Brain Energetics and Neuronal Activity

Applications to fMRI and Medicine

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R G Shulman and D L Rothman
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Foreword

My experiences over the past 30 years have led me to the realization that there are two kinds of researchers in the biological sciences: those who almost immediately recognize the centrality of metabolism to a full understanding of biological function and those who do not. The latter group always seems to represent a larger proportion of biological scientists, so that the focus of biological research tends to drift from studying metabolism into various phenomenological directions, until one realizes, as one inevitably does, that metabolism, particularly the metabolism of cellular energy production, what is often termed 'intermediary metabolism' is the key to inter-level analysis of biological systems. Metabolism provides the bridge from the genetic to the cellular and from the cellular to the organismic. Sooner or later, therefore, the hard search for truth comes back to addressing and unraveling the complexities of intermediary metabolism and abandoning the quest for a 'magic bullet'. This return to a focus on metabolism is becoming unavoidable for scientists studying the influence of gene expression patterns on biological function. Likewise, as the chapters in this book document, cognitive neuroscientists searching for an understanding of the mechanisms by which neuronal assemblies process information can not avoid acquiring a deeper awareness of brain energy and intermediary metabolism. Brain structure – function relationships can only be clearly seen through a metabolic lens.

As I neared completion of my doctoral research in the Laboratory of Cerebral Metabolism at Cornell University Medical College in 1984, I confronted the metabolism versus non-metabolism dichotomy head on. Although devoted to studying brain energy metabolism, my mentors advised me to forsake such studies and become more 'molecular' if I intended to have a viable research career. They had my best interests at heart; in their eyes, the era of traditional metabolic neurochemistry was on the wane. The classic approach of using metabolic encephalopathies (including ischemia, hypoxia, and hypoglycemia) to probe how perturbations in cerebral oxidative energy metabolism altered conscious states (e.g. lethargy, stupor, and coma) was becoming passé. To be fair, my advisors were not entirely incorrect. The general emphasis in neurochemical studies in the 1970s and 1980s concentrated on detailing how the supply of glucose and oxygen for energy metabolism supported neuronal function. The key observation (not yet made but described fully in this volume) needed to advance the field was the critical link between the need to sustain the high rate of energy metabolism and its driving force – glutamate neurotransmitter turnover.

Luckily, the solution to my career dilemma revealed itself while I browsed in the library. Serendipity, that often unacknowledged contributor to careers and discoveries, led me to one of the most important books I have ever read: *Biological Applications of Magnetic Resonance* edited by R.G. Shulman (1979 Academic Press New York, San Francisco and London). Shulman's volume profiled several directions of research using nuclear magnetic resonance (NMR) spectroscopy to study metabolic reactions *in vivo*. It was immediately apparent to me that this approach, if applied to the brain, might be able to discover the Holy Grail of neurochemistry – the ability to monitor the metabolic correlates of neural information processing. *In vivo* NMR spectroscopy, because of its ability to monitor energy metabolism in the intact brain, *in vivo*, in real time, provided a novel approach for investigating the all-important tight coupling of cerebral metabolism and neuronal function. Not surprisingly, Bob Shulman's laboratory at Yale University was

already actively pursuing this line of research – and soon after provided me a new intellectual home. NMR spectroscopy breathed renewed life into the pursuit of what remains a somewhat elusive goal – unraveling the complex inter-relationships among cerebral function, cerebral structure, and cerebral metabolism.

How closely the traditional tools of neurochemistry can monitor changes in the concentrations of intermediary and energy metabolites and accurately mirror real time *in vivo* levels is hampered by the unique dependence of brain function on blood flow and cerebral metabolism as thoroughly documented in the chapter by Sokoloff. The few seconds of hypoxia, ischemia, or hypoglycemia inflicted during the time between obtaining brain tissue for study and doing the study unavoidably alters the concentrations of energy metabolites and destroys the functional cyto-architecture. During the 1970s a significant percentage of neurochemists' research efforts were directed toward developing ingenious methods to minimize experimental post-mortem changes of intermediary and energy metabolite concentrations. Researchers pursued every strategy to freeze the metabolite levels by limiting the effects of ischemia and hypoxia so as to mirror the *in vivo* concentrations present at the experimental time of interest Techniques such as *in situ* freezing preserved the structural integrity of the brain tissue, but risked uneven rates of inactivation of metabolic processes, particularly for deeper brain structures. The 'brain blowing' technique introduced by Bud Veech, a rather extreme apparatus using air pressure to extrude the brain from the cranium, provided shorter extraction and freezing times, but destroyed the anatomy. What was needed, if the field was to progress, were methods that could monitor blood flow and metabolism linked to normal cognitive and behavioral states in the intact brain, *in vivo*, in real time.

The development of the 2-deoxyglucose (2-DG) autoradiography technique in the laboratory of Louis Sokoloff in the 1970s offered neurochemists the ability to measure regional rates of glucose utilization in animal models and to begin to link metabolism with physiology in the absence of induced pathology. Soon thereafter, positron emission tomography made possible, for the first time, measurement of regional cerebral metabolic rates for glucose (FDG-PET) and oxygen (O-18 PET) in human subjects in real time as they engaged in behavioral tasks.

For studies correlating regional changes in cerebral metabolism with regional changes in neural activity, 2-DG autoradiography and PET have some limitations. The analysis of 2-DG autobiographic data occurs in brain slices, post-mortem. Each individual animal in an experimental design provides data on cerebral metabolic rates for many different brain regions but for only a single time point. Human PET studies provide measurements of cerebral metabolism with excellent temporal series and spatial resolution, but the short-lived radio-isotope labeled compounds needed to conduct the experiments limited research opportunities. Furthermore, the need for intravenous injections of radio-isotopes limited the subject pool and essentially eliminated the possibility of pursuing certain types of studies, such as those involving healthy children. Additionally, 2-DG autoradiography and FDG-PET are not identical in every aspect, somewhat complicating comparisons between human and animal studies. *In vivo* NMR spectroscopy offered an alternative approach which allowed direct comparison of animal models with human subjects and was also not dependent on the use of radioactive tracers.

As the development of *in vivo* NMR spectroscopic techniques for metabolic measurements was rapidly progressing, other applications of MR physics allowed scientists to obtain remarkably detailed anatomical images of the intact, living, human body. The brain images produced by magnetic resonance imaging (MRI) were particularly stunning; they revealed the 3-D complexity of the brain at a degree of resolution far surpassing that of contemporaneous imaging devices such as CAT scans. In the late 1980s, the physics of MRS and MRI led to the discovery of what could, arguably, be considered one of the 20th century's most powerful advances in the ability to monitor brain activity in the intact subject. In 1990 Seiji Ogawa and colleagues at Bell Laboratories published the first study using BOLD MRI, often referred to in the literature as functional magnetic resonance imaging or fMRI, to monitor ongoing brain activity related to ongoing behavior.

The ability to use PET and fMRI measurements to localize cognitive processes within a particular volume of neural tissue is based on the assumption that functional neuronal activity, and therefore metabolism, increase when a region is involved in the neural computations that implement a cognitive task. The functional neuronal activities involved in the communication of information between neurons include neurotransmitter synthesis, packaging, and release followed by the subsequent generation of action potentials. As explained above, the energy required for these and other brain processes is provided almost exclusively by oxidative glucose metabolism. Regions of increased functional energetic demand are identified by the corresponding increase in glucose metabolism, or the coupled parameters of the cerebral metabolic rate for oxygen (CMRO2) and the cerebral blood flow rate (CBF). Depending on the radio-labeled tracer used, PET can measure glucose consumption, oxygen utilization, or CBF. Interpreting the fMRI BOLD signal changes is somewhat more complicated because BOLD is sensitive to both $CMRO_2$ and CBF.

The cognitive neuroscience community quickly embraced BOLD fMRI despite the pitfalls of equating changes in a signal comprising several complex physiological parameters to changes in neuronal 'activity'. Cognitive neuroscience emerged as a unique academic discipline in the late 1980s from the deliberate efforts of scientists interested in identifying the neural correlates of cognition by integrating the experimental and theoretical traditions of cognitive psychology, systems and computational neuroscience, linguistics, and philosophy. BOLD fMRI seemed ideally suited for pursuing cognitive neuroscience's stated goals. Cognitive neuroscientists' enthusiasm for fMRI studies led to a rapid and exponential growth in institutional MR centers. These centers concentrated their resources on developing methods for marrying psychological studies to functional imaging. Unfortunately, their lack of interest in pursuing metabolic or mechanistic research meant there was not a corresponding commitment to nurturing brain research with *in vivo* NMR spectroscopy.

The lack of an appreciation by many cognitive neuroscientists (particularly those entering the field primarily trained as psychologists or systems neuroscientists) of the centrality of energetics to understanding how information is processed in the brain has resulted in a cognitive neuroscience that acts as though there has not been a century of effort expended on unraveling the mechanisms coupling metabolism, blood flow, and function in the brain. Cognitive neuroscientists interpreting changes in the BOLD signal as direct measurements of the neural correlates of human cognition and behavior without understanding the energetic and metabolic correlates of neural activity stand on theoretically shaky ground. It is the energetic and metabolic correlates of neuronal information processing that are coupled to the hemodynamic signals.

There are several important questions integral to the understanding of the neural basis of cognition that cannot be answered with functional imaging studies but that can be investigated with MR spectroscopy. Functional neuroimaging studies proceed from an assumption that the observed signal changes reflect neuronal activity, even though other cell types, most notably astrocytes, are known to be important contributors to overall brain energy metabolism and neurotransmitter function. Pierre Magistretti and his colleagues have published a series of papers identifying the astrocyte as the primary site of cerebral glucose consumption. Magistretti has proposed that astrocytic glucose uptake is mechanistically coupled to neuronal energy requirements via non-oxidative glycolysis to provide energy for removing glutamate, an excitatory neurotransmitter 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. The chapter by Sibson summarizes the *in vivo* MRS results from rat and human brain detailing the flux from glucose to glutamate that support and extend Magistretti's findings. ^{13}C NMR spectroscopic studies have shown that the rate of astrocytic glutamate uptake increases with glucose metabolism at a close to 1:1 stoichiometry.

In vivo MRS findings offer insights into the underlying nature of the BOLD signal. This statement does not imply that MRS research should be embraced *merely* because it offers the hope of 'quantitating' BOLD. Rather, as is made clear by several chapters in this volume, MRS functional neuro-energetic studies offer

cognitive neuroscientists the ability to pursue an entirely new level of analysis of brain function. The series of PNAS papers by Hyder *et al*., described in detail in this volume, provides a biological underpinning for theoretical models of unconscious and conscious information process such as that proposed recently by Stan Dehaene and Jean Pierre Changeux. Slowly but surely, cognitive neuroscience is becoming aware that the small physiological differences detected by BOLD imaging are the mere tip of an iceberg. Functionally, the high rate of ongoing neuronal activity, representing close to 80 % of total cerebral energy metabolism, may be of greater interest. *In vivo* MRS studies, together with fMRI, allow us to build an integrated science of brain/behavior by bridging from cognition to cerebral oxidative energy metabolism and by linking neuronal information processing with glutamate neurotransmitter function.

It is rare to discover a single volume that both summarizes and celebrates decades of biological research while also pointing out a path to the future. In 20 years I have come across only two. Not surprisingly, they both emphasize that we cannot understand biological function without understanding metabolism and both are edited by Bob Shulman.

Susan Fitzpatrick
September 2003
http://anestit.unipa.it/reference/bibliography/snacc/Metab3.html

Section A

Background

1

Introduction

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This book illustrates and celebrates recent NMR investigations of brain, identifying its molecular constituents and following their metabolic activities *in vivo*. This physical, spectroscopic method is anchored in its two origins – its present technical methods descended from Bloch and his colleagues and its present metabolic information derivable from the simple, beautiful NMR experiments on water of Bloembergen, Purcell and Pound. Water continues as an endlessly fascinating material and in the present volume plays the central role in MRI and fMRI experiments. From the beginning NMR studies have depended upon interaction between atomic nuclei and their chemical environment and have been valuable reporters of these environments. These interactions have been readily interpreted because of two simplifying characteristics of the NMR phenomena. First spin $\frac{1}{2}$ nuclei of the common elements, e.g. hydrogen, carbon and phosphorus, interact very weakly with their environment relative to the strong forces they can experience in available laboratory magnetic fields. Accordingly environmental interactions affecting chemical shifts, multiplet structure and relaxation times, being weak compared to these Zeman interactions, are perturbations and as such are readily and quantitatively evaluated. The second simplifying property that allows reliable information about the nuclei's surroundings is that NMR phenomena, in contrast to most spectroscopic methods, take place in the ground electronic state, and thereby provide a well-established basis for interpreting the perturbations by the molecular environment. As a quantitatively understandable measure of molecules and their interactions NMR has grown continually in these years. It has become a prime method

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for the study of matter – in chemistry, condensed matter physics, geology, biochemistry and in all studies of materials.

Studies of brain metabolism are playing a growing role in neuroscience. Cerebral metabolic pathways date back to the exciting beginnings of physiological chemistry, when Charles Sherrington proposed that 'the blood supply of any part of the cerebral tissue is varied in accordance with the activity of the chemical changes which underlie the functional activation'. Activities of brain were then to be explained by the same criterion that physiologists were using in exploring the chemical basis of muscle and heart. Methods for measuring cerebral inputs of glucose and oxygen that have been developed since 1950 have established the basic dependence of cerebral energetics upon the oxidation of glucose. Cerebral blood flow has been measured by similar methods so that neurophysiology has shown that blood flow follows energy consumption and thereby links brain chemistry with the organism.

In more recent years non-invasive methods of nuclear magnetic resonance and positron emission tomography (PET) have played increasingly important roles in brain studies. Magnetic resonance imaging (MRI) has revealed cerebral anatomy with great specificity and has helped to localize the more chemical findings of PET, functional magnetic resonance imaging (fMRI), and magnetic resonance spectroscopy (MRS). These metabolic methods have now been developed to the point that their findings contribute substantially to brain science.

This book describes recent contributions of magnetic resonance methods to studies of brain energetics and function. The value of magnetic resonance, particularly MRI, has been evident from its first human applications. High-resolution non-invasive images moved clinical studies into a better-informed era. A little more than a decade after, fMRI made parallel advances in the localization of stimulated brain activity. MRS, particularly in measurements by ^{13}C-label flow, has provided information relating brain metabolism and neurotransmitter flows. The contributions of ^{13}C MRS to brain studies form the unifying theme underlying this book. The insights into metabolism, provided by MRS and PET, have depended to some extent upon the great powers of MRI and fMRI while, in turn, they have led to some re-interpretation of these imaging methods. Together these magnetic resonance methods have opened new understandings of brain function and now offer new directions for research.

Although in retrospect a smooth research path can be traced through the past decades leading to a metabolic model of brain function, nonetheless certain obstacles to research planning unique to the brain had to be overcome. The very nature of the work done by the brain was not as obvious as in other organs. Muscle work was readily defined and measured as force through a distance. Nor did muscle power expenditures, easily measured as the rate of work, present any conceptual difficulties. The chemical basis of cerebral work, like the analogous work done by the heart, is now being expressed by the studies presented in this book, in similar terms, as molecules and ions pumped across gradients by ATP. We have obtained this analogous definition and quantitation of brainwork from the experimental (Sibson) and theoretical (Laughlin) research described in Chapters 5 and 7.

However, these very recent results had to overcome paradigmatic ideas about the unimportance of energy for brainwork that had been formed on the basis of very few studies. Until recently the generally accepted belief in the field, from a comparison with the squid axon, considered only $\sim$1 % of brain energy production to be devoted to neuronal firing. Only when the multi-synapses of the mammalian brain were specifically included did calculations support the MRS results that nearly all the brain energy was devoted to neuronal firing. The negligible role assumed for cerebral energetics had been reinforced by the suggestion that stimulated brain activity was supported by non-oxidative glycolysis despite the main energy source being the oxidation of glucose. The recent stoichiometries showing efficient use of glucose oxidation for energy had to overcome these widespread views.

Perhaps the more strongly held obstacles came from residual phenomenological dualism. It was difficult to realize that the thinking process of mind required work, based upon the hydrolysis of ATP, just as

a muscle did work when lifting weights. This traditional difficulty in formulating brainwork slowed the efforts to calculate such work energetically. But that relationship has now been quantified as described in the following pages, and provides a sturdy foundation for the study of the more complex aspects of brain. As this understanding advances it leads to consideration of the interactions between these aspects of neuroscience with the broad field of psychology. To what extent does modern psychology further our understanding of neurophysiology or, conversely, in what way does the understanding gained about brain activity and energetics advance psychological conceptualization? Functional imaging of brain localizes brain activity non-invasively and quickly – but how does this methodology illuminate our understanding of brain activity? And how does understanding energy consumption help to expand the scope of functional imaging? Finally, underlying all these questions, how does brain energy metabolism relates to neuroscience, the study of brain components, structure and action at the cellular and molecular level?

Out of the broad fields that are presently actively studying the functioning brain, the studies reviewed here have focused upon a simple concept, namely brain energy consumption and neuronal activity are chemical processes. Brain activity is not merely powered by chemical reactions – that activity, in fact, consists of chemical reactions. Brain energetics compares the production and use of energy, usually mediated through ATP, in which glucose is metabolized. Neuronal activity is the sum of chemical reactions by which information is processed. This activity we show can be profitably followed by measurements, *in vivo*, of the rates of chemical reactions. Chemical reactions *in vivo* do not occur in isolation, as they do in a test tube. *In vivo* all reactions are linked to others and the linked reactions form a network that is conveniently defined as metabolism. In the brain the chemical reactions of ion fluxes across membrane, neurotransmitter processing and the electrical actions of neuronal spiking are all coupled to energy consumption and the control of metabolism.

The unique perspective of this book derives from the ability to measure cerebral metabolic fluxes, in the living human and animal brains, by *in vivo* NMR methods. These quantitative assessments of metabolite concentrations and rates of reaction, *in vivo*, provide the unique, strong data set, that has allowed metabolism, studied this way, to provide a platform for novel insights into biological function.

1.1. BACKGROUND

The first four chapters provide background information of a historical and technical nature. The NMR studies of the brain, which form the core of this book are discussed in the early chapters. NMR methods, magnetic resonance spectroscopy (MRS) have measured *in vivo* concentrations and kinetics of molecular metabolites. These small molecules, e.g. glutamate, glutamine, lactate, glucose, etc., are studied as the components of energetic and neurotransmitter pathways. The early physiological studies of cerebral glucose and oxygen uptake established the chemical basis of brain energetics, showing that glucose oxidation is the main energy source, have been summarized by Lou Sokoloff, *a pioneer of this field*, in Chapter 2. Dr. Sokoloff's development of the deoxyglucose tracer extended these studies, which originally were primarily based on arterial venous difference measurements, to high resolution mapping of brain functional activity based upon glucose uptake and flow. This deoxyglucose mapping remains the gold standard to which more recent non-invasive modalities such as fMRI are compared. In addition these studies also formed the basis for the development of PET studies of human glucose metabolism using the deoxyglucose tracer. A key finding in these studies, the understanding that glucose oxidation provides the brain energy, was re-inforced and confirmed *in vivo* by ^{13}C MRS measurements of the flux from labeled substrates particularly 1-^{13}C glucose, through the (tricarboxylic acid) TCA cycle. Numerical values of the cerebral metabolic rate of oxygen consumption obtained from these MRS measures of TCA cycle flux agreed with the results from the deoxyglucose autoradiography and PET methods in animals and humans respectively.

Chapter 3 by Robin de Graaf introduces NMR methods used in the book. NMR of cerebral metabolism obtains quantitative measurements by combining the regional specificity of imaging methods with the

chemical identification and quantitation of metabolites which has been developed extensively by high resolution NMR. The description is at a simple level, suitable for anyone who has taken (and remembers some of) a course in organic chemistry. From this base more advanced methods used in MRS studies are developed and are referred to throughout the other reports.

Since the core of quantitation lies in hypothesizing and evaluating a model of *in vivo* fluxes, this subject is reviewed in Chapter 4 by Graeme Mason. 'Modeling' of data is used in a very positive way in these studies, in that the 'model' represents bundled hypotheses about the *in vivo* reaction pathways. As such the models serve to evaluate fluxes from data but also serve to test the hypothesis about the parameter being evaluated. In a particularly simple application, a model predicts that the value of a particular flux must be the same (within experimental error) when that flux is measured in different, independent experiments. Values of rates obtained by modeling the data depend upon statistical fits of the model to the data. A simplified, yet informative description of such calculation forms a central feature of this chapter.

1.2. ENERGY AND NEUROTRANSMISSION

Section B includes descriptions of the experimental and theoretical determinations of *in vivo* metabolic rates, energy consumption in the form of oxygen usage and neuronal work done by neurotransmission and spike activity. A key novel finding of this work is that energy consumption is coupled to the release of the neurotransmitter glutamate upon neuronal firing and its subsequent recycling through glutamine. This flux was proposed in the 1970s and was established as the major pathway by the ^{13}C experiments. In Chapter 5 by Nicola Sibson, studies are described in which ^{13}C-labeled glucose and other labeled precursors are followed into glutamate and glutamine pools in human and animal brains by *in vivo* ^{13}C MRS. The rate of label appearance in glutamate has provided a measure of the tricarboxylic acid cycle flux V_{TCA}, which is a direct measure of neuronal energy consumption. The flux into the glutamine pool, measured in the same experiment by *in vivo* ^{13}C MRS, is used to quantitate the rate of glutamate neurotransmitter release and recycling. The quantitation shows that there is a one-to-one change of glutamate neurotransmitter flux with neuronal glucose oxidation, i.e. for each additional molecule of glutamate released as a neurotransmitter there is one molecule of glucose oxidized in neurons. This stoichiometry allows changes in the cerebral rate of energy consumption to be converted directly into change of neuronal signaling, and provides key information for understanding the energetic and molecular mechanisms which couple glutamate neurotransmission to energy consumption. The proposal linking the *in vivo* results to the cellular findings of Magistretti and coworkers that astroglial glutamate uptake depends on glycolytic ATP are described. It provides a chemical measure of neuronal activity, which has previously been undefined, and in conformity with the message of this book, shows that the rate of neuronal activity is defined by the flux through a metabolic pathway.

These total fluxes of glutamate to glutamine neurotransmitter cycling reflect the sum of glutamate and GABA neurotransmitter flow. GABA is the most abundant inhibitory neurotransmitter in the cortex. Chapter 6, by Kevin Behar and Douglas Rothman, shows how GABA neurotransmitter release and cycling can be distinguished from the total glutamate to glutamine cycling. The contributions of GABA to the total glutamate plus GABA neurotransmitter cycling flux has been shown to be significant. The chapter describes how the use of other labeled substrates, in particular labeled acetate, allows this distinction to be made, and sets the stage for future studies to understand the relationship between inhibitory and excitatory neurotransmission and energetics, as well as subcellular metabolic compartmentation of GABA synthesis.

In Chapter 7, Laughlan and Attwell review their energy budget for glutamate neurotransmitter cycling. Taking literature values for the different steps in the pathway of neuronal firing with glutamate release and cycling they have broken the energy requirements into different steps. Most of this energy is consumed in pumping the neuronal ion gradients after firing with approximately equal energies devoted to pre- and post-synaptic pumping. As expected the energy requirements for the chemical steps of glutamate release and

conversion to glutamine and recycling back into the glutamate vesicles is small compared to the neuronal energy requirements. Assuming the glutamate concentration of vesicles, the efficiency of neuronal firing and based on a firing rate of 4 Hz, they find reasonable agreement with the observation by ^{13}C MRS that a large majority of the energy is devoted to neuronal activity and only ~20 % is devoted to non-functional neuronal energy needs commonly called housekeeping. Their energy budget provides a valuable framework for relating energy consumption, measured by ^{13}C MRS methods to detailed energetic sub-processes. It also provides a framework for studies combining MRS and MRI with inhibitors of specific energetic sub-processes to test the understanding of the energetic basis of function in detail.

The next two chapters present very recent NMR methods for measuring the basic neuroenergetic parameter, CMR_{O2}. High magnetic fields have increased NMR sensitivity so that direct ^{17}O NMR experiments can determine the rate of oxygen consumption by following the label flow to $H_2^{17}O$, as shown in Chapter 8 by Wei Chen. These quite direct measurements of oxygen usage are a substantial advance in sensitivity over previous $^{1}HNMR$ methods using indirect detection of $H_2^{17}O$ relaxation times. Furthermore they have potentially similar spatial and temporal resolution as PET CMR_{O2} measurements, with the advantages of more accurate quantitation and the ability to perform multiple repeated studies due to the absence of radioactive isotope. Careful studies of this direct measurement of CMR_{O2} have elucidated the methodology of similar PET measurements.

The BOLD fMRI, is known to depend upon complex interactions of NMR relaxation times, CMR_{O2}, CBF and Cerebral Blood Volume (CBV). In Chapter 9, Hyder describes his method of determining ΔCMR_{O2} from BOLD measurements. This required associated measurements of ΔCBF by a spin tagging imaging experiment, of ΔCBV by the use of iron contrast reagents and of the spin-spin relaxation times. Values of ΔCMR_{O2} agreed with values obtained by an indirect $^{1}HNMR$ detection of the ^{13}C turnover of glutamate. The high resolution results of this method, giving increments of ΔCMR_{O2} can, by connections to absolute CBF – spin tagging results, be converted to absolute values. Values of ΔCMR_{O2} obtained this way are compared with the values measured by the flows of ^{13}C-labeled glucose in the same experiments. This calibration brings together functional imaging and metabolic energetics. In place of the phenomenological connections offered by other investigators between functional imaging signals and arbitrarily selected variables, this calibration relates the fMRI signal mechanistically to a meaningful neurophysiological parameter. Once CMR_{O2} is derived from the BOLD signal in this way, the relationships between energy and neuronal activity established throughout the earlier chapters become available with the temporal and spatial resolutions of the calibrated BOLD measurement. This connection forms the basis of interpreting the BOLD effect in later chapters. Both of these newer methods of determining CMR_{O2} are shown to agree with classical methods of oxygen consumption with significant advantages over earlier methods.

In Chapter 10 single electrode recordings of neuronal firing rates are regionally coordinated with values of CMR_{O2} obtained from calibrated BOLD measurements. Neuronal firing rates, or spiking, are for many the ‘gold standard’ of neuronal activity. To measure these rates, individual neurons are identified in the rat somatosensory cortex, located during forepaw stimulation by BOLD experiments, and their firing rates followed through changes in depth of anesthesia and during sensory stimulation. The ensemble of neurons, followed in this way, correlates deployments of the neuronal population with the total energetics. Changes during the stimulation are shown to involve the majority of neurons in the voxels, rather than a selected few. Implications of these results for neuronal function are explored here, and in several of the subsequent chapters.

1.3. CLINICAL BEGINNINGS

Applications of magnetic resonance results to epilepsy and the psychiatric diseases clearly indicate directions for future study. Mesial temporal lobe epilepsy, one of the most common and debilitating forms, has

been shown by these measurements in conjunction with PET and microdialysis to be a disease in which the energetics and the glutamate neurotransmitter cycling have been impaired. In Chapter 12, Petroff and Spencer describe applications of ^{13}C MRS to the glutamate glutamine cycling in the hippocampus. They show that the degree of neuronal loss/damage in the hippocampus correlates well with the degree of impairment. A model is proposed in which astroglial metabolic impairments lead to reduced glutamate uptake, explaining the elevated extracellular glutamate (and resultant hyperexcitability) during even the quiescent interictal periods between seizures.

The concentration of NAA is shown by Hetherington *et al*. in Chapter 11 to depend upon the energetic state of the mitochondria NAA decreases, reflecting a decrease in mitochondrial energetics, are proposed to be accompanied by more rapid release of glutamate. The flux from neurons is measurable by ^{13}C MRS. Combining the results from this two pronged study of temporal lobe epilepsy we see that (1) the epileptic focus has lower NAA; (2) this reflects decreased mitochondrial energetics which (3) leads to a more rapid release of glutamate, thereby suggesting a cause for the (4) high concentrations of extracellular glutamate previously observed. An additional mechanism that presumably contributes to the high glutamate levels is seen in (5) the decreased rate of glutamate neurotransmitter cycling in the epileptogenic focus. Clearly these novel, sturdy *in vivo* insights offer interventional opportunities for preventing epileptic seizures.

From another direction the concentration of GABA has been shown to correlate quantitatively with the mood of normal subjects. The variation of GABA levels during alcoholism and in the subsequent withdrawal period has promise for understanding features of addiction. In the more daunting problems of depression and schizophrenia, preliminary results of neurotransmitter activity give promising correlations. The approach from magnetic resonance as from so many directions is mainly confined to phenomenological correlations. The problem faced by magnetic resonance in studying mental illness resembles that of other physical methods, immersed as they all are in the cyclical problem of having to define the problem while studying it. The definite information about the depressed condition reported by Graeme Mason in Chapter 11 provides an exciting starting point for future studies.

1.4. BRAIN AND MIND

Several implications of the metabolic studies for the planning and interpretations of fMRI experiments are proposed in the book. The interpretations of fMRI experiments in light of these results are discussed in this section. The metabolic results are presented in six chapters of Section B and interpreted in Chapter 16. They emphasize that the differencing methods used in fMRI experiments, measure changes in neuronal activity between the baseline and stimulated conditions. fMRI by discarding the baseline activity neglects the majority of neuronal activity which has been measured in Chapters 5 and 10 where it was shown to participate in the brain's response to the stimulus. One implication for the usual functional imaging signal is that, while increases or decreases of signal show activity changes in the region, still the absence of changes does not mean the absence of activity. Furthermore the magnitude of the change, being dependent upon the baseline activity, does not measure the magnitude of brain activity during the task.

Chapter 14 applies some of these concepts to survey and evaluate fMRI studies of declarative memory in humans. The conventional differencing method has been employed in the experiments surveyed and information about the pathways of memories reviewed to show how differencing, if used carefully, can illuminate mental processes. It is suggested that the absence of incremental signals in the hippocampus during traditional memory tasks arises from the high baseline activities, in accordance with the ^{13}C MRS results. The wide variety of enriched memory tasks that can provoke incremental fMRI signals are examined closely in the search of a unifying conceptual basis.

Chapter 15 reviews selective fMRI results to explore the accomplishments and limitations of localizing activations. Beyond the static mapping of brain responses this chapter investigates effects of the changing

nature of the response, of the task and of the subject upon the meaningfulness of the results. Chapter 16 considers the consequences of the present metabolic findings for the investigation of complex brain activities including subjectivity. It emphasizes how the quantitative description of baseline neuronal activity, reached in the early chapters, should not be ignored by any psychology of mind. In the resting awake state, there is a high level of cortical energy consumption dedicated to neuronal signaling. It is suggested that the high baseline activity is caused by wide neuronal connections which support consciousness. These results describe a brain that accommodates and is intrinsically dependent upon subjective experience. The metabolic findings of highly active baseline activities are proposed to provide boundary conditions for a view of mind in which subjectivity and consciousness are not neglected.

The last chapter summarizes conclusions reached in the book, and suggests possible future directions.

2

Energy Metabolism in Neural Tissues *in vivo* at Rest and in Functionally Altered States

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2.1. INTRODUCTION

The biochemical pathways of energy metabolism in brain are in most respects like those of other tissues, but special conditions peculiar to the central nervous system produce obstacles to studies of intermediary metabolism of the brain *in vivo*. For example, the blood–brain barrier restricts the exchange of substrates and products between brain and blood, and, consequently, biochemical reactions normally operating within the brain tissues are often not detectable with traditional *in vivo* methods based on exchange of substrates and metabolites between the blood and brain. *In vitro* studies best serve to identify pathways of intermediary

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metabolism, biochemical mechanisms, and potential rather than actual performance, but valid identification of the ultimate substrates and products of cerebral energy metabolism and their rates of utilization and production under conditions of normal brain function can be obtained only *in vivo*.

The first definitive studies of cerebral energy metabolism *in vivo* began with the development of the nitrous oxide method by Kety and Schmidt (1948). This method made it possible to determine the rates of cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$). Originally designed for use in man it was also applicable in animals, and, furthermore, it could be used in the unanesthetized state, a particularly important requirement to study the normal functioning brain. The method was based on the indirect Fick principle which states that, when a chemically inert tracer (i.e. one that is neither consumed nor produced by a given tissue) is introduced into the circulation, the rate of change in its content in any tissue is equal to the difference between the rates at which it is brought to that tissue in the arterial blood and removed from it in the venous blood. The method originally employed low concentrations of nitrous oxide as the tracer but was subsequently adapted for use with inert radioactive gases, e.g., ^{85}Kr, ^{79}Kr, and ^{133}Xe. Inasmuch as application of the Fick principle requires sampling of both arterial and representative cerebral venous blood for determination of their tracer concentrations, cerebral arteriovenous differences for a variety of substrates and products of cerebral metabolism could also be measured and their rates of utilization or production by the brain calculated as the product of these arteriovenous differences and CBF.

Numerous studies with this method established that cerebral energy metabolism is extraordinarily active in the unanesthetized state (Clarke and Sokoloff, 1999). For example, in normal, conscious, young adult men the brain comprises only about 2 % of total body weight, but it alone consumes about 20 % of the total resting body oxygen consumption. Studies with the method also established that glucose is not only an essential but normally the almost exclusive substrate for the brain's energy metabolism. Under normal conditions the brain utilizes glucose in almost stoichiometric amounts with the oxygen required for the complete oxidation of glucose to CO_2 and H_2O (Table 2.1). For complete stoichiometry 6 mol of O_2 are consumed and 6 mol of CO_2 produced per mole of glucose utilized, i.e., a molar ratio (O_2/glucose ratio) of 6.0; the respiratory quotient (R.Q.) (ratio of CO_2 production to O_2 consumption) is then 1.0. Under normal conditions cerebral glucose utilization (CMR_{glc}) not only accounts for all the O_2 consumed, but exceeds the rate for complete stoichiometry with $CMRO_2$ by about 20 %, and the measured O_2/glucose ratio is about 5.5 (Table 2.1). In ketotic states, such as those due to starvation, fat-feeding, diabetes, etc., the blood levels of the ketone bodies, D-β-hydroxybutyrate and acetoacetate, are elevated, and these can substitute partly but not completely for glucose as the substrate for the brain's

Table 2.1. Rates of and relationship between cerebral oxygen consumption and glucose utilization in normal, conscious, young adult men

Function	Rate
O_2 consumption (μmol/100 g of tissue/min)	156
Glucose utilization (mmol/100 g of tissue/min)	31
O_2/glucose ratio (mol/mol)	5.5
Glucose equivalent of O_2 consumption (μmol/100 g tissue/min) (assuming 6 mol O_2 per mol of glucose)	26
Excess of total glucose utilized above glucose oxidized (mmol/100 g of tissue/min)	5
CO_2 production (μmol/100 g of tissue/min)	156
Cerebral respiratory quotient (R.Q.)	0.97

oxidative metabolism (Owen *et al*., 1967; Krebs *et al*., 1971); in such cases the O_2/glucose ratio rises above 6.0.

Numerous studies with the nitrous oxide method and its derivatives demonstrated that cerebral energy metabolism declines in parallel with reduced levels of consciousness. For example, $CMRO_2$ was moderately lower in conditions in which the sensorium was impaired but not to the level of unconsciousness, e.g., diabetic acidosis, moderate hypoglycemia, cerebral arteriosclerosis, etc., and markedly depressed in conditions with complete unconsciousness or coma, e.g., general anesthesia, diabetic coma, insulin coma, hepatic coma, and cerebral ischemia due to increased intracranial pressure caused by brain tumors (Kety, 1950; Clarke and Sokoloff, 1999). All these were, however, pathological conditions in which energy metabolism was probably reduced due to disrupted intracellular biochemical processes, insufficient substrate supply, or lesser demand for energy because of pathologically depressed neuronal functional activity. In physiological and pharmacological states with obviously altered mental functions, not necessarily altered levels of consciousness, such as in schizophrenia, LSD intoxication, mild alcoholic inebriation, sedation, mental exercise during solving of arithmetic problems, etc., no changes in $CMRO_2$ were found. The failure of the nitrous oxide method to detect a relationship between energy metabolic rate and functional activity in the brain was undoubtedly due to the fact that it measured only average rates of oxygen and glucose utilization in the brain as a whole and not in the specific regions of the brain where the functional activities were localized.

2.2. LOCAL CEREBRAL ENERGY METABOLISM

The capability to determine local rates of energy metabolism in specific regions of the brain *in vivo* first became available with development of the 2-[^{14}C]deoxyglucose ([^{14}C]DG) method (Sokoloff *et al*., 1977). This method measures local rates of glucose utilization simultaneously in all regions of the nervous system. Furthermore, it is fully applicable to unanesthetized animals, thus avoiding the complications of alterations of neural functions and metabolism by anesthetic agents. Because of the close to stoichiometric relationship between oxygen and glucose utilization in brain, glucose utilization is generally as good a measure of cerebral energy metabolism as oxygen consumption. 2-[^{14}C]Deoxyglucose (2-[^{14}C]DG) was used because it is an analog of glucose with which it competes for blood–brain transport into the brain and for phosphorylation by hexokinase to their hexose-6-phosphate derivatives. Unlike glucose-6-phosphate, however, which is very rapidly metabolized further via the glycolytic and tricarboxylic pathways ultimately to H_2O and CO_2, 2-[^{14}C]DG-6-phosphate can be metabolized to some extent to 2-[^{14}C]DG-1-phosphate, 2-[^{14}C]DG-1-6-phosphate, and ^{14}C-labeled glycogen, but all the products of 2-[^{14}C]DG phosphorylation remain trapped in the cells. Consequently, all the labeled products of 2-[^{14}C]DG phosphorylation accumulate with time and are retained within the cells with negligible loss for considerable lengths of time. From a kinetic analysis of the behavior of glucose and tracer concentrations of 2-[^{14}C]DG in brain an operational equation was derived with which to compute the local rates of CMR_{glc} from the measured times courses of arterial plasma glucose and 2-[^{14}C]DG concentrations and the local tissue isotope concentration at the end of the experimental period, usually 30–45 min. Local ^{14}C concentrations in identifiable anatomical structures throughout the brain are determined by the use of quantitative autoradiography (Figure 2.1), a technique originally developed for measurement of local cerebral blood flow in animals with chemically inert, diffusible radioactive tracers (Landau *et al*., 1955; Freygang and Sokoloff, 1958). The autoradiographs obtained with the 2-[^{14}C]DG method can be converted by computerized image-processing techniques into color-coded quantitative images of the brain in which local rates of CMR_{glc} are encoded in the color scale. These processed autoradiographic images provide, therefore, the means to visualize local rates of glucose utilization throughout the brain exactly where they occur with a spatial resolution of 100–200 μm (Smith, 1983a).

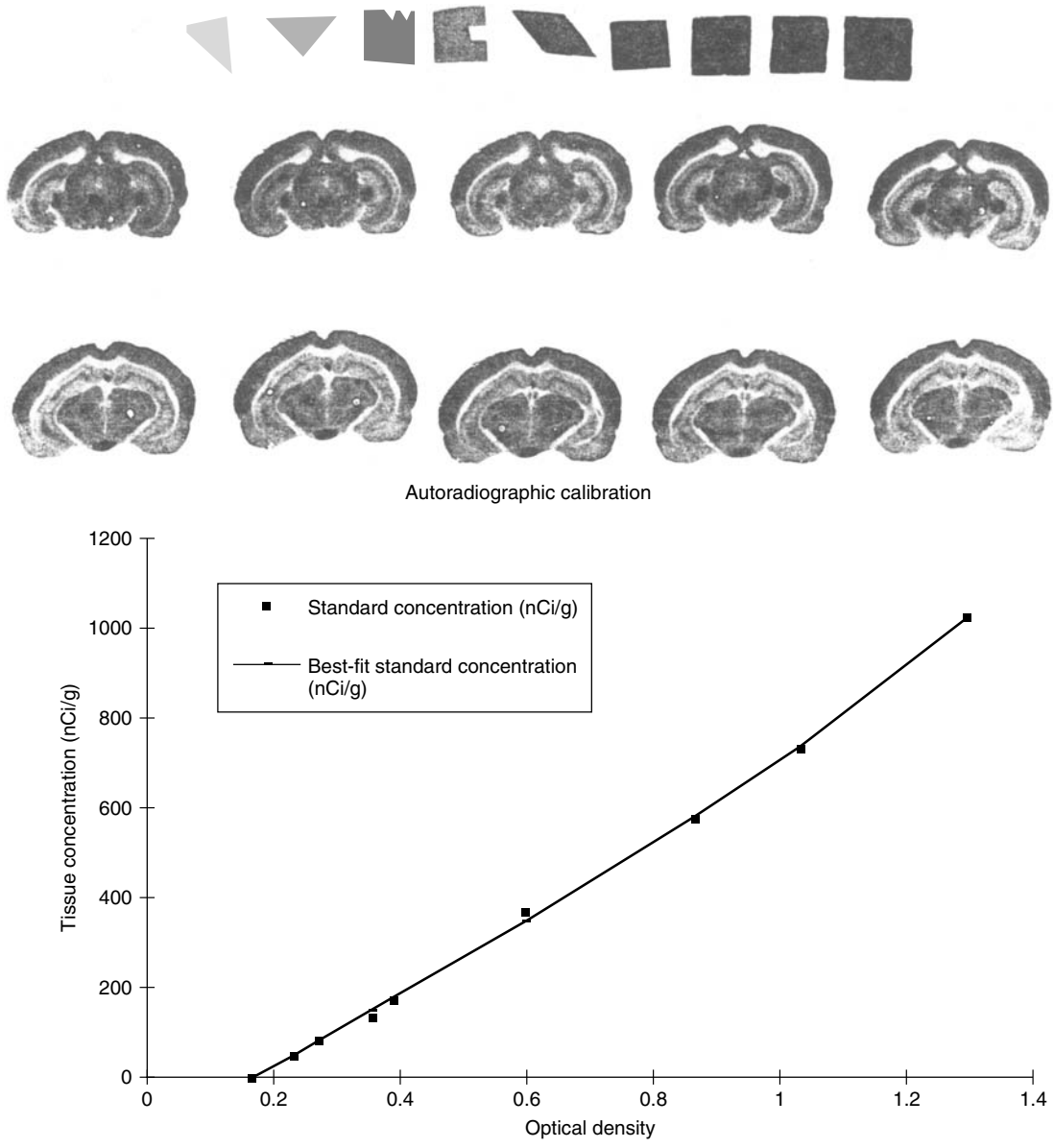

Figure 2.1. [^{14}C]DG autoradiographs of conscious rat brain and of calibrated [^{14}C]methylmethacrylate standards as well as the calibration curve between optical density and tissue ^{14}C concentrations derived from densitometry of the standards.

Autoradiography is, of course, not applicable to man. To adapt the [^{14}C]DG method for use in man it was necessary to introduce a γ-emitting radioactive label into 2-deoxyglucose to allow external detection of the tracer. This was done by attaching ^{18}F to the carbon in the 2-carbon position of 2-deoxyglucose to produce 2-[^{18}F]fluoro-2-deoxy-D-glucose (^{18}FDG), and the local tissue concentrations of label were determined by external scintillation counting with a single photon emission scanner in place of autoradiography (Reivich

et al., 1979). ^{18}F is a positron emitter, and the ^{18}FDG method was subsequently adapted further for use with positron emission tomography (PET) (Phelps *et al*., 1979). PET provides better accuracy and spatial resolution than single photon detection although its spatial resolution in the mm range is still at least an order of magnitude below the 100–200 μm of the autoradiographic [^{14}C]DG method.

Applications of the [^{14}C]DG and ^{18}FDG methods in both animals and man showed that CMR_{glc} varies widely in the various structures of the brain. It is much higher in gray matter than in white matter and is generally reduced by anesthesia (Table 2.2) (Sokoloff *et al*., 1977). It was also clearly established that neuronal functional activation stimulates energy metabolism in neural tissues just as it does in other tissues (Sokoloff, 1981). The magnitude of the increases in glucose utilization is quantitatively related to the intensity of functional activation. For example, retinal stimulation by randomly timed light flashes raises local CMR_{glc} in proportion to the logarithm of the light intensity in those structures of the brain that receive direct projections from the retina (Figure 2.2). (Miyaoka *et al*., 1979; Sokoloff, 1981). Electrical stimulation of the cervical sympathetic trunk or sciatic nerve increases CMR_{glc} linearly with spike frequency in the

Table 2.2. Local rates of cerebral glucose utilization in rats in the conscious state and under light thiopental anesthesia (means ± SEM)

Structure	Conscious (6)	Anesthetized (8)	% Effect
	Gray Matter		
Visual Cortex	111 ± 5	64 ± 3	−42
Auditory Cortex	157 ± 5	81 ± 3	−48
Parietal Cortex	107 ± 3	65 ± 2	−39
Sensory-motor Cortex	118 ± 3	67 ± 2	−43
Lateral Geniculate Nucleus	92 ± 2	53 ± 3	−42
Medial Geniculate Nucleus	126 ± 6	63 ± 3	−50
Thalamus: Lateral Nucleus	108 ± 3	58 ± 2	−46
Thalamus: Ventral Nucleus	98 ± 3	55 ± 1	−44
Hypothalamus	63 ± 3	43 ± 2	−32
Caudate-Putamen	111 ± 4	72 ± 3	−35
Hippocampus: Ammon's Horn	79 ± 1	56 ± 1	−29
Amygdala	56 ± 4	41 ± 2	−27
Cochlear Nucleus	124 ± 7	79 ± 5	−36
Nuclei of Lateral Lemniscus	114 ± 7	75 ± 4	−34
Inferior Colliculi	198 ± 7	131 ± 8	−34
Superior Colliculi	99 ± 3	59 ± 7	−40
Superior Olivary Nucleus	141 ± 5	104 ± 7	−26
Vestibular Nucleus	133 ± 4	81 ± 4	−39
Pontine Gray Matter	69 ± 3	46 ± 3	−33
Cerebellar Cortex	66 ± 2	44 ± 2	−33
Cerebellar Nuclei	106 ± 4	75 ± 4	−29
	White Matter		
Corpus Callosum	42 ± 2	30 ± 2	−29
Genu of Corpus Callosum	35 ± 5	30 ± 2	−14
Internal Capsule	35 ± 5	29 ± 2	−17
Cerebellar White Matter	38 ± 2	29 ± 2	−24

Note: The effects of anesthesia were statistically significant in every structure ($p < 0.05$).
From Sokoloff *et al*. (1977).

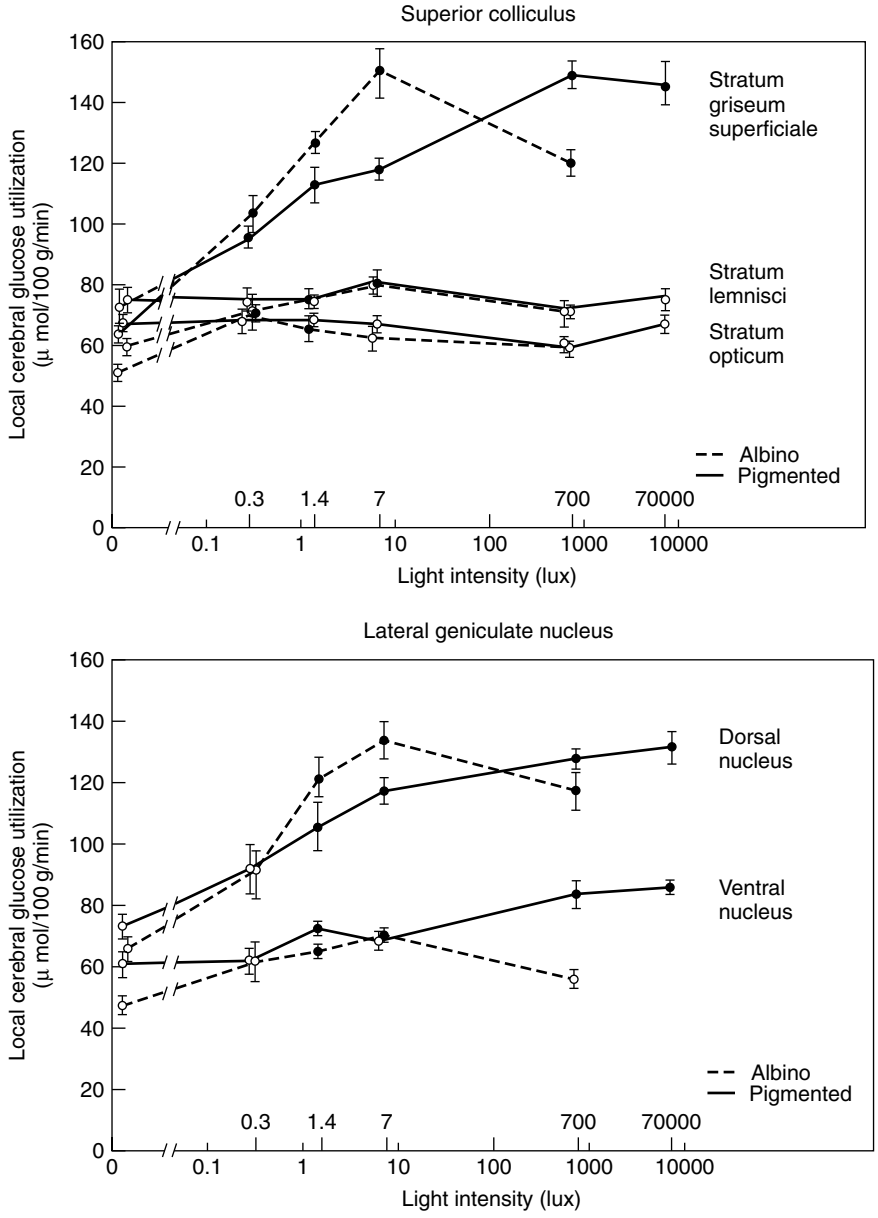

Figure 2.2. Rates of glucose utilization as a function of intensity of retinal illumination with randomly timed light flashes in various layers of superior colliculus and lateral geniculate nucleus of dark adapted albino and pigmented rats. From Miyaoka *et al*. (1979).

superior cervical ganglion (Yarowsky *et al*., 1983) and dorsal horn of the lumbar spinal cord, respectively (Figure 2.3) (Kadekaro *et al*., 1985). The glucose consumed per action potential can be determined from the slope of the straight line and equals 0.4 nmol/g of tissue per spike in the superior cervical ganglion and 0.6 nmol/g per spike in the dorsal horn of the lumbar spinal cord (Figure 2.3).