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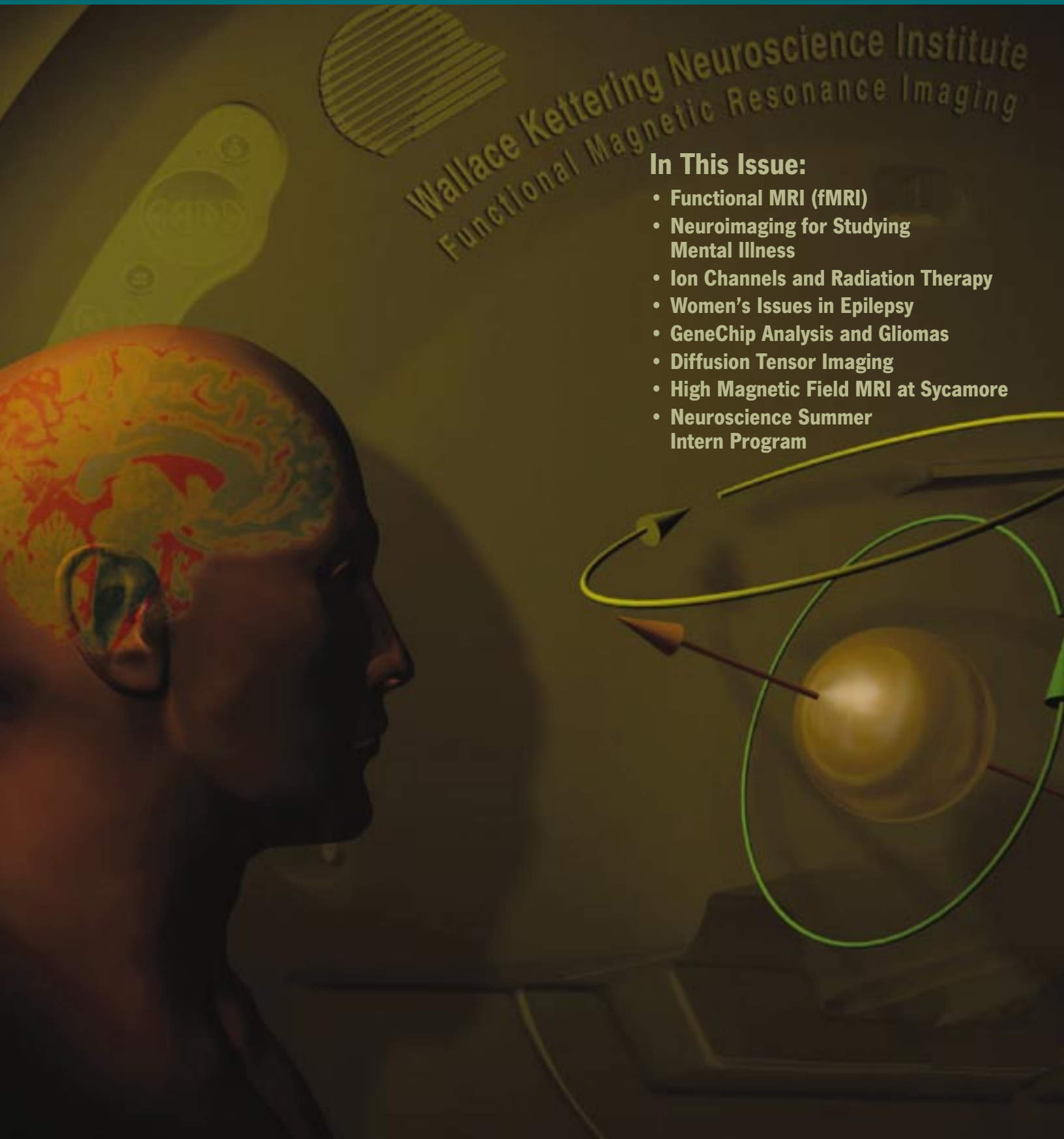
Neuroscience Directions

A Publication of The Wallace-Kettering Neuroscience Institute



In This Issue:

From the Executive Director, WKNI:

Gerald Szkotnicki,

Executive Director,
Wallace-Kettering
Neuroscience Institute

It is said that nothing is moving faster than neuroscience, which is unique among clinical specialties due to the applicability and relevance of a broad range of scientific and technical advances. These advances continue to occur in the field at an ever-increasing rate, and they can become part of the routine regimen available to healthcare providers only after overcoming a large number of validation studies, and economic and other challenges. This gap between what is possible and what is available and practical is addressed only through clinical trials and research, which means that only those facilities that have a research infrastructure can offer the latest options to patients. In addition, since health insurance companies rarely cover the cost of new technology, sponsored research and clinical trials may be the only way to access such care. This is why clinically relevant research is integral to achieving the mission of the WKNI and Kettering Medical Center.

From the Editor:

Lenora Gray, M.D., FAAN,

Editor, Co-Medical Director
of Sleep Disorders Center,
Kettering Medical Center

This issue of the Wallace-Kettering (WKNI) Neuroscience Directions highlights several newer technologies which are useful in a broad spectrum of neurologic disorders, especially in diseases where no clear structural abnormalities are found, such as schizophrenia.

- Functional MRI (fMRI) is a technique that images physiologic changes in particular areas of the brain in response to specific activities, such as movement or visual tasks, and combines this information with structural MRI images, comparing normal to abnormal responses.
- Diffusion Tensor Imaging (DTI) is able to delineate abnormal changes in specific white matter axonal tracts by comparing them to normal pathways. Diagnosis, treatment planning, and research in schizophrenia, multiple sclerosis, strokes, tumors, epilepsy, and other brain diseases benefit from both fMRI and DTI.
- Positron Emission Tomography (PET) scanning, which produces a functional image, is greatly enhanced by using new radioactive tracers to locate and quantify abnormalities in specific regions of the brain. This has been very useful for research on schizophrenia and other mental illnesses.
- GeneChip analysis can profile abnormal gene activity in brain tumors (gliomas) to improve the treatment of these malignant tumors.
- Ion channel abnormalities in neurons are being studied for methods to enhance the death by radiation of malignant cells selectively, while sparing normal neurons.

Part V in the epilepsy series covers problems specific to women. This fall, WKNI will hold a symposium on schizophrenia research. Details are included on page 21. As always, your comments and input are welcome. I can be contacted through WKNI. My e-mail is lgray26@aol.com.

From the Medical Director, WKNI:

Theodore Bernstein, M.D.,

Medical Director,
Wallace-Kettering
Neuroscience Institute

The utility and value of a technical innovation are rarely clear from the outset. It takes the desire to improve methods and outcomes for innovations to become changes in the way things are done day to day. Maintaining this openness to change and continuously seeking new approaches are two of the hallmarks of medical centers of excellence, and are core values held by the physicians and staff involved in WKNI. As obvious as this might appear, it remains a challenge in the highly regulated and scrutinized environment that we, as healthcare providers, work in. I would like to take this opportunity to thank the many benefactors of WKNI, and the people behind the scenes, who make this pursuit of beneficial change possible.

Cover art by Bilal Ezzeddine, Ph.D.

Functional MRI at KMCN/WKNI

Mehdi Adineh, Ph.D., Bilal Ezzeddine, Ph.D.

Wallace-Kettering Neuroscience Institute (WKNI) is a recognized leader in defining the standards of patient care in the use of neuro-imaging techniques. WKNI possesses an array of state-of-the-art research tools for Magnetic Resonance Imaging. Physicians, scientists, nurses and technicians at WKNI are advancing the current standards of care, diagnosis and treatment for patients with brain and spine disease using sophisticated technology. Technological advancement at WKNI rivals the best neuroscience centers in the world.

Magnetic Resonance Imaging (MRI) and functional MRI (fMRI) are imaging techniques that take advantage of the magnetic properties of atomic nuclei to create images of different bodily tissues and compounds. MRI distinguishes different tissues like fat, bone, muscle, white and gray matter, and cerebrospinal fluid from each other. In contrast to the structural images, fMRI images changing blood flow in the brain over time.

This change in blood flow correlates with the change in activity of the neurons. When a region of brain is active, that region begins to use more oxygen, depleting the amount of oxygen in the surrounding blood vessels. In response, these blood vessels dilate to increase blood flow and oxygen supply to the working neurons (a process known as the hemodynamic response). This hemodynamic response changes the properties of surrounding water molecules, which in turn changes the signal that can be detected by the MRI scanner. The change in signal intensity is a relative one, representing the blood flow that differs from a baseline blood flow. During a typical fMRI, a subject is first asked to fixate their eyes on a point and to relax their mind. If the subject is able to do this, the image of baseline blood flow will show the blood flow in the subject's brain when it is not engaging in any particular kind of activity. While the living brain is never inactive, certain cognitive tasks require that specific areas become significantly more active than when they are at rest. Comparing resting images to activated images can be used to find out what parts of the brain are needed to do a specific task. The task may be as simple as tapping one's finger or passively viewing images, or it may involve complex problem solving. When the image of blood flow in the baseline condition is subtracted from the task-related blood flow, what remains is a picture representing the areas where blood flow changed in response to the task (Figure 1).

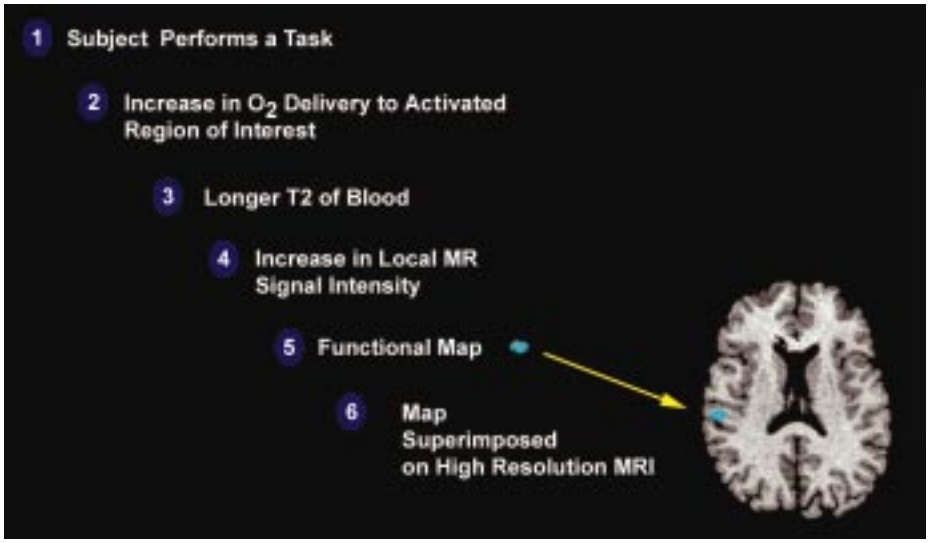


Figure 1.

Mehdi Adineh, Ph.D.,

is a Research Associate/ MRI Physicist at WKNI. Dr. Adineh is a Ph.D.-level scientist with extensive expertise in the application of Magnetic Resonance techniques for biomedical research.

Bilal Ezzeddine, Ph.D.,

is the Project Director of Medical Imaging at Kettering Medical Center. He has dual Ph.D.s in computer science and biomedical engineering from the Ohio State University. He has 14 years of experience in computer hardware and software, specifically as applied to medical image acquisition, processing, and visualization.

Figure 2.

The images produced by MRI and fMRI require the use of complex software and powerful computers. This equipment does mathematical transformations and statistical analyses of the raw data from the scanner and transforms it into images that look like brains. By themselves, fMRI images simply look like colored blobs, and give little or no indication of the location in the brain where blood flow increased. To know precisely what parts of the brain were active, the software must align the fMRI image with an anatomical MRI, a process known as co-registration. The software can also now correct for distortion in the images, correct for head motion, and construct 3-D images of the whole brain. WKNI scientists are working with General Electric (GE) Medical System's researchers to automate these aspects of fMRI data processing (Figure 2).

Advantages of fMRI

Advantages of fMRI over other functional mapping techniques include:

1. Repeatability and Safety – a single subject can be scanned as often as needed without exposure to radioactive isotopes or harmful substances of any kind.
2. Short Scan Times – an fMRI session may last as little as 10 – 15 minutes.
3. High Resolution – functional images generally have an in-plane resolution of 1.5X1.5 mm.

Another advantage of fMRI is that the same subject can be studied multiple times in a longitudinal (long-term) design. Other functional-imaging techniques require injections of radioactive isotopes and cannot be repeated frequently. However, radioisotope functional activation techniques are valuable tools that serve many neuroscience applications.

Uses of fMRI

Functional activity of the brain determined by fMRI is being used for cognitive mapping of anatomically distinct processing areas in the visual cortex and Broca's area (associated with speech and language-related activities). In addition, fMRI is being used to view complex cognitive tasks such as recognition, emotion, comprehension, memory, learning, motivation and attention.

This approach has been used to investigate how healthy subjects learn new complex tasks and, more importantly, to aid in early diagnosis of neurological diseases like stroke and epilepsy. The clinical relevance of fMRI has also been strengthened by recent experiments exploring the neurobiological basis of specific rehabilitation

strategies intended to aid the recovery of function. The use of fMRI for the pre-surgical mapping of eloquent cortex also suggests that it plays an important role in the routine management of patients with neurological disease. fMRI has been used to aid the understanding and treatment of multiple sclerosis, schizophrenia, depression, bipolar disorder, Parkinson's disease and addictive illnesses. Current research by Thickbroom et. al. (2003) involving auditory and visual stimuli is being used to map language areas with fMRI. The development of new methods of language localization will lead to new insights into the brain circuitry underlying disorders like aphasia and dyslexia. Bonaffini et. al. (2002) found fMRI useful for distinguishing between potentially salvageable and irreversibly damaged tissues in the brains of stroke and tumor victims (Figure 3).

Current Projects and the Future Direction of WKNI

"Brainwave"

A time-consuming and often subjective step in the use of fMRI is the analysis of data. As previously outlined in this article, the images must be reconstructed and corrected for variables like head movement. Currently, the data output of an fMRI could take up to a day for manual analysis. In an effort to provide better care for patients and a more practical tool for research, the Wallace Kettering Neuroscience Institute has been involved with GE Medical Systems to develop automated software. This software, known as "Brainwave," efficiently and accurately analyzes data that is acquired from the fMRI, and automatically runs it through the steps necessary to produce clear, accurate pictures of brain functions. Researchers from surrounding universities, including the Ohio State University, the University of Cincinnati, and Wright State University (WSU), have visited WKNI to view these capabilities. WKNI has worked to become one of the primary locations in the U.S. for the display of Brainwave software. The benefits of this software will revolutionize the use of fMRI in research and patient care.

Schizophrenia

Using the rich variety of brain-imaging resources found at WKNI, and the cadre of scientists supporting these technologies, our hope is to reveal new information about several areas of the brain thought to be involved in schizophrenia, including the striatum, the prefrontal cortex and the thalamus. The imaging technologies are capable of revealing the white-matter tract architecture of the brain (responsible for relaying neural signals) and imaging gross neural anatomy and activity. Obtaining an assortment of images from schizophrenic patients and control subjects, and examining them "in the context of" comprehensive psychiatric and neurocognitive information, we will be better equipped to characterize disease-related brain abnormalities than ever before.

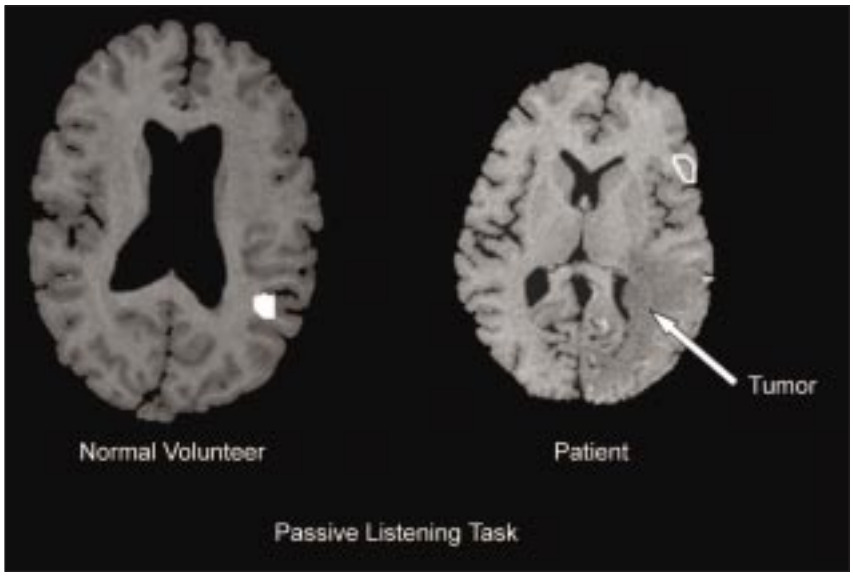


Figure 3.

Brain Mapping/Localizing Function (Figure 4)

1) Epilepsy
2) Alzheimer's disease
3) Schizophrenia
4) Visual cortex
5) Motor cortex
6) Complex cognitive functions (i.e., memory, learning, comprehension, attention, language).

Visual Motion Perception, fMRI and Schizophrenia

The ability to process motion is one of the most important visual capacities that we have, and one that may reveal something significant about schizophrenia. Schizophrenic patients exhibit impaired smooth-pursuit eye tracking (the ability to smoothly follow a moving target) and other motion-processing deficits. Chen et. al. found evidence that schizophrenic patients have trouble with velocity discrimination when velocity cues are separated from nonmotion cues like contrast. Perception of the direction of motion is also defective in schizophrenics, and a more recent study showed that these patients were impaired in estimating the speed and spatial trajectory of a moving target. This evidence suggests that some of the specific impairments affecting schizophrenic patients are located within the network of motion processing. Both visual areas V5 and V1 are likely involved in this task. Understanding the brain regions involved in motion processing and developing tasks that specifically tap into the processing of natural motion used for pursuit eye movements will help identify the nature of the deficiencies found in motion processing of schizophrenia.

A current study undertaken at WKNI is using fMRI to look for the anatomical/physiological correlates of the observed perceptual abnormalities within the primary visual cortex (V1). The stimulus in the fMRI experiment will be a signal dot moving at a fixed velocity and direction through the center of gaze amid a background of random-motion dots. We expect to find a characteristic pattern of activation when normal patients are exposed to the moving stimulus, and a specific discrepancy between the activation patterns of schizophrenic and control subjects.

Characterizing specific activation pattern idiosyncrasies in schizophrenics could greatly expand the power of pursuit paradigms to aid in the diagnosis and treatment of schizophrenia. Activation patterns could become useful in tracking drug response, course of illness and differential diagnosis as well as important familial markers.

In the first phase of a long-term series of such activation studies, we have demonstrated the feasibility of performing such experiments. Our primary long-term objective is to integrate the advanced neuropsychological findings relating to visual motion perception with fMRI to better

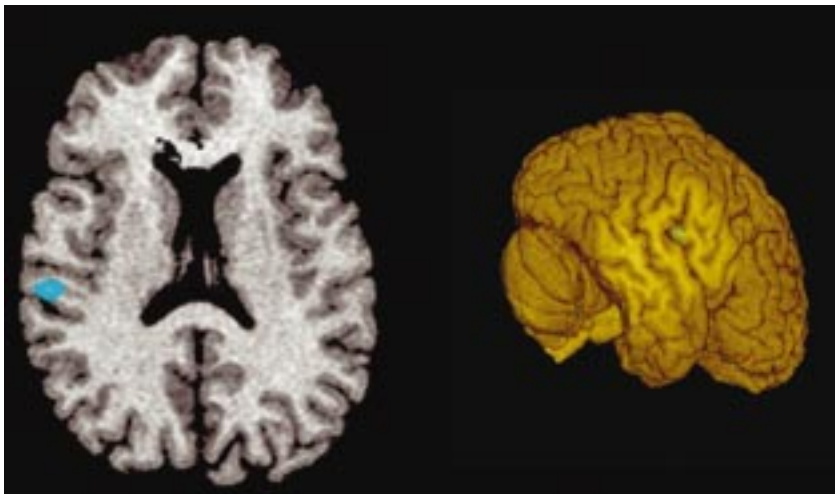


Figure 4.

understand aberrant neural networks characterizing schizophrenia. Our colleague at WSU, Dr. Watamaniuk, is a leader in the field of visual motion perception. He has developed a mathematical model that describes how cells in our visual system respond to motion. Prior to joining WSU, he was a research fellow at the Smith-Kettlewell Eye Research Institute in San Francisco (Figure 5).

The Future of fMRI

Neurosurgery relies on a precise delineation of the structural and functional aspects of the brain, making fMRI very significant for neurosurgical planning. The structural and functional topography of the brain differs from individual to individual, and the need for individualized maps of brain function is enhanced when the presence of a tumor alters the typical functional role of a brain region, or when the tumor resides in an area with a function that is uncertain. These areas may include association or language-related cortex. A wide variety of investigators has reported fMRI results consistent with PET, cortical stimulation and magneto-encephalography, serving to confirm that fMRI does indeed provide a source of precise functional and structural information for neurosurgery.

With the ability to image the entire 3-D volume of the brain, fMRI is capable of isolating many simultaneous and coordinated brain events. These include aspects of higher-level cognitive functioning that have not previously been scrutinized with such precision and represent some of the remaining frontiers in neuroscience.

fMRI has proven itself useful for neurosurgical planning, better assessment of risk for individual patients, improved seizure localization and better understanding of the physiology of neurological disorders. We look ahead to these and other emerging applications as the benefits of this technology become incorporated into current and future patient care.

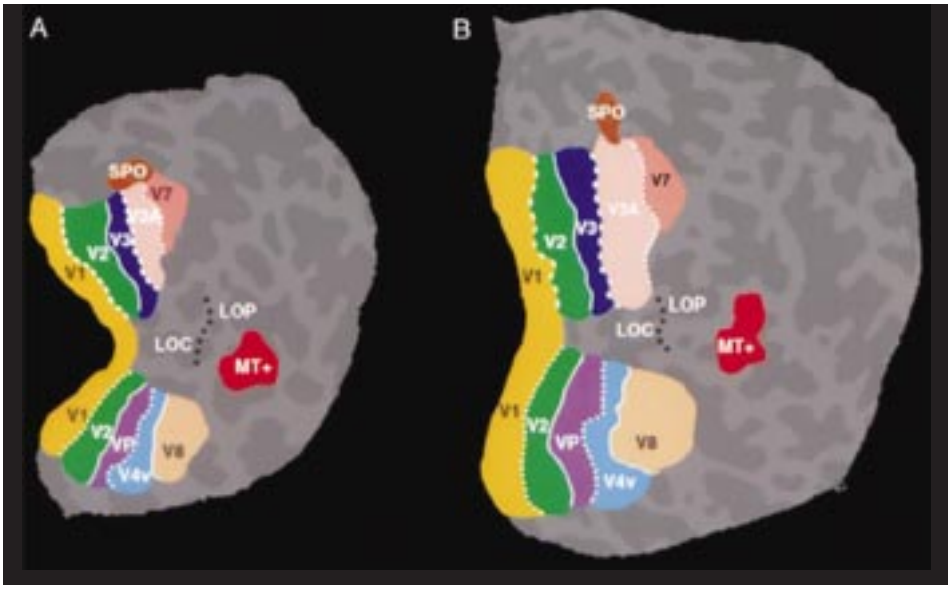


Figure 5.

Acknowledgment: The support of the United States Air Force, Air Force Research Laboratory (AFRL/HEOP), Air Force Material Command, under cooperative agreement F33615-98-2-6002 is gratefully acknowledged.

Expanding Neuroimaging to Study Mental Illness

Brad Christian, Ph.D.

Brad Christian, Ph.D.,

is a Medical Physicist in the PET/Nuclear Medicine Department at Kettering Medical Center Network. His work focuses on 3-D PET imaging and the development of mathematical models to study neuroreceptors. His Ph.D. is from the University of Wisconsin, which was followed by a fellowship at Harvard University with one of the pioneering PET groups.

The field of medical imaging has made a large impact on almost all specialties in medicine, allowing the radiologist to visualize broken bones, the cardiologist to assess coronary blockage and the oncologist to track tumor growth. In the near future, medical imaging may arm the psychiatrist with the ability to objectively diagnose mental illness, see the "cleaving" of neural circuits, guide the clinicians with the most effective course of therapy and monitor improvements in patient brain function. Positron Emission Tomography (PET), which has already established itself as an invaluable tool in whole body tumor imaging, is also proving to be a powerful *device* in studying changes in brain chemistry often implicated in mental illnesses, such as schizophrenia.

I felt a cleaving in my mind -
As if my brain had split -
I tried to match it - seam by seam -
But could not make them fit.
The thought behind, I strove to join
Unto the thought before -
But sequence ravelled out of sound
Like balls - upon a floor.

- Emily Dickinson

Schizophrenia is a devastating mental disorder that afflicts approximately 1% of the world population, not discriminating for wealth, race or social status, and has been documented since the beginnings of written history. Our understanding of this disease has improved greatly. Owing to scientific research, schizophrenia is no longer believed to be deservingly cast upon an individual as punishment for sinful ways, or equally misguided, inflicted upon those with weak moral character unable to muster up the will power to overcome their ailments. Modern science has revealed that schizophrenia is caused by a disruption in the neurochemical systems of the brain, though the mechanisms and causes are largely unknown. The ability of PET imaging to study these miniscule quantities of chemicals may provide valuable insights for a greater understanding of the underlying processes.

It is now well known that drugs that are effective for treating the psychotic symptoms accompanying schizophrenia, may block the dopamine receptors in the brain. This has lead to the "dopamine hypothesis of schizophrenia," predicting overactive transmission in the brain's dopamine receptors. Our research in studying schizophrenia has focused on using neuroimaging methods to study the dopamine systems of the brain. By attaching positron-emitting atoms to a particular chemical or drug (termed as a radiotracer) and imaging the brain

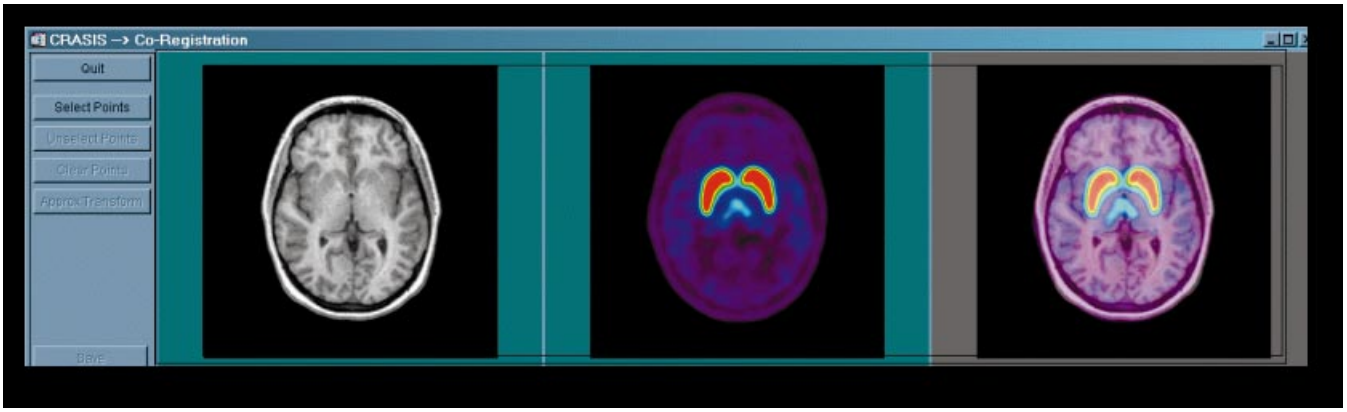


Figure 1.
A transaxial view of [F-18] fallypride distribution in the human brain. The PET image (center) is co-registered to an MRI image (left) and displayed as an overlay image (right).

with a PET scanner, it is possible to follow the path of the radiotracer in the body and see where it is incorporated into a biochemical process (Figure 1).

By developing chemicals that are exquisitely selective for the dopamine receptors in the brain, it is now possible to visualize "dopamine functional regions" of the brain that have never been seen before (Figure 2). When PET imaging of the dopamine receptors is used in combination with antipsychotic medications, it is possible to directly measure the effect of the medication at the dopamine regions in the brain. This will allow the psychiatrist to fine tune the best course of therapy for each patient (Figure 3).

Continued advances in neuroimaging technology, and the rapid development of new radiotracers for studying brain chemistry, place us at a very exciting time for studying the neurochemical basis of schizophrenia. Hopefully, the day is close when neuroimaging will allow us to truly visualize the split in the brain's wiring, and a course of therapy can be chosen to sew it back together.

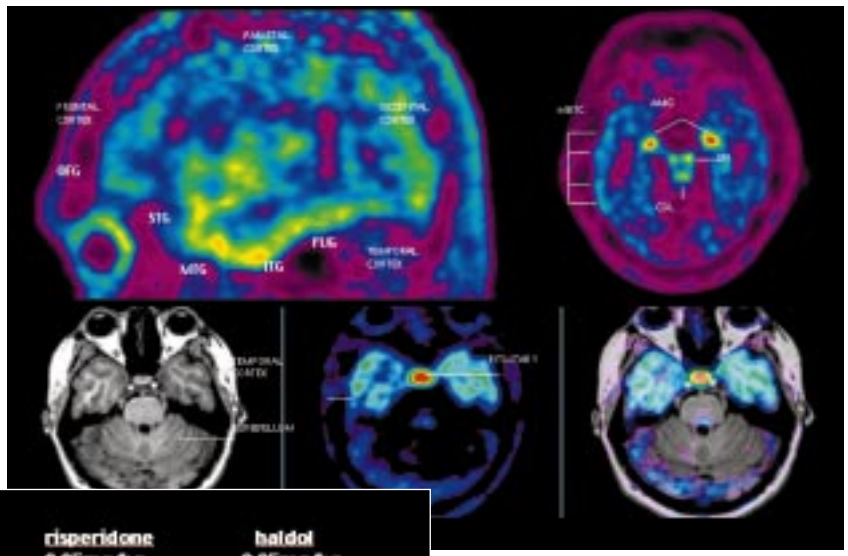


Figure 2.
Regions of the brain with very small amounts of dopamine receptors can now be visualized using [F-18] fallypride PET imaging.

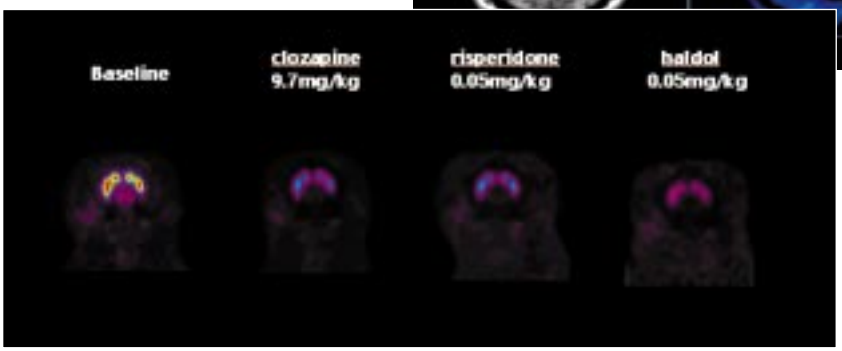


Figure 3. The effects of antipsychotic medications on the uptake of the PET radiotracer ([F-18] fallypride) binding. The image on the left shows normal uptake of the radiotracer (baseline) and the three images on the right reveal that less uptake of radiotracer is seen because of competition by the antipsychotic medication.

Acknowledgment: The support of the United States Air Force, Air Force Research Laboratory (AFRL/HEOP), Air Force Material Command, under cooperative agreement F33615-98-2-6002 is gratefully acknowledged.

Ongoing WKNI Research Projects

Epilepsy Imaging Project

Robert Simkins D.O., FACN

We are studying the utility of 11C-flumazenil PET scanning in temporal lobe epilepsy. Patients with medically intractable partial epilepsy who are studied with 11C-flumazenil PET scans are also undergoing extensive diagnostic investigations, including video/EEG monitoring, high-resolution MRI scanning and 18FDG PET scans, as part of a pre-surgical evaluation of their medically intractable epilepsy. Flumazenil attaches to the benzodiazepine receptors in the brain. The benzodiazepine receptors are a component within the GABA receptor complex. While we do not have a way of directly imaging the actual GABA receptors with PET scanning, this technique does give us an indirect way of imaging the GABA receptors. GABA receptors are known to be affected in temporal lobe epilepsy. The absolute numbers of GABA receptors and/or their functional binding characteristics are diminished in epileptic temporal lobe tissue. We are studying the utility of 11C-flumazenil PET scanning for localization of epileptic foci. In addition, we are investigating changes in 11C-flumazenil binding distant from the primary epileptogenic focus. Specifically, where are the remote changes in binding located and what are their relationships to the primary epileptogenic focus? Are the remote changes reversible after resection of the alleged primary epileptogenic focus? Does the finding of abnormalities in 11C-flumazenil binding remote from the suspected primary focus imply additional independent epileptogenic foci and a poor prognosis for seizure control?

Ion Channels and Improved Radiation Therapy

James Olson, Ph.D.

All cancer treatments are designed to damage or remove tumor cells, while allowing normal tissue to survive. Radiation treatment focuses beams of high-energy particles on the tumor, with a reduced dose of radiation given to adjacent normal cells. Chemotherapy exploits slight differences in the biochemistry of normal and cancerous cells in order to selectively poison the tumor. However, normal cells are often in the path of the high-energy radiation beam or may be adversely affected by the chemotherapeutic treatment. Thus, to avoid excessive damage to normal cells, the magnitude of the therapeutic dose delivered to the patient is limited. As the dose is reduced, a larger number of viable cancerous cells will remain after the radiation or chemotherapy treatments, thus increasing the risk that the tumor will reappear.

Research at the Wallace-Kettering Neuroscience Institute (WKNI) and other centers has suggested that combining radiation and chemical treatment methods may enhance the selective killing of tumor cells, and thus provide more effective and safe cancer therapies. This interaction between therapeutic modalities is explored in detail in a basic research project headed by Drs. James Olson and Viney Jain and funded by the Wallace-Kettering Neuroscience Institute. The researchers are examining novel drug treatments that may specifically sensitize tumor cells for damage by radiation treatment or protect cells from injury.

Drs. Olson's and Jain's research builds on recently discovered information about the biochemical mechanisms of cell death. Cells injured by radiation and other causes initiate a built-in self-destruct program called apoptosis (Figure 1). During this process, the outer cell membrane opens specific ion

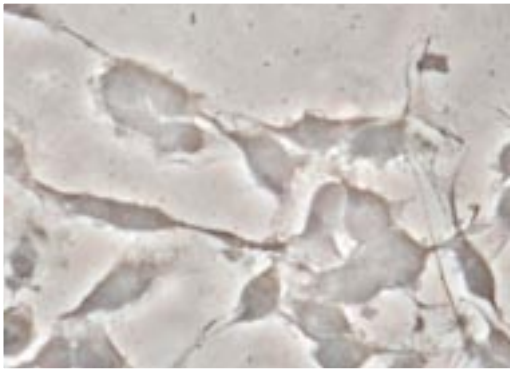


Figure 1A. Phase contrast photograph of brain cancer cells.

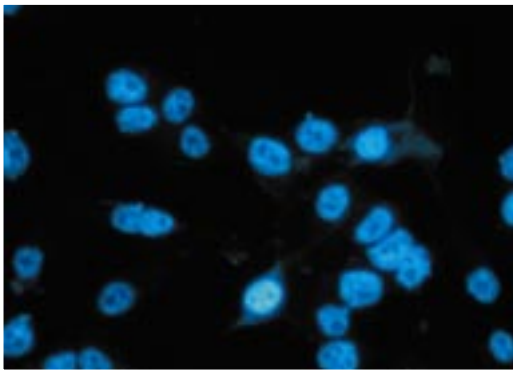


Figure 1B. The nucleus of each cell in Figure 1A is identified with a blue fluorescent stain.

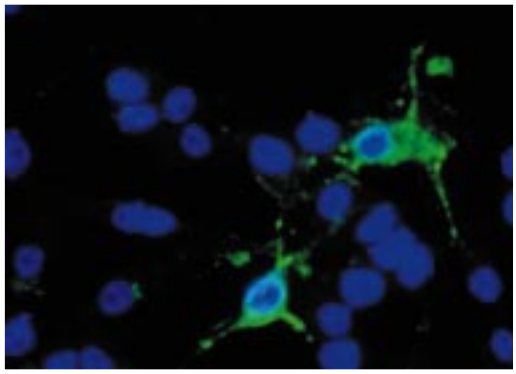


Figure 1C. Cells injured by oxidative stress (simulating damage produced by radiation) are identified by a green fluorescent stain, to indicate which cells have begun the process of programmed cell death called apoptosis.

James Olson, Ph.D.,

is a Professor and the Research Director in the Department of Emergency Medicine and also a Professor of Anatomy and Physiology at Wright State University.
In addition to funding from WKNI, Dr. Olson's research is supported by two grants from the National Institutes of Health. He graduated from Cornell University with a Bachelor of Science in Engineering Physics and obtained his Ph.D. in Biophysics from the University of California at Berkeley. Following postdoctoral training at Stanford University Medical School, he took a faculty position at Tulane University Medical School. Dr. Olson moved to his present position at Wright State University in 1986.

channels. This causes the cell to lose intracellular electrolytes and shrink in size. Subsequently, the cell activates enzymes that digest its internal proteins and chop up its long DNA molecules into shorter pieces. Eventually these processes bring the metabolic machinery of the cell to a halt and the cell dies. Several researchers have shown that by inhibiting the initial opening of the membrane ion channels, the programmed progression to cell death is blocked.

To bring this new information to bear upon the treatment of patients with brain tumors, Drs. Olson and Jain are examining membrane ion channels of human cells from normal brain and from brain tumors. Their attention is focused on channels involved in cell death following radiation treatment. Because the regulation of membrane channels in normal and tumor cells is different, the researchers anticipate that drug treatments can be designed to block the function of channels in normal tissue, but not those of tumor cells. Alternatively, certain drugs may selectively increase the activation of membrane channels in irradiated tumor cells. Such treatments would protect the normal brain cells from injury, while increasing the number of tumor cells to undergo the programmed cell death of apoptosis. For example, after protecting normal cells from apoptosis with a drug treatment, the dose of radiation used to kill cancerous cells may be increased, thus causing greater damage to the tumor and reducing the risk of it reappearing. These results would directly impact patients at Kettering Medical Center Network – and throughout the world – by providing a more selective means to destroy tumor cells while maintaining the viability and function of normal brain tissue.

Ongoing WKNI Research Projects

New High Magnetic Field MRI Coming to Sycamore

Peter Roe, B.S., Mehdi Adineh, Ph.D., John German, M.D., Bilal Ezzeddine, Ph.D.

As technology races ahead, changing the world faster and faster, the world of MRI is forever changing and improving. Sycamore Hospital will be installing a new high magnetic field MRI system this summer. The new magnet will be a 1.5 Tesla short bore system, which will feel much like an open magnet but with superior imaging qualities. The opening of the magnet is flared and larger than many MRI systems and combines the accuracy of a tunnel scanner with the comfort of an open MRI. The integrated panoramic array allows simultaneous scanning with up to four coils. The advantage is revolutionary: you do not have to take the patient off the table to change coils for more than 95% of all studies. Given the improved technology in the gradient strengths and imaging coils, this magnet will provide Sycamore with state-of-the-art MRI capabilities, and high-resolution images when they are needed.

Clinically this new MRI can provide higher resolution capability with stronger and more precise images for diagnosis. It includes new arrays of neuroimaging sequences to allow greater diagnostic capability and accuracy. This more powerful system produces images faster, which adds to the comfort of the patient.

Women's Issues in Epilepsy, Part V of V

Phillip White, M.D.

Until recently, very little attention has been given to the special concerns of women with epilepsy. Drug studies largely exclude women because of the unknown effects of new agents on pregnancy. From anecdotal experience, there does not appear to be any clear differences in women's compared to men's responses to epilepsy medications; but there are clear differences in side effects, which make some agents better than others for certain groups of women. Approximately one-half of women who suffer from epilepsy will experience some change in the frequency or timing of seizures throughout the changes in their menstrual cycle, during pregnancy, and after menopause. We think these differences are due to estrogen's effects on seizures, but this is still a very complicated subject with no clear answer. One of the primary concerns of women of child-bearing age is the effects of these medications on the developing child. Unfortunately, while we know that there is an increased risk of birth defects with the older medications, there is still much that we do not know about the newer medications. This article addresses some of these concerns.

Antiepileptic Medications for Women With Epilepsy

Other articles in this series have addressed the proper use and potential side effects of both newer and older antiepileptic medications. While there is no evidence that epileptic syndromes in women differ from men, there are reasons to consider using other medications than the older "traditional agents" for certain women. Older agents, such as Phenytoin, Carbamezepine and Phenobarbital, are the traditional choices for partial onset seizures. However, these medicines induce the liver to break down other medications and nutrients. In particular, they cause the liver to metabolize estrogens rapidly, decreasing the effectiveness of estrogen-containing birth control pills. Three options to counteract this problem would be to use (1) higher dose estrogen-containing birth control pills, (2) progesterone (such as DepoProvera shots) or (3) an alternative antiepileptic drug. In addition to estrogens, these older agents also cause the liver to handle calcium and vitamin D differently, resulting in a higher risk of osteoporosis. The newer antiepileptic medications do not affect the liver in the same way and are now preferred by most epilepsy experts treating women.

Valproic acid is the "traditional" choice for patients with primary generalized epilepsy. However, this agent may cause considerable weight gain, making it less desirable for many women. Also, valproic acid carries the strongest association with severe birth defects, limiting its use in women of child-bearing years. And thirdly, though still uncertain, there is concern that valproic acid may contribute to the development of polycystic ovarian disease in some women. While valproic acid is an excellent agent for primary generalized epilepsy, for women of child-bearing years, one of the newer medications, such as Lamotrigine, Topiramate, Levitiracetam or Zonisamide is a better choice.

Phillip A. White, M.D., is board certified in neurology and clinical neurophysiology. He received his medical degree from Baylor College of Medicine, Houston, Texas. In addition, he received his neurology residency and postdoctoral training in clinical neurophysiology and epilepsy from Baylor College of Medicine. Dr. White was an Assistant Professor of Neurology and ran the Epilepsy Clinic at Baylor College of Medicine.

Seizures and the Menstrual Cycle

Between 30%-40% of women report a catamenial pattern (seizures associated with the menstrual cycle) to their epilepsy. Numerous animal studies show that estrogen lowers the threshold for seizures, thereby making seizures more likely to happen. This may account for seizures occurring around the time of menses and explain why estrogen replacement increases seizure frequency in some women. However, progesterone treatment is only occasionally effective for control of catamenial seizures, hysterectomy is ineffective for control of seizures, and seizure frequency may increase, decrease, or stay the same during pregnancy and menopause. The interactions between hormones and seizures are, therefore, unclear. We are also uncertain how knowledge of these interactions will translate into effective therapies. At this point, we should probably view the menstrual cycle as an organizer of when seizures occur rather than a cause of their occurrence.

Epilepsy and Pregnancy

The overwhelming majority of children born to women with epilepsy are normal and healthy. However, there is an increased risk of birth defects and need for special education in children born to women with epilepsy, even when no medicine is taken. For women taking antiepileptic medication, the risk for birth defects and mental slowing in their children is even higher. The older medications increase these risks by about 4-6%, with valproic acid being the most likely drug to cause a major fetal malformation. Phenytoin, Carbamezepine, Phenobarbital, and Primidone have all been associated with greater risks of birth defects; and taking more than one medicine also increases this risk. The newer medications appear to have a lower risk of birth defects in animal studies, but we do not yet know the full impact on humans. Of the newer agents, we have the most information about Lamotrigine during pregnancy. This medication appears to be safer than the older drugs, but this is only preliminary information at best. All of the newer agents should be considered "unknown" regarding their effects during pregnancy. Despite our uncertainties, the newer antiepileptic agents (particularly Lamotrigine) are generally preferred.

During pregnancy, seizure frequency increases in one-quarter to one-third of women with epilepsy. Should a woman have a generalized tonic-clonic seizure, there is a risk of her baby being injured, not receiving enough oxygen, or being spontaneously aborted. Because of these concerns, convulsions (tonic-clonic seizures) are considered more dangerous to both mother and child than exposure to antiepileptic medications. Despite the concerns of birth defects expressed above, treatment appears better than the alternative.

All antiepileptic agents are present in breast milk. The concentration in breast milk is, however, much lower than in the blood stream (the concentration the baby was exposed to during pregnancy), even with Lamotrigine, which may be concentrated at a higher level than other medications. Every mother must decide for herself if she wishes to breast-feed; but there is no absolute reason to avoid it because of using antiepileptic medication.

Generally, treatment of women with epilepsy does not differ significantly from that of men. There are, however, special concerns that must be taken into account when treating women, especially during child-bearing years. Most physicians avoid valproic acid and try to use one of the newer antiepileptic agents. All women of child-bearing age should take folic acid to help minimize the risk of birth defects. Pregnant women should be treated with a single antiepileptic medication when possible.

GeneChip Analysis and Gliomas

Steven Berberich, Ph.D.

Although surgery and radiation for patients with malignant cerebral gliomas can prolong survival for a few months, the long-term outlook is dismal. As part of a multi-center phase II clinical trial, researchers at the Wallace-Kettering Neuroscience Institute (WKNI) at Kettering Medical Center are evaluating a therapy that uses a glucose analogue (2-deoxyglucose, 2DG) to make tumors more sensitive, and surrounding tissue less sensitive, to radiation. Although the therapy has shown promise, there is wide variation in tumor response.

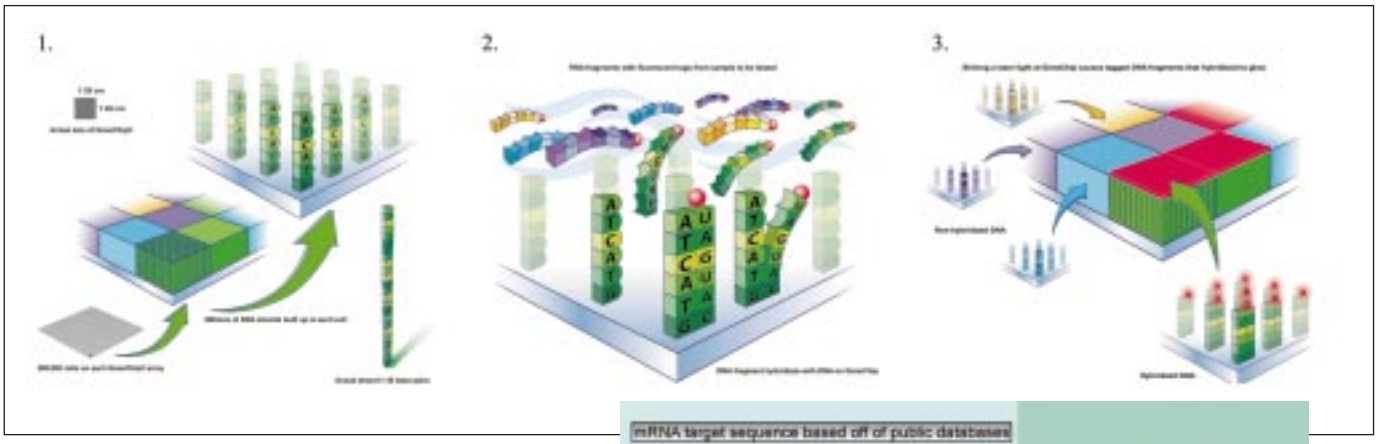
To better understand the variation in tumor response, WKNI has supported studies led by Dr. Viney Jain, WSU Emergency Medicine, and Dr. Steven Berberich, Director of the Gene Expression Laboratory at WSU's School of Medicine. Using Affymetrix "GeneChips" - which contain small DNA oligomers representing thousands of human genes (Figure 1) - these researchers have been systematically examining how 2-deoxyglucose affects the changes of gene expression in cultured human gliomas. They hope the gene expression changes they uncover will be useful in future clinical trials investigating correlations between treatment-induced changes and clinical response in a large patient population. Establishing a significant correlation would lead to development of predictive assays for therapy response and individualization of therapy for optimal benefit to the patients, according to the researchers.

This novel technology of determining changes of gene expression using DNA arrays or GeneChips is one of the new technologies that has developed out of the completion of the human genome project. The pharmaceutical industry has begun to use the technology to develop new drugs, as well as potentially tailoring drugs based on a person's genetic makeup. In a few years, pathologists and other physicians are likely to be using DNA arrays for diagnosing diseases and selecting treatments most appropriate for individual patients.

Steven Berberich, Ph.D., is the current Director of the Gene Expression Laboratory at Wright State University's School of Medicine and is an Associate Professor in the Department of Biochemistry and Molecular Biology. His laboratory studies the regulation of the p53 tumor suppressor protein and is funded by the National Cancer Institute.

Figure 1. Affymetrix GeneChip™.

Figure 2. Cartoon of the steps to detect those genes that are expressing mRNA (active). 1. Each probe cell or feature contains approximately 1 million identical oligonucleotides of a particular sequence specific for a given gene. 2. Labeled cRNA probe generated from a given sample binds to its "matching" oligonucleotide sequence on the GeneChip. 3. The amount of bound cRNA is determined by capturing the fluorescence intensity for each probe cell.



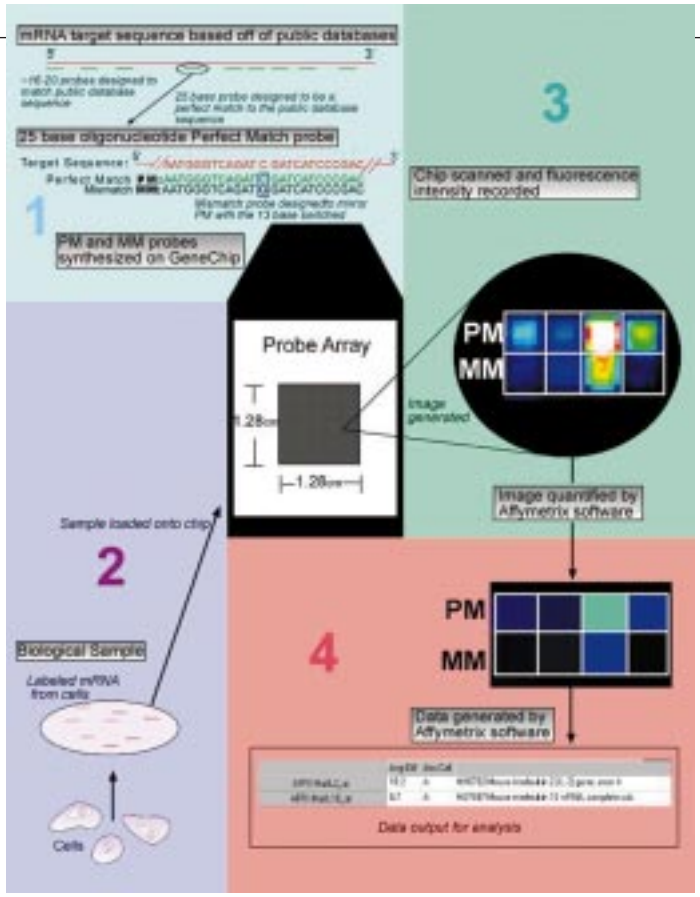


Figure 3.
Outline of a gene expression profiling experiment. 1. Small DNA molecules (oligonucleotides), specific for the gene of interest, are identified and placed on the GeneChip probe array. 2. RNA is isolated from tissue or cells and used to create labeled cRNA that is loaded into the GeneChip. 3. The amount of cRNA that binds to the various oligonucleotides (probe features) is determined by fluorescence. 4. Using computer programs, the fluorescence images are digitized and used in data analysis programs to develop a profile of gene expression.

Affymetrix GeneChips are available now for a host of species. The human GeneChip being used in the 2DG project contains over 12,000 different genes, each identified as a series of spots or "features" arranged on a grid. Each feature is approximately 24 microns wide. This array of DNA molecules provides a fixed reference in an assay that begins with isolating messenger RNA from cell or tissue samples (Figure 2). The method is as follows. The RNA is converted to a probe, then applied to the chip. Probe from RNA expressed by specific genes in the sample bind to their counterparts on the chip (Figure 3). Excess probe for RNA that hasn't matched with genes on the chip is washed off, and the chip is placed in a laser scanner that measures the fluorescent intensity of the features. Each feature's fluorescence is proportional to the amount of probe present, which is proportional to the amount of RNA present in the sample. Figure 4 represents the fluorescence pattern seen with a GeneChip containing over 12,000 human genes.

The resulting gene expression profile indicates which genes were active and inactive in the sample. The gene expression profile represents a "snapshot" in time, documenting gene expression at the moment when the RNA was isolated. When examining cells stimulated with a compound like 2-deoxyglucose the snapshot depends on the dose and time of treatment. While many of the cell's genes that are expressed will be unaffected by 2-deoxyglucose, the others that do fluctuate in response to 2-deoxyglucose represent new insights into the biological effects that lead to radiosensitizing malignant gliomas.

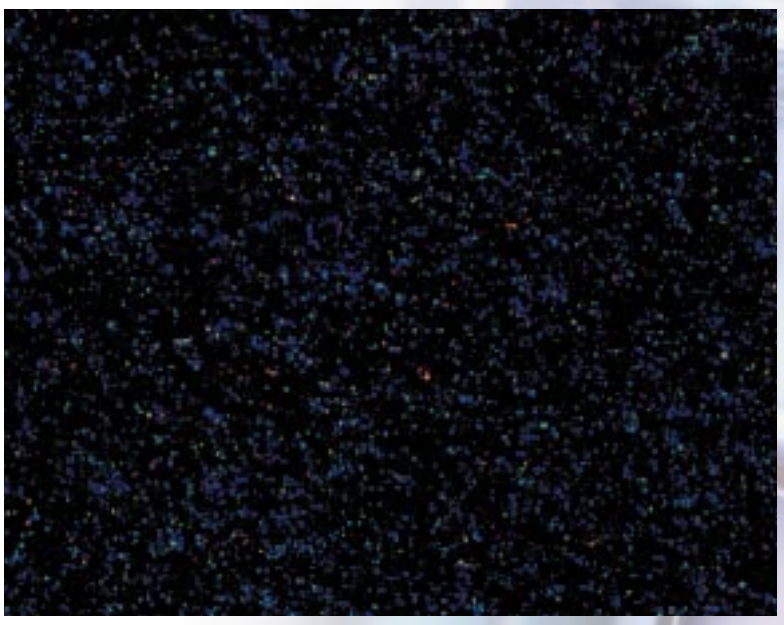


Figure 4. HG_U95Av2 GeneChip image.

Diffusion Tensor Imaging (DTI)

Mehdi Adineh, Ph.D.

Mehdi Adineh, Ph.D.,
is a Research Associate and MRI Physicist at WKNI.

What Is DTI?

Brownian motion describes the properties of water diffusion that are affected by the nature of the medium in which it occurs. Diffusion within biological tissues reflects both tissue structure and architecture at the microscopic level. In brain tissue, the motion of water molecules can be restricted in many ways (i.e., by interacting with tissue components). Anisotropic diffusion describes how water diffusion in the white matter of the brain is restricted in random directions. Observing this type of diffusion provides a good means of characterizing the local structure in tissues like muscle and the white matter of the brain.

The first attempts to detect diffusion with MR within the brain led to the development of diffusion weighted imaging (DWI) and, more recently, to the development of multidimensional assessment of diffusion data known as diffusion tensor imaging (DTI). Gray matter, white matter and CSF (cerebrospinal fluid) in the brain can easily be identified using the current, conventional MRI. However, the appearance of white matter is difficult to accurately observe and quantify. DTI can be used to explore anisotropic diffusion, and to more precisely analyze the white matter of the brain (Figure 1).

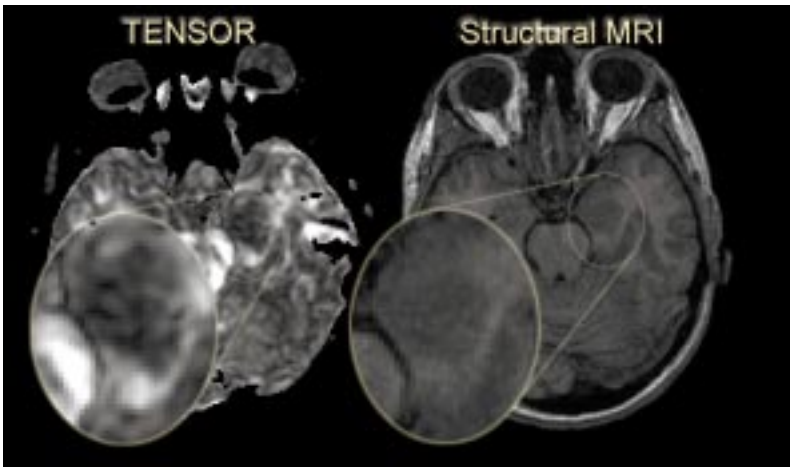


Figure 1.

Diffusion MR imaging was introduced in 1986, but was plagued with technical constraints like motion sensitivity. This limitation provided a major incentive to develop faster sequences that were more accommodating in terms of bulk motion. The development of diffusion sensitive pulse sequences basically followed two directions: echo-planar imaging methods, which acquire a complete image within a single shot, and navigator methods, which acquire images in multiple shots, and use navigator MR signals to detect and correct the bulk motion.

In the mid '90s, a more complex technique to describe multidirectional water diffusion, DTI, was introduced to the field of MR diffusion imaging. The term "tensor" was taken from physics and engineering, where it had been used to describe tension forces in solid bodies. In MR diffusion tensor imaging, is a tensor that describes diffusion in all spatial directions. The importance of this technique is that it can be used to make detailed, 3-D maps of nerve pathways in the brain.

What Is the Role of DTI in Neurological Assessment?

In the absence of any barriers, diffusion is equally probable in all directions. Using DTI, a disrupted flow of water can be used to locate places where there might be an underlying abnormality. The DTI scan can be compared with the scans of people without neurological disorders.

Epilepsy:

DTI may be a useful tool for gathering information about the nature and severity of epilepsy in individuals whose pathology is difficult to characterize with MRI. This technique gives further useful information about the brain and is a major step forward in the ability to pinpoint the causes of epilepsy, which may lead to successful surgical treatment.

Arfanakis et. al. (2002) suggest that DTI may determine the extent of damage to white matter structures of temporal lobe epileptics. Rugg-Gunn et. al. (2002), using DTI to examine a patient with clinical temporal lobe epilepsy not detected using MRI, found an area of abnormal diffusion in the right frontal lobe, which was identified and surgically resected.

Multiple Sclerosis:

Tensor diffusion imaging detects changes in the white matter of the brain in multiple sclerosis (MS) patients that are not visible in standard MR images. Standard MR images can show plaques (the areas of white matter affected by the disease), but plaques typically become visible only during the more advanced stages of the disease. In addition, MR images can appear somewhat normal while the patient is experiencing severe symptoms of MS. The images are not always correlated with the current progression of the disease. Tensor imaging allows a more accurate detection of abnormalities in MS patients, which may aid early diagnosis and treatment of the disease.

Werring et. al. (1999) found that DTI does in fact detect structural changes in seemingly normal white matter and does discriminate between focal lesions of varying severity. Horsfield et. al. (1998), using DTI in patients with MS, found an increase in the diffusion coefficient of water molecules in the plaques of patients with MS, compared to healthy brains.

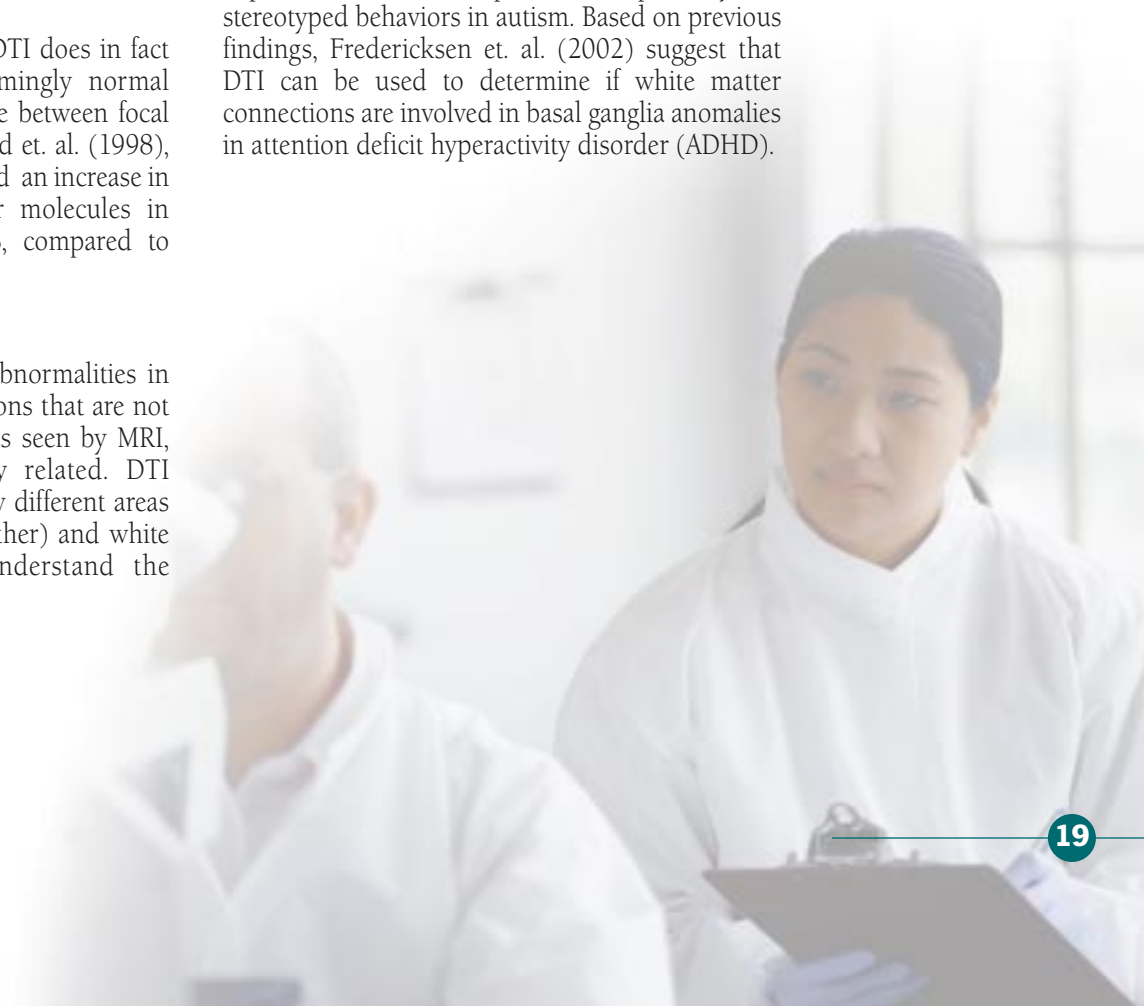
Schizophrenia:

DTI can explain neural circuitry abnormalities in schizophrenia that affect brain regions that are not necessarily structurally proximal, as seen by MRI, but are nonetheless functionally related. DTI investigates brain connectivity (how different areas of the brain interact with one another) and white matter fiber tracts to better understand the neuropathology of schizophrenia.

When 10 men with schizophrenia and 10 healthy men were scanned using DTI and MR structural imaging, significant differences were found, indicating widespread alterations in brain white matter integrity, but not necessarily in white matter volume Lim et. al. (1999). In order to verify the hypothesized disturbance in frontal-striatal-thalamic circuitry in schizophrenia, Buchsbaum et. al. (1998) compared results obtained from diffusion tensor images with Positron Emission Tomography (PET) scans and revealed convergent evidence for diminished frontal-striatal connectivity.

Developmental Diseases:

DTI can be used to investigate developmental diseases such as attention deficit disorders and autism. Tensor imaging can reveal neural abnormalities linked to these diseases, and aid in diagnosis and treatment planning. Cody et. al. (2002) suggest examining frontal-striatal connections with DTI to measure the integrity of the white matter connections between the caudate, the pre-frontal cortex, and other regions, in order to explore the relationship of these pathways to stereotyped behaviors in autism. Based on previous findings, Fredericksen et. al. (2002) suggest that DTI can be used to determine if white matter connections are involved in basal ganglia anomalies in attention deficit hyperactivity disorder (ADHD).



Active Tensor Imaging Program at the Wallace-Kettering Neuroscience Institute

Here at WKNI, we have added this powerful new tool to our growing collection of imaging techniques. We boast a 25-direction DTI with automated processing, ensuring timely data analysis. We are using DTI to investigate diseases such as schizophrenia, MS and epilepsy. By using this technology, we are ensuring better patient care for the Dayton area.

Multiple Sclerosis:

Scientists from WKNI and Yale University are currently using DTI to more precisely characterize white matter abnormalities caused by MS, which could lead to more accurate diagnosis and treatment of specific subtypes of MS (Figure 2).

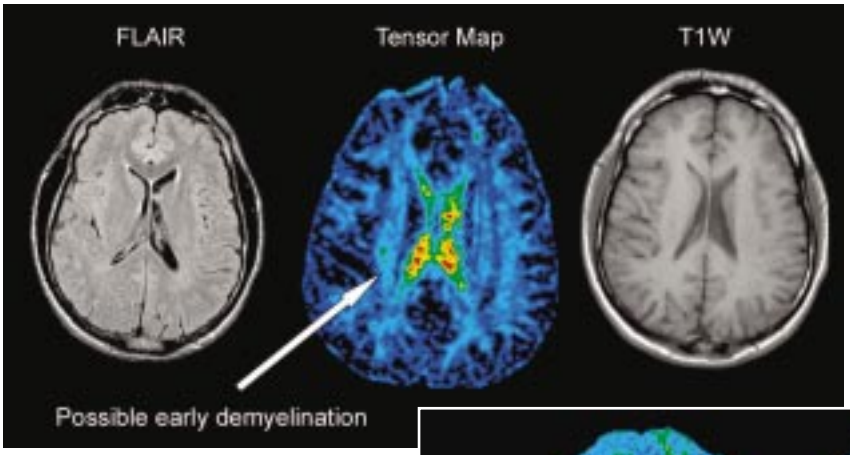


Figure 2.

Epilepsy:

For many epileptics, seizures can be found to originate from a very specific area of the brain (called an epileptic focus). WKNI is testing to see if DTI can be used to localize seizure foci when they cannot be delineated by other means (i.e., MRI or neuropsychological evaluation).

Schizophrenia:

WKNI researchers are combining the use of PET and DTI to further the understanding of how the caudate (a dopamine-rich region of the brain implicated in schizophrenia) communicates with a broader network of brain areas. DTI can be used to investigate the connections between the caudate and other areas, and how these connections might be interrupted. Silenced communication between these areas of the brain would lead to topographical disorganization of function (Figure 3).

Using these imaging techniques in conjunction with each other is unique to WKNI, and will enable our researchers to study schizophrenia in detail, extending our knowledge about the neural circuits linked to schizophrenia.

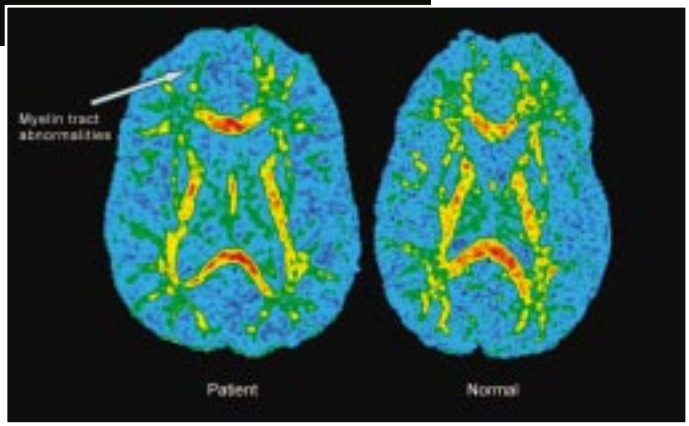


Figure 3.

Future Directions of DTI

Critical evaluation of the diffusion of water in neural tissues is already being investigated in many diseases, such as MS, autism, and others. DTI can produce intricate images of soft-tissue structures that can aid doctors in studying development, degeneration, disease and aging in soft tissue. The implementation of this technique at very high field strength (3 Tesla MR) and characterization of imaging features will extend diffusion tensor capabilities in the future (Figure 4).

Acknowledgment: The support of the United States Air Force, Air Force Research Laboratory (AFRL/HEOP), Air Force Material Command, under cooperative agreement F33615-98-2-6002 is gratefully acknowledged.

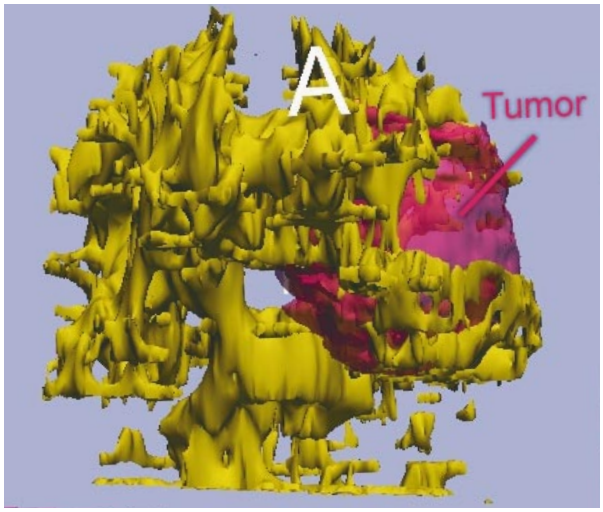


Figure 4. Fractional Anisotropy

Ongoing WKNI Research Projects

Boonshoft Schizophrenia Symposium

The Wallace-Kettering Neuroscience Institute's Boonshoft Schizophrenia Center - in collaboration with the Wright State University School of Medicine's Department of Psychiatry - will present the Fall Schizophrenia Symposium on October 28, 2003, from 8:30 a.m. to 5 p.m. The event will be held at Sinclair Community College's David H. Ponitz Conference Center, located at 444 West Third Street in downtown Dayton. For additional information, please call Aaron Murray at (937) 395-8227.

Neuroscience Summer Intern Program at WKNI

In order to provide students an opportunity to gain experience in the field of medical research, Wallace-Kettering Neuroscience Institute created a summer internship program, which is in its third year of existence. The program allows students to gain hands-on experience in many aspects of medical research by working with medical professionals. Through the summer of 2003, 13 students have participated in this program and expressed overall satisfaction with their time spent here. The students have come from a diverse background of varying areas of study at different institutions. Some of the interns from the past and present are discussed here:

Katie Kimble is currently a graduate student in the field of human factors psychology at Clemson University. She was an intern at WKNI in the summer of 2001 and spent her time looking at fMRI studies that focused on mapping language areas of the brain, including Broca's area and Wernicke's area.

"I learned a great deal at WKNI – about how to do research and conduct studies. It was a very interesting and fun experience!"

John Maffie, currently attending Cornell University to complete a degree in biology and chemistry with a concentration in psychology, is applying to medical schools and M.D./Ph.D. programs. Primarily, during his time at WKNI, he worked with Bingzhi Shi adapting a synthesis method for F18-flurocholine, a PET tracer to use at the nuclear chemistry lab with a dramatically longer half-life, enabling it to be transported to nearby hospitals for use in tumor detection. In addition, John assisted Mike Kent, looking at the role of antiphospholipid in multiple sclerosis, and helped Mehdi Adineh, researching, writing and editing grant applications while observing fMRI and MRI work.

"Working at WKNI gave me the unique opportunity to see and participate in a diverse cross-section of scientific research in a medical setting."

Robert Nauman (Tom) graduated from the University of Dayton in May of 2003 with a psychology major and a philosophy minor. He plans to apply to neuroscience Ph.D. programs to attend in the fall of 2004. He began working for WKNI in the summer of 2002, and continued his internship this summer. He has worked on developing a battery of language paradigm presentations to be used with functional MRI (fMRI). He also had the opportunity to work with Mike Kent doing immunohistochemistry.

"Mehdi and Mike and all of the MRI techs are great people to work with. I've been interested in functional imaging and I'm glad I've had the opportunity to learn about it firsthand."

Leslie Mathewson will graduate from Antioch College in May of 2004 with a major in biochemistry and plans to apply to medical schools. She recently began interning at WKNI. She is working with Mike Kent, performing scientific literature searches and investigating multiple sclerosis and antiphospholipid antibodies using neuroimaging and neuroimmunological methods in detecting phospholipid changes in the hippocampus of patients with epilepsy.

"I've expanded my knowledge of multiple sclerosis and epilepsy, and I've really gotten a chance to apply my classroom lab experience to an actual project."

Ryan Mace is currently attending the University of Maryland with a focus in business and pre-law. After college, he plans on applying to law schools in the Washington D.C. area. He is interning this summer, working on the *Neuroscience Directions* publications for WKNI, and updating the current WKNI website.

Christina Hochwalt is currently studying zoology and neuroscience at Miami University. During her time here, she learned how the grant process worked. Her major project was a grant for a smoking cessation program for teens at high risk. Under the assistance of Dr. Kent, she performed immunohistochemistry and histology and observed staining of slides in research studies of patients with epilepsy and MS.

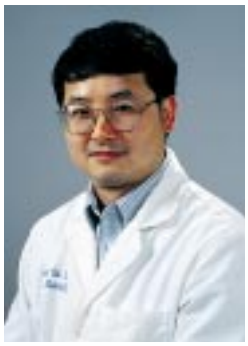
Jeremy Olson is currently a student at Harvard University, and is considering physics or biophysics studies in graduate school. As a WKNI intern, he researched and worked on a study to optimize techniques for assaying the quantities of phospholipids in the brain, which would eventually be used on hippocampal tissue biopsies.

Brittany Cleveland worked as a student intern during the summer of 2001. She edited the final report for WKNI's Cooperative Agreement with the Department of Defense. She currently attends Baylor College as a pre-med major.

Heather Singleton is a graduate student in the field of human factors and experimental psychology at the University of Dayton. After completing her Master's in May of 2004, she plans to continue research work, and may move to the field of sensory research and usability testing. Heather has worked on scientific literature reviews and on IRB proposals for research projects. She is creating paradigms (or tasks) to use with fMRI for mapping various regions of the brain.

"Working on many diverse projects while I've been here has really helped me develop as a professional. I truly enjoy the work that I do, and the people that I work with during my time here."

WKNI Scientist Profile



Bingzhi Shi, Ph.D.,

Radiochemist, PET/Nuclear Medicine Department, Kettering Medical Center Network.

Bingzhi Shi, Ph.D., has been a Radiochemist in the PET/Nuclear Medicine Department at Kettering Medical Center Network since 1998. He is currently conducting research in the development of radiopharmaceuticals for the study of dopamine receptor and transporter systems. He received his Masters in Organic Chemistry from Beijing Polytechnic University and a Ph.D. in Organic Chemistry at Iowa State University. After receiving his Ph.D., he stayed at Iowa State as a research associate in PET until 1994. He then went on to complete a postdoctoral fellowship in PET at Emory University School of Medicine. After finishing his fellowship, Dr. Shi stayed on as a faculty member for the Department of Radiology until December 1997.

Along with his responsibilities to produce routine clinical radiopharmaceuticals, Dr. Shi has implemented an improved method for the production of F-18 Fdopa, gold standard PET tracer for Parkinson's disease. He has contributed to various WKNI research projects by adopting automated production of C-11 methionine and C-11 choline for tumor diagnosis, C-11 flumazenil for seizure focus of epilepsy sponsored by the U.S. Department of Defense, and F-18 fallypride for schizophrenia research sponsored by the Boonshoft Schizophrenia Center.

Dr. Shi's research interests center on applying synthetic radiochemistry technique to develop PET radiopharmaceuticals. He is currently involved in the design and synthesis of PET biological probes for the investigation of neurotransmission systems, especially dopamine neurotransmission systems. Recent efforts include the development of PET radioligands of dopamine receptor agonists and dopamine transporter, and their potential application to the studies of neuropsychiatric illnesses, including schizophrenia. Dr. Shi has published a dozen peer-reviewed journal articles and numerous meeting abstracts and symposium proceedings. He serves as a member of the KMCN radioactive drug research committee.

Dr. Shi lives in Mason with his wife, Yili Wang, and their children, James and Chelsea.

Coming in future issues ...

- **Spinal Disorder Surgery Techniques**
- **Vertebroplasty**
- **Highlights of the Boonshoft Schizophrenia Symposium**
- **Frameless Stereotactic Surgery**
- **Gamma-Knife Surgery and MR Spectroscopy**
- **Neurotoxins**
- **Neuroscience Specialty Nursing**
- **Software for Image-Guided Spine Surgery**

References and Bibliographies for all articles can be found on our website at www.wkni.org

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