Magistretti, P. J., and Pellerin, L. (1996) Cellular bases of brain energy metabolism and their relevance to functional brain imaging: evidence for a primary role of astrocytes. *Cerebral Cortex* **6,** 50–61.

Mata, M., Fink, D. J., Gainer, H., Smith, C. B., Davidsen, L., Svaki, H., Schwartz, W. J., and Sokoloff, L. (1980) Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. *J. Neurochem.* **34,** 213–15.

Nicholls, J. G., Martin, A. R., and Wallace, B. G. (1992) *From Neuron to Brain.* Sinauer: Sunderland, MA.

Oldendorf, W. H. (1971) Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Am. J. Physiol.* **221,** 1629–39.

Phelps, M. E., and Mazziotta, J. C. (1985) Positron emission tomography: Human brain function and biochemistry. *Science* **228,** 799–809.

Raichle, M. E. (1998) Behind the scenes of functional brain imaging: A historical and physiological perspective. *Proc. Natl. Acad. Sci., USA* **95,** 765–72.

Reivich, M., Alavi, A., Wolf, A., Fowler, J., Russell, J., Arnett, C., MacGregor, R. R., Shiue, C. Y., Atkins, H., and Anand, A. (1985) Glucose metabolic rate kinetic model parameter determination in humans: The lumped constants and rate constants for [18F]-fluorodeoxyglucose and [11C]-deoxyglucose. *J. Cereb. Blood Flow Metabol.* **5,** 179–92.

Reivich, M., Kuhl, D., Wolf, A., Greenberg, J., Phelps, M., Ido, T., Cassella, V., Fowler, J., Hoffman, E., Alavi, A., Som, P., and Sokoloff, L. (1979) The [18F]-fluoro-deoxyglucose method for the measurement of local cerebral glucose measurement in man. *Circulation Res.* **44,** 127–37.

Robinson, P., and Rapoport, S. I. (1986) Glucose transport and metabolism in the brain. *Am. J. Physiol.* **250,** R127–36.

Schwartz, W. J., Smith, C. B., Davidsen, L., Savaki, H., Sokoloff, L., Mata, M., Fink, D. J., and Gainer, H. (1979) Metabolic mapping of functional activity in the hypothalamo-neurohypophysial system of the rat. *Science* **205,** 723–5.

Sibson, N. R., Dhankhar, A., Mason, G. F., Rothman, D. L., Behar, K. L., and Shulman, R. G. (1998) Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc. Natl. Acad. Sci., USA* **95,** 316–21.

Siesjo, B. K. (1978) *Brain Energy Metabolism.* Wiley: New York.

Sokoloff, L. (1977) Relation between physiological function and energy metabolism in the central nervous system. *J. Neurochem.* **29,** 13–26.

Sokoloff, L. (1981) The relation between function and energy metabolism: its use in the localization of functional activity in the nervous system. *Neurosci. Res. Prog. Bull.* **19,** 159–210.

Sokoloff, L. (1991) Relationship between functional activity and energy metabolism in the nervous system: Whether, where and why? In: *Brain Work and Mental Activity: Quantitative Studies with Radioactive Tracers,* pp. 52–67. Eds. N. A. Lassen, D. H. Ingvar, M. E. Raichle, and L. Friberg. Munksgaard: Copenhagen.

Sokoloff, L., Reivich, M., Kennedy, C., Rosiers, M. H. D., Patlak, C. S., Pettigrew, K. D., Sakurada, O., and 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.

Thomas, R. C. (1977) The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. *J. Physiol.* **273,** 317–38.

Tsacopoulos, M., and Magistretti, P. J. (1996) Metabolic coupling between glia and neurons. *J. Neurosci.* **16,** 877–85.