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The Evelyn F. McKnight Center for Age-Related Memory Loss
2006 Progress Report

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Progress Report, December 2006

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It is proposed that the best way to approach the key issue of cognitive decline in humans is through imaging. This can be accomplished at the University of Miami through the development of a center for Molecular Imaging, with a focus on monitoring cognitive decline at the functional level in the human brain that occurs with normal aging and in neurological diseases. In the current period we developed a cohesive plan that would give the proposed Center a structure, vision, methods, and approaches that would make it highly competitive at the national level. Our recommendation is that this proposed new Center should be focused on the development of new neuroimaging technologies that would permit a substantial advance in age-related cognitive research. The development of such techniques and their application to age-related cognitive alterations would bring together several areas of clinical expertise in the confluent fields of imaging, cognition, aging and memory research. The primary instrumentation necessary for such a cross-disciplinary effort will include nuclear medicine and magnetic resonance based molecular imaging technologies, which are described below.

Development Plan

Major instrumentation for the imaging center is planned, including a MRI/PET (3 Tesla, with multinuclear capabilities); a Cyclotron; and a MR Hyperpolarizer. This instrumentation, and the associated personnel, would be developed in three phases:

Phase 1: MRI installation; The MR Hyperpolarizer would be purchased and methods developed using an existing small animal MR instrument;
Phase 2: Multinuclear upgrade added to the 3T MRI and hyperpolarized MRS studies in animal models will be extended to this instrument.
Phase 3: PET insert implemented; Cyclotron and associated radiochemistry and MR chemistry facilities.

The total cost for this instrumentation is estimated at $9.5 million and additional funds will be required for the site preparation. This will require that additional sources of funding be developed. Since the University of Miami will be placing a large order for instrumentation purchases to equip the new hospital, it is anticipated that research agreements can be reached with a major instrumentation manufacturer to support the research instrumentation. In addition, agreements may be established with a local radiopharmaceutical company and other departments at the University to reduce the cost of the cyclotron and personnel.

Scientific Direction

Dr. Andrew Maudsley, Ph.D. has been provisionally identified as Scientific Director of the Center. Additional recruitment efforts will proceed to obtain additional senior scientific personnel, notably, a clinical director in the area of PET neuroimaging; a MR contrast agent development scientist; and technical support for the development of hyperpolarized MR spectroscopy.

Dr. Andrew Maudsley research career has focused on development of MRI and in vivo MR spectroscopy. Dr. Maudsley completed a Ph.D. in physics in 1976 (under the direction of Sir Peter Mansfield, Nobel Prize winner, 2003), during which he implemented one of the first MRI methods and obtained the first MRI from the human body, which was of his finger. Following his Ph.D. work he did a postdoctoral fellowship (with Professor Richard Ernst, Nobel Prize, 1991), working in the area of heteronuclear NMR spectroscopy. In 1979, Dr. Maudsley obtained a faculty position in the department of radiology at Columbia Presbyterian Medical Center, New York, where he worked on the construction of the first 1.5 Tesla MRI instruments located in a clinical setting. Over the next several years, Dr. Maudsley and colleagues published several early papers in the area of technique developments for MRI and in vivo MR spectroscopy, including the development of spectroscopic imaging methods and the first sodium and phosphorus imaging in animals and humans. In 1987, Dr. Maudsley joined the faculty at the University of California, San Francisco, where he continued development of MR spectroscopy methods for examination of brain diseases in humans, including areas of stroke, epilepsy, and traumatic brain injury. This work is now being continued at the University of Miami, where he moved in 2002.

Dr. Maudsley has over 110 publications and 8 patents. He is currently principal investigator of four grants awards from the National Institutes of Health, has received a Gold Medal award of the International Society for Magnetic Resonance in Medicine, and is currently serving on the Medical Imaging study section of the National Institutes of Health.

Introduction to Molecular Imaging

The term of molecular imaging can be applied to a number of emerging imaging methodologies, all of which aim to map biological processes occurring on the cellular or sub-cellular level. These may include including kinetic analyses, for example, measuring the rate at which a single metabolic process takes place. A common feature of most molecular imaging methods is the use of exogenous molecular probes, or biomarkers, that are targeted to a specific compound associated with the metabolic process to be monitored.

Because molecular imaging methods monitor metabolic processes in living tissue they provide diagnostic information that is complimentary to high-resolution structural images (e.g. MRI or CT) and which is more sensitive to detecting metabolic changes occurring with disease or injury that may precede any structural changes. Example applications for clinical research include monitoring gene expression; molecular interactions; measuring metabolic flux rates and changes with disease; following implanted stem cells; and optimizing drug and gene therapies (reviewed in (1,2)). Important clinical applications include diagnosis of neurodegenerative diseases and cancer.

Historically, molecular imaging is most widely associated with nuclear medicine, primarily PET and SPECT imaging, and indeed this remains a primary technological development area for this rapidly evolving field; however, other technologies based on magnetic

resonance imaging (MRI) and spectroscopy (MRS) also offer considerable potential. Both of these modalities enable non-invasive measurements from deep within the human body; the instrumentation is widely available in the clinical setting; and they are exceptionally well suited for clinical translation of molecular imaging methods. It is proposed that the combined development and clinical translation of these molecular imaging methods in a single location, and with a focus on ageing research, will provide a unique resource. This proposal therefore concentrates on the following molecular imaging technologies:

Positron Emission Tomography (PET)
MR Spectroscopy (MRS) and Hyperpolarized MRS
Targeted MRI contrast agents

When applied to molecular imaging, a common feature of these technologies is the development of molecular marker compounds. Such compounds combine two functions: the first is a molecular component that interacts with a target compound or metabolic process of interest; and the second is a signaling component that can be detected and spatially located with the body. The targeting components may be designed to attach to a specific protein or binding site, or to take part in a specific metabolic process, and there exists common aims to the design of molecular probes for all of the molecular imaging methods proposed. The common development of these different imaging methods in a single location will therefore promote active cross-fertilization between different groups of investigators.

The selection of molecular imaging modalities in this proposal is based around several new technological developments. These methods, and recent technological advances in these fields, are briefly reviewed here:

MRI/PET:

Positron Emission Tomography (PET) is based on the detection of the radioactive decay from an isotope contained within an agent introduced into the body. A number of radioisotopes can be used, the choice of which is dependent upon the rate of decay and the associated availability of a cyclotron at the same site in order to use radioisotopes with very short half-lives. The most widely applied method for clinical applications uses the Fluorine-18 isotope in fluoro-deoxyglucose (FDG) to monitor brain energy metabolism (Figure 1); however, numerous molecular probes have been developed that target many proteins or receptor sites (Figure 2).

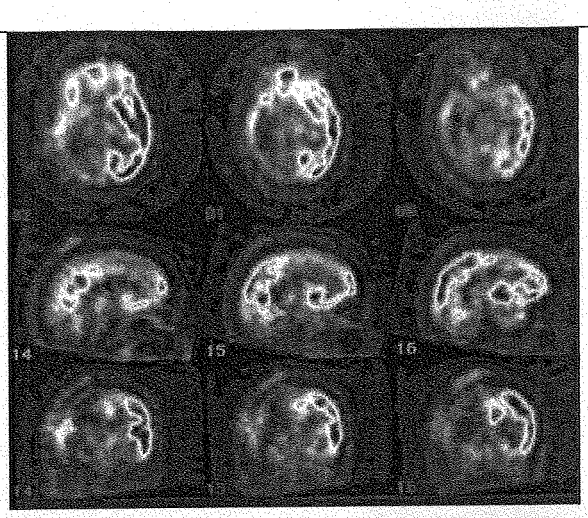

Figure 1.

Example F18-FDG PET scan of a patient with traumatic brain injury. The areas shown in red indicate normal energy metabolism, whereas severe reduction in metabolism is indicated in other brain regions as a result of injury.

This data was obtained at the University of Miami.

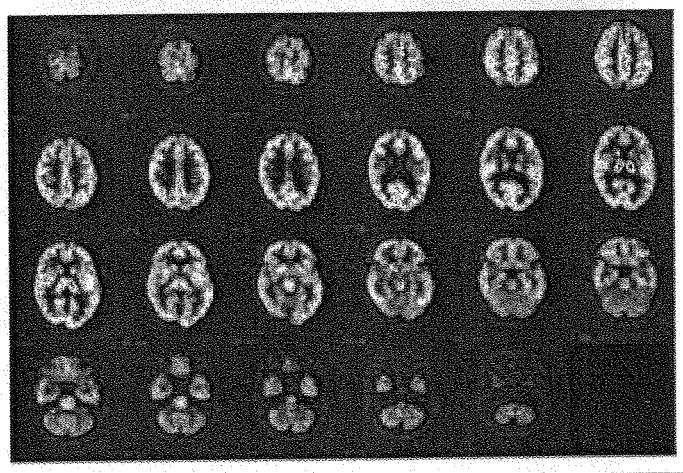

Figure 2.

Example PET image of the brain obtained using C11-clorgyline, a marker for monoamine oxidase, an important enzyme involved in regulating neurotransmitter concentrations. From Fowler et al (3).

The sensitivity of PET is very high, enabling compounds to be detected with concentration as low as 10^{-12} Moles/Liter, and with the latest generation of technologies, spatial resolutions on the order of 2 mm are possible. A recent advancement in the PET imaging technology is the combination with structural imaging; which not only provides both metabolic and structural information from the same region, but also enables improved image reconstruction of the PET data by incorporating information derived from the structural images. Currently this dual-modality approach is widely implemented with PET/CT; however, the combination of PET with MRI represents a technological breakthrough that is about to be made available by the instrument manufacturers. While the driving force behind the combination of PET with MRI is viewed as a method to reduce radiation dose, over PET/CT, for the purpose of this proposal this is more importantly considered to be an outstanding approach of combining PET-based molecular imaging with MRS-based molecular imaging. Since different metabolic targets can be mapped by each imaging modality, their combined use in a single subject potentially opens up many additional applications, particularly for studies of the human brain.

A suitable PET/MRI unit based will be made available within 2 years. This unit is based on a 3-Tesla MRI instrument, which is a field strength that is suitable for clinical MRS studies, and the PET unit will be provided as an insert that can be added to an existing MRI scanner. In addition, the unit can be supplied with a multinuclear MR option to enable advanced MRS studies to be implemented, including the developing area of hyperpolarized MR molecular spectroscopy, which is described below. This would represent a unique instrument on which

structural imaging and multiple molecular imaging modalities may be implemented in a single subject, in a single study.

MR Spectroscopy and Hyperpolarized MRS:

Magnetic resonance methods are based on the manipulation and detection of the precessing magnetic moment of certain nuclei, and this phenomenon is exploited for MRI using detection of the proton nucleus, primary located in water. MRI produces high-resolution images with superb discrimination of different tissues, and is widely used for clinical studies. The related MR spectroscopy (MRS) method extends the technique to provide a chemical analysis of the tissue. This information is complimentary to the structural information in a MRI, and is of considerable interest for biomedical studies.

When used for in vivo applications, the MRS measurement remains limited in sensitivity and its use is typically restricted to detection of metabolites with concentrations greater than 10^{-3} moles/liter and to tissue volumes of approximately 1 cc or greater. MRS data can be obtained in vivo from several nuclei, with protons and phosphorus-31 being the most common and carbon-13 being used for specialized studies where C-13 enriched compounds, such as glucose, are administered to a subject. The observation of protons provides the greatest sensitivity and this can be effectively as an imaging method as shown in Figure 3, which shows data obtained using new metabolic imaging methods developed at the University of Miami and implemented on a 3-Tesla MRI instrument.

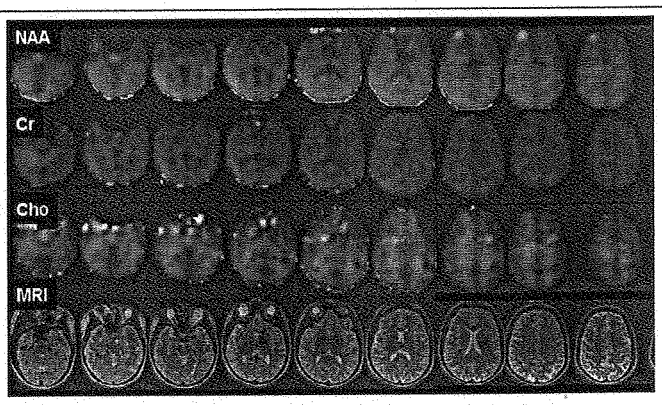

Figure 3.
Example images of the brain obtained using proton MR spectroscopic imaging, which maps distributions of N-acetyl-aspartate (NAA), creatines (Cr), and cholines (Cho), which are compounds that provide valuable diagnostic information on neuronal status and brain metabolism. The corresponding MRI is shown on the lower row.

While MRS can be considered a true molecular imaging method, and does not require administration of an exogenous marker, the measured compounds generally represent steady-state concentrations as opposed to monitoring dynamic molecular processes. Nevertheless, considerable metabolic information can be monitored using MRS and these methods are widely applied for clinical diagnostic studies, especially for studies of the brain.

The nuclear magnetization detected in the conventional MR measurement actually represents a minute fraction of that theoretically available. For molecules in thermal equilibrium at room temperatures, most of the nuclei are co-aligned in a minimum energy state such that their magnetization vectors cancel and only one nucleus in 100,000 actually contributes to the detected MR signal. Although sensitivity can be improved by going to higher magnetic field strengths, these efforts are costly and fundamentally limited. A radically different approach to improving detection sensitivity is to increase the degree of nuclear magnetization using methods termed hyperpolarization, and recent developments have demonstrated that increased levels of magnetization can be produced in compounds that can then be introduced into the body, in an

approach analogous to the PET agents. Initial investigations used hyperpolarized gasses to image the air spaces of the lung, but more recently, it has been demonstrated that small quantities of water-soluble compounds of biological relevance can be generated with high degrees of polarization. These new developments mean that the sensitivity of the MRS measurement of endogenous compounds can be extended to concentrations on the order of 10^{-7} to 10^{-8} Moles/liter, which opens up new and potentially highly significant avenues for developing MR molecular markers for applications such at measuring tissue metabolism and alterations with disease.

Although MR detection sensitivity can be enhanced by factors of $>10^4$ using hyperpolarization, it remains a considerable challenge to achieve this in tissue and the development of suitable molecular imaging agents is the subject of intense research effort. First, the nuclear spin relaxation rates of these compounds significantly limit the amount of time available to do the measurement. By transferring the enhanced magnetization to nuclei such as C^{13} and N^{15} these relaxation rates are lengthened, but it remains likely that the lifetimes of the enhanced polarization will remain at a few minutes. Although the measurement may be repeated by reintroduction of the agent this nevertheless limits the metabolic pathways that can be investigated by this technology. These characteristics also mean that high-speed acquisition methods are required and that the MRI instrument has multinuclear capability. An additional challenge is to get sufficient amounts of the hyperpolarized compound to the target area within the limited timeframe. For metabolic studies in the brain, it will be necessary that the target compound not only crosses the blood-brain barrier but also enters the cell and is metabolized within this short time period.

The area of hyperpolarized MRS represents a new, and as yet unproven, technology, although it is also an area that is the subject of major research efforts by the medical imaging instrumentation manufacturers (most prominently by GE-Amersham). However, there are compelling arguments to include this technology in this proposal, including that: a) this represents the forefront of a potentially dramatic new tool for diagnostic and basic biomedical research applications; b) it can be implemented in a relatively small laboratory for a modest startup cost; c) that the success of this technology will likely hinge on the development of suitable molecular markers, the development of which will undoubtedly benefit from the synergistic interaction with the other molecular imaging methodologies included in this center; and d) the data acquisition methods required are modifications of the standard MRS methods, which will also be implemented on the PET/MRI instrument.

Example uses of hyperpolarized MRS include the polarization of a metabolically active compound, such as C^{13}-glucose or pyruvate, which is then injected into a subject and the subsequent metabolic pathways monitored. The power of this method therefore lies in monitoring metabolic pathways, using approaches similar to those already developed for studies of cerebral glucose and glutamate metabolism or measurements of TCA reaction rates following administration of C^{13}-enriched glucose (4-6). To date, the following compounds of interest have been detected with hyperpolarized MRS in vivo, either as the injected compound or as a metabolic product: Glucose, Lactate, Pyruvate, Choline, Alanine, and Acetate. Potential applications of hyperpolarized MRS remain highly speculative, but will almost certainly include monitoring neurotransmitter and tumor metabolism.

Targeted MR Contrast Agents:

The use of exogenous compounds to enhance MRI contrast has been widespread for many years, for example using compounds containing gadolinium. These compounds perturb the magnetic field on a molecular scale, resulting in a decrease of the MR relaxation rates (T_1 and T_2) of the surrounding water. Due to the extent of the magnetic susceptibility gradient and the effect of water diffusion around the molecule, there is an amplification of the effect of these agents, resulting in a detection sensitivity equivalent to micromolar concentration levels. More recent developments have demonstrated that MRI contrast agents based on compounds such as superparametric iron oxide nanoparticles (SPIOs) can provide greater sensitivity, enabling the development of targeted MR contrast agents suitable for molecular imaging, with applications include tracking gene expression and labeled stem cells (7). Currently, these techniques have been widely applied in animal models, an example of which is shown in Figure 4; however, extension to human studies is widely anticipated.

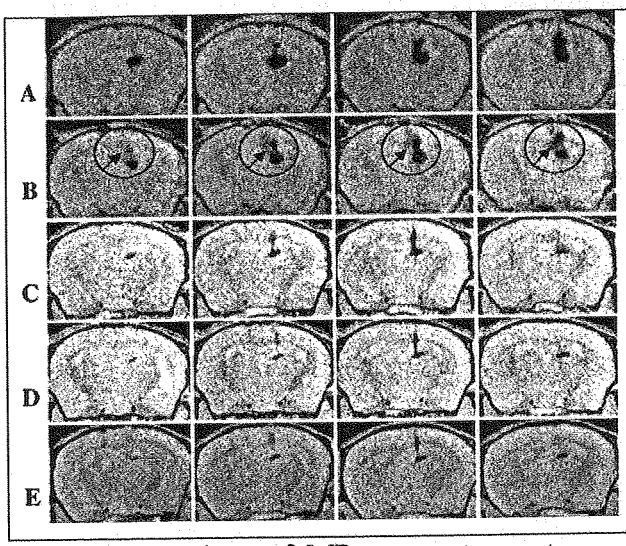

Figure 4.

Example of the use of parametric MR contrast agents to monitor brain cell migration. The figure shows the MRI from an adult mouse brain after implantation of SPIO-labeled stem cells, at 1 (a), 7 (b), 16 (c), 21 (d) and 32 days (e) after transplantation. Arrows point to the hypointense areas indicating the migration of cells in the cortex and hippocampus. From Magnitsky et al. (8).

One class of MR contrast agents are designed become active only in response to a specific enzyme or chemical compound, and these so-called magnetic relaxation switches offer the potential to monitor dynamic events. Other variations of such markers include the ability to measure pH, chemical exchange, or exchange of tissue-bound water with the bulk water pool. Modifications of these methods based on modification of MRI contrast can therefore provide additional probes into the molecular environment with the cell.

Applications of Molecular Imaging for Age-Related Research

This type of exciting new methodology makes it now possible to investigate high-level cognition such as language comprehension, problem-solving, visual thinking, and executive processes through the use of state-of-the-art imaging techniques. The overall goal of such a body of new research would be to develop theories of cognition that are grounded in and account for brain activity. In simpler terms, our goal is to explain how cognition and cognitive decline emerge from brain function.

The use of a combination of existing and newly emerging imaging techniques would bring the proposed new McKnight Center to the forefront of cognitive neuroscience, which is aimed at unraveling the neural mechanisms that underlie mental activity, such as cognition, perception, motor control, attention and language. In the early 1990s, the possibility to study cognition in humans was revolutionized by the advent of functional brain imaging that created the possibility to obtain detailed images of the 'working' human brain during sensory, motor and cognitive tasks. The spatial and temporal resolution provided by imaging technology and its non-invasive character are some of the main strengths of this type of approach. In the last years, brain imaging research has contributed to clarify the organizational principles of the human brain and the neural substrates of cognitive, sensory, perceptual, and motor processes. These new insights not only foster theory development, but also provide much needed understanding of the causes and treatment of diseases of the central nervous system and developmental learning disorders. Very recently, imaging is providing scientists with the ability to test in living human beings theories and models of various types of cognition and interaction in relation to the underlying neurobiological mechanisms. The combination of imaging techniques proposed here significantly enhances the toolkit of neuroscientists, psychiatrists, neurologists, and psychologists to study brain mechanisms of cognition which constitutes one of the main methodological challenges in the field. The synergy between methods development and applications will make cognitive brain research at the University of Miami McKnight Center regionally and nationally recognized. This proposed Center will embark on this type of integrated research activity with a clear focus on the brain organization and connectivity of specific cognitive functions in normal adult and aging subjects as well as in cognitive dysfunction throughout the life span.

Summary

The proposed imaging center will make available complimentary molecular imaging methods, based on PET and MRI, for studies of metabolic changes associated with aging. These capabilities will greatly enhance the neuroimaging facilities at the University of Miami. A particularly unique feature of this center will be the combination of expertise in the area of design and synthesis or molecular probes. Although the different molecular imaging methods will require different agents, there will be significant commonality in the synthesis and pharmacokinetics of these agents, and undoubtedly cross-fertilization between these imaging disciplines. This proposed structure has the potential to become an invaluable national resource for the discovery of the brain substrates for age-related changes in human cognition.

References

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University of Miami
Evelyn F. McKnight Center for Age Related Memory Loss Fund
Summary Analysis at Market
June 2006-September 2006

	Market Value
Beginning Balance - 6/1/06	$4,102,395
Gift - July 2006	$875,000
Investment Return - 6/1-9/30/06	169,511
Ending Balance - 9/30/06	$5,146,906