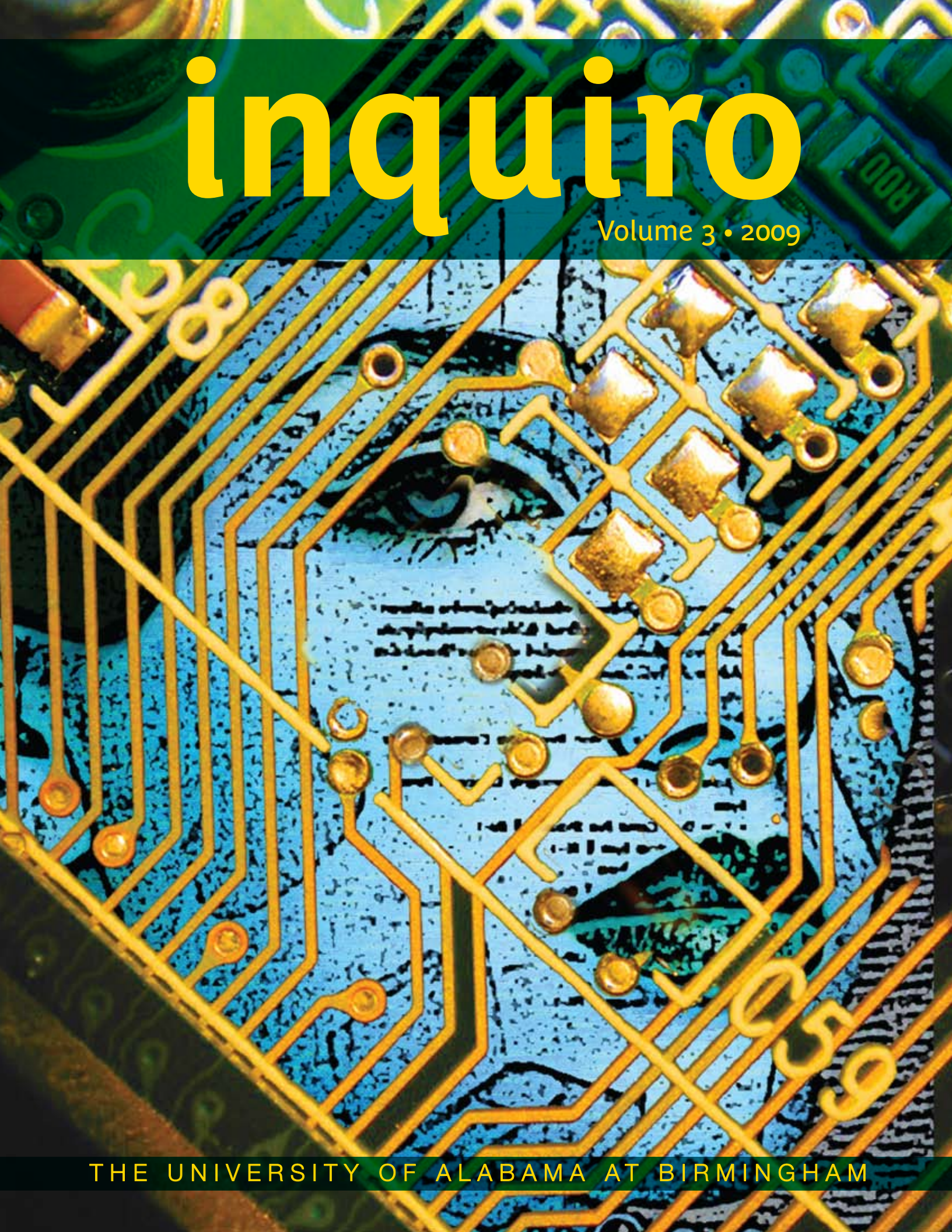
inquiro

Volume 3 • 2009



THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

This is an internal document of

UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Front cover art:
Anna Stein, psychology major

Inside cover art:
Jackson Echols, art studio major specializing in photography

inquiro

Volume 3 • 2009

Founded and staffed by undergraduate students at the University of Alabama at Birmingham, *Inquiro* is an annual research journal produced as an outlet for the publication of undergraduate scientific research. UAB is an excellent undergraduate research university, and with the addition of a journal such as *Inquiro* in which to publish their findings, the package is complete. Any undergraduate student at UAB, as well as any student participating in a summer program at the university, is eligible to submit research. The rights to every paper published in *Inquiro* are retained by the author, leaving each individual free to submit to and publish in a larger national journal or magazine. Students are invited to submit research papers, short reports derived from posters or research narratives throughout the year.

from the editors

Inquiro: to search; to know.

Curiosity about the natural world has been a defining trait of our species since the beginning of time. Ancient civilizations all over the world developed methods to harness the power of nature and to explain the mysteries of the universe. The human spirit of discovery has survived millennia and flourishes now more than ever before. As we uncover the secrets of the human genome, the laws of modern physics, and the delicate balance of our environment, we embark on unprecedented journeys into the unknown. Furthermore, it is a journey which allows us to escape national borders, age differences, and even language barriers. This journey is for all humanity.

This journey of inquiry is like gravity: its potency pulls us in to discover the answers of the unknown. Because of the atmosphere at the University of Alabama at Birmingham (UAB) and its dedication to scientific research, many students have been enticed by the world of research, like Andrew and I. However, we don't just stop there; we don't just stop at finding solutions. We want to share our findings, our passion, with our peers. Why do research if you don't share it with others to allow for further discoveries and improvements? *Inquiro* is our solution.

The first issue of *Inquiro* was published in 2007 when Andrew and I were in our first year in college. We realized quickly what an impressive journal it was because it solely involved undergraduates as contributors and editors. Our first step was to join the 2008 *Inquiro* editorial board. As staff writers our sophomore year, we were deeply involved in the process of editing and reviewing submissions, writing articles, or conducting interviews. This was not our first time being part of a publication committee: in high school, Andrew was involved his school's yearbook and I was involved in my school's literary magazine. Despite our prior experience that rooted our joy for producing publications, working on Inquiro was perhaps more satisfying and exhilarating because of its relatedness to scientific curiosity, being a part of a process that displayed our peers' hard work to the community.

After a successful second issue of *Inquiro*, we wanted to remain a part of the editorial board. Our growing passion for the journal and constant need to seek the next tier of challenges motivated us to apply for the Chief Editor positions. Receiving this position, we were overwhelmed with excitement to play an integral part in the publication of the journal. We had ambitions of making the journal bigger and more diverse, a feat we like to think we have accomplished.

Inquiro, now in its third issue, has exceeded all of the expectations set by the inaugural issue. This journal is admired and supported by all students, faculty, and administration. UAB, more than any other institution in the state, encourages students to participate in research, and students are certainly responding. This year, not only did we have more involvement and submissions than previous years, but we had more diversity in submissions as well. For instance, in this issue, we have published papers on neuroscience, we have more research narratives outside of the editorial board, and we have

accepted our first computer science paper. With more high-quality submissions also came more competition for acceptance. The success of *Inquiro* is a testament to the unique atmosphere at UAB that encourages undergraduate students to participate in research.

Because UAB is research-oriented, *Inquiro* was established from the need of an outlet through which UAB undergraduates can display their ground-breaking research. Every year, students have the opportunity to work in research labs, whether it is through summer programs, departmental honors, or independent studies. Although many university departments hold research symposiums throughout the year, such as the annual UAB Expo held in April, it is rare for students to have the opportunity to display their work before peers and faculty from other disciplines, as well as to the university community as a whole. These symposiums provide undergraduates ample opportunity to perfect their presenting skills; however, *Inquiro* allows undergraduates to polish their scientific-writing skills. While undergraduates may work in labs for a few semesters or a summer, it is unusual for students to publish their work in internationally peer-reviewed journals, simply due to time constraints.

This third issue of *Inquiro* helps to embody the unyielding legacy of the University of Alabama at Birmingham. This journal gives students the opportunity to partake in the scientific process and prepare their research for publication. Each paper is reviewed by at least one or two faculty members, so that students get a feel for submission and revision processes, preparing students for the graduate and professional world. The concept of the undergraduate journal has previously been embraced by other universities such as Harvard, Columbia, and Yale. With the continued success of *Inquiro*, UAB students now have the opportunity of ascending to the undergraduate publishing ranks with the best and brightest students in the nation. Please join us as we blaze the trail for the future of undergraduate research at the University of Alabama at Birmingham!

—Andrew Buie and Shweta Patel
Chief Editors

inquiro

Volume 3 • 2009

table of contents

Letter from the Editors 2
Science News 4

Research Narratives

Finding a Lab: My Niche 10
The Incision into the Realm of Science 12
Scientific Research? Child's Play... 14
Transitional Research for Undergraduate Students 17
You're Not in High School Anymore 19
Adventures in Deutschland: Laboratory Learning 21

Faculty Interview: Dr. James Ward, Mathematics 23

Short Reports

Accelerating Lossless Data Compression with Graphics Processing Units 24
A Preliminary Characterization of Btbd9 Knockout Mice 28
The Effect of Mimetic Peptide 4F on Paraoxonase-1 30

Faculty Interview: Dr. Thane Wibbels, Biology 34

Papers: Biology

A *Caenorhabiditis elegans* Mutagenesis Screen to Identify Candidate Human Cystic Kidney Disease Genes 37
Environmental Tobacco Smoke: Role in Progression of Diabetic Nephropathy 46
Evaluating Sex Ratios of Hawksbill Hatchlings on St. Croix, U.S. Virgin Islands 50
Spatial Analysis of Environmental Factors Related to Lyme Disease in Alabama Using NASA Earth Obeservation Systems 57

Paper: Biomedical Engineering

Single Walled Carbon Nanotubes as a Regenerative Substrate in Spinal Cord Injury 70

Faculty Interview: Dr. Jacqueline Nikles, Chemistry 76

Paper: Chemistry

Microcrystal Analysis of Cocaine Hydrochloride and Added Adulterants 78

Faculty Interview: Dr. J. David Sweatt, Neuroscience 80

Papers: Neuroscience

Cognitive Abilities and Cortical Activity: a functional MRI Investigation of Figurative Language Comprehension 82
Obese Women with Greater Impulsivity Show Reduced Executive Function Brain Activation During Delay Discounting 85

Faculty Interview: Dr. Bradley Newcomer, Physics 96
Student Feature: UAB's 2009 Goldwater Representatives 97
Inquiro staff 98
Submission Guidelines for 2010 99
Acknowledgments 100

science news

Melting on Mount Kilimanjaro

Natalie Mitchell

Snow on the peaks of one of the world's tallest mountains is melting at an extremely rapid rate and may disappear by the 2020s. According to scientists, dark rocks surrounding the ice on Mount Kilimanjaro in Tanzania absorb sunlight, causing an increased melting rate of ice on the mountain. Findings published in *Proceedings of the National Academy of Sciences* (November 2009) report that the melting is occurring at an alarming rate. From 1912 to 1953, ice coverage declined by only 1.1 percent per year; however, from 1989 to 2007, that rate increased to 2.4 percent per year. An astounding eighty-five percent of the ice cover has vanished since 1912. Studies have also indicated that one quarter of the ice cover present in 2000 had disappeared by 2007.

Current debate centers on whether Mount Kilimanjaro's ice loss stems from melting caused by global warming or from increased sublimation. However, no definite conclusion can be made regarding the role of either human activity or climatological influences on the melting. Researchers do stress that that the melting seen on the mountain is paralleled by melting occurring in ice fields elsewhere throughout the world, including South America and Indonesia. Thus, more conclusive research must be conducted in order to generate a clearer picture of this startling situation.

New Gene Therapy to Repair Damaged Lungs for Transplantation

Timmy Wang

It is a widely known fact that a shortage of organs, such as kidneys, lungs, and livers, exists in the United States. As a result of this shortage, waiting lists of patients hoping for a second chance at life from organ transplantation continue to grow. However, cutting edge research from Toronto's University Health Network may provide a way through gene therapy to actually repair lungs that would have once been discarded due to damage and/or inflammation of the airways. This especially bodes well for the supply of lungs for transplants as only 15% of lungs from organ donors are qualified for transplant. The therapy was devised in a two step process experiment by Dr. Shaf Keshavjee, the chief of lung transplant at University Health Network. The first step began by taking damaged lungs from pigs as well as 10 donated human lungs. These lungs were each placed into a dome which mimicked the internal temperature, nutrients, and oxygen concentration of the human body. Essentially, the dome was able to keep the lung cells alive outside of the body. The second step was the introduction of a gene known as interleukin-10 (IL-10), which, when expressed, is able to prevent the inflammation of the lungs cells and any further damage of the lungs as a transplant is taking place. This second step began by first inserting the interleukin-10 gene into an adenovirus. The adenovirus was placed in the airways of the lung to allow the cells to take in the virus along with the interleukin-10 gene. From the study, the research team found that the when the gene-treated lungs were transplanted into pigs, there was a significant improvement in the lungs' ability to exchange carbon dioxide and oxygen within four hours of the transplantation compared to that of an untreated lung transplantation. Dr. Keshavjee also noted that it may be possible "to transduce the cells in the lungs to become little IL-10 factories. It's personalized medicine for the organ, if you will." This would mean that the lungs will also not have any post-surgery inflammation. As of yet, there have been no human lung transplants using this IL-10 gene therapy treatment as most specialists believe that more studies need to be conducted on animals before starting human trials. Additionally, others, such as Indiana University's Dr. David Wilkes, have noted that previous studies using the adenovirus as a vector for gene therapy has caused some side effects. However, Dr. Keshavjee made note that the adenovirus disappeared after it delivered the gene into the lung cells, meaning there was less chance of side effects. Overall, if proven effective, both sides agree that this new treatment would improve the lives of many more patients who are still waiting on the list.

World's Most Powerful X-ray Laser Created

Natalie Mitchell

Initial experimentation has begun using the world's most powerful X-ray laser, the Linac Coherent Light Source (LCLS). Located at the Department of Energy's SLAC National Accelerator Laboratory, the LCLS will provide researchers with the ability to illuminate objects at extraordinary speed. The machine can resolve the size of atoms at ten billion times the brightness of any other X ray source.

→ *top of page 5*

Nearly 40 years ago, a two mile linear accelerator (SLAC) was built to study the building blocks of the universe. With distinct modifications to this accelerator, the LCLS was formed and allows scientists to investigate energy science, structural biology, physics and assorted other fields.

After the SLAC Linac accelerates short pulses of electrons to 99.9999999 percent the speed of light, the LCLS takes them through a 100 meter stretch of alternating magnets that force the electrons to move back and forth emitting X-rays. The X-rays become synchronous with the electron pulses, resulting in the world's brightest X-ray laser pulse. The laser pulses combine as many as 10 trillion X-ray photons into a pulse that is 100 femtoseconds in duration (the time it takes light to travel the width of a human hair). Experiments using this instrument have revealed aspects regarding the basics of atomic physics. Specifically, researchers have been able to completely strip neon atoms of all their electrons due to the extreme brightness of the laser beam.

Five other instruments are being planned for the LCLS to gain understanding of how ultra-bright beams interact with matter. Future experiments will create stop-action movies of molecules in motion. This will ultimately allow scientists to watch chemical bonds forming and breaking in real time.

Father of Green Revolution Dies at 95

Aaron Neal

On September 12, 2009, the world mourned the loss of plant scientist and Nobel Peace Prize laureate Norman Borlaug. Though his name never reached household use like scientists Einstein, Fleming, or Curie, Borlaug is considered by many to be the greatest person to have ever lived. "Norman E. Borlaug saved more lives than any man in human history," said U.N. World Food Program Executive Director Josette Sheeran. "His heart was as big as his brilliant mind, but it was his passion and compassion that moved the world."

Born in 1914 to the descendants of Norwegian immigrants, Borlaug spent most of his youth working on his family's farm near Protivin, Iowa. In 1933, he enrolled at the University of Minnesota where he subsequently received his Bachelor of Science degree, Master of Science degree, and Ph.D. in plant pathology and genetics. In 1944, Borlaug accepted an appointment organizing and directing the Cooperative Wheat Research and Production Program in Mexico, a joint undertaking by the Mexican government and the Rockefeller Foundation.

To combat the rapid spread of newly emerging wheat rusts, parasitic plant fungi capable of destroying entire wheat harvests, Borlaug developed hardy, disease-resistant varieties of wheat through crossbreeding and genetic engineering. A mere twenty years after arriving in Mexico, his wheat varieties increased the country's wheat harvest six-fold, allowing Mexico to become self-sufficient and a net exporter of the crop. Borlaug's immense success quickly gained the attention of other nations, leading to the spread of his wheat varieties and the transformation in global agriculture known as the "Green Revolution."

In 1970, the Nobel Peace Prize committee recognized Norman Borlaug's efforts by selecting him for the award. "More than any other single person of this age, he has helped provide bread for a hungry world. We have made this choice in the hope that providing bread will also give the world peace."
While Borlaug's efforts have saved over one billion lives, he encountered harsh criticism for his approach. Many opponents of the Green Revolution considered Borlaug's genetic cross-breeding of plants to be unnatural or to have negative effects. Others criticized his emphasis of large-scale, input-intensive farming techniques over the subsistence farming countries typically relied on. Borlaug took these concerns seriously, though he dismissed many critical Westerners by saying, "If they lived just one month amid the misery of the developing world, as I have for fifty years, they'd be crying out for tractors and fertilizer and irrigation canals and be outraged that fashionable elitists back home were trying to deny them these things."

Despite his enormous success, Borlaug remained humble and dedicated to solving the world's hunger problem. He took a distinguished faculty position at Texas A&M University in 1984, where he continued developing disease-resistant crops in addition to teaching students and advocating the elimination of global hunger. "I want to see science serve a useful purpose to improve the standard of living for all people," Borlaug said. "You can't build a peaceful world on empty stomachs and human misery."

The legacy of Norman Borlaug is undoubtedly felt on every continent by millions and millions of people every day. It is estimated that every day, half of the world's population, over three billion people, consume grain descended from Borlaug's wheat varieties. As the father of the Green Revolution, Borlaug did more to advance agricultural self-sufficiency and sustainability than anyone before him, especially in under-developed nations. "Dr. Norman Borlaug's life and achievements are testimony to the far reaching contribution that one man's towering intellect, persistence, and scientific vision can make to human peace and progress," said Indian Prime Minister Manmohan Singh. "One of Dr. Borlaug's favourite quotations was to 'reach for the stars'. In doing so, Dr. Borlaug helped millions of people escape from a life of hunger and deprivation."

Laser-scanning Microscope Images Brain Cells In Freely Moving Animals

Courtney Sparkman

Source: www.sciencedaily.com

Scientists at the Max Planck Institute for Biological Cybernetics in Tubingen, Germany have designed a Laser-Scanning microscope small enough to be worn by freely moving rats. This new technology will provide critical information for studies of attention and perception processes because of the way that neurons can now be observed:

"We need to let the animal behave as naturally as possible if we want to understand how its brain operates during interaction with complex environments. The new technology is a major milestone on the way to helping us understand how perception and attention work," says Jason Kerr, lead author of the study.

This new, non-invasive microscope uses a high-powered pulsing laser and fiber optics to scan cells beneath the surface of the brain. Insertion of electrons, therefore, is not necessary. The lightweight, miniaturized laser scanning microscope images fluorescent neurons while animals are awake and active.

Attention and perception are complex processes that involve the utilization of senses simultaneously to construct our view of the world. In the past, it has been difficult for researchers to study such processes because information about attention and perception are dependent on the observance of meaningful signals from groups of neurons while the organism is in motion. Past study methods include presenting a restrained animal with movies, images, and scenes while observing brain activity.

Cancer Vaccinations

Ashruta Patel

"Cancer is projected to become the leading cause of death worldwide in the year 2010," with increases in the number of cancer incidences and death rates. There are many types of cancer that result from an uncontrollable growth of abnormal cells. A significant amount of research is conducted on cancer to help discover possible treatments for its prevention, one of which includes implementing the use of cancer vaccines. Cancer vaccinations could increase the immune system's ability to protect the body from infections related to cancer-causing viruses. On the other hand, cancer vaccines have the potential to depress immune functions, risking the contraction of many more diseases.

Cancer vaccines can significantly decrease the occurrence of virus strains in the body by enhancing the function of the immune system. Vaccines are being considered for cancer treatments because of their medicinal properties capable of boosting the immune system's natural ability for protection against various foreign microbes, such as bacteria, fungi, parasites, or viruses. Once a cancer vaccine is injected, the immune system eliminates the substances from the body and develops a memory to protect the system from future threats posed by cancerous cells. White blood cells help the vaccine stimulate the immune system by protecting the body against any abnormal or damaged cells. Successful advances of such vaccines have been made, and implementing their use has led to a decreased number of patients suffering from certain cancer-causing virus strains.

There currently are two cancer preventative vaccines approved by the U.S. Food and Drug Administration (FDA) which have been responsible for cancer not developing in healthy people. There are

vaccines against the hepatitis B virus and types 16 and 18 human papillomavirus (HPV), which can cause liver and cervical cancer, respectively. Both of these vaccines consist of harmless viruses that "take a substance from a cancer cell's surface and attach it to something the immune system already recognizes as foreign," indirectly training the immune system to kill something. The increased use of these vaccines has led to positive outcomes because of their ability to stimulate the immune system. A study conducted with vaccinated women that were not infected with HPV-16/18 showed a high efficacy and proven ability to reduce the incidence of intraepithelial lesions. Not only does the HPV vaccine have the ability to significantly reduce cervical cancer rates, but it is also associated with other health benefits. Chronic infections can arise from one or both of the virus types, which are associated with cancers of the anus, penis, and oropharynx. If one vaccine can assist in a better immunity towards other cancers, continued tests and studies could provide further health advantages.

Additional research using experimental vaccinations for prostate cancer, melanoma (skin disease), lymphoma, and neuroblastoma (childhood tumor) all gave positive results over past treatments such as surgery, chemotherapy, or radiation. The side effects vary from patient to patient and the type of vaccine; however, most side effects reported include mild and limited inflammation. Thus, with accurate experimental designs, cancer vaccines can be promising for many other virus strains because science reports are readily available to back up improvements.

Cancer vaccines increase immune response against cancer cells already present within the body. Vaccines target viruses that cause the cancer rather than cancer cells themselves. Although cancer vaccines have made progress and improvements, additional research still needs to be devoted to the field to eliminate any uncertainties with recent or future discoveries.

Although, cancer vaccinations have been studied for several years, not many advances have been made in the past. Cancer comes from our own cells, making it difficult for the immune system to distinguish normal cells from cancer cells. Despite the discovery of two cancer vaccines, it is difficult to develop effective ones with numerous types of cancer existing. Some cancers "can escape detection by the immune system or weaken natural immune responses against cancer cells." The immune system is the body's only defense against disease, and a temporary immunity from the vaccine can make it vulnerable to developing other infections. The chemicals contained within vaccines can depress the immune system, the virus can depress immune function, and foreign DNA/RNA from tissues can depress immunity.

Vaccinations can reduce immunity in many ways. They contain immuno-suppressing chemicals and heavy metals which can alter the function of white blood cells and deplete the body of vital nutrients. Even though many studies have been conducted, it is difficult to consider vaccines as the best preventative medications for cancer. The lengths of the benefits are unknown, and many vaccines for certain cancers need to be looked at individually for each patient. The vaccines become impractical in this case, and the costs of executing such practices are high.

There are numerous ways cancer can be prevented in a practical fashion. Instead of individually treating each cancer patient and putting money into drug companies for questionable vaccines, increasing education and outreach efforts could be more advantageous. For example, employing regular screening for cervical cancer and eradicating barriers for individuals to access all health services could lower cancer incidences knowing that third-world countries have a greater number of cancer rates. By simply improving lifestyle, cancer deaths can significantly decrease. Changes to lifestyle include not smoking, eating a diet rich in fruits and vegetables, protection from the sun's rays, or practicing safe sex. For instance, the HPV infection can be reduced by practicing safe sex; however, a vaccine against it provides optimal protection. In contrast, the HPV vaccine has many implications related to its use.

The human papillomavirus is common in many women worldwide, yet the vaccine is not effective in women who already have the virus. The vaccine is predominantly used in North America, where cases are low, and an alternative method of prevention can be done by regular pap smears. Countries that require greater attention do not have the vaccine marketed because of the high cost for the series of vaccinations per person. Although there was a significant amount of time and effort designated to discover the vaccine, there is no proof that the Gardasil will prevent cervical cancer. Many of the experimental tests were performed on women whose ages did not realistically match with the average diagnosis age of cervical cancer. For this reason, Gardasil's manufacturer includes "no claims to proof of cervical cancer prevention should be made" and "vaccine has not been tested for its own ability to cause cancer." These statements signify that the vaccine does not have enough evidence to consider it efficient in the long run. Cancer vaccinations have many positive and negative outcomes associated with their use. Cancer is a growing cause of concern, and efforts to prevent or treat the illness should be continued. The discovery of vaccines has helped many individuals from contracting the virus, and further experiments may lead to a more efficient approach. Despite all the drawbacks involved with vaccinations, it appears more probable to continue testing scientific studies to maintain the progression of cancer research. Perhaps, persistent studies could lead to a universal vaccine capable of sufficing the most prevalent cancers.

References

American Cancer Society. "Cancer Projected To Become Leading Cause Of Death Worldwide In 2010." ScienceDaily 9 December 2008. 12 September 2009 <http://www.sciencedaily.com/releases/2008/12/081209111516.htm>

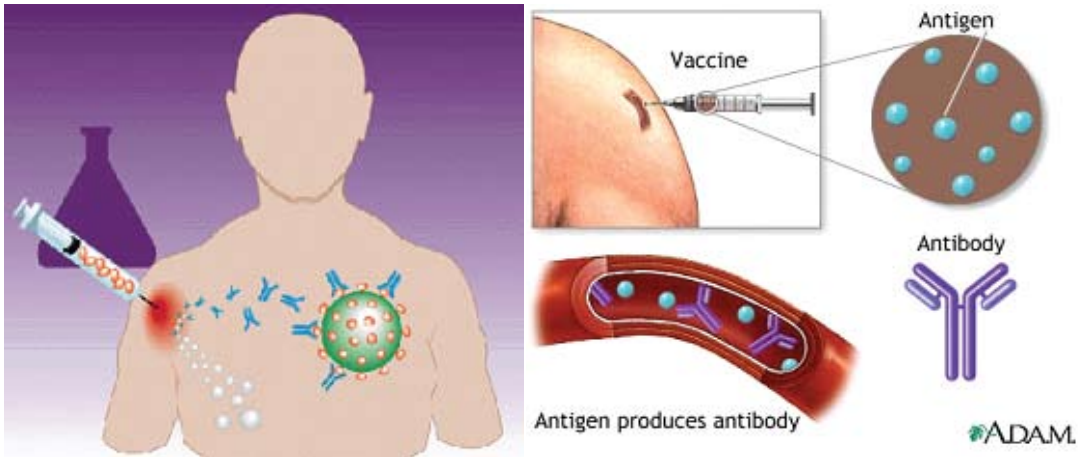


Figure 1 – the processes that occur when a vaccine is injected into the body

Encarta Encyclopedia. "Cancer (medicine)".Encarta Online Encyclopedia 2009. 2009. 15 September 2009. <http://encarta.msn.com>

Hakim and Dinh. "Worldwide Impact of the Human Papillomavirus Vaccine." Springer Science and Business Media. Houston: The Methodist Hospital,2009.

Mercola. "Vaccines and Immune Suppression." 2009. 10 September 2009. <http://www.mercola.com/article/vaccine/immune_suppression.htm>

MSNBC. "Cancer vaccines offer new way to fight disease." 31 May 2009. 10 September 2009. <http://www.msnbc.msn.com/id/31019270/ns/health-cancer>

National Cancer Institute. "Cancer Vaccines." 17 March 2009. 12 September 2009. <http://nci.nih.gov/cancertopics/factsheet/Therapy/cancer-vaccines>

Richardson and Rex. "Proposed Childhood Cervical Cancer Vaccine Mandate: Bad Policy, Questionable Science." January 2007. 12 September 2009. <http://www.whale.to/vaccines/rex.html>

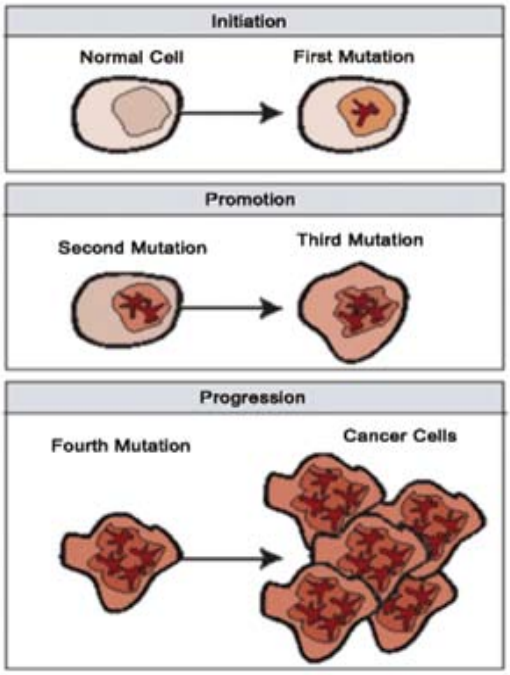


Figure 2 – mutation steps in cancer cells

iPS Cells Yield a Live Mouse

Atbin Doroodchi

Consider making a live animal from their skin cells. It sounds cool and scary at the same time. Induced pluripotent stem cells (iPS) are stem cells that are derived from non-pluripotent cells, i.e. skin cells. Since 2006, the year when iPS cells were discovered, scientists have tried to generate a live animal from iPS. In the summer 2009, scientists made a live mouse from a group of iPS cells in a petri dish. Fanyi Zeng, one of the investigators from Shanghai University, told The Scientist magazine that he had created 27 live mice from 37 iPS cell lines. The researchers told The Scientist that the mice had some abnormalities that weren't described in the paper. In the second study, which was published in the journal of *Cell: Stem Cell*, Dr. Shaorong Gao of the National Institute of Biological Sciences created four live iPS mice from five iPS cell lines, one of which survived to adulthood; according to the paper, it was completely healthy. Yet the production of a live animal from iPS is not as effective as it should be. Only half of the iPS cell lines in Zeng's study yielded live mice. Also, one out of five iPS cell lines in Gao's study resulted in a live animal. In my opinion, this technique still needs to be improved. These studies showed that iPS cells have the potential of being as powerful and pluripotent as embryonic stem cells.

Nobel Prizes are Awarded

Atbin Doroodchi

The 2009 Nobel prizes were announced in October. The Nobel Prize in Chemistry was awarded to Venkatraman Ramakrishnan, Thomas Steitz, and Ada Yonath for their studies of the structure and function of ribosomes. Ribosomes are essential cellular components responsible for translating messenger RNA into complex proteins. In Physics, the prize was shared between Charles Kao, for his groundbreaking achievements regarding light transmission through optic fibers, and the scientists Willard Boyle and George Smith for their invention of the charged coupled devices (CCD) sensor, an imaging semiconductor circuit. Fiber optic cables consist of glass or plastic fibers that carry light along their pathway, and they are used extensively in medicine, the military, and telecommunications. CCD is a device used to displace electrical charges, usually from within the device to an area where the charge can be manipulated. CCD is used in digital cameras, allowing the captured image to be converted to a digital signal. The Nobel Prize in Medicine was awarded to Elizabeth Blackburn, Carol Greider, and Jack Szostak for their discovery of how chromosomes are protected by telomeres and the enzyme telomerase. Telomerase rebuilds the tips of chromosomes and ultimately determines the life span of cells. Their finding is extremely important in many fields, particularly the study of genetic diseases and cancers.

Carol Greider

Thomas Steitz

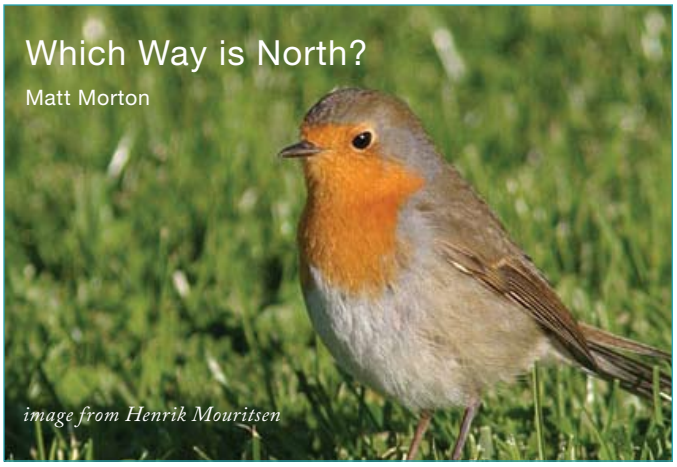
George Smith

Artificial Germ Cells May Hold the Key to Studies of Early Human Development

Timmy Wang

Recently at Stanford University, researchers have been able to control and differentiate embryionic stem cells into germ cells. In certain cases, the germ cells were able to mature even further into spermatids. Previously, the research had only produced immature versions of germ cells, but researchers at Stanford were able to push the differentiation process into the creation of viable germ cells through a complete meiosis. The research team hopes that these germ cells will be a great help in the study of meiosis in human cells as well as in the early development of the human embryo since the only research conducted in this area has been mice embryos. This sets a limitation regarding the knowledge of what humans know about their own reproduction as the reproductive genes in humans are unique, according to Dr. Reijo Pera, professor of obstetrics and gynecology at Stanford University of Medicine. The team plans on continuing research in order to produce a human oocyte. If this becomes possible, the team hopes that the research will be able to further help infertile couples, who make up 10 to 15 percent of all infertile couples, that are unable to produce their own sperm or eggs. This will allow couples to have their own children as well as help prevent the possibility of birth defects, such as Down's Syndrome, that may arise due to incorrect meiosis of the germ cells. However, this does not mean that the purpose of this research is to make artificial children as the current germ cells do not contain human DNA but rather foreign DNA, known as transgenes. It would be considered unethical to make children who have foreign DNA. Due to this concern, Dr. Pera says that the research team is still "waiting for guidelines and regulations regarding how to go about using artificial germ cells." Yet she remains hopeful that these germ cells will be able to accelerate research in future projects.

science news



Which Way is North?

Matt Morton

image from Henrik Mouritsen

Each year, countless birds migrate to more acceptable living conditions with the changing of the seasons. They do so by using a previously misunderstood 'sixth sense' that allows them to detect the Earth's inherent magnetic field and orient themselves accordingly. Until recently, many researchers attributed this ability to iron-based receptors in cells of the upper beaks of migratory birds. However, a recent study published in *Nature* suggests that much of this magnetic sense is the result of specialized light-sensing cells of the eye that send signals to a light-processing part of the brain known as cluster N. Henrik Mouritsen of the University of Oldenburg in Germany claims that special proteins, cryptochromes, in birds' eyes play a large role in light-dependent magnetic sensing. When struck by light, the proteins produce a pair of molecules known as free radicals. Unpaired electrons on the free radicals have a spin property that may be susceptible to magnetic fields. Signals generated by these electron spins may then travel to cluster N where the brain interprets the signal and informs the bird which direction is North. To test this hypothesis, Mouritsen and his team caught 36 migratory European robins, all of which were able to correctly orient themselves under both natural and induced magnetic fields. In one group of robins, the trigeminal nerve, which connects the beak cells to the brain, was severed. In the other group, researchers damaged brain cells in cluster N known to receive light signals from cells of the eye. Those robins with the severed trigeminal nerve were still able to orient correctly, but those with damage to cluster N were not. Since information was unable to travel from the beak to the brain in the group of birds with the severed trigeminal nerve, this study suggests that the beak cells and trigeminal nerve play little, if any, role in orientation, though Mouritsen thinks the beak cells may still be responsible for detecting minute changes in magnetic field strength along the north-south axis. The study has important implications for conservation efforts focused on the relocation of migratory species. These birds often return to their migratory grounds after relocation, but if scientists are able to understand exactly how birds navigate then conservationists may be able to fool birds into permanently relocating to safer, more habitable environments.

research narrative

Finding a Lab: My Niche

Toral Patel

Before coming to the University of Alabama at Birmingham (UAB), my career goal had always been to be a doctor. Not sure about the specialty, I held onto the options of being a cardiologist, endocrinologist, or oncologist. Swayed by family and my interest in helping people through the use of medicine, I decided to pursue Molecular Biology and a pre-medical degree...only to realize that at UAB I would be inspired by research.

Spurred by a genuine curiosity in developing and answering questions that could increase our understanding of the world and enable us to save lives, I began a search for laboratories I could become a part of. I looked for opportunities in which I could fulfill my desire to travel the bridge between practical science and a clinical setting. With no knowledge of the office of undergraduate research or faculty at UAB, I narrowed my interests to the pathogenesis of cancer and cardiovascular disease. From there I began an online search and came to the page of Dr. Dale Benos, Ph.D., Chair and Professor in the Department of Physiology and Biophysics. I was intrigued by his molecular-based research in evaluating potential mechanisms involved in the pathogenesis of cancer, human immunodeficiency virus (HIV), and cystic fibrosis. I learned of and established techniques such as tissue passaging, cell counting, basic light and fluorescent microscopy, wound-healing or scratch assays, quantification by spectrophotometry, Real Time-PCR, restriction digests, gel extraction, subcloning, and minipreps of bacteria. I began to understand what a true hands-on experience in science was as I applied what I had just been taught.

With the thrill of being able to perform experiments and develop new findings in a particular field, I volunteered in the Department of Physiology at The University of South Alabama with Dr. Mary Townsley, Ph.D. After being shown a few of the basics, I was given responsibilty for my own research project in determining the presence of certain TRP isomers in whole lung samples and pulmonary microvascular endothelial cells (PMVEC). Specifically, I examined TRPV1 and TRPV4 through PCR and gel electrophoresis. My findings informed Dr. Townsley's own research project, but more important the work allowed me to think independently and to apply what I knew without someone's assistance. Being able to work alongside researchers who were eager to share their passion with students and heighten our experiences in research was invaluable. The support and guidance I received ultimately persuaded me to consider a Ph.D. in the future.

While working in both Dr. Benos and Dr. Townley's labs, I re-

alized that there was an interdisciplinary if not multidisciplinary nature of science and research. In order to further understand research, I ventured into the world of research seminars and conferences, where posters, powerpoint presentations, lectures, and national meetings embodied the work of many individuals and greatly displayed the collaboration of many disciplines. These seminars and meetings featured research in everything from physics and biomedical sciences to biology and biochemistry. There were many scientific implications of the experiments done by other people, but none of them seemed to satisfy my own desire to experience the entire adventure from writing a proposal to running experiments and evaluating data.

While participating in seminars during the fall semester of my sophomore year, I came across a laboratory in the Department of Medicine that used molecular-based techniques to work on projects for understanding the role of novel synthetic drugs (specifically peptides) in atherosclerosis. Branching medicine and coronary artery disease together, I was intrigued by the approach and began to familiarize myself with the laboratory and the research projects. After a year in the lab, I began my own research project under the supervision of Dr. David Garber, Ph.D. I have been able to work with Dr. Garber in determining the effect of the mimetic peptide 4F on the enzyme paraoxonase-1 (PON-1). I was given the opportunity to work with human cell lines, primary mouse hepatocytes, and animal models. Working in the Atherosclerosis Research Unit is great as I use an interdisciplinary approach to determining the effects of the peptide and the requirement of the enzyme for the anti-oxidative effects of peptide 4F. I have been able to take what I learn from my classes in molecular genetics, biochemistry, and organic chemistry and incorporate it into my understanding of this topic. Today, I can look back on my journey from not quite understanding research to finding my niche in a lab that bridges both basic science and medicine and excels in developing novel therapeutic interventions that I could one day use as a physician.

I advise, if not encourage, students with curiosity and eagerness to explore new areas to try research. Go into a lab and learn its techniques and experience thinking independently and incorporating everything you have learned from your classes. Even if you are not interested in lab-bench science, other opportunities for research in other fields can be found on campus and can allow you to gain an appreciation for how scientific or medical research is conducted.

The best part of research for me has been getting involved in behind the scene research that might be applied to medical practice in the future. Even though my journey in research has led me through many labs and through much searching into my own personal motivation and determination, there are many programs at UAB through which students can explore fields of science, enhance their creativity and intelligence, and work with nationally and internationally claimed researchers.

The Incision into the Realm of Science

Chase Taylor
& Jason Neeley

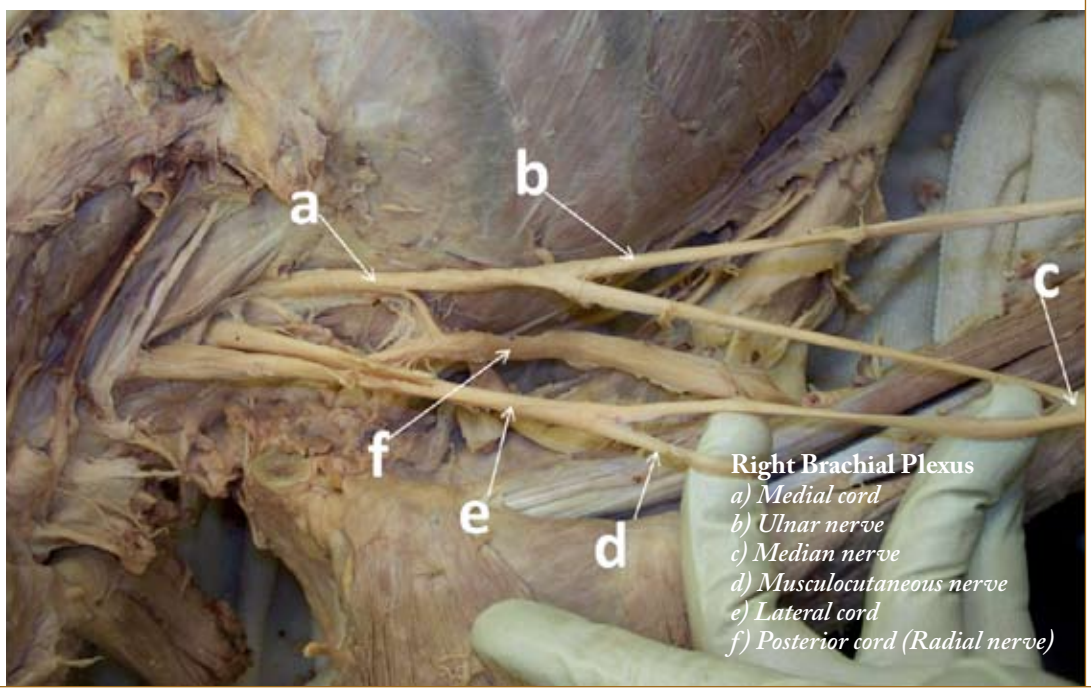
Suddenly, in the midst of mid-afternoon, my cell phone rings with the tune of a familiar number. I answer the phone with a friendly, "Hello, Leslie," not knowing what to expect. I was soon asked to enroll in a newly restructured advanced dissection class with Dr. Dana Peterson and Leslie Hendon as the instructors. "Oh my, I have a full schedule this semester, there is no way I can do this," I replied with great apprehension.

"I think that you can handle it, it will be an awesome experience. Besides it will take your mind off the rest of your classes and at the same time, you will be earning credit. And, you will have so much fun!" Leslie exclaimed.

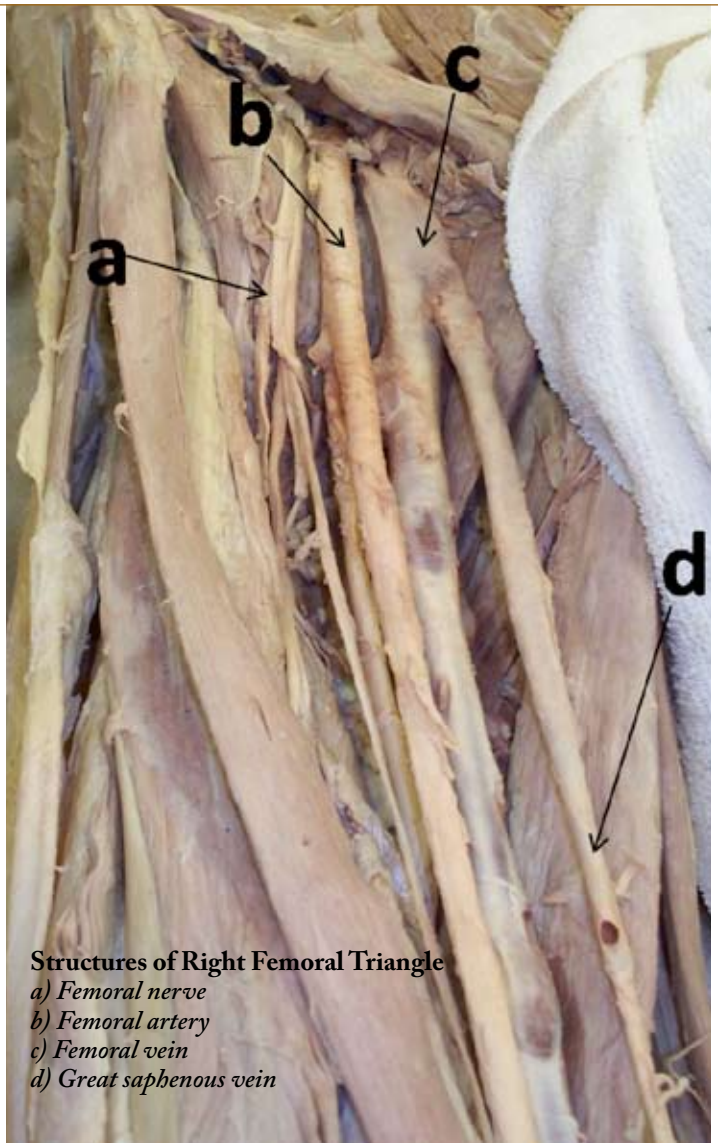
As the conversation continued, I began to think to myself, "Why not? I already have so many positive academic activities; why not dive into another course that I can be passionate about." As a result, I answered, "Okay, okay, I will do it!" It was that particular telephone call that would add hours to my fall semester class schedule and a new excitement in life.

When my partner Jason Neeley and I unzipped the cadaver bag for the first time, I was overwhelmed with emotion. I was there. I was about to dissect a human cadaver and I am not even in medical school, let alone a doctor of any sort – just an undergraduate student that has a passion for medicine and all the surprises it offers. I soon grasped the scalpel with a nervousness that I had never felt before. It was as if I had fallen asleep and had begun to dream just as a child would if they had discovered the pot of gold at the end of the rainbow and didn't know what to do. As time passed and the integument was removed, there were several incisions that I made that were deeper than I had wanted, but as I glanced at the hour hand on my wrist watch during our initial lab session, I discovered that Jason and I had already been in the lab for four hours. It was now time to go home and get some rest before my eight o'clock class the following day.

Lying there in bed that Wednesday night reflecting back on my first time with a scalpel in my hand, I was both a happy and frustrated young man. I had made an incision in the skin of the posterior torso with the intention of removing the integument from around the area of the trapezius and latissimus dorsi while leaving behind the superficial nerves and veins. No more did I advance the length of my incision in the medial to lateral direction than I realized I had severed the latissimus dorsi. Not only was this a terrible feeling to have on the first night in the lab, but it also made me realize that there is a little more to dissecting a cadaver than simply slicing away until revealing musculature as perfect as the anatomy diagrams from my dissection book. Although I was not careless with my dissection, I learned that there are certain precautions and techniques that must be practiced in the laboratory. Also, I concluded that there would be challenges that I would have to overcome in order to gain the most from this course. For instance, I had never held the tools that one uses to dissect or manipulate the human body. The only information that I came into lab knowing was what I had read from the required textbook, *Grant's Dissector*, and the knowledge I had gained from the human anatomy course that I had taken previously. I initially followed the instructions of which instruments to use during my dissection, but I quickly realized that this was a unique experience



Right Brachial Plexus
a) Medial cord
b) Ulnar nerve
c) Median nerve
d) Musculocutaneous nerve
e) Lateral cord
f) Posterior cord (Radial nerve)



Structures of Right Femoral Triangle
a) Femoral nerve
b) Femoral artery
c) Femoral vein
d) Great saphenous vein

and that in order for my dissections to be perfected, I would need to develop my own unique methods. In doing so, I have learned that the careful execution and time that I put into each dissection is precious.

Trust me, there were times we would much rather have chosen to be at home on the weekends watching college football and visiting with friends and family, but we were committed to our dissection efforts.

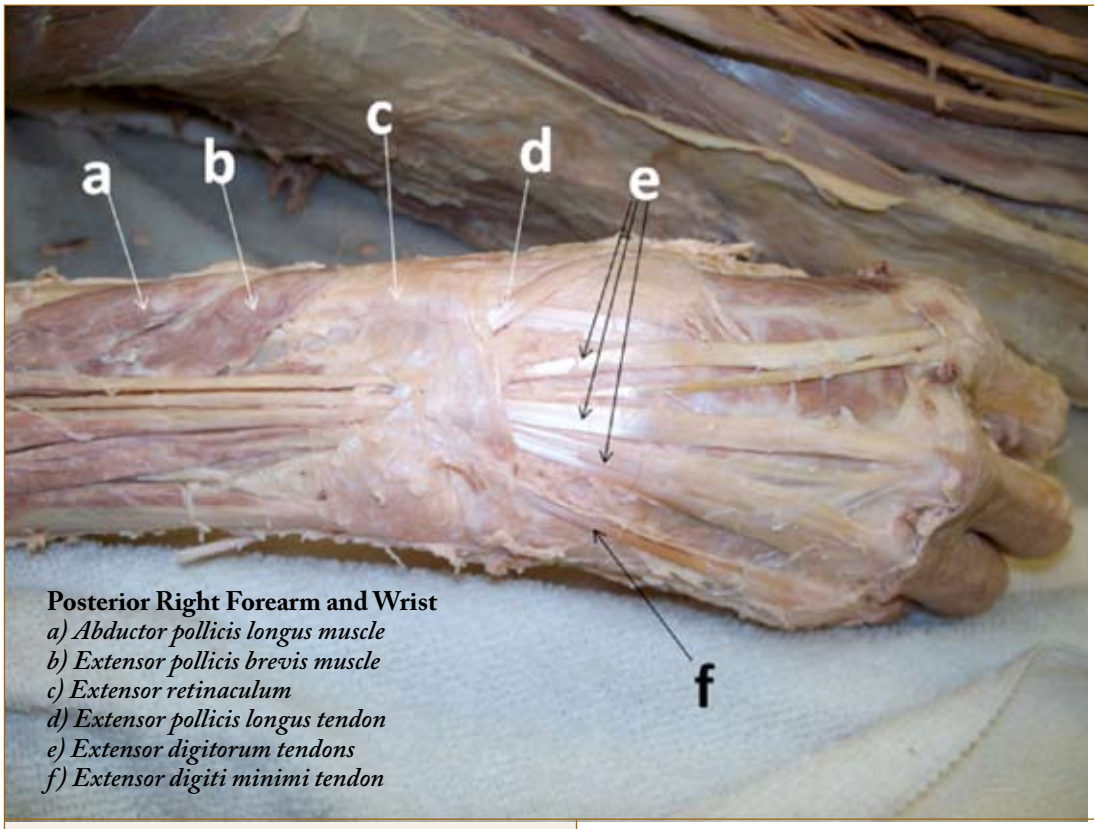
As we acquired a new respect for the value of the hands-on experience gained during our time in lab, another aspect of our lab experience began to hit us in a very profound way. The reality that total strangers donate their bodies to science so that we might gain intellectual insight struck both Jason and I as an invaluable gift. I never imagined that taking a biology class in college would change my view on science so drastically. It is only science – how sentimental could it be? Well, in fact, it has the ability to be very sentimental. It is one thing to know that my family supports my future academic goals, but knowing there are people in the community that I have never met or even knew existed, that care just as much, if not more, that are invested in our futures as health care professionals, is truly inspiring. There were times in the lab when I was exhausted and wanted to lay down my scalpel and go home, but I always remembered how much this research meant to the individual who donated his/her body to science and realizing this inspired me to maintain concentration and carry on with the tasks for that day. Overall, the immense appreciation that Jason and I gained from this course was moving and life-changing.

Nevertheless, aside from the newly acquired dissection skills and the new appreciation for science, our work continued to be mostly tedious. It was paramount never to rush any dissection procedure, whether it was removing the superficial fascia from the skeletal muscles or separating the individual nerves of the brachial plexus. In addition, it became particularly important to be aware of what was in the immediate proximity of the area being dissected. If these structures are not clearly understood it is easy to reveal the unforgiving nature of the scalpel. However, our time spent dissecting progressed with great speed. For example, I went to the lab one morning at six o'clock with the intent of spending a couple of hours dissecting and leaving in time to take a shower and make it to class by ten. However, I suddenly realized that four hours had already passed and there was now no time to shower before class started. Needless to say, I went to class smelling of formaldehyde, but with the glow of satisfaction from the opportunity to gain greater understanding and appreciation of human anatomy.

...I was overwhelmed with emotion. I was there. I was about to dissect a human cadaver and I am not even in medical school, let alone a doctor of any sort – just an undergraduate student that has a passion for medicine and all the surprises it offers.

So, Jason and I continued on with countless hours of dissection on numerous occasions inside the cadaver lab, studying and examining every aspect of the cadaveric anatomy that we revealed. As a result, we were able to turn what were initially amateurish, casual explorations, into impressively detailed dissections. However, our expertise did not come without cost. We spent approximately fifteen to twenty hours per week in the lab in order to meet the goals that we had set for ourselves, in addition to the many study hours the rest of our courses demanded for success.

→ *top of page 14*



Posterior Right Forearm and Wrist
a) Abductor pollicis longus muscle
b) Extensor pollicis brevis muscle
c) Extensor retinaculum
d) Extensor pollicis longus tendon
e) Extensor digitorum tendons
f) Extensor digiti minimi tendon

The decision to enroll in an advanced cadaveric dissection course, BY 398, with instructors Dr. Dana Peterson and Leslie Hendon, has been one of the most valuable and rewarding choices of my academic career. UAB is one of the few institutions in the nation that offers a cadaveric anatomy course to undergraduates. I would encourage all those who have decided on a future career in science or medicine to consider enrolling in the advanced dissection class, so that they too, might uncover their own passion for science and research.

research narrative

Scientific Research?

Harrison To

hild's Play…

Lunchtime is long past due. The cafeteria's indistinguishable chatter has grown weary. The early morning ritual PB & J sandwich mommy fixed for me has been devoured, my Capri-Sun drained, and applesauce inhaled. My yellow Pokémon lunchbox is a pigsty of breadcrumbs, Ziploc bags, empty cartons, and a half-eaten cookie. Time drags by. Anticipation mounts as the teacher approaches. One by one, my classmates rise and line up behind one another. ***RECESS TIME!!***

Recess was always my favorite part of the day. It was my chance to escape the uniformity of the classroom where every student read the same books, spelled the same words, and worked the same math problems. During recess time, I was at total liberty to immerse myself in the sand pit where I manipulated my surroundings to dig the deepest trenches or build the biggest forts, castles, caves–whatever my creativity could harness. The jungle gym was a rainforest of equipment–decked with slides, swings, stairs, ropes, and poles–waiting to be explored from every aspect. Grasshoppers, ant colonies, and ladybugs in the field were specimens to be collected and observed. Every fallen branch, mud clot, and pinecone was a resource to personify my imagination. Recess was when I flourished. I dreaded getting older, because I would eventually be forced to relinquish the freedom recess provided me and enter the monotonous realm of sipping coffee all day, talking about the weather, and working in the "grown-up world."

Having been raised in a small college town where my dad was a biological engineering research mentor, I always felt that performing scientific research was somehow a passage of manhood for me–I hated it. I used to sit in my dad's office each evening after school and overhear his students

conversing with one another about their research as I mined through the puzzles in the latest issue of Highlights or played on my Gameboy.

"Hey, Tim, how do you plan to handle sustained injuries with the limited healing capacity of the cartilage?"

"Well, I figured since the tissue is avascular, the invasion of undifferentiated MSC's might…"

….*Blah, blah, blah, blah.* As I meandered the hallways warding off the Green-Goblin and ridding the world of evil with my spidey senses and indestructible spider webs woven together in my mind, I often stumbled upon the students' presentation posters displayed on the wall. *Production of Hyaline-Like Cartilage by Bone Marrow Mesenchymal Stem Cells in a Self-Assembly Model* (Elder, 2008). The diction was a foreign language to me, composed strictly of convoluted words and abbreviations that only left me totally discombobulated and tongue-tied whenever I tried to follow. If becoming of age meant that I had to face that abominable nemesis called Research, I would kindly welcome the Green-Goblin into my life any day.

I'm a freshman in college now, and my passage into manhood has already dawned upon me in the form of neurology. I entered the lab for the first time bracing myself with the few general science courses I had under my belt, ready to get tossed and turned by the storms of adulthood. The lab was a wreck–maybe the storms had already passed and I could leave. My mentor approached–maybe not. Either way, resignation seemed welcoming. With a wide grin stretched across his face, my mentor greeted me, his pleasant tone immediately throwing my expectation for turbulence off course.

"Hi, Harrison! I'm Dr. Li. Welcome to the lab."

He apologized for the cluttered workbenches, mentioning that they were currently performing their annual inventory check. Dr. Li then blatantly told me that I would not be performing any actual lab work for my first semester. I would simply be reading up on lab-related material and observing the researchers' techniques and habits so I could begin actual work later on. Good, he was cutting me a break. Maybe I had hope after all. After meeting his research assistants, Dr. Li handed me a stack of research articles to read as an introduction to the lab. For the first time since I came to college, I gazed into the eyes of evil on top of the stack: *Generation and characterization of Dyt1 ΔGAG knock-in mouse as a model for early-onset dystonia* (Dang, 2005). The little optimism I had accumulated from my first minute with Dr. Li was immediately crushed as my childhood horrors resurfaced, plunging me into a sea of despair with nothing but a useless stack of paper

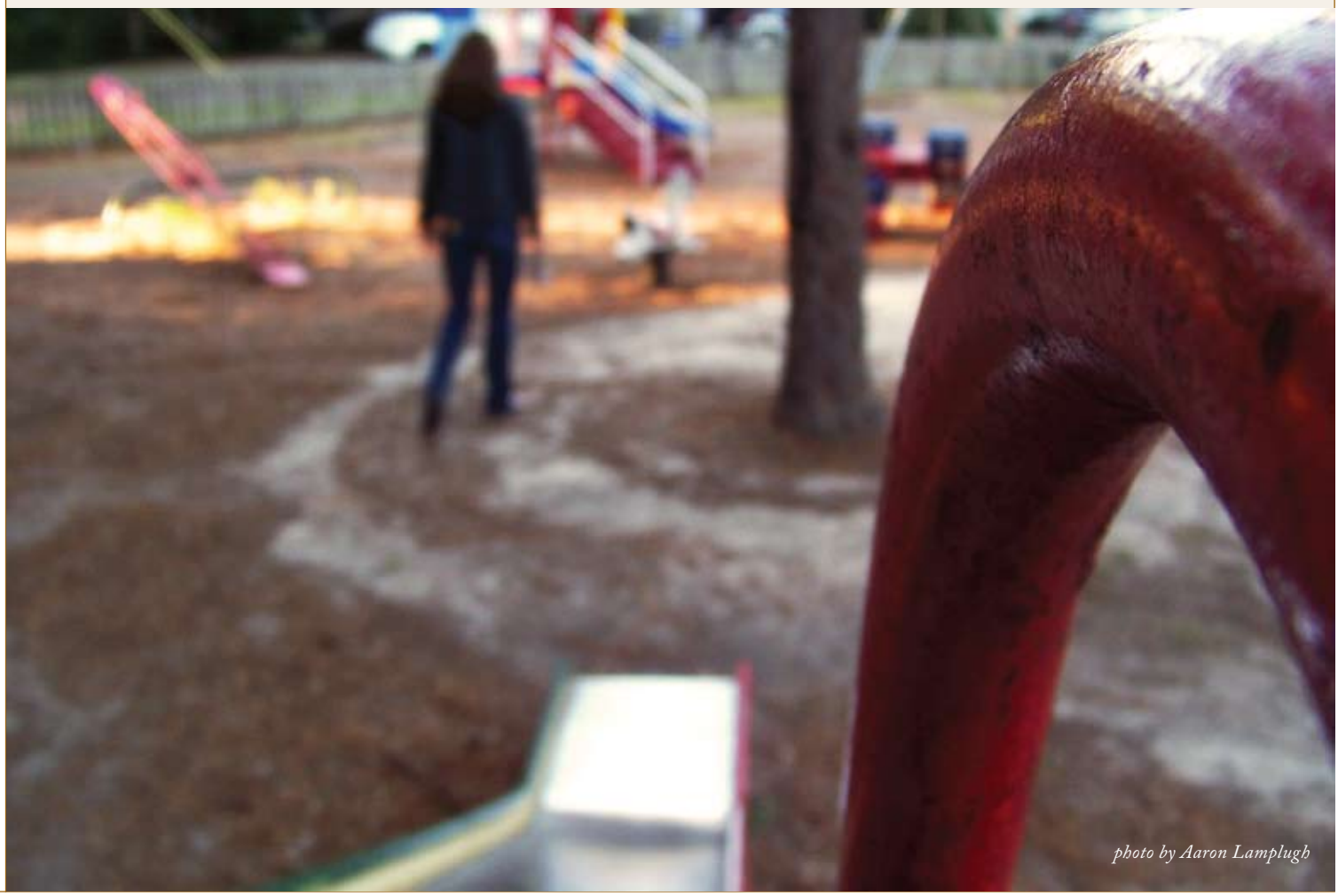


photo by Aaron Lamplugh

and a few staples to save me. Those gigantic words seemed just as horrifying as when I was in the third grade, except this time I was actually expected to know, or at least learn, what they all meant. I then looked back up at Dr. Li, forced a smile, and replied, "Thank you."

Two weeks passed, and I grew more familiar with life in the lab; however, I still loathed my days in the research facility. I attended the weekly lab meetings where the workers presented updates to one another. Each meeting was an epic battle to stay awake as my attention span was burned to ash during the first two minutes of each presentation by seemingly indiscernible diagrams and terminology. I always forced myself to pretend that I learned something in the end so as not to look stupid. I soon gave into my shame as I admitted to myself that I could not maintain this facade for a whole semester. I raised my white flag as I openly confessed to Chad, one of the research assistants, that I had no earthly idea about what was going on in the lab. To my surprise, he simply chuckled and replied, "Neither did I the first two semesters I was here." I showed Chad the research papers Dr. Li gave me the first day, and he patiently sat with me and broke down each of the papers into their individual units, filling me in with general concepts and common terminology in neuroscience. Gradually, the crashing waves of research and scientific articles that had attacked me my whole life began to recede. Within a few hours the whole research process seemed elementary. All those articles simply said was, "Hey, if I put this disease in a mouse and find changes in its brain activity, maybe I can revert this activity, apply this finding to humans, and find a cure for the disease." As my time in the lab passed, my understanding of research deepened. I learned that the actual execution of a lab blueprint is an extensive succession of experiments that can take decades to complete. Before a disease can be studied, it must first be placed into countless specimens. Upon having been properly transmitted, the condition must be diagnosed through a myriad of recordings and tests for symptoms both anatomically and physiologically at the molecular level. Next comes the treatment, followed by human studies where the cycle then starts over. Of course, this is all assuming no errors were made in the process. By the end of the day, I was exhausted from having absorbed so much information. But I reigned victorious over a looming enemy finally conquered. So now that I'm in college, I guess I'm about to become a part of that grown-up world I always feared entering. Ironically, I feel just as much a kid as I did during my playground days. I still despise the bitter taste of coffee with the passion of a thousand suns. My conversations with people might have become a little duller, having been polluted with grueling homework assignments, the weather, and yes, research. However, all those group "study" sessions, spontaneous 3:00 a.m. Waffle House runs, parties not beyond recollection, and endless waves of resulting college drama make my life far from droning. I have yet to learn what it's like to work for a living, but my experience in the research lab has broadened my horizons tremendously. Do I plan to do research for a living? I don't know. Do I understand all that complicated diction that baffled me as a kid? Nope–don't think I will for a while either. Albert Einstein once stated, "The whole of science is nothing more than a refinement of everyday thinking" (Abrajano, 2007). After having been exposed to the world of research, I think I kind of know what he meant and how it can be applied to research in general. The most difficult feat to overcome in performing scientific research is simply obtaining a firm grasp of the concepts and terms in a particular field of science through a procession of education. Once you reach that level of understanding where you are able to freely manipulate your scientific knowledge, the research laboratory that was once an enemy lair becomes a playground. The abyss of mesenchymal stem cells is sand at your fingertips and transgenic mouse breeding a jungle gym. Anticipation mounts. It's recess time.

So now that I'm in college, I guess I'm about to become a part of that grown-up world I always feared entering. Ironically, I feel just as much a kid as I did during my playground days. I still despise the bitter taste of coffee with the passion of a thousand suns.

Works Cited

Dang, Mai., Fumiaki, Yokoi., McNaught, Kevin., Jengelly, Toni-Ann., Jackson, Tehone., Li, Jianyong., Li, Yuqing (2005). *Generation and characterization of Dyt1 ΔGAG knock-in mouse as a model for early-onset dystonia.* Illinois: *Science Direct.*

Elder, Steven., Cooley, Avery., Borazjani, Ali., Sowell, Brittany., To, Harrison., Tran, Scott (2008). *Production of Hyaline-Like Cartilage by Bone Marrow Mesenchymal Stem Cells in a Self-Assembly Model.* Mississippi: *Tissue Engineering.*

Abrajano, Joseph (2007). *Cover to Cover.* New York: *Einstein Journal of Biology and Medicine.*

Translational Research for Undergraduate Students

Nicole Guyette and Karin Tran

Undergraduate research provides students with opportunities to gain a broader perspective on the scope of the field of science. Students introduced to research at an earlier stage are able to explore areas they may have never previously considered. Presently, professional schools are encouraging undergraduate students to participate in research, anticipating that more students will return and accept faculty positions as clinical researchers. Therefore, undergraduate students interested in scientific research should consider the possibility of researching with their professional school of interest.

As founding officers of the Pre-Optometry Club at UAB, Karin Tran and I learned about the possibility of research at the school of optometry through Keely Stewart, our advisor for the organization and student affairs representative for the UAB School of Optometry. I had never done research before and wanted to explore the research aspect of optometry. Karin, on the other hand, spent her previous semester conducting research with Dr. Thane Wibbels but wanted a more optometry-related research experience. After visiting different laboratories, we both decided to work with Dr. Rod Fullard, whose translational research (moving basic science into clinical practice) focuses on dry eye studies, tear collection, and cytokine analysis. We spent the spring semester of our junior year assisting Dr. Fullard's Ph.D student Lucy Kehinde with her research on varying cytokine levels in extended versus daily contact lens wear. The tear collection training and monitoring required for this study enabled us to gain patient interaction experience. In addition, we were able to utilize the pipetting techniques we had learned from multiple chemistry labs.

Currently, Karin and I are conducting our own independent research study as part of the honors biology research program, validating the tear levels for chemokines Interleukin 8 and IP-10 measured by Cytometric Bead-Based Assay (CBA) with the corresponding levels for identical duplicate samples by enzyme-linked immunosorbent assay (ELISA). Some researchers believe that both chemokines have misleading tear concentrations as a result of assay interference by tear components. By comparing the two assay types for each chemokine, our studies will provide validation of the CBA measured tear levels. The basis of our projects entails collecting nonstimulated and stimulated tear samples from twenty participants in order to run the CBA and ELISA.

Getting Involved in Undergraduate Research

To gain a broader perspective on how research can help undergraduate students learn about the range of possibilities of their professional school choice, we interviewed two UAB School of Optometry faculty members, Dean John Amos and Dr. Keshia Elder. The UAB School of Optometry houses world-class investigators. As such, it provides ample research opportunities for undergraduate students. When asked about his view on undergraduate research at the school, Dean John Amos of the UAB School of Optometry said that he strongly supports the idea of getting interested students involved in the many research opportunities the school has to offer. Previously, students were responsible for seeking out research op-

portunities by individually contacting research professors at the school. However, with the newly established Pre-Optometry Club at UAB, Dean Amos believes the organization will serve a critical role in recruiting more students to research at the school as an alternative to the undergraduate campus. The club creates relationships between pre-optometry students and the faculty at the school of optometry, allowing students to gain a broader view of the different areas of research in order to find one that best fits their interests. For pre-optometry students, especially, researching at the optometry school would be more relevant to their career interests. Dean Amos further believes that participation in undergraduate research at the school of optometry will provide a more in depth understanding of basic and clinical research that may lead to a future concentration in a research career.

According to ocular surface disease investigator Dr. Keshia Elder, "Undergraduate research allows students to get a good taste of what research is like, so they can decide if they are interested without becoming fully committed." Her statement describes exactly how undergraduate research has provided us with the opportunity to attain a fast-track Master of Science degree. Being able to use our undergraduate honors research project as the basis of our Master of Science project has relieved the pressure

Undergraduate research allows students to get a good taste of what research is like, so they can decide if they are interested without becoming fully committed.

of balancing the Master of Science program with the optometry program. Undergraduate research experience at the UAB School of Optometry has also made us more competitive as prospective optometry students because of the relationships we have formed with researchers and professors. Although Karin and I are both pursuing the O.D/M.S. degree, students do not have to be pre-optometry to participate in research at the school. The Department of Vision Science offers Master of Science and Ph.D. programs for those who are interested solely in vision research.

Overall, research is a learning experience, and we advise students to explore undergraduate research. If students already have a professional program of interest, they should experience the clinical side of that program through research. Researching through the professional school builds a network of connections among the faculty, thereby creating a more competitive application. Research could be an unknown interest waiting to be discovered by the right student.

You're Not in High School Anymore

Russell K. Fung

As a senior in high school, chemistry was always my favorite science subject to study. Whether learning about Gauss's law or how to work a stoichiometry problem, I took pleasure in learning the wide range of chemistry topics taught at Hoover High School. When it came to working in chemistry lab, however, things couldn't be less enjoyable. Each week, I performed a chemistry lab by following a set of instructions and then wrote up a lab report. This experience led me to believe that labs were all about following instructions, replicating a set of procedures that had been performed previously. If all I was doing in a lab was replicating a set of procedures, though, then what must professional researchers do in their laboratories? As luck would have it, my question was answered when I received the opportunity to work in a research facility at UAB.

Lab experience at UAB

I first considered joining a research lab after talking to a friend who had worked in a research facility at UAB as a junior in high school. Given my experience with labs at the time, replicating similar procedures for a whole summer didn't seem all that appealing. But I was encouraged to apply and work at the research lab anyway, with the idea that I would be exposed to a "real" research facility at UAB. The summer before entering college, I entered my first research facility at the Atherosclerosis Research Unit (ARU) at UAB, placed to work as a laboratory assistant under Dr. Vinod Mishra.

I soon realized that Dr. Mishra is an excellent laboratory mentor who has made astounding achievements in the biochemical sciences. He is a researcher who was previously involved in the development of different biophysical methods in India that allow for the study of peptides and lipids. At UAB, Dr. Mishra is part of the Atherosclerosis Research Unit, headed by Dr. G.M. Anantharama. The unit is divided into different areas of expertise but specializes in studying a cardiovascular disease known as atherosclerosis. Dr. Mishra's areas of study include the functions of lipoproteins, specifically the effect of High Density Lipids (HDL) on atherosclerosis. Working under Dr. Mishra, I was able to learn about his line of work and perform many sub-projects that relate to his project as a whole. Although many of these tasks do require me to follow a set of instructions, to the best of my knowledge, most of these instructions were probably not completed previously, since most of the protocols were instructions that I had created myself.

My first day working at the lab was quite an experience. I was asked to prepare a diluted chemical sample of peptides that was to be analyzed in a spectrometer. I realized that I needed the knowledge of past high school chemistry lectures for me to be able to perform this task. Without knowing about molarity or stoichiometry, I would not have known how to perform this simple task. In addition, a spectrometer was needed to find the absorption spectrum of a peptide sample. Since I had learned how to operate one in my high school chemistry class, I could perform the task with ease. To make an agarose gel, for example, I needed to know the concept of molarity for measuring the exact amount and concentration of agarose. Dr. Mishra routinely made sure I understood the necessary background information before a test was performed. This in turn has helped me to understand the reasons why the test was performed at all for a given sample and what was happening as I followed the protocol.

Of course, if all tests were conducted the way they are in high school, research would take forever. At the lab, many tedious steps of calculation are simplified with new techniques and methods, oftentimes performed by computers rather than by hand. For instance, it is reasonable to find an absorption spectrum of one sample with a spectrometer manually. However, by using a computer, multiple absorption spectrums can be found for different samples in less time. In high school labs, there's no rush to produce results from simple replicated experiments with a known outcome. Each technique is performed and explained thoroughly such that the concept is comprehendible. Many errors will be made, though they are acceptable as long as they are understood by the student. But in a research facility, experiments are performed to answer specific research questions that are possibly unknown to the scientific community. Unlike high school labs, errors in procedures will not only affect the reliability of the results, but will also waste valuable resources. Each procedure must also be performed swiftly so that the results can be evaluated to produce the next set of research questions that needs to be answered. Therefore, many basic methods are maximized by machines in order to reduce experiment time and produce relatively quick and errorless results.

During my time at the research facility, I've learned several different techniques that have helped me to obtain the data necessary for different types of analysis for lipids and peptides. Many of these techniques include the makeup of different assays that test the concentration of factors added to the lipoprotein samples. Some of these assays include PON, PAF-AH, and DCFA assays that test for different factors such as cholesterol and phospholipids for each sample. Many of these new techniques are not taught in high school laboratories, and the samples don't come readily available.

In a professional lab, each sample is made separately each time to avoid contamination and chemical changes over time. Precision takes a great deal of time and is needed to obtain satisfactory results. Even with the technology today, some samples take hours to prepare and usually come in small volumes. In addition, some tests take hours to complete at a time. Making one mistake could require a repetition of an experiment, which may take up a lot more time than needed to finish the experiment!

Science as a Team

Working in a research laboratory at UAB gave me a new perspective on laboratory work and research in general. Many other student lab assistants and researchers also worked in other labs within the ARU. It's interesting how the concept of teamwork is seen in real life settings within the lab. I used to think research was something performed by a single person. But if that was the case, then science would hardly be advancing at the rate it is now. The Atherosclerosis Research Unit best portrays the elements of teamwork within a research environment. Different areas of expertise are separated into their respective groups to study the different factors that may influence cardiovascular diseases. However, even with different perspectives of study, it is the interactions between the different groups that essentially show the dependency between labs. . For instance, Dr. Mishra might prepare peptide samples that may potentially have an effect on the disease, while Dr. Handattu, a researcher in a separate lab within the Atherosclerosis Research Unit, would verify the sample by testing them on mice. The level of collaborative effort within the lab has shown me what it means to work as a team and how much more efficient the process of research can be than when working individually.

From a career perspective, I've always thought of scientific research as a long and tedious process, repeating the same tests over and over again. But while some of that is true, research also allows researchers to think independently about which steps to take. Unlike high school laboratories where old experiments are repeated, I learned to think critically about a completely new procedure in order to obtain the necessary results to progress. In short, I think research is almost like a never-ending challenge, always looking for ways to advance to the next step in an experiment. As Dr. Handattu from the ARU would say, "For every one step you take in research, you'll have to take three steps back."

From a career perspective, I've always thought of scientific research as a long and tedious process, repeating the same tests over and over again. But while some of that is true, research also allows researchers to think independently about which steps to take. Unlike high school laboratories where old experiments are repeated, I learned to think critically about a completely new procedure in order to obtain the necessary results to progress.

According to Dr. Mishra, it is important and highly recommended for undergraduates to be actively engaged in research, regardless of their major. Research experience is a benefit for students working in the sciences because they are exposed to multiple areas of sciences. Dr. Mishra proposes to anyone who is interested in the sciences to find a few categories of interest before finding a mentor. This way, the best match for a research mentor can be found. Entering UAB as a Science and Technology Honors Program student, I realized the vast amount of research opportunities and resources there were available to me as a freshman. Although I am currently not working in a laboratory with a mentor, I will be looking forward to my next lab experience at UAB!

Adventures in Deutschland: Laboratory Learning

Elise Ottenfeld

One December day after working at a Chemistry Open House, my research mentor, Dr. David Graves of the chemistry department, caught me in the stairwell and tossed "Want to go to Germany?" my way. Five months later, I was working overseas with a doctoral student on his research project. Better yet, I was being paid.

From May until August 2009, I participated in the DAAD-RISE program. Funded by the German Academic Exchange Service, the Research Internships in Science and Engineering program allows North American and United Kingdom undergraduates to apply to work on specific projects based upon their areas of study. Students apply to a total of three projects offered by different German Ph.D. students, and then the RISE committee pairs students with projects based upon their preferences. As a chemistry major, I applied to work on a biochemistry project that aimed to characterize the structure of the DNA fragmentation factor or DFF protein. DFF, as I learned from the description written by my PhD student Daniel Kutscher, was the major nuclease responsible for degrading the genome during apoptosis or cell

death. For some time I had been learning about apoptosis and its relationship to DNA from the work I performed in Dr. Graves's lab in conjunction with Dr. Katri Selander at the Comprehensive Cancer Center; so I was more than interested in the opportunity to learn the full process of cell death.

My journey began in Berlin where I spent two weeks with a group of fellow RISE students traipsing through the basics of the German language as well as the historical city. Seemingly no time passed before I was onto Giessen, my home for the next three months and the site of my research group. After one day of rest, Daniel began immersing me into workings of the Justus-Liebig University-Giessen's Biochemistry department. The first day he explained the basics of my project: I was to use polymerase chain reaction (PCR) techniques to systematically change the amino acid sequence of the DFF45 subunit of the DFF protein (DNA fragmentation factor), also called ICAD (Inhibitor of caspase activated DNase), for the mouse protein and then use them in enzymatic assays in order to determine the sites important in its chaperon function, leading to proper folding of the nuclease subunit, DFF40, of DFF. It sounded so simple!

What I learned in the following weeks was that making protein variants was anything but simple. Even though making protein variants was problematic from the beginning, during my three month stay I successfully learned techniques such as DNA mutagenesis, protein expression and purification, and DNA cleavage experiments. Additionally, I learned how to make and run several types of electrophoresis gels and to whip up a new batch of buffers in a heartbeat. Even though our experiments would not always work, I carried them out independently - trying, failing, and learning on my own - with Daniel nearby to consult on my results and to offer helpful wisdom for next time. However, laboratory techniques were not the only thing I learned during my stay; twice a week the entire lab group of around 30 people met to either discuss and review a paper or learn how the projects were going from various lab members. Though I did not accomplish any lab work during the seminars, I found them helpful for learning how to critique a journal article properly, something that I with my love of English literature had always abhorred. Group seminars also helped me get to know the people I was working with, and to learn that shuffling into morning seminar clutching a cup of coffee is a universal bonding experience.

Overall, my summer experience helped me develop my analytical skills with regard to both practice and theory, which I have brought home to my lab work at UAB. While working in a lab at home was always the most intriguing part of my studies, working in a lab at Justus-Liebig allowed me the opportunity to truly absorb what it means to be a researcher. Everyone I encountered was more than helpful in pointing me the right way and, with no language barrier for everyone spoke English, in laughing with me while I fumbled with my German. I strongly encourage all science students, no matter what your goals may be at this point, to seek out the opportunity to work in a research lab, not necessarily for the elaborate techniques you'll learn but for the value of patience and teamwork you'll experience.

...my summer experience helped me develop my analytical skills with regard to both practice and theory, which I have brought home to my lab work at UAB. ...working in a lab at Justus-Liebig allowed me the opportunity to truly absorb what it means to be a researcher.

faculty interview: mathematics

An Interview with James Ward, Department of Mathematics

Ashruta Patel

This interview was conducted with Dr. James Ward, who is currently my Calculus Professor. Dr. Ward has been associated with UAB and research for the past 20 years; his efforts have provided many insights in mathematical concepts. I had the opportunity to discuss his career interests as a faculty member and what suggestions he has to offer potential undergraduates passionate to pursue a future occupation in any form of research.

Q) How did you become interested in research?

A) Research is a way of learning about the world, a way of finding what is true. It is also a way of living. I had been interested in mathematics and science even as a child. Of course, family influences played a role. My family often engaged in philosophical discussions and debates, and this encouraged analytic thinking and openness to new ideas. By the time I went to college, I was interested in mathematics, science, literature, and philosophy. But mathematics had a special appeal to me. After taking calculus, I was advised to take a topology course in my sophomore year. Topology is a subject concerned with very general, abstract, notions related to geometry and calculus. In the course we, the students, were given mimeographed notes containing only definitions and statements of theorems. Our job was to prove the theorems. If someone found, or thought they found, a proof, they would present it to the class. That was the whole course; there were no lectures, no books, so it was like doing mathematics research. I loved it and would spend hours, even days, working on a single question. Here at UAB our Advanced Calculus course is run in much the same way. After that topology course, I was pretty sure I wanted to be a mathematician. My advisor, Dr. Jack Roth, who taught me topology, abstract algebra, logic and foundations of mathematics, also influenced me. He was a remarkable man, both a mathematician and an award winning artist. I think mathematics, science and the arts are complementary, and Jack Roth exemplified that. He encouraged my further studies in mathematics. Later, in graduate school and after, there were other influences and a particular direction for my research.

Q) Where did you do your undergraduate and graduate studies?

A) University of South Florida

Q) How long have you been at UAB, and what persuaded you to come here?

A) I have been here since 1989, and before that I was at the University of Alabama. While at Tuscaloosa, I had a lot of contact with the mathematics department at UAB. UAB seemed to offer an excellent atmosphere for my research, which is in nonlinear analysis and differential equations, by having faculty with similar research interests. In addition, the people in the mathematics department were very intent on building a strong research department to attract good students. I think we have succeeded in that goal.

Q) Please give a basic description of your current research interests/projects?

A) Much of my work has been in non-linear analysis and differential equations and how solutions relate to the structure of the equations. Another interest of mine is in bifurcation theory. A system exhibits a bifurcation if the type or number solutions change as a parameter changes. For example, a stable equilibrium state might suddenly lose stability, with the stability transferred to another, new, equilibrium, or even to a periodic solution. Bifurcation phenomena are observed in physics, chemistry, and biology, especially physiology, and elsewhere. For example, the bending of a beam under a force and the firing of a neuron are bifurcation phenomena. Topological notions are fundamental in the study of bifurcation and nonlinear equations. Thus my early training in topology probably helped! For the past several years, I have been working with some excellent Chilean mathematicians, Raul Manasevich and Marta Garcia-Huidobro, on some problems involving nonlinear differential equations and bifurcation. Recently we decided to look at some mathematical modeling questions in biology and sociology, so that is a new direction.

Q) Have you ever worked with undergraduates?

A) Yes, I have often worked with mathematics fast-track students at UAB.

Q) What advice would you give to undergraduates considering research activities both now and later as a career?

A) Take the initiative. Do lots of outside reading in your subject and related areas. Find other students with similar interests. Make your interests known to your professors and adviser. The UAB science and mathematics faculty are generally very open to working with undergraduates. It may also help to get involved with seminars. Ideally, the student should be seriously interested in the subject and shouldn't be doing something just for credentials.

short report

Accelerating Lossless Data Compression with Graphics Processing Units

R.L Cloud, M.L. Curry, H.L. Ward, A. Skjellum, P. Bangalore

Abstract

Huffman compression is a statistical, lossless, data compression algorithm that compresses data by assigning variable length codes to symbols, with the more frequently appearing symbols given shorter codes than the less. The work to be presented is a modification of the Huffman algorithm which permits data to be decomposed into independently compressible and decompressible blocks, permitting concurrent compression or decompression on multiple processors. We implemented this modified algorithm on a NVIDIA GPU using the CUDA API as well as on a current Intel chip and the performance results are compared, showing higher performance compression and decompression on the GPU.

Introduction

Lossless data compression is important in application domains and usage environments where bandwidth or storage limitations may negatively impact application or system performance. Generally classifiable into statistical or dictionary methods, lossless data compression algorithms can range widely in compression speed and efficiency (compression factor). Certain algorithms, especially the more efficient, can be quite computationally expensive, and as the data processing needs of current scientific endeavor continue to scale with more rapidity than storage or bandwidth, compression becomes increasingly necessary, but questions remain as to how to accelerate it and how to do so without consuming a large amount of computational resources.

The use of graphics processing units (GPUs) for general purpose computation, i.e. problems outside the graphical domain, is a relatively recent development. First this was achieved though third party toolkits, e.g. Stanford's BrookGPU, but even more recently have GPU manufacturers themselves begun to offer general purpose tools which give the programmer a lower level communion with the chip than earlier GPGPU programming interfaces which are built upon OpenGL and DirectX. One of these, and currently the most prominent, is the Compute Unified Device Architecture(CUDA) from the NVIDIA corporation. The potential benefits of GPUs in general purpose computation are great, but potential must be emphasized, more so even than for parallel programming on the x86. To achieve anywhere near the theoretical maximums in performance on the GPU, the computation patterns underlying a solution's algorithm must be very near to the traditional usage of the GPU; a prospective algorithm's implementation on the GPU should be, in order of importance to performance, highly data parallelizable, logically simple, and have relatively many computations to memory accesses. In essence, to use the GPU to maximum effect, the abstractable computation patterns underlying a solution should be co-linear to the GPUs original task, graphics rendering. Our problem domain, I/O, while it does not perfectly fit these criteria, has already benefited from GPUs to enhance storage redundancy [5]; we attempt now their utilization in lossless data compression

One major difficulty here in achieving good speedup with slim negative side effects is that lossless data compression algorithms can generally not be, in their unaltered form, thought of as highly parallelizable. Indeed, if one wishes to express these algorithms in parallel, one often needs to consider tradeoffs between compression efficiency and performance. Nevertheless, we hope to effectively demonstrate that it is possible to come to a reasonable middle ground with respect to coding acceleration and efficiency loss.

Huffman Encoding

Statistical methods of data compression perform analysis on the nature of the data to make intelligent decisions about how it can be represented more efficiently in compressed form. The Huffman encoding algorithm falls within this genus and operates by counting the appearance of every distinct symbol in the uncompressed data, then representing the more frequent with shorter codes than the less frequent. Every symbol in the data is replaced with its code, and if the data is non-random, i.e. a few symbols appear with greater frequency than others, compression can be achieved. The Huffman compression algorithm is old by the standards of our science [6], but is still used, and has the attractive quality of being a primitive of several more modern and common algorithms, e.g. Deflate [1] and potentially the algorithm described by Burrows and Wheeler [4].

Parallel Huffman Coding

There is literature on parallel Huffman coding and of varying goals, ranging from the actual construction of Huffman codes in parallel [3], [2], to [7] which addresses details of decomposition for parallel Huffman decoding and demonstrates some moderate decoding speedups while maintaining optimally encoded data by making use of the observation that Huffman codes can frequently synchronize. Because of limitations in our architecture, we must try to create the simplest encoding routine possible. In doing this we make a minor modification to the output of the Huffman algorithm.

A modification is necessary because of the nature of Huffman codes, i.e. they are of a variable length; an encoded data string is composed of these codes packed together in a nature where bit codes can cross byte boundaries. Simple decomposition of the encoded data stream into blocks of static size would result in the practical certainty that decoding would take an erroneous path, which is discussed in some detail in [7]. One counter to

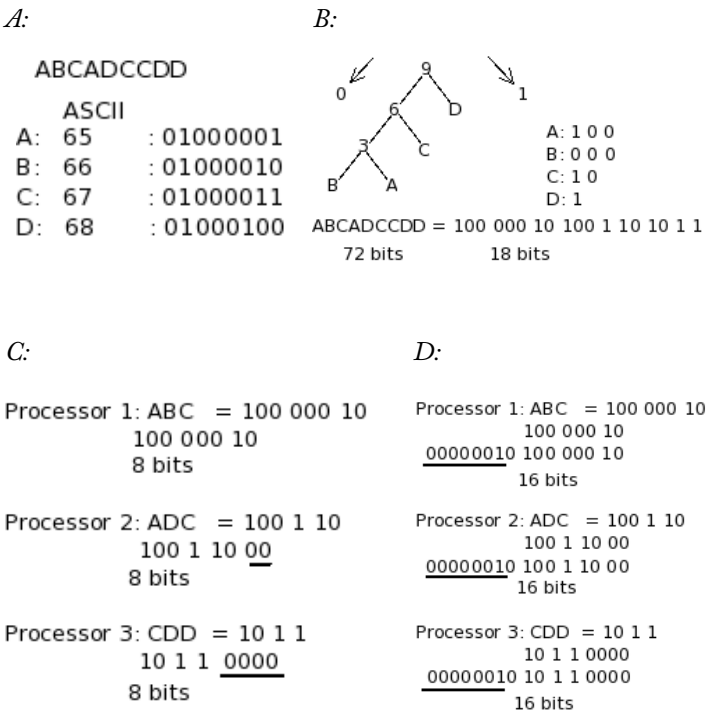


Figure 1: A: The original ASCII encoded string. B: The binary tree and encoded representation of the original string. C: Decomposing the string into three symbol blocks and adding packing bits to the nearest byte. D: The addition of a length delimiter at the start of the block. Single bytes are used for the overhead in the diagrams for simplicity. In our implementation, we pack the block to four bytes and use a four byte integer to represent the block length.

this is to pack the blocks to byte boundaries, introducing some size overhead. One more change is necessary. Because the codes are of variable lengths, even if we encode a constant number of symbols in each block, the resulting length of the encoded block will vary, sometimes dramatically. For this reason, we must encode an indication of where the block starts and ends. Our approach is again simple; at the start of the encoded block we give the length of the block which is known by making an additional pass over the unencoded block and summing the lengths of the code representation of the symbols. Our implementation stores this length as an unencoded four byte integer for simplicity, and because of this and the requirements of our architecture, we pack the blocks to four byte boundaries.

The overhead of our modifications range therefore, from between 32 bits and 63 bits per block, with the variation being because if the size of the encoded block is evenly divisible by four bytes, it is unnecessary to add packing bits to its tail. This overhead naturally becomes less significant as the length of the block is increased, which is indicated in the figure measuring block size against overhead. The time required for summing the block lengths is measurable but undramatic and most noticeable when comparing the runtimes of a sequential block encoder to a sequential traditional(non-block) encoder.

To parallelize decoding, it is sufficient to build a table of offsets into the encoded data from these block length delimiters. The computation threads on the GPU can then index into the encoded data and decode in parallel, storing decoded data in a sequential array indexable by thread and block numbers.

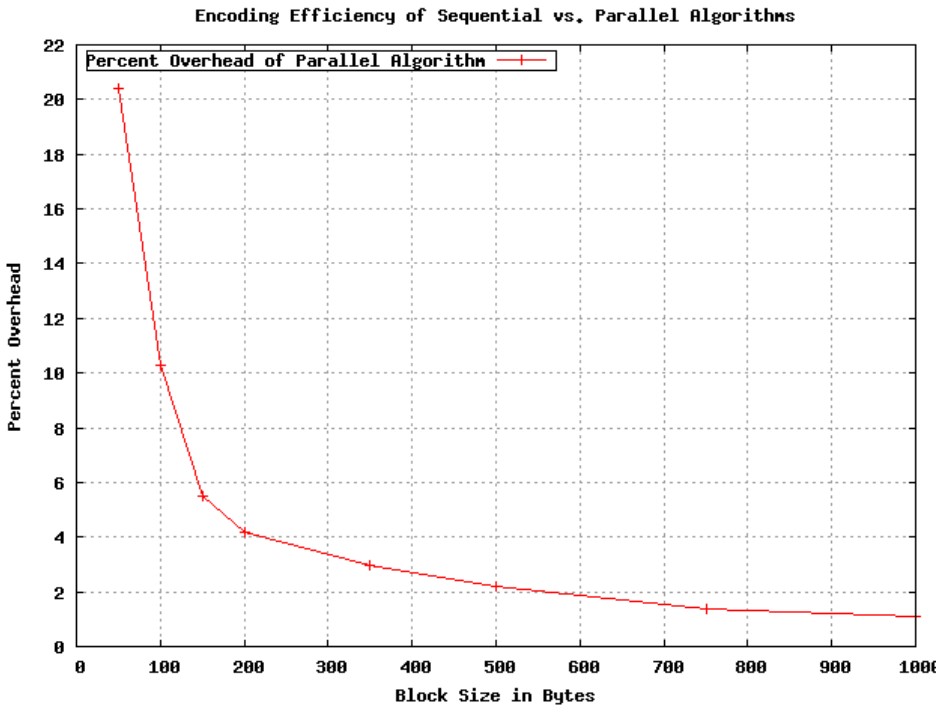


Figure 2: The size overhead of using the parallel Huffman algorithm graphed against the block size. The number of bytes overhead per block remains a constant, so as the block size increases the overhead becomes less significant. At large block sizes, the overhead per block can be less than one percent.

Performance Comparisons

Encoding

Acceleration over our sequential implementation was achieved for both encoding and decoding. This comparison is most meaningful in terms of throughputs, the amount of data which can be encoded or decoded per second. Following is the comparison of our sequential encoder to our parallel GPU encoder and a parallel CPU encoder programmed with OpenMP. The GPU used in these experiments is the NVIDIA GeForce GTX 285 with 240 cores at 1.5 GHz, and the CPU used is the Intel Core i7 Extreme Edition 965 with four cores at 3.2 GHz. Despite the GPU having 60 times the number of cores as our CPU, the differences in throughput between the GPU encoder and the OpenMP encoder are not dramatic. This paradox can be largely resolved by recalling that the architecture of the

GPU was developed for the SIMD, single instruction multiple data, programming model while our CPU was developed with MIMD, multiple instruction multiple data, in mind.

The processors in the GPU are organized into 30 groups of 8 cores. Each group of cores is known as a multiprocessor and contains a single control unit and a small amount of high speed memory shared between the cores in the multiprocessor. The control unit broadcasts an instruction to all the cores, and optimal performance can only be achieved when every core can execute it. If, for example, the instruction is a branching statement, then there is a likelihood that some cores will not follow the jump, and in this case, some cores must remain inactive until they either themselves satisfy the branching instruction or control passes beyond the branching sections of the code. Therefore, in the worst case, when only one core can satisfy the jump and the other seven are left idle, our GPU behaves more like a crippled 30 core shared memory MIMD machine with a slow clock speed and no automatic memory caching. Our encoder consists of complicated branching statements for the bit manipulation which makes worst case behavior relatively likely. This also illustrates that in heterogeneous programming environments, one must be very aware of the strengths and weaknesses of the various architectures so that programming effort can be directed where benefits are most likely to be found.

Decoding

Our decoding routine consists of reading bits and traversing a binary tree repeatedly for each code string. This contains branching instructions, but markedly fewer than the encoding routine, and the factor of acceleration on the GPU is greater than that of the encoding routine. Also interestingly, the measured increases in throughput from using OpenMP on the CPU, compared to the sequential implementation, are even better than linear by number of cores on the CPU. By launching increasing numbers of threads, we can hide latency by issuing more memory requests. In this way, we saw continued performance improvements through increasing thread counts up to 8. Intel's Hyper-Threading technology assists significantly in this.

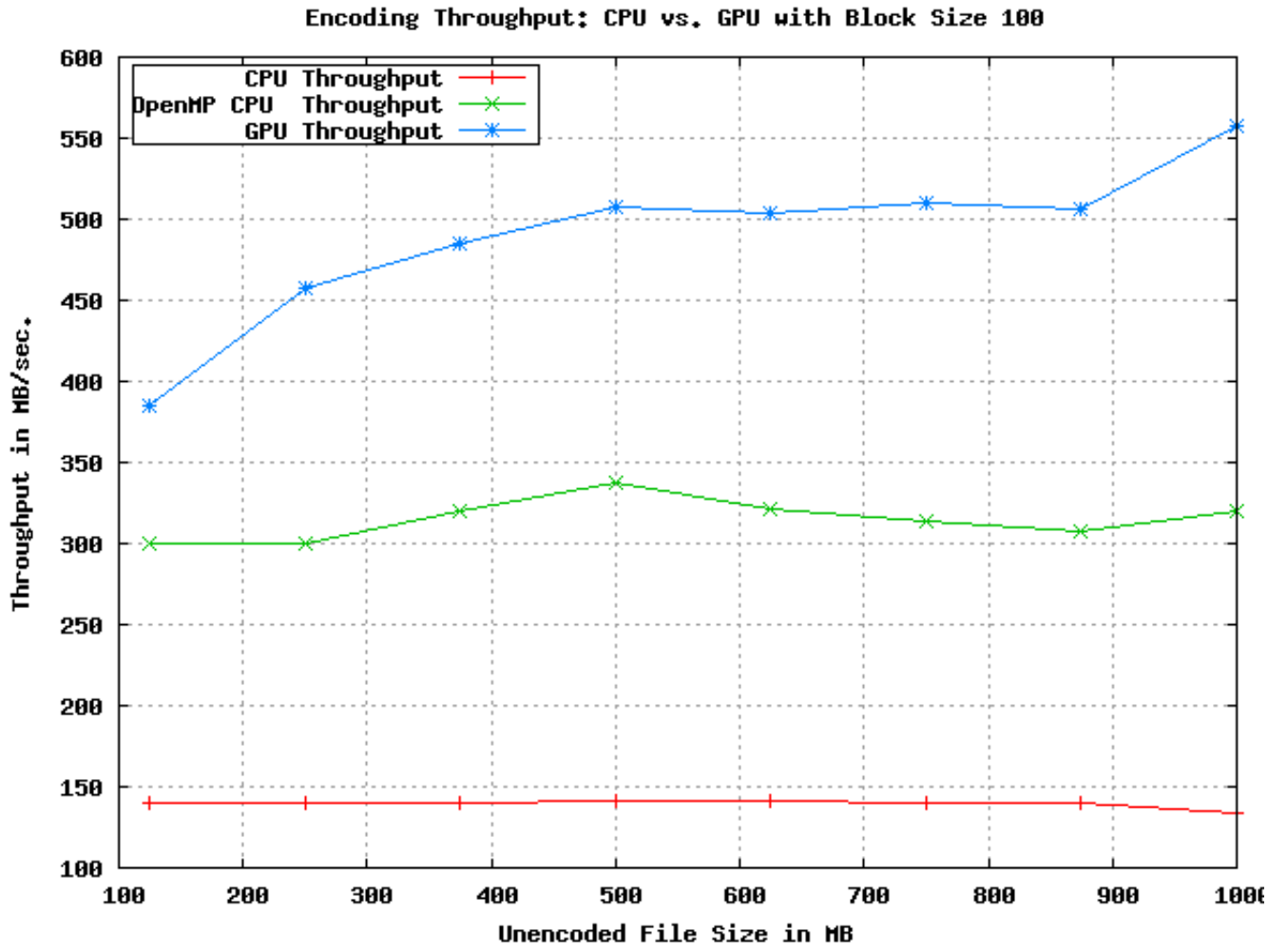


Figure 3: We saw superior performance with the GPU based encoder compared to our multi-core CPU encoder and our single threaded CPU implementation

Conclusions

The data presented here suggests that the strengths of the GPU architecture are robust enough to give performance benefits to applications which, while data parallel, still have a not insignificant level of logical complexity. Optimal use of the GPU's SIMD cores requires the complete elimination of divergence within warps, which, in practicality, requires the complete absence of if statements from the GPU sub-routine; however, sub-optimal performance, through the emulation of MIMD, can still be acceptable. Despite the large number of divergent threads in a warp, our encoder kernel is capable of throughputs, sans memory transfer times to and from the GPU, in excess of 4 GB/sec. Total encoding throughputs using the GPU are weighed down by the need to transfer data to and from the card; however, in an online system, or when encoding very large amounts of data, this could be somewhat ameliorated by using asynchronous data transfers with the GPU to fully exploit bus resources while encoding.

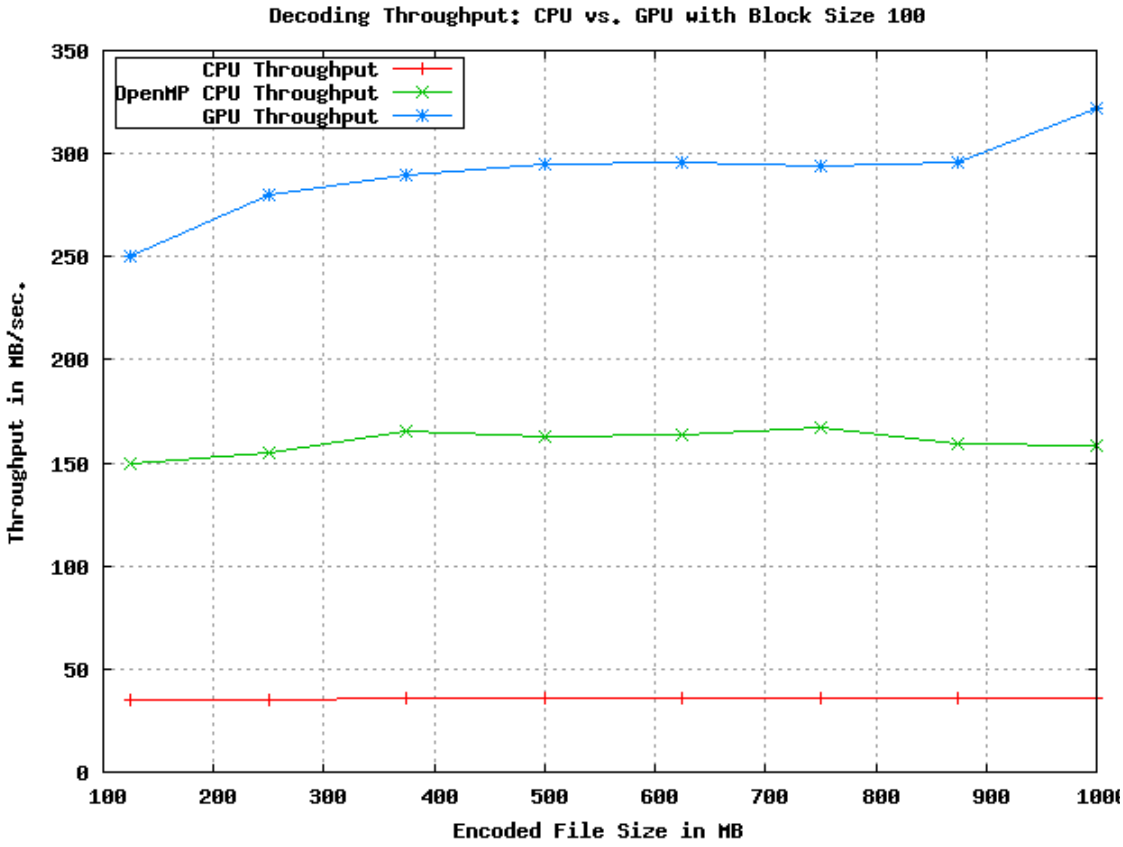


Figure 4: Again, our GPU based decoder gave better performance than both CPU decoders.

Realistically, current performance levels for our GPU encoder and decoder do not warrant the use of the program as a standalone encoding system. The Huffman algorithm itself is not the best choice for such purposes and even the strengths of the GPU do not make up for the algorithm's deficiencies. However, our encoding system could be used as an auxiliary process to a GPU application. Much greater coding performance than that shown in the above figures could be seen were the data to be encoded already on the GPU.

References

[1] M. Adler, Deflate algorithm. http://www.gzip.org/algorithm.txt.

[2] M. J. Atallah, S. R. Kosaraju, L. L. Larmore, G. L. Miller, and S.-H. Teng. Constructing Trees in Parallel. In SPAA '89: *Proceedings of the first annual ACM symposium on Parallel algorithms and architectures*, pages 421–431, New York, NY, USA, 1989. ACM.

[3] P. Berman, M. Karpinski, and Y. Nekrich. Approximating Huffman Codes in Parallel. *Journal of Discrete Algorithms*, 5(3):479–490, 2007.

[4] M. Burrows and D. J. Wheeler. A block-sorting lossless data compression algorithm.Technical report, Digital SRC Research Report, 1994.

[5] M. Curry, A. Skjellum, H. Ward, and R. Brightwell. Accelerating Reed-Solomon Coding in Raid Systems with Gpus. *IEEE International Symposium on Parallel and Distributed Processing*, pages 1–6, 2008.

[6] D. Huffman. A Method for the Construction of Minimum-Redundancy Codes. *Proceedings of the IRE, 40:1098–1101*, 1952.

[7] S. T. Klein and Y. Wiseman. Parallel Huffman Decoding with Applications to Jpeg Files. *The Computer Journal*, 46:487–497, 2003.

short report

A Preliminary Characterization of Btbd9 Knockout Mice

Mark P. DeAndrade, Chad C. Cheetham, Fumiaki Yokoi, Yuqing Li
Department of Neurology, Center for Neurodegeneration and Experimental Therapeutics (CNET)

Abstract

Family and twin studies strongly support a genetic contribution to the pathogenesis of Restless Legs Syndrome (RLS). Two independent studies published recently suggest that the BTBD9 gene plays a role in RLS. We have created a line of Btbd9 mutant mice that mimic the mutation reported in RLS patients by an insertion of a gene trap vector into the Btbd9 gene. The Btbd9 knockout mice were born in a Mendelian ratio suggesting the knockout is not lethal. However, the knockout mice showed a retarded growth pattern and were approximately 20% smaller. Our preliminary experiments show that the knockout mice had periodic wakefulness in sleep and had increased pain sensitivity.

Introduction

Restless Leg Syndrome (RLS) is a disorder that is manifested at rest by periodic movements in sleep and unpleasant sensations deep inside the legs that are relieved partially with movement. RLS has been associated with the central dopaminergic system and iron metabolism. Genome-wide association studies have implicated several genes in RLS including BTBD9. In the mouse, Btbd9 is expressed almost ubiquitously in the brain and the rest of the body and expressed both during development as well as in adults. The function of Btbd9 protein is not known, however the family of proteins it belongs to has function in transcriptional regulation, ion channels, and protein ubiquitination. Additionally, the protein family has important dopaminergic and glutamatergic functions in the brain.

The human BTBD9 protein is 544 amino acids long while the mouse Btbd9 protein is 612 amino acids long. The extra 68 amino acids in the mouse protein are located at the N-terminal of the protein, produced by an additional exon with a methionine start codon. Excluding the 68 amino acids of the N-terminal addition, the remaining 544 amino acid sequences share 95.4% amino acid residue identity and 96.5% similarity. The high homology between mouse and human BTBD9 proteins makes it feasible to model human RLS in mice by manipulating Btbd9 gene in embryonic stem (ES) cells.

The goal of the present study is to understand the function of Btbd9 protein by using Btbd9 knockout mice. Here we analyzed the sensory and circadian function of the mutant mice. Our research should provide insight into the pathogenesis of RLS and eventually new therapeutic treatments for RLS.

Methods and Materials

Generation of Btbd9 knockout mice. Considering the large proportion of mutations in humans is contained within the 6th intron of the BTBD9 gene, we looked for mutations in the mouse homolog gene, Btbd9. We found a gene trap clone, BayGenomics RRE078, which contained a promoterless β-geo gene inserted inside the 6th intron. The β-geo protein is a fusion protein of β-galactosidase and neomycin selection gene. The fusion protein has the activity of both β-galactosidase and neomycin. The β-geo gene contains a stop codon at the 3' end thus causing an alternative splicing when inserted into a gene (Figure 1).

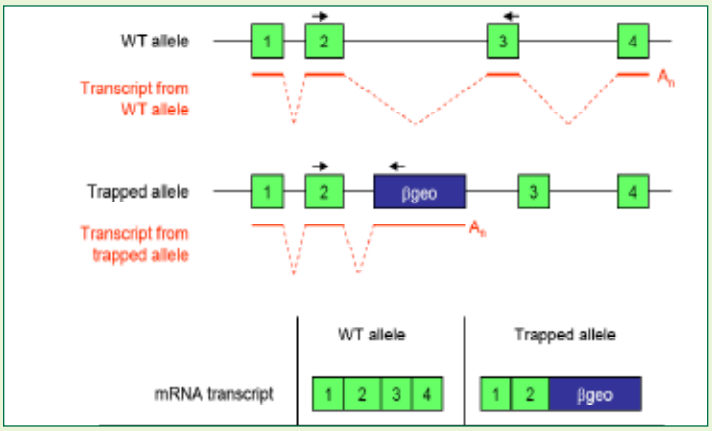


Figure 1. Diagram to show insertional mutations in mouse ES cells and the production of truncated fusion proteins (from www.mmrrc.org). Red lines: predicted splicing patterns in WT and trapped allele.

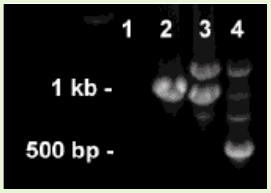


Figure 2. LA PCR genotyping of Btbd9 KO mice. Template DNA used: 1 and 3 from a KO mouse, 2 and 4 from wild type ES cells. Primers used for lanes 1 and 2 generate a predicted band of 1 kb (lane 2) and primers used for lanes 3 and 4 should produce a band of 500 bp. Top three bands in lanes 3 and 4 are non-specific PCR reaction products. Both reactions failed in lanes 1 and 3.

We obtained the ES cell clone containing this insertion and confirmed the insertion of site by 5'-RACE RT-PCR sequencing (data not shown) and the results showed that the insertion is in intron 6. The ES cells were then injected into C57BL6 blastocytes, from which we obtained 4 chimeras. One of the chimeras transmitted the mutation to germline which was confirmed by PCR genotyping of the tails (data not shown). These heterozygous mice born from that chimera were then bred to start building up a colony.
In order to determine the approximate location of the insertion site within intron 6, 19 pairs of primers spaced

about 10 kb apart within the intron covering the entire 179.223 kb intron 6 and exons 6 and 7 were designed. Each pair of primers then underwent long-arm (LA) PCR using an LA PCR kit (TaKaRa). The LA PCR was conducted using templates from control wild type DNA and DNA from a knockout mouse. After a series of LA PCR reactions we narrowed down the insertion site to approximately 500 bp (Figure 2).

Wheel running. Animals were housed in a plastic cage equipped with a steel running wheel (Lafayette Instruments). The cages contained little bedding with one nestlet as to avoid blockage of the wheel. Food and water were accessible *ad libitum*. Wheel revolutions were measured by a small sensor. Signals were registered on a computer using a data acquisition system. The mice were monitored continuously for 7 days with a 12 hr light/12 hr dark cycle. The data were imported to ClockLab for analysis of circadian activity.

Tail flick. The distal half of the animal's tail was placed on a platform under a heat lamp. The lamp was rapidly heated up to a temperature of approximately 49-53°C and the time taken to vigorous reflex withdrawal of the tail was measured. The cut-off time for this test in the absence of a withdrawal was 15s to prevent tissue damage.

Statistical analysis. Statistical analyses were performed using SAS Analyst (version 9) for wheel running distance and tail flick latency. Data was analyzed using ANOVA taking into consideration genotype, sex, age, and weight in the models. Significance was assigned at $P < 0.05$.

Results

The Btbd9 knockout mice were born in a Mendelian fashion and appeared to be healthy, suggesting that the Btbd9 knockout mice are not lethal. It was found that the body weight of the male homozygous knockout mice was approximately 78% of their control heterozygous male littermates (Figure 3; $p = 0.078$). The body weight difference was maintained at least up to 5 months of age. There was no significant body weight difference between wild type and Btbd9 heterozygous knockout mice.

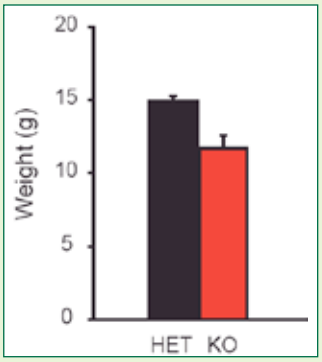


Figure 3. Body weight of Btbd9 mutant mice. HET: Btbd9 heterozygous mice; KO: Btbd9 knockout mice.

To determine if there was a sensory alteration in the heterozygous Btbd9 mice we used the tail flick experiment. The heterozygous mutant mice exhibited a significantly reduced latency to exhibit a withdrawal reflex compared to their wild type litter mates, by approximately 53 percent (Figure 4; $p = 0.0183$).

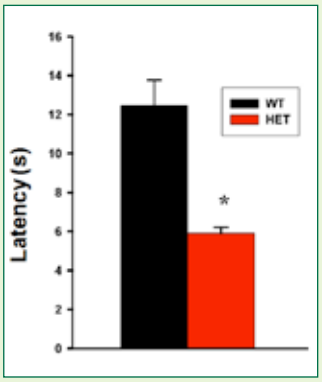


Figure 4. Tail flick experiment for pain sensation. WT: Wild type mice; HET: Heterozygous mutant mice.

For wheel running we wanted to analyze the data for hyperactivity and periodic wakefulness during the day as mice are nocturnal. Data from the wheel running experiment showed that the heterozygous Btbd9 mutants had a significant increase in wheel running activity during the day compared to that of the wild type mice ($p=0.0295$). The heterozygous mice showed an activity score of 130 while the wild type mice had a score close to 0. Additionally, the typical heterozygous mice appeared to have disrupted day activity (Figure 5). The typical heterozygous mutant mouse had brief, regular active periods during the day from 6:00 AM to 6:00 PM.

Discussion

We created a line of Btbd9 knockout mice by inserting a gene trap vector inside intron 6 of the Btbd9 gene. Using long range PCR we have identified an approximate location of the insert and confirmed the insert of the vector in the Btbd9 knockout mice. The knockout was not lethal and the mice have survived more than 6 months, albeit with a slower growth.

Preliminary behavioral and physiological experiments in the heterozygous mice have shown hyperactivity and periodic wakefulness during the day. Additionally, the heterozygous mice showed increased pain sensitivity suggesting a sensory abnormality in the mice. This coincides with the sensory and circadian abnormalities that are found in human patients with RLS.

Further experiments will be conducted to test for periodic leg movements in sleep (PLMS), decreases in dopamine and its metabolites, and decreases in iron and its transporter proteins in the Btbd9 knockout mice.

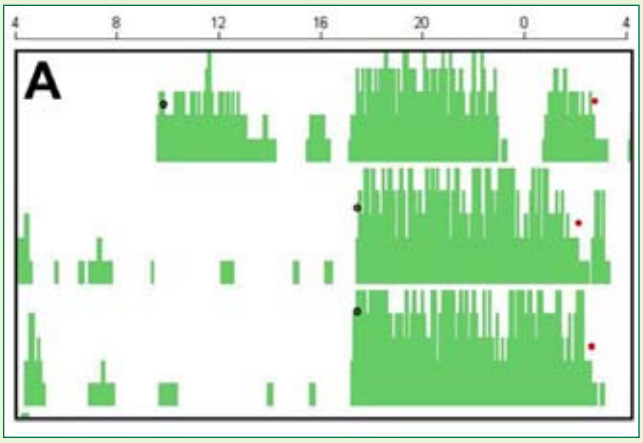
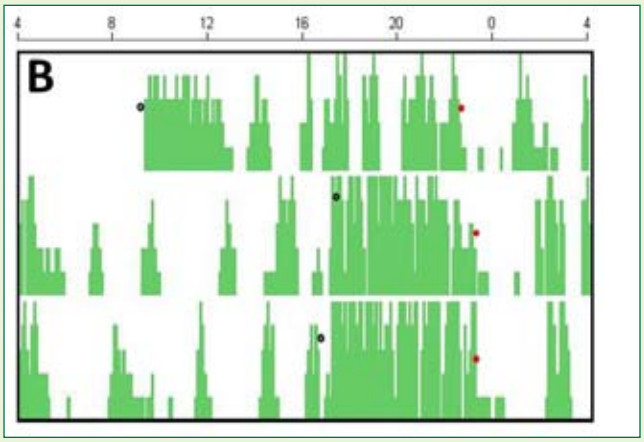


Figure 5. (A-figure above) A typical activity plot across three

days for a wild type mouse. (B-figure below) A typical activity plot across three days for a heterozygous mutant mouse, which includes periodic periods of wakefulness. Red dots signify the approximate time the mouse goes to sleep and the black dots predict the approximate time the mouse wakes up predicted by ClockLab. Heights of the bars are amount of activity.



Acknowledgments

Special thanks to Dr. Thomas van Groen, Miki Jinno, Jennifer Neighbors, and Veena Ganesh for technical assistance throughout the project. Additionally, thank you to Dr. Yuqing Li for his support and guidance.

References

1. Allen, R. P., Barker, P. B., Wehrl, F., Song, H. K., and Earley, C. J. (2001). MRI measurement of brain iron in patients with restless legs syndrome. Neurology 56, 263-265.
2. Allen, R. P., Walters, A. S., Montplaisir, J., Hening, W., Myers, A., Bell, T. J., and Ferini-Strambi, L. (2005). Restless legs syndrome prevalence and impact: REST general population study. Arch Intern Med 165, 1286-1292.
3. Clemens, S., Rye, D., and Hochman, S. (2006). Restless legs syndrome: revisiting the dopamine hypothesis from the spinal cord perspective. Neurology 67, 125-130.
4. Connor, J. R. (2008). Pathophysiology of restless legs syndrome: evidence for iron involvement. Curr Neurol Neurosci Rep 8, 162-166.
5. Stogios, P. J., Downs, G. S., Jauhal, J. J., Nandra, S. K., and Prive, G. G. (2005). Sequence and structural analysis of BTB domain proteins. Genome Biol 6, R82.
6. Winkelmann, J., Schormair, B., Lichtner, P., Ripke, S., Xiong, L., Jalilzadeh, S., Fulda, S., Putz, B., Eckstein, G., Hauk, S., et al. (2007). Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. Nat Genet 39, 1000-1006.

short report

The Effect of Mimetic Peptide 4F on Paraoxonase-1

Toral Patel, Dr. David Garber Ph.D.
Department of Medicine, Atherosclerosis Research Unit

Abstract

Even though there have been many advances in the diagnosis and treatment of coronary artery disease (CAD), CAD remains to be the major cause of deaths in the U.S. In humans, CAD is inversely related to levels of high density lipoprotein (HDL) cholesterol. The "quality" of HDL is just as important as the HDL levels. The major component of HDL, apolipoprotein (apo) A-I, appears to be largely responsible for the atheroprotective qualities of HDL. Apo A-I has been postulated to possess eight α-helical sequences. The majority of these sequences form class A structures that can be mimicked by several 18-residue peptide analogues. One such peptide, peptide 4F, has been found to inhibit atherosclerosis in atherosclerosis-susceptible mouse models. Also, peptide 4F increases paraxonase-1 (PON-1) activity in HDL in mouse models. PON-1 is an enzyme to which many of the anti-oxidative properties of HDL have been credited. The purpose of this study is to determine the effect of mimetic peptide 4F on PON-1 and provide insight into the mechanism by which peptide 4F effects PON-1. To examine the effects of 4F on PON-1, apo E null mice were treated with peptide L-4F and plasma and livers were harvested. The expected result was an increase in PON-1 activity in the plasma; however, preliminary results were not as expected and further experiments must be done to establish a conclusion.

Introduction

In spite of the advancement of treatments for coronary artery disease, the mechanisms in which the drugs prevent atherosclerosis are still unknown. It has been established that high levels of low density lipoproteins (LDL) and low levels of high density lipoproteins (HDL) contribute significantly to the development and progression of cardiovascular diseases (Parthasarathy, 2008). High density lipoprotein (HDL) is seen as one of the most important protective factors against atherosclerosis. Apolipoprotein (apo) A-I, the major component of HDL, is also inversely associated with coronary artery disease (Wilson, 1988). The protein apo A-I consists of 234 amino acids forming eight α-helical sequences that form class A structures. The manufacture of apo A-I is difficult and expensive, and therefore, research has been directed towards finding smaller peptide mimetics that produce similar results to apo A-I but are easier to manufacture and administer.

Peptide 4F was not homologous to the amino acid sequence in apo A-I but provided similar secondary structure, and also

contained 4 phenylalanine (F) residues on the hydrophobic face (Datta, 2001; Navab, 2005). Compared to apo A-I, it only consisted of 18 amino acids instead of 234 amino acids and 4 phenylalanine groups that provided high biological activity (Figure 1).

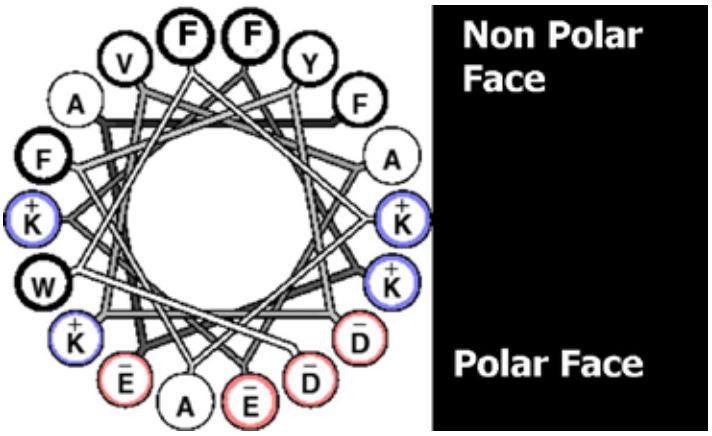


Figure 1: The Class A structure of peptide 4F

Paraoxonase-1 (PON-1) is an enzyme synthesized in the liver as an integral membrane protein (Bradshaw, 2005) and is secreted into the blood stream as a lipid vesicle precursor of nascent HDL (Oda, 2001; Deakin, 2002). Once in the plasma, PON-1 binds to apo A-I and is then found on the HDL complex (Figure 2). Studies have also shown that PON-1 is reduced in atherosclerosis and cardiovascular disease models and patients. Many antioxidative qualities of HDL have also been ascribed to PON-1. Previous research has shown that PON-1 destroys lipid hydroperoxides (LOOH), degrades oxidized LDL phospholipids, reduces accumulation of oxidized lipids in LDL, hydrolyzes oxidized LDL associated compounds, and inhibits both LDL and HDL oxidation (Florentin, 2008).

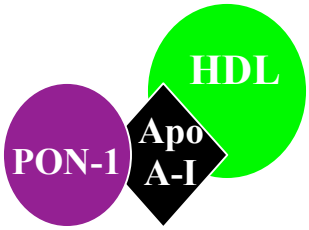


Figure 2: HDL complex with paraoxonase-1

After discovering the increase of PON-1 activity as a result of administration of peptide 4F, questions began to arise on the mechanism by which peptide 4F affected PON-1. Increased levels of PON-1 in the plasma can result either from increased synthesis of the enzyme in the liver or increased acceptor sites in the plasma. In order to increase the synthesis of PON-1, the peptide must affect mRNA levels by interacting with elements associated to the PON-1 promoter. These interactions would affect PON-1 expression and could therefore be hypothesized as a mechanism for 4F modulation. We hypothesize that 4F affects PON-1 by directly causing apo A-I to bind to PON-1 in nascent pre-β HDL and increasing PON-1 activity or by enhancing the genetic expression of the enzyme (or both) (Figure 3).

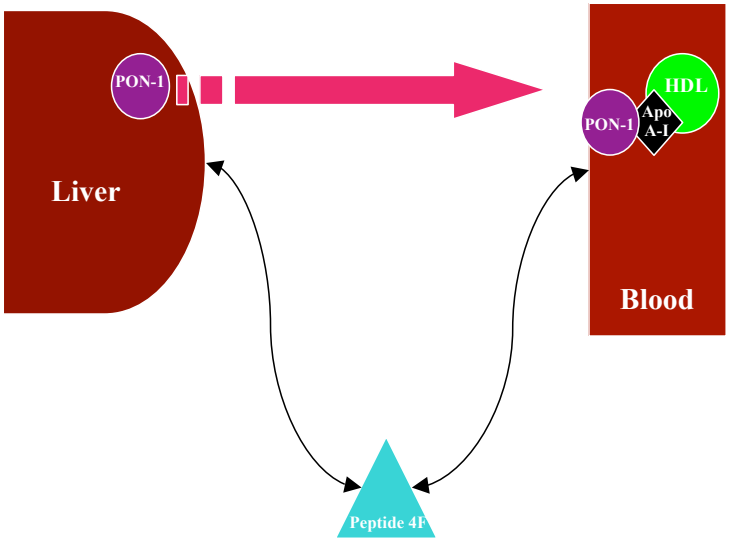


Figure 3: Hypothesized mechanisms of the effect of peptide 4F on PON-1

Methods

Subjects

30 six week old female apoE null mice were purchased from Jackson laboratories (Bar Harbor, ME). These mice were fed standard mouse chow diet (Ralston Purina). All mice were housed 3 per cage in autoclaved, filter-top cages under 12:12 hr light/dark cycle. Food and water was available ad libitum. All procedures were performed in accordance with the University of Alabama at Birmingham Institutional Animal Care and Use Committee with national regulations and policies.

Peptide synthesis

Peptide L-4F (i.e. Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH2) was synthesized by solid phase method using an automated solid phase synthesizer as previously described (Datta, 2000; Datta, 2001).

Injection Protocols

Peptide administration started at 7 weeks of age. Chronic administration of the peptide for 4 weeks would determine the chronic effects of the peptide. A baseline bleed was done when animals were 7 weeks of age to determine total plasma cholesterol levels. Animals were divided into 2 groups; n=15 per group. A

control group of 15 animals were injected with 200 µL of 0.9% saline everyday for 4 weeks. An experimental group of 15 animals were injected with 50 µg of L-4F (.5mg/ml concentration) i.p. everyday for 4 weeks. Mice were euthanized under xylazine/ketamine anesthesia for cardiac puncture blood collection and organ harvesting. Portions of livers were fixed in phosphate-buffered 4% formaldehyde for at least 24 h before freezing sections. Other portions were immediately placed in RNAlater (Qiagen) and stored at -80°. Blood was placed in heparinized microcentrifuge tubes and centrifuged (30min 12rpg) to separate the plasma.

Paraoxonase-1 activity

2 µL of whole plasma (for plasma PON-1 activity) was mixed with 200 µL of PON buffer (100 mmol/L Tris containing 2 mmol/L CaCl2, pH 8.0) containing paraoxon (1 mmol/L O,O-diethyl-O-p-nitrophenylphosphate) (Sigma) and the rate of formation of 4-nitrophenol over a period of twenty minutes was determined spectrophotometrically at 405nm. Blanks were included to correct for the spontaneous hydrolysis of paraoxon. The assay was performed in a 96-well plate (Costar) and readings were taken every 2 minutes.

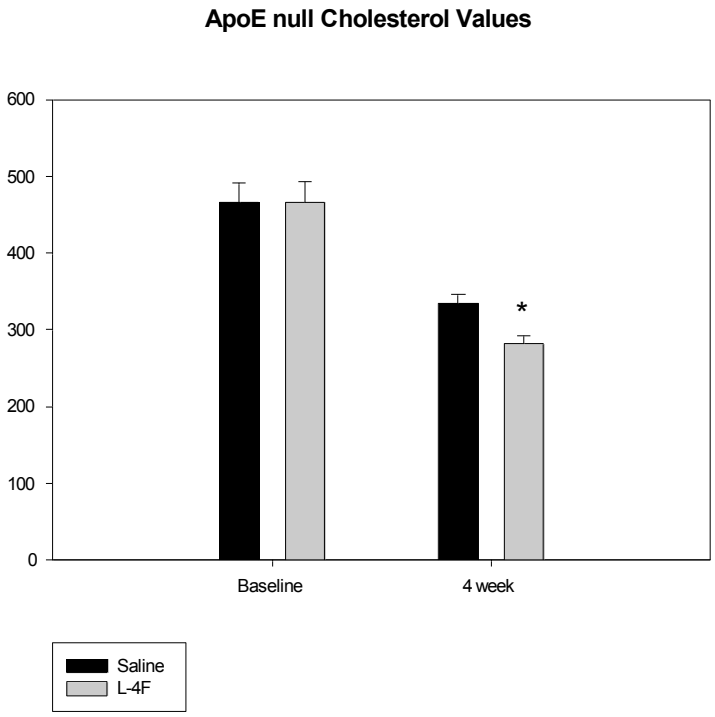
Cholesterol Levels

5 µl of standard/sample was mixed with 200 µL of Cholesterol reagent (cat # TR1342 from ThermoDNA, Arlington, TX) in a 96-well plate (Costar). The samples were incubated for 30min at room temperature. The absorbance was measured at 505nm vs. reagent blank.

Results and Discussion

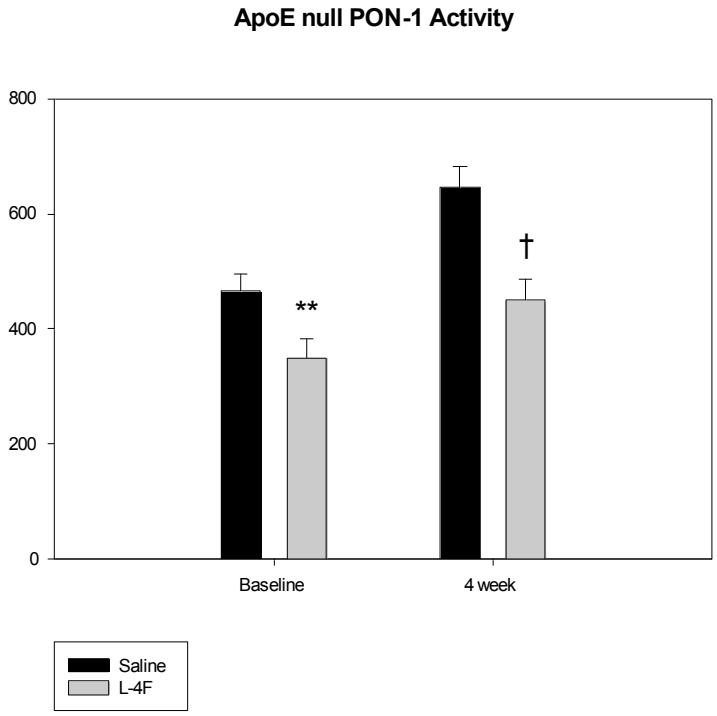
Administering 50µg of L-4F for 4 weeks significantly (*p<0.002) decreased cholesterol levels in the L-4F group compared to the control group (Figure 4). Previous studies have indicated that L-4F does not alter cholesterol levels. However, in the current experiment there was a decrease in cholesterol levels in L-4F treated mice.

PON-1 activity increased in both the control and the L-4F groups, but there was significantly (**p<0.02; †p<0.001) greater PON-1 activity in the control group compared to the L-4F group at both baseline and final measurements (Figure 5). This significant difference between the control group and experimental group at the baseline makes the interpretation of the data difficult. However, PON-1 activity varies between subjects in the natural environment, making it difficult to standardize both groups. Previous studies have exhibited a greater increase in the L-4F groups than the control groups. The PON-1 activity experiment provided results contradictory to those previously reported (Datta, 2001; Navab, 2005). Future studies will be needed to determine the anomaly that caused these results and the amount of PON-1 activated.



Cholesterol Levels

*Figure 4: Apo E null cholesterol levels. apoE null mice were injected with 50µg of L-4F or saline (n=15 in each group) and plasma cholesterol levels were compared to standards of 75, 150, and 300. The mice were bled at the beginning of 4 weeks and after 4 weeks of treatments. Cholesterol assays display a significant decrease of cholesterol levels in L4F treated mice than in control mice. The results shown are Mean±SD. *p<0.002 vs Control.*



PON-1 activity

*Figure 5: (bottom of page 32) Apo E null PON-1 activity. apoE null mice were injected with 50µg of L-4F or saline (n=15 in each group) and plasma PON-1 activity was determined. The mice were bled at the beginning of 4 weeks and after 4 weeks of treatments. PON-1 assays show an increase in PON-1 activity during the 4-week period of treatment with the peptide L4F. The results shown are Mean±SD. **p<0.02; †p<0.001 vs control.*

Limited Results

Due to this being an ongoing study and unexpected results, there are limited results based on the methods proposed. However, the next step is to conduct immunohistochemistry and Real-Time-PCR on histological liver sections and begin experiments with a human hepatocyte cell line, HepG2. We hope to discover a peptide and mRNA interaction causing an increase in genetic expression of PON-1 gene and an increase of enzyme PON-1 levels within the liver. With the cell line, we expect to find an increase of PON-1 secretion into the media and also an increase in genetic expression of the PON-1 gene.

Conclusion

Peptide 4F affects PON-1 activity and levels but conclusive data is yet to be determined.

References

Bradshaw, G., A. Gutierrez, J. H. Miyake, K. R. Davis, A. C. Li, C. K. Glass, L. K. Curtiss, andR. A. Davis. Facilitated replacement of Kupffer cells expressing a paraoxonase-1 transgene is essential for ameliorating atherosclerosis in mice. Proc. Nat. Acad. Sci. USA 2005;102:11029-11034

Datta, G., M. Chaddha, S. Hama, M. Navab, A. M. Fogelman, D. W. Garber, V. K. Mishra, R. M. Epand, R. F. Epand, S. Lund-Katz, M. C. Phillips, J. P. Segrest, and G. M. Anantharamaiah. Effects of increasing hydrophobicity on the physical-chemical and biological properties of a class A amphipathic helical peptide. J. Lipid Res. 2001;42:1096-1104

Deakin, S., I. Leviev, M. Gomaraschi, L. Calabresi, G. Franceschini, and R. W. James. Enzymatically active paraoxonase-1 is located at the external membrane of producing cells and released by a high affinity, saturable, desorption mechanism. J. Biol. Chem. 2002;277:4301-1308

Florentin, M., Liberopoulos, E., Wierzbicki, A., Mikhailidid, D. Multiple actions of high-density lipoprotein. Current Opinion in Cardiology. 2008;23:370-378

Navab, M., G. M. Anantharamaiah, S. Hama, G. Hough, S. T. Reddy, J. S. Frank, D. W. Garber, S. Handattu, and A. M. Fogelman. D-4F and statins synergize to render HDL anti-inflammatory in mice and monkeys and cause lesion regression in old apolipoprotein E null mice. Arterioscler. Thromb. Vasc. Biol., 2005;25:1426-1432

Oda, M. N., J. K. Bielicki, T. Berger, and T. M. Forte. Cysteine substitutions in apolipoprotein A-I primary structure modulate paraoxonase activity. Biochemistry 2001;40:1710-1718

Parathasarathy, S., Litvinov, D., Selvarajan, K., Garelnabi, M. Lipid peroxidation and decomposition-Conflicting roles in plaque vulnerability and stability. Biochimica et Biophysica Acta 1781. 2008: 221-231

Wilson, P. W., R. D. Abbott, and W. P. Costelli. High density lipoprotein cholesterol and mortality. The Framingham Heart Study. Arteriosclerosis 1988;8:737-741

faculty interview: biology

The Preservation of Sea Turtles: *An Interview with Dr. Thane Wibbels*

Timmy Wang

Recently, I had the privilege to interview Dr. Thane Wibbels about his journey in research, his involvement in UAB, and his current research. As an associate professor at UAB, Dr. Wibbels works in multidisciplinary research related to comparative reproductive physiology, specifically on temperature-dependent sex determination in reptiles. Since 1993, Dr. Wibbels has helped undergraduates obtain research experience in his lab and in this interview he provides some advice to undergraduates who are interested in starting research.

Q) Where and how did you get started with research?

A) As an undergraduate, I was very interested in the broad spectrum of sciences. As a child, shows like Jacque Cousteau and the National Geographic Channel fascinated me and instilled in me a love for biology. This is what I chose to study as an undergraduate along with zoology courses with the thought of potentially getting into teaching and research.

Q) So you didn't start research as an undergraduate?

A) As an undergraduate, I did not do any research. I was at the University of Nebraska where there were very few undergraduate research jobs. It was not like here at UAB, where we have a multitude of students working in undergraduate research at any given time. It was difficult, and I did apply for one or two positions. What they needed though was one person per lab at the most, which basically meant you were competing with the entire student body for that one position. I actually began research after I started my Master's degree. I will say, however, that had I started research as an undergraduate, it really would have given me a head start on what it was all about. This way, I had to figure all those things out as a Master's student rather than getting the experience beforehand as an undergraduate when there was less pressure. That is one of the reasons why I believe in furnishing opportunities to the undergraduate students so that they get a feel for what research is like.

Q) Did you know specifically what area of research in which you wanted to specialize?

A) Well, let's put it this way, I did not anticipate studying sea turtles, but I was very much interested in marine biology. When I started applying for a Master's degree program, I was applying to schools that had strong marine biology programs. That's how I ended up attending the University of Houston. At this institution there was a marine science program, and, as it turned out, Galveston, Texas was the place where they were raising sea turtles. Once I arrived, I started working with the turtles, and that developed into my Master's degree. At this time, I was commuting between Galveston and Houston depending on whether I was taking courses or working with sea turtles. I got to know people who studied sea turtles in various locations, and I started studying the whole idea of sea turtles. We were raising 2,000 sea turtles a year when I was there. So my job included taking care of the sea turtles, which I would do that in the morning, while in the afternoon I would do my research - studying the orientation behavior of turtles.

Q) Your research was specifically on orientation behavior of sea turtles. Was it at all related to what you are looking at currently about temperature-dependent sex determination?

A) That came later. We were actually trying to save a species that was coming close to extinction. In fact, it was during this time that scientists performed some of the first studies on temperature-dependent sex determination in sea turtles. So, at the

time, we didn't know any information on the subject.

Q) Do you remember which species you were trying to save?

A: It was the Kemp's Ridley species. As part of our study, we raised about 2,000 turtles to dinner plate size so that we could release them with a greater chance of their survival.

Q) So where did you go for your Ph.D.?

A) I then went to Texas A&M University because I met a person who was one of the world's foremost experts on sea turtles, Dave Owens. He was studying the hormone cycles that controlled the migration, reproduction, and production of thousands of eggs in a nesting season and other things of that nature. I went and worked with him and worked towards my Ph.D., which included a number of projects. We did work in Mexico and in Florida, but my favorite project was six months of studying sea turtles out in the Great Barrier Reef in Australia.

One of the great benefits of UAB is that it has a large amount of research opportunities for undergraduates. …If you are going into research as a career, then participating in research as an undergraduate will allow you to have a head start.

Q) What types of thoughts were you experiencing when working so closely with these creatures?

A) As I was raising and releasing turtles, thoughts of how this project was going to help propagate the species and prevent them from extinction kept entering my mind. During this time, I started learning about turtles in general and their unique life history. They have been around for a hundred million years and were really long lived in that some of the species took 10 to 50 years to reach maturity while floating around in the ocean. Then, once they reach maturity, they migrate long distances back to their nesting beaches and become tremendous reproductive machines for that season. Not only nesting once, turtles have been found nesting two, three, four, five, even six times depending on the species. So these types of things fascinated me about this interesting animal from a nature standpoint, a life history standpoint. That an animal can actually perform these feats is really fascinating and captivates one's imagination.

Q) Was it during this time, while having these thoughts come about and while experiencing deep fascinations, that you decided that you wanted to go into this type of research as a career?

A) I will say that it was a gradual evolution. It is just one of those things that you are not only interested in but you also see all these hurdles that you have to get over. So I took the hurdles one at a time, and I began thinking about the first thing I was going to do, get my Master's degree. Then, if that worked out, I would probably go ahead and get a Ph.D. If I got a PhD, then I would vie for a faculty position. I was setting goals one step at a time based on my fascination with the science. As a matter of fact, I have always been driven by science, but not to the point where it's something that I worry about doing so that I can make a living. It became something I was always trying to do because I liked it. What has ultimately driven my career has been kind of an interest in science and actually getting to do something that I really enjoy doing.

Q) Did you stay at Texas A&M to do your post-doctoral research?

A) I finished my Ph.D. at Texas A&M, studying the adult reproductive endocrinology in sea turtles. Changing the focus of my research, I decide to attend the University of Texas at Austin to do my post-doctoral work with two people who were experts on temperature-dependent sex determination, David Crews and James J Bull. The question of what controlled reproduction in these fascinating animals sparked my curiosity. At that time, it was becoming evident that all of these sea turtles had temperature-dependent sex determination. I realized that I had been studying the adult aspects of reproduction and that it would be really interesting to study the other end of the spectrum: the actual development of the reproductive system since they have this temperature-dependent sex determination.

Q) What actually brought you to UAB, and how long have you been here?

A) While I was doing my post doc at the University of Texas, I began checking out faculty positions at various universities. The department of biology at UAB had a position that was just the exact type of thing I was looking for – a department of biology that was very organismally oriented. I had read a number of publications from there like that of Ken Marion. He had been studying adult reproductive endocrinology in Musk turtles. Thus, he had been doing some of the same type of stuff that I had been doing, only I had been doing it in sea turtles. So I thought that this would be a very nice academic atmosphere. I came here in 1993.

Q) What is your current research focused on?

A) My current research is multidisciplinary. It centers on temperature-dependent sex determination in reptiles. In particular, it focuses on implications on the conservation of endangered species. Branching out from this, I have one person studying the molecular aspects: what kind of genes temperature may be turning on. I have other researchers who are just finishing studying what sex ratios are being produced in sea turtle populations in various locations. One person is working down in Mexico, while another is looking at stuff in Florida. The main concept is this: If the turtles do have this temperature-dependent sex determination, are they producing a one-to-one sex ratio, or, if not, what are they producing and is that good for the survival of the species? I also have another person who just came on board studying giant leatherback sea turtles that are going extinct in the Pacific. He is from Indonesia where some of the last strongholds of leatherback nesting beaches can be located. He is running a hatchery program on the beach to make sure that they are producing the correct sex ratios. Finally, I have one other person, Andy, who is studying a turtle that occurs here in the salt marshes of Alabama, the diamondback terrapin. It was once very abundant in Alabama, but they are now very scarce. We have found pockets of them in various locations but nothing the way it would have been a thousand years ago. We are primarily looking at where they nest and how we can try to initiate the recovery of that species in Alabama.

Q) Do you have any advice for students seeking to get involved in research: what they should do or how they should seek out these opportunities?

A) Absolutely. I think that the best thing they can do is to start by visiting the web page of the department of interest. Word of mouth is also another great way to discover these opportunities. Instructors often talk about research and can help an undergraduate find something that they are interested in. One of the great benefits of UAB is that it has a large amount of research opportunities for undergraduates. UAB is set up so that you can find various research opportunities, and you can contact that researcher and explain to them that you are very interested in what they are working on, asking if it would be possible to work in their lab. I have at least ten people per semester working in my labs due to this type of communication, and so I have seen where it is a great opportunity for students. If you are going into research as a career, then participating in research as an undergraduate will allow you to have a head start. If you are not going into research, you will get at least a feel for what research is like.

A *Caenorhabiditis elegans* Mutagenesis Screen to Identify Candidate Human Cystic Kidney Disease Genes

Rachel M. Stupay, Corey L. Williams, Svetlana Masyukova, and Bradley K. Yoder
Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294

Abstract:
Cilia are membrane bound, microtubule-based organelles involved in cellular activities ranging from sensory perception to motility and fluid movement. These cilia project from almost every cell type in the body. Defects in cilia proteins cause the autosomal recessive developmental disorders Nephronophthisis (NPHP), Joubert Syndrome (JBTS), and Meckel-Gruber Syndrome (MKS) in humans; however, in most cases, the disrupted genes have not yet been identified. Many of the known NPHP, JBTS, and MKS genes are conserved in the nematode Caenorhabiditis elegans, raising the possibility that this simple model organism can be used to understand the human disorders. Single mutations in NPHP or MKS genes in C. elegans have minimal affects on ciliogenesis; however, we have found that combinations of NPHP and MKS gene mutations alter cilia formation and positioning. Here, this redundant requirement of NPHP and MKS genes was utilized in a forward EMS mutagenesis screen in C. elegans to identify novel candidate genes involved in human NPHP or MKS. Mutagenesis was performed on nphp-4(tm925) mutant worms and progeny lacking properly formed cilia were isolated for analysis. Genes disrupted in this screen are being identified by whole genome sequencing and confirmed by transgenic rescue experiments. Ultimately, NPHP and MKS families will be screened for potential mutations in the human homologs of genes identified from this screen.

Introduction:
The autosomally recessive Nephronophthisis (NPHP)-associated disorders are heterogenic and affect a variety of organs. Also termed ciliopathies, these genetic disorders result from mutations affecting proteins of largely unknown function that localize to the cilia or base of the cilia (basal bodies). In NPHP, cysts will form within the corticomedullary border of the kidney. These symptoms tend to be isolated to the kidneys with renal interstitial infiltration in addition to fibrosis, and basement membrane disruption along with tubular atrophy. The most severe NPHP-related ciliopathy is known as Meckel-Gruber Syndrome (MKS). MKS patients traditionally do not live past birth, or are naturally aborted earlier. The additional symptoms of this autosomal recessive lethal disorder are central nervous system malformations, occipital encephalocele, post-axial polydactyly, bowing limbs, severe heart malformations, and hepatic developmental defects. There is extensive genetic overlap between MKS and NPHP with distinct mutations identified in shared genes. This indicates that disease severity, and thus, clinical diagnosis, is influenced by which gene is affected, the nature of the mutation in that gene, and the genetic background of the patient. Unfortunately, the causative lesion in most MKS and NPHP patients remains unidentified. Identifying the missing genes involved in these disorders is critical to ultimately understanding the cellular and molecular basis of the disease, and in turn, developing possible genetic therapeutic strategies. [6]

A large number of NPHP and MKS genes are conserved in the nematode *C. elegans* (Figures 1 and 2). Whereas humans have primary cilia extending from the majority of their cells, *C. elegans* only have sensory cilia extending from a subset of their neurons in the head (amphids) and tail (phasmids). The cilia have a basal body complex that anchors the cilium axoneme to the plasma membrane and cytoskeleton. The basal bodies function in the assembly of proteins that are involved in intraflagellar transport (IFT) in addition to initializing ciliogenesis. IFT is a critical component of cilia formation and it mediates the trafficking of proteins along the cilia axoneme. When mutations occur within genes encoding the basal body and IFT components in mice, symptoms of the aforementioned ciliopathies (diseases associated with ciliary defects) will result. In contrast to the critical requirement of cilia for mammalian development, these cilia function primarily as sensory organs of the worm and are not essential for the viability of the organism. The nonessential nature of the sensory cilia, along with the genetic malleability of *C. elegans,* facilitates the analysis of interactions between the large number of NPHP and MKS gene homologs with relation to cilia structure and/or function. [4,6]

Biochemical and genetic analyses have indicated that MKS and MPHP proteins form two distinct but potentially interacting complexes at the base of the cilia. Solitary mutations in genes in one complex will not cause a visible defect in cilia morphology in *C. elegans*; there will only be a visible adverse effect when a combination of disruptions in both complexes exists. This combination causes a striking deactivation of cilia function to be induced. The disruption of cilia morphology in *mks;nphp* double mutant worms is most easily observed via a dye-filling assay in which the animals are exposed to a hydrophobic fluorescent dye. If cilia structure is normal, the dye is taken in through the cilia membrane and spreads throughout the sensory neurons. In the absence of properly formed cilia, the dye cannot stain the neurons. This phenomenon is referred to as dye-filling defective (Dyf) phenotype. Any combination of mutations in the currently known *mks* genes with the *nphp-4(tm925)* mutation results in the Dyf phenotype (Table 1, Figure 3). Based on this phenomenon,

we hypothesized that novel candidate *mks* genes could be targeted in a mutagenesis screen for Dyf isolates in the context of the *nphp-4(tm925)* mutation. Once the successful mutants are identified from the *C. elegans* screen, a homolog in the human genome might then be identified and screened in ciliopathy patients in whom a causative mutation has not been found. [6,7]

Procedure / Results:

An ethyl methane sulfate (EMS) mutagenesis screen was performed on *nphp-4(tm925)* mutant worms to introduce additional mutations into the genome with the intent to target novel genes that affect cilia morphology in the context of the *nphp-4* mutation. The EMS mutagenesis was used because of its ability to generate nonsense mutant alleles by implementing deletions or rearrangements in the nucleotide base-pairs.

From this EMS screen, ~170 mutant strains with a Dyf phenotype were initially obtained. This phenotype is not observed in any of the non-mutagenized *nphp-4(tm925)* worms. Each strain was then backcrossed with N2 Bristol males (wild-type) to eliminate any background mutations in the genome and to determine the dependency of the Dyf phenotype on the *nphp-4(tm925)* allele. Once they laid F1 progeny, the mutagenized parents were removed from the plates and the hermaphrodite progeny were dye-filled. Worms with a wild-type phenotype that were able to uptake dye (dye-fill) were selected. These were then allowed to self-fertilize. The parents were then removed and from the F2 progeny, dye-filling-defective (Dyf) worms were selected provided the collection of progeny exhibited a Mendelian ratio of 1 Dyf:15 wild-type (Figure 4). If the progeny had followed a ratio of 1 Dyf:3 non-Dyf, this would be indicative of a single gene recessive trait acting independent of the *nphp-4(tm925)* mutation. By having a ratio of 1 Dyf:15 non-Dyf, there is evidence that the genes are assorting independently from each other, and a homozygous recessive genotype for both traits is required for a Dyf phenotype. Again these progeny were allowed to self-mate. Their progeny were then dye-filled to verify the Dyf phenotype was transmitted to 100% of the progeny, indicative of a mutation to homozygosity. If hermaphrodite progeny possess a true homozygous recessive phenotype, they will only be able to produce homozygous recessive offspring. The outcrosses to the N2 wild type worms were repeated three times. Mutant strains that segregated with the 1:15 ratio were then genotyped to verify they were homozygous for the *nphp-4(tm925)* mutations. From the screen, we obtained strains 39.3, 91.3, and 5.3 showing a Dyf phenotype that was dependent on the *nphp-4(tm925)* mutation. The mutant strains' cilia was then imaged to verify the delocalization and non-functionality of the ciliary fibers (Figure 5). The outcrossed Dyf strains retaining the *nphp-4(tm925)* mutation were then mapped to a chromosome using SNP analysis.

For this project, I focused on lines 5 and 39 for further analysis. A SNP map, also referred to as bulk segregate analysis, was constructed to identify the locus of the mutations on the *C. elegans* genome. Briefly, the mutagenized strain was outcrossed with the Hawaiian (SNP variant) strain. SNPs variants between the Bristol N2 and Hawaiian strains were identified via a Polymerase Chain Reaction (PCR) across regions of each chromosome. Eight SNP variants on each chromosome were selected having different *DraI* restriction endonuclease digestion profiles which can be detected on gel electrophoresis analysis. For this analysis the *nphp-4(tm925)* novel gene mutagenized strain on the N2 Bristol background were crossed with the Hawaiian strain. All the progeny from this cross will have a random mix of SNP-*DraI* digest profiles. Subsequent progeny from the F1s of the N2/Hawaiian cross where the SNPs segregate randomly were then grouped according to the Dyf phenotype and SNP analysis was performed. N2 derived SNPs located near the new mutation will be enriched in the worms with the Dyf phenotype, while SNPs located distant from the mutation or on distinct chromosomes will have an equal N2 and Hawaiian ratio due to random segregation. When these reactions are run in an electrophoresis gel, the mutation can be narrowed down to a specific region on a chromosome. [3]

Briefly, from the F2 progeny ~50 worms that were Dyf and ~50 worms that were non-Dyf were selected to be lysed. The Dyf and non-Dyf worms were then added to two separate tubes, lysed and genomic DNA was isolated. SNP regions were amplified by PCR and the DNA was digested by a *DraI* restriction endonuclease. The digested DNA was separated by agarose gel electrophoresis. The gel was imaged to obtain the respective banding pattern for each strain.

The SNP mapping revealed a mutation on chromosome II for both strain 5 and strain 39. The mutation in *nphp-4(tm925)* on chromosome V was also evident in the SNP map (Figure 6). Together, the mapping data indicate the requirement of mutations in both genes to obtain a Dyf phenotype. Complementation analysis was then conducted by crossing lines 5 and 39 resulting in progeny that were all Dyf, thus indicating that the mutations in these lines are in the same gene. These results verify the phenomenon previously hypothesized that novel candidate genes, which could possibly be homologous to those undiscovered *mks* genes, may be identified through a mutagenesis screen with an *nphp(tm925)* already present. These results indicate that we have identified a mutation in a novel gene which functions along with NPHP4 to regulate cilia formation. Based on the previous data, we predict that this new gene will be a strong candidate in human MKS patients for which the underlying genetic cause is not yet known.

Future endeavors:

The next major goal is to identify the gene mutated in lines 5 and 39. For this we are using a Whole–Genome Sequencing approach. Deep-sequencing will be performed on mutant genomes of both line 5 and 39 followed by bio-informatic

Figure 1: Alignment of Homo sapiens NPHP4 with Caenorhabiditis elegans nphp-4

Figure 1 continued...

```
hsNPHP4          LRQGRPAVQASHELEVVATEYEQDNMVVSGDMLGFGRVKPIGVHSVVKGRLHLTLANVGH 829
ceNPHP-4         YRRGREAVQLFIQCPVVDTSLDTS--------------------SKAGAFLYMRVANIGF 747
                  *:** ***   :  ** *. : .                     * . . *:: :**:*.

hsNPHP4          PCEQKVRG---CSTLPPSRSRVISNDG-ASRFSGGSLLTTGSSRRKHVVQAQKLADVDSE 885
ceNPHP-4         PSGNTYDLSSSSSSLTTTRSNVNSGQGTVVRRLTSSIRLNEEGPHSYRIHAKPLPGNSGV 807
                 *. :.      .*:*..:**.* *.:* . *   .*:  . .. :.: ::*: *.. ..

hsNPHP4          LAAMLLTHARQGKGPQDVSRESDATRRRKLERMRSVRLQEAGGDLGRRGTSVLVRQSVRT 945
ceNPHP-4         GLDRFLTAQR----LDIQQRHEQLFNENSLDKIRQWNDLKEGFNFSDN-----KEIAQKF 858
                     :**  *     :  .*..:  ....*:::*. .  : * ::. .      . : :

hsNPHP4          QHLRDLQVIAAYRERTKAESIASLLSLAITTEHTLHATLGVAEFFEFVLKNPHNTQHTVT 1005
ceNPHP-4         IFEEELAAYKKLRYESKPAKLLEAVFKGITSCHQINPSFGEKVFFEFPLENYNSEPINCT 918
                  . .:* .    * .:*. .: . :  .**: * ::.::*   **** *:* :.   . *

hsNPHP4          VEIDNPELSVIVDSQEWRDFKGAAGLHTPVEEDMFHLRGSLAPQLYLRPHETAHVPFKFQ 1065
ceNPHP-4         IEFDDEALKPVFDAEEWKFYKTVNKVTTPSEKQMMRQT-TDRIEICLQPGDVLFIPFIYD 977
                 :*:*:  *. :.*::**: :* .  : ** *::*::   :   :: *:* :. .:** ::

hsNPHP4          SFSAGQLAMVQASPGLSNEKGMDAVSPWKSSAVPTKHAKVLFRASGGKPIAVLCLTVELQ 1125
ceNPHP-4         AFFFPNDAFNMYST----------------------KVVFRRWDTKEPLAILDLHVHRR 1014
                 :*   : *:   *.                        *: * .  :*:*:* * *. :

hsNPHP4          PHVVDQVFRFYHPELSFLKKAIRLPPWHTFPGAPVGMLGEDPPVHVRCSDPNVICETQNV 1185
ceNPHP-4         NFLLQHSVTFICETSGNWEKQLVLPP----------MARDRRVLSCRCSDPSVRLTVRNA 1064
                  .:::: . *     .  :* : ***          *  :   :  *****.*   .:*.

hsNPHP4          GPGEPRDIFLKVASGPSPEIKDFFVIIYSDRWLATPTQTWQVYLHSLQRVDVSCVAGQLT 1245
ceNPHP-4         T--LQQIVGFTTYSGETNDRKTFLLLMYSDHYQTRLMATWKITILPFFNVDVRSIVGQTT 1122
                      : : :.. ** : : * *::::***:: :    **:: : .: .*** .:.** *

hsNPHP4          RLSLVLRGTQTVRKVRAFTSHPQELKTDPKGVFVLPPRGVQDLHVGVRPLRAGSRFVHLN 1305
ceNPHP-4         RLHLLVHRRSEHDGVPDDLLKVYTASGCMKVVDSVLTERTPTATIDFTPNFIGTKKLVVS 1182
                 ** *:::  .    *     :    .   * *  : .. .    :.. *   *:: : :.

hsNPHP4          LVDVDCHQLVASWLVCLCCRQPLISKAFEIMLAAGEGKGVNKRITYTNPYPSRRTFHLHS 1365
ceNPHP-4         VVNTNTLKLERGFLVYGKSEAPRITQKFVIQIPSSD-EAIRKRIPIRNPYGLPKTFRITT 1241
                 :*:.:  :*  .:**   .. * *:: * * :.:.: :.:.***.  ***   :**:: :

hsNPHP4          DHPELLRFREDSFQVGGGETYTIGLQFAPSQRVGE-EEILIYINDHED-KNEEAFCVKVI 1423
ceNPHP-4         SNSDIVKITDSLLSVPPMGKLPCEMYFVKNTHLQKNIETLLYISDAETYVQEEAYSITLA 1301
                 .:.::::: :. :.*    . .  : *. . :: :  * *:**.* *   :***:.:.:

hsNPHP4          YQ-- 1425
ceNPHP-4         FEAS 1305
                 ::
```

Figure 2: Alignment of Homo sapiens MKS5 with Caenorhabiditis elegans mks-5

Figure 2 continued...

```
hsMKS5      IHINKVTFSSEVLQASGDKEPVT------FCTYAFYDFELQTTPVVRGLHPEYNFTSQYL 662
ceMKS5      INIPTPRVNTDLSVKLINVKPSPSLTSKFFFSLEFFDFQLETTPIMDAKQHNMDFTTVYD 838
            *:* .  ..:::     : :* .      * :  *:**:*:***:: . : : :**: *

hsMKS5      VHVNDLFLQYIQKNTITLEVHQAYSTEYETIAACQLKFHEILEK-SGRIFCTASLIGTKG 721
ceMKS5      VLVSNLLIHYLQTNGIVIEMYRPASDCYKLLAAATISLIPLFEDSVLRKFCSEIMLKSVD 898
            * *.:*:::*:*.* *.:*:::. *  *: :**. :.:  ::*.   * **:  :: : .

hsMKS5      DIPNFGTVEYWFRLRVPMDQAIRLYRERAKALGYITSNFKGPE----HMQSLSQQAPKTA 777
ceMKS5      TGVEMCTLRYEIEVSQPISDSFKKFKKSEMARNMLPLQLENEDTEDTNFDPLTIMVNRVV 958
               :: *:.* :.:  *:.:::: :::   * . :. :::. :    :::.*:  . :..

hsMKS5      QLSSTDSTDG----------NLNELHITIRCCNHLQSRASHLQPHPYVVYKFFDFADHDT 827
ceMKS5      GLDTFGKDPSTEFCIVDEFLSFSPYFTDFSTSSEIRSKRDCYIPKIDIARNLFATSSISF 1018
             *.: ..  .           .:.  .  :  ...::*: .   *:  :. ::*  :. .

hsMKS5      AIIP-SSNDPQFDDHMYFPVPMNMDLDRYLKSES--LSFYVFDDSDTQENIYIGKVNVPL 884
ceMKS5      FLIENIPRQDGVIATLHLPLHPLCKLGGSIKGTFPMLDTDGRPSSVSLDLCLIWKHEIPS 1078
             :*   ..:  .   :::*:    .*.  :*.    *.     .* : :   * * ::*

hsMKS5      ISLAHDRCISGIFELTDHQKHPAGTIHVILKWKFAYLPPSGSITTEDLGNFIRSEEP--E 942
ceMKS5      FFLKHEPKEP-LKEVKDTPILPQPVRRTSKEFVVTPVKEAELHDAEPTSMPPKAPEPTTA 1137
            : * *:   . : *:.*    *  . :.  :: .: : :   :    :*  .   :: **

hsMKS5      VVQRLPPASSVSTLVLAPRPKPRQRLTPVDKKVSFVDIMPHQSDETSPPLEDRKEISPEV 1002
ceMKS5      PLRRLSTDSSDTSFSHSSKDLFSPPTNPQTYDYEIPAVTPALVDSDGEEEADRIVFDDDD 1197
             ::**.. ** :::  :.:       .*   . .:  : *   *. .    **  :. :

hsMKS5      EHIPEIEINMLTVPHVPKVSQEGSVDEVKENTEKMQQG-----KDDVSLLSEGQLAEQSL 1057
ceMKS5      DEIESVSAVSSQRDPEPLEVPERQVENLPSPEDTPRPSDPLKPNGTNESKESTPVTQRSV 1257
            :.* .:.         *    * .*::: .  :. : .     :.  .  ..  ::::*:

hsMKS5      ASSEDETEITEDLEPEVEEDMS---ASDSDDCIIPGPISKNIKQSLALSPGLGCSSAISA 1114
ceMKS5      DKTDDVAPVDPELEPESGPEPEPVVESEPNEVAETEEDRKRELKTEELKSLLGALPPIAK 1317
             .::* : :  :****   : .    *:.::   .    *.  ::  *.. **. ..*:

hsMKS5      HCNFRL-PGSSDFPASASQVDGITG------ACHHSQPSEKIRIEIIALSLNDSQVTMDD 1167
ceMKS5      PRNIPVGPIALTEQPEATRQQGSTGRILFTDPLHFSVPPSESSSTSSPRRAEKAPVPLPD 1377
              *: : * :    ..*:: :* **       . *.* *..:      .   :.: *.: *

hsMKS5      TIQRLFVECRFYSLPAEETPVSLPKPKSGQWVYYNYSN-VIYVDKENNKAKRDILKAILQ 1226
ceMKS5      YEGHSLIKVRKPLSPTDKDVLEPNMKVSIQLETFELVPGSSLTPLTREETTFFVDWVFLD 1437
               : ::: *    *:::  :.     * *   ::       .   .:::.  :  .:*:

hsMKS5      KQEMPNRSLRFTVVSDPPEDEQDLECEDIGVAHVDLADMFQEGRDLIEQNIDVFDARADG 1286
ceMKS5      FTNEQSKSTIFDFPRRPQEMVDIRYTKEYTLTRGQLSLLDQWIRASIKFELTIIKISPGD 1497
              :  .:*  * .   * *  :    ::  ::: :*: : *  *  *: :: ::.  ...

hsMKS5      E---GIGKLRVTVEALHALQSVYKQYRDDLEA------------------- 1315
ceMKS5      EEELGFGSLILVPNNTQNKSFVIDVYDRSGIVQAEMTLTLHFSRALIEQLT 1548
            *   *:*.* :. :  :  . * . *  .  .
```

Dye-filling Defective Mutants

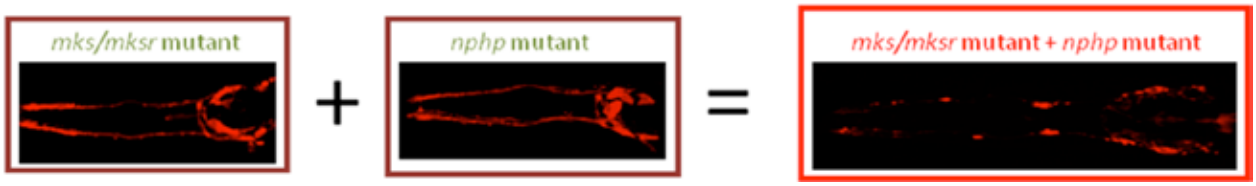


Figure 3 (above): mks mutant worms and nphp mutant worms individually dye fill normally. Only when there was a combination of an mks mutation along with an nphp mutation in the same worm did a dye-filling defect arise.

Figure 4 (below):Compilation of current mutant strains being studied. Strains 5, 39, and 91 were all isolated from this study and mapped to chromosome II. This showed that the induced mutation localized separately from the nphp-4(tm925) mutation along with inducing the Dyf phenotype.

Strain	Phenotype	Phenotype F1	Genotype Cross #1	Genotype Cross #2	Genotype Cross #3	Current Stage	Chromosome Location
yhw3	faint 1:15	wt	tm925	tm925	tm925	mapping	Chr IV
yhw 4	dyf		tm925	heterozygous		frozen	
yhw5	dyf	Wt	tm925	tm925	tm925	mapped	Chr II
yhw9	faint	Wt	tm925	tm925	tm925	mapping	
yhw10	faint					cross #1/ mating	
yhw12	very faint	Wt	tm925	tm925	tm925	mapping	
yhw14	dyf	Wt	tm925	tm925	tm925	cross #3 / genotyping	
yhw15	faint ~ 1:15	Wt	tm925	tm925	tm925	mapped	Chr V
yhw17	faint ~ 1:15	Wt	tm925	tm925	tm925	mapped	Chr V
yhw19	faint ~ 1:10	Wt	tm925	tm925	tm925	mapped	Chr V
yhw20	dyf	Wt	tm925	tm925	tm925	cross #3 / genotyping	
yhw22	dyf	Wt	tm925			cross #2 / genotyping	
yhw24	mix	Wt	tm925	tm925	tm925	cross #3 / genotyping	
yhw26	dyf	Wt	tm925	tm925	tm925	mapped	Chr X
yhw34	dyf	Wt	tm925	tm925	tm925	mapping	?
yhw35	dyf	Wt	tm925	tm925	tm925	mapping	
yhw36	faint ~ 1:15?*	Wt	tm925	tm925	tm925	mapping / outcross	
yhw39	dyf ~1:15?	Wt	tm925	tm925	tm925	mapped	Chr II
yhw40	dyf	Wt	tm925	tm925	tm925	mapping	
yhw62	dyf	Wt	tm925	tm925		cross #3 / genotyping	
yhw64	faint*	Wt	tm925	tm925	tm925	mapping	
yhw65	faint	Wt	tm925	tm925	tm925	mapped	Chr IV
yhw66	dyf	Wt	tm925	tm925	tm925	mapped	Chr IV
yhw 67			tm925	heterozygous		frozen	
yhw71	faint ~ 1:10	Wt	tm925	tm925	tm925	mapped	Chr X
yhw73	dyf	Wt	tm925			cross #2 / genotyping	
yhw91	very faint		tm925	tm925	tm925	mapped	Chr II
yhw103	dyf / roll					cross #1	
yhw 106	dyf		heterozygous				
yhw111	dyf	Wt	tm925	tm925		cross #3 / mating	
yhw112	dyf	Wt	tm925	tm925		cross #2 / genotyping	
Yhw 115	DYF		tm925	heterozygous		frozen	
yhw128	dyf < 1:15	Wt	tm925	tm925	tm925	mapped	Chr II
yhw129	dyf	Wt	tm925	tm925	tm925	mapping	
yhw130	dyf	Wt	tm925	tm925	tm925	mapping	
yhw131	dyf	Wt	tm925	tm925	tm925	mapping	
yhw146	dyf	Wt	tm925	tm925		cross #3 / mating	
yhw153	dyf	Wt	tm925			cross #2 / genotyping	
yhw165	very faint	Wt	tm925	tm925	tm925	mapped	Chr IV
yhw166	dyf	Wt	tm925	tm925	tm925	mapping	

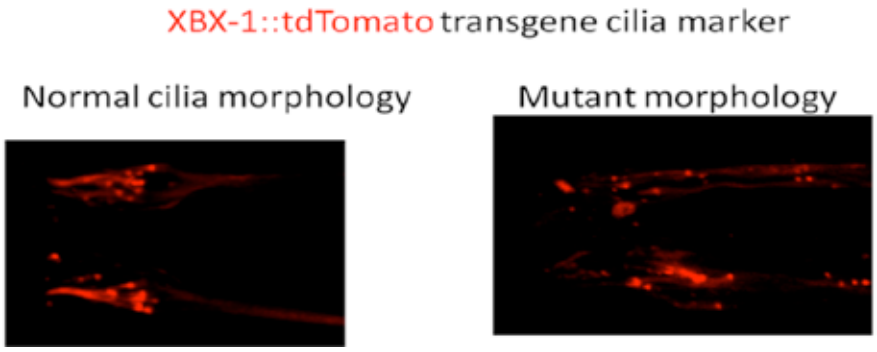


Figure 5: Morphology of the wild-type and experimental mutant strain expressing XBX-1::tdTomato cilia imaging marker. The mutant cilia morphology is noticeably misaligned. This accounts for the Dyf phenotype.

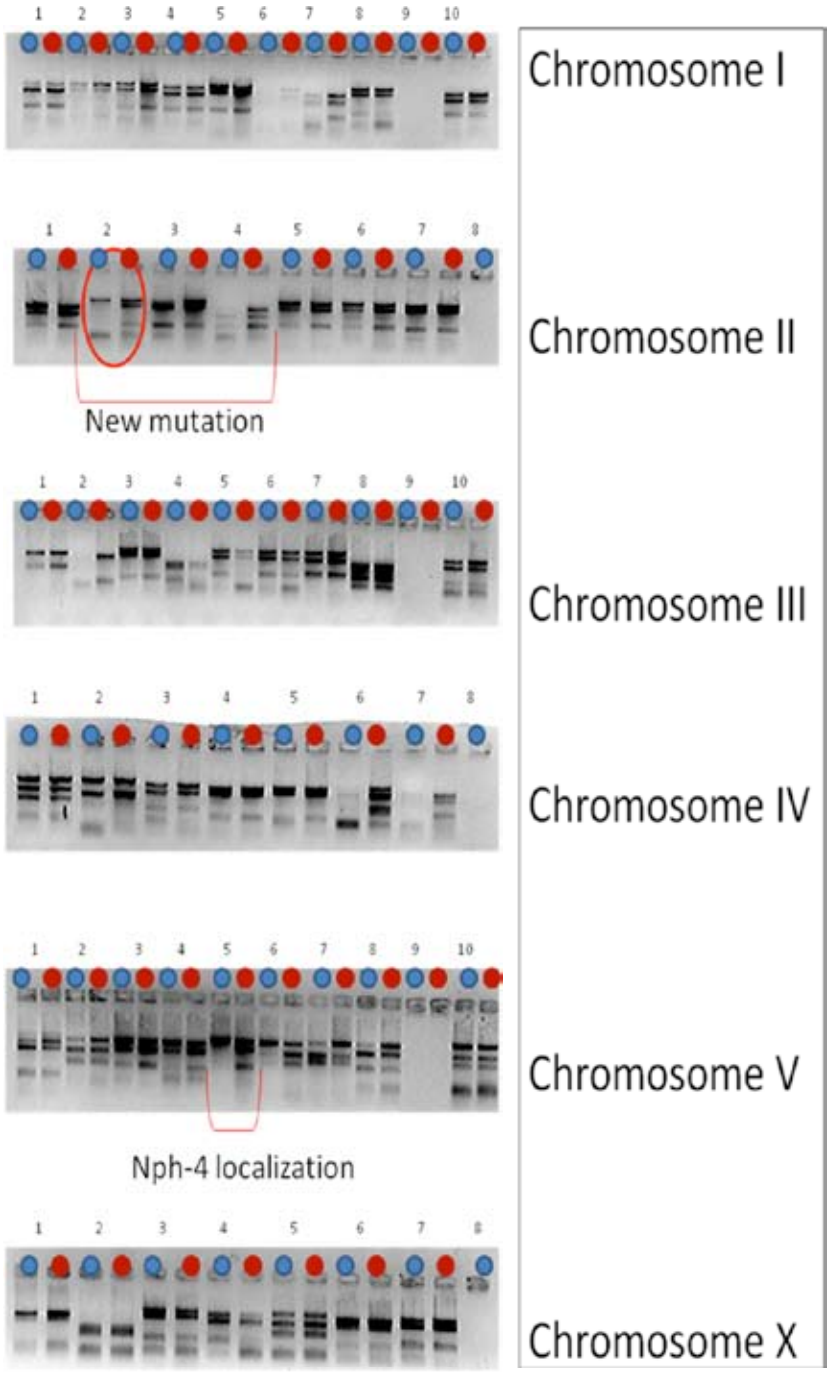


Figure 6: SNP mapping of strain 39.3. Visible mutations occurred on Chromosome II and V. nphp-4(tm925) localizes to chromosome V and the induced mutation localized to Chromosome II.

Dye-filling in NPHP/MKS compound mutant *C. elegans*

	mks-1	*mks-3*	*mks-5*	*mks-6*	*nphp-4*
mks-1	WT	WT	ND	ND	Dyf
mks-3	WT	WT	ND	ND	Dyf
mks-5	ND	ND	WT	ND	Dyf
mks-6	ND	ND	ND	WT	Dyf
nphp-4	Dyf	Dyf	Dyf	Dyf	WT

Table 1: Of the compound mutations studied solely between mks genes, there was a consistent wild-type phenotype. Induced compound mutations in an mks gene in combination with the nphp-4(tm925) resulted in a Dye-filling defective phenotype (Dyf).

analysis to uncover the molecular identity of the mutations. After sequencing, transgenic rescue experiments will be performed to confirm the identified mutant gene is responsible for the Dyf phenotype. Following this confirmation, the localization of the corresponding protein and its role in regulating cilia assembly can be explored. Further studies will involve screening for mutations in this gene in human patients with cilia-related disorders. [3,5]

References

1. Bialas, N.J., Inglis, P.N., Li, C., Robinson, J.F., Parker, J.D., Healey, M.P., Davis, E.E., Inglis, C.D., Toivonen, T., Cottell, D.C., Blacque, O.E., Quarmby, L.M., Katsanis, N., Leroux, M.R. (2009). Functional Interactions Between the Ciliopathy-Associated Meckel Syndrome 1 (MKS1) Protein and Two Novel MKS1-Related (MKSR) Proteins. *J Cell Sci* 122, 611-24.

2. Davis, M.W., Hammarlund, M., Harrach, T., Hullett, P., Olsen, S., Jorgensen, E.M. (2005). Rapid Single Nucleotide Polymorphism Mapping in *C. elegans*. *BMC Genomics* 6:1, 18.

3. Hillier, L. W., Marth, G. T., Quinlan, A. R., Dooling, D., Fewell, G., Barnett, D., Fox, P., Glasscock, J. I., Hickenbotham, M., Huang, W., Margrini, V. J., Richt, R. J., Sander, S. N., Stewart, D. A., Stromberg, M., Tsung, E. F., Wylie, T., Schedl, T., Wilson, R. K., and Mardis, E. R. (2008). Whole-Genome Sequencing and Variant Discovery in *C. elegans*. *Nature Methods* 5:2, 183-188.

4. Jorgensen, E. M., and Mango, S. E. (2002). The Art and Design of Genetic Screens: Caenorhabiditis elegans. *Nature Publishing Group.* www.nature.com/reviews/genetics. 3. 353-368.

5. Shen, Y., Sarin, S., Liu, Y., Hobert, O., and Pe'er, I. (2008). Comparing Platforms for C. elegans Mutant Identification Using High-Throughput Whole-Genome Sequencing. *PLoS ONE* 12, e4012. 1-6.

6. Williams, C. L., Winkelbauer, M. E., Schafer, J. C., Michaud, E. J. and Yoder, B. K. (2008). Functional Redundancy of the B9 Proteins and Nephrocystins in Caenorhabiditis elegans Ciliogenesis. *Mol Biol Cell* 19, 2154-2168.

7. Winkelbauer, M. E., Schafer, J. C., Haycraft, C. J., Swoboda, P. and Yoder, B. K. (2005). The *C. elegans* Homologs of Nephrocystin-1 and Nephrocystin-4 are Cilia Transition Zone Proteins Involved in Chemosensory Perception. *J Cell Sci* 118, 5575-87.

Environmental Tobacco Smoke: Role in Progression of Diabetic Nephropathy

David M Obert[1], Ping Hua[1], Meagan E Pilkerton[1], Wenguang Feng[1], Edgar A. Jaimes[1,2]

[1]Renal Division, University of Alabama at Birmingham, and [2]Birmingham VA Medical Center.

Abstract:

Clinical studies suggest that smoking is a risk factor in the progression of chronic kidney disease, including diabetic nephropathy (DN). The mechanisms involved, however, are not completely understood. We have previously demonstrated that nicotine, one of the compounds present in large amounts in tobacco smoke, promotes mesangial cell proliferation and fibronectin production. In this study, we hypothesized that exposure to environmental tobacco smoke (ETS) promotes the progression of diabetic nephropathy by increasing the expression of cytokines such as TGF-β, resulting in increased mesangial expansion and matrix deposition.

Six-week-old diabetic (db/db) mice were divided into two groups. The experimental group (n=12) was exposed to ETS at a concentration of 30 mg/m3 for 6 hrs/day, 5 days/week for eight weeks. The control group (n=8) was not exposed to smoke. Urine was collected before euthanasia for albumin by ELISA, and creatinine measurements by mass spectrometry. After euthanasia, the kidneys were harvested for morphometric analysis and Western blot analysis. Serum was saved for cotinine measurements using ELISA.

ETS exposure resulted in significant mesangial expansion that was accompanied by concomitant increases in TGF-β and fibronectin expression. There was, however, no difference in albumin urinary excretion between the two groups. Serum levels of cotinine found in the ETS group were similar to those found in smokers.

Introduction

The prevalence of type 2 diabetes mellitus in the United States has doubled since 1990.[1] There are about 24 million people in the United States with diabetes, of which 16.11 million have been diagnosed with type 2 diabetes.[2] A complication of diabetes, diabetic nephropathy, is the most common cause of end stage renal disease in the United States, and is also associated with increased cardiovascular morbidity and mortality.[3] Clinically, it is characterized by increased protein excretion in urine and progressive decrease in glomerular filtration rate (GFR). Pathologically, it is characterized by an initial phase of glomerular hyperfiltration followed by glomerular hypertrophy, mesangial expansion, and increased deposition of extracellular matrix (ECM) proteins such as fibronectin and collagen. Fibrosis and progressive mesangial expansion induce irreversible changes in the structure and function of the glomeruli, effectively reducing the glomerular filtration surface,[4] and eventually resulting in glomerulosclerosis and interstitial fibrosis.[5]
Several clinical and experimental studies have demonstrated the role of transforming growth factor beta (TGF-β) in the pathogenesis of chronic kidney disease, including diabetic nephropathy.[6] This cytokine is largely pro-fibrotic, and plays a significant role in diabetic nephropathy by increasing the production of ECM components in the glomerulus.

The purpose of this study was to investigate the mechanisms by which tobacco smoke causes accelerated progression of existing diabetic nephropathy. While clinical studies have shown that cigarette smoking is an independent risk factor in the progression of chronic kidney disease and diabetic nephropathy,[7] the mechanisms involved are not yet known. Additionally, while studies based on large population samples have in fact found a strong correlation between tobacco smoke exposure and progressive kidney disease in subjects with preexisting nephropathy,[8,9,10,11] some studies have found no association between tobacco smoke exposure and progressive renal failure.[12,13,14,15]

It is important to understand the mechanisms by which tobacco smoke promotes diabetic nephropathy, as this could lead to the development of new therapies or preventative measures in diabetic individuals who are exposed to tobacco smoke, whether through direct inhalation or through secondhand exposure. Secondhand smoke is a major and widespread health concern in the United States, with around 126 million people receiving exposure annually.[16] Of this group, about 49,000 will die prematurely due to secondhand smoke exposure.[17] In these studies we hypothesize that exposure to tobacco smoke worsens the progression of diabetic nephropathy by increasing the severity of extracellular matrix deposition and increasing the expression of the pro-fibrotic cytokine TGF-β.

Methods

Environmental Tobacco Smoke (ETS) Exposure

For these studies we used six-week old db/db (diabetic) mice (Jackson Labs), which were exposed to either room air (n=8) or to ETS (n=12) for eight weeks. ETS exposures were performed at the Center for Health and the Environment at the University of California-Davis. Mice assigned to ETS were exposed to tobacco smoke from Research Cigarettes (University of Kentucky) at a concentration of 30 μg/m^3 for six hours per day, 5 days a week. This concentration was chosen in order to obtain mice smoke exposure levels similar to those of human smokers.[18] After eight weeks of exposure to either room air or ETS, the mice were euthanized, and kidneys harvested for histology and molecular biology. Serum was saved for cotinine measurements.

Measurement of Glomerular Surface Area
Light microscopy of PAS-stained sections (5 µm) was used for morphometric analysis. The surface area and mesangial area (μm^2) of a minimum of 20 glomerular sections from each animal were determined from digital images using the Image-Pro Plus 4.5 software (Media Cybernetics). Glomerular and mesangial surface areas were measured from digital images by tracing around the perimeter of the glomerular tuft (glomerular area) and around the perimeter of intraglomerular PAS positive material (mesangial area). Glomeruli that were incomplete, distorted, tangentially sectioned, or globally sclerosed were excluded from analysis. The analysis software was calibrated to a stage micrometer.

Western blotting
Briefly, kidney cortex homogenates were separated by SDS-PAGE (8% acrylamide gel) under reducing conditions and transferred to a nitrocellulose membrane (Hybond ECL). Blots were incubated for 1 h with rabbit anti-murine polyclonal antibody to fibronectin at a 1:200 dilution (Sigma), TGF-β at a 1:1000 dilution (R&D Systems), or a polyclonal antibody to β-tubulin at 1:200 dilution (Santa Cruz). After washing, the blots were incubated with goat anti-rabbit antibody (Santa Cruz) for one hour, and the signal detected by horseradish polymerase-catalyzed chemiluminescence.

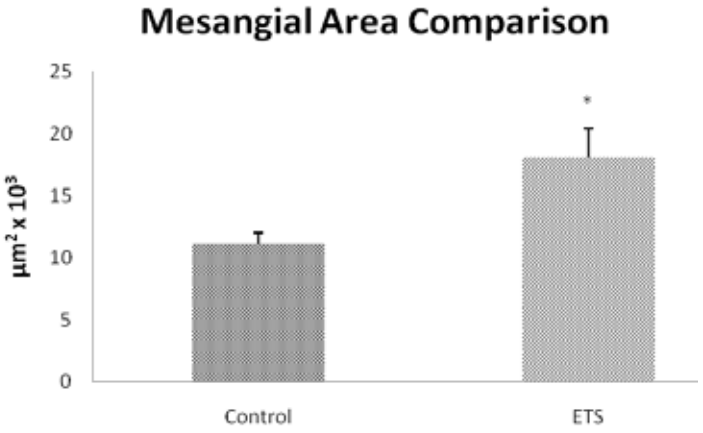
Serum cotinine measurements
Cotinine was measured in serum by ELISA (E-101-25, Bethyl Labs) following the manufacturer's instructions.

Urinary albumin excretion
Urine collections were performed the day prior to sacrifice, and urine saved at –20° C. Urinary albumin concentration was measured by ELISA (E-101-25, Bethyl Labs) following the manufacturer's instructions. Urinary albumin excretion was adjusted for urinary creatinine, which was measured by tandem mass spectrometry (Biochemical Genetics Laboratory, University of Alabama at Birmingham).

Statistical analysis
Data is expressed as mean ± SEM. For statistical comparisons, a student's unpaired *t*-test was used (Microsoft Excel).

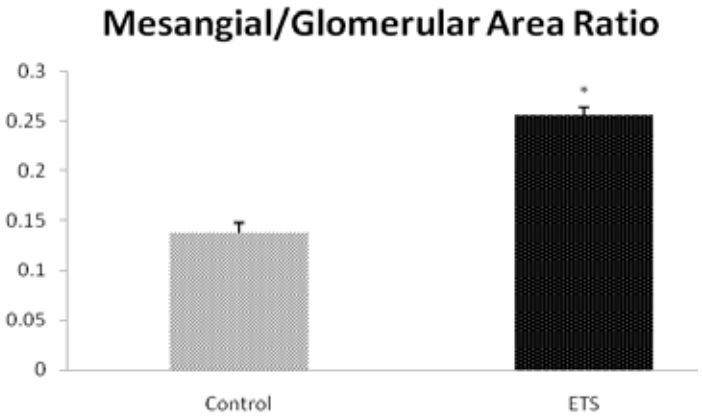
Results
Effects of ETS on mesangial expansion in diabetic mice
Morphometric analysis in PAS-stained sections demonstrated a significant increase in both total mesangial area (Figure 1) and the mesangial/glomerular area ratio (Figure 2) in mice exposed to ETS, suggesting that ETS exposure worsens mesangial expansion and extracellular matrix deposition in a mouse model of diabetic nephropathy. To determine whether these changes were accompanied by changes in the expression of the extracellular matrix protein fibronectin[19] and the pro-fibrotic cytokine TGF-β,[20] we performed Western blot analysis in renal cortex homogenates from air-exposed and ETS-exposed diabetic mice. As shown in Figures 3 and 4, ETS exposure resulted in significant increases in the expression of both fibronectin and TGF-β as assessed by Western blot. In the aggregate, these findings demonstrate that chronic ETS exposure increases mesangial expansion in diabetic mice.

**Note: the following figures include all control group subjects (n=8) and all experimental group subjects (n=12). In choosing the sample size for each group, it was asserted that more subjects should belong in the experimental group due to the variability of data in animal testing. This increased number allowed a more accurate representation of the experimental group data.*



*P < 0.05 versus control group.

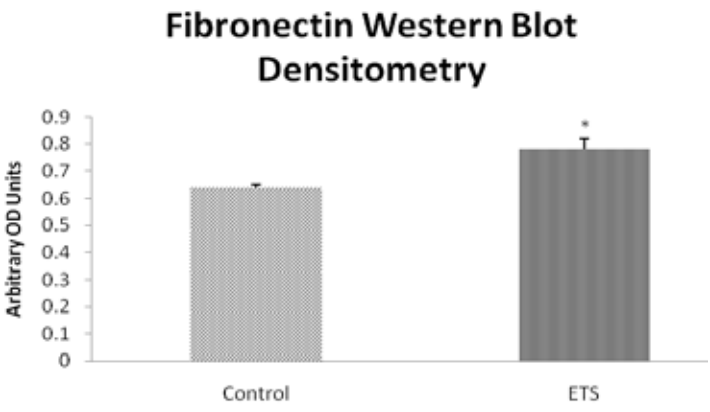
Figure 1: The ETS-exposed group demonstrated a significant increase in mesangial area.



**P < 0.05 versus control group.*

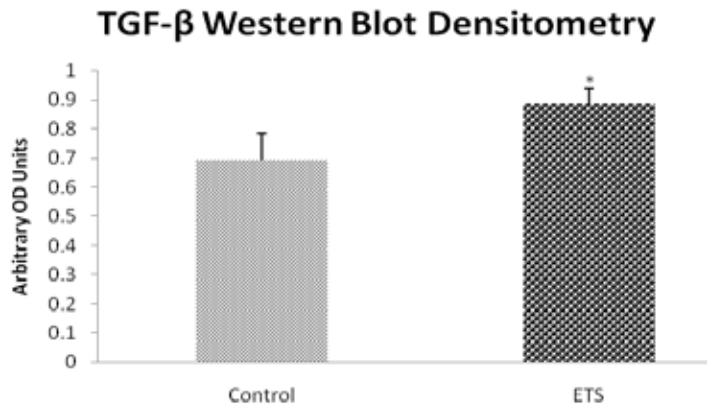
Figure 2: The ETS-exposed group had a significantly larger mesangial-to-glomerular area ratio than control.

Effects of ETS on urinary albumin excretion
To determine whether ETS-induced mesangial cell expansion was associated with changes in urinary protein excretion, we measured albuminuria in air-exposed and ETS-exposed diabetic mice. As shown in Figure 5, air-exposed diabetic mice had urinary albumin excretions in the range reported by others in db/db mice with diabetic nephropathy,[21] however, and in contrast with our morphometric analysis and Western blot analysis, ETS exposure did not significantly modify the urinary excretion of albumin.



*P < 0.05 versus control group.

Figure 3: The ETS-exposed mice showed significantly increased fibronectin expression.



*P < 0.05 versus control group.

Figure 4: TGF-β expression was significantly increased in the ETS group compared to control.

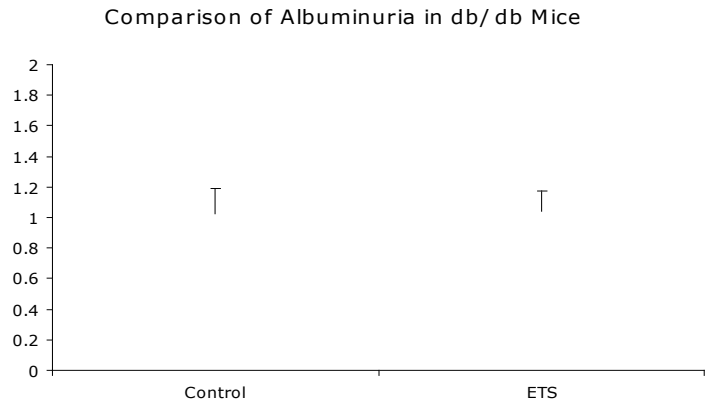


Figure 5: Albuminuria levels were not significantly different between control and ETS groups.

Cotinine levels in ETS-exposed mice

We measured the serum levels of cotinine, a stable metabolite of nicotine, as a marker of ETS exposure. The ETS group had measured plasma cotinine levels of 80.6 ng/ml (± 7.45 SEM), while the control group exhibited virtually undetectable levels. ETS exposure resulted in levels of cotinine similar to those found in the plasma of smokers.[22]

Discussion

These studies demonstrate that long-term ETS exposure worsens the severity of diabetic nephropathy as assessed by mesangial expansion and extracellular matrix deposition in a well- validated mouse model of diabetic nephropathy. ETS exposure, however, did not significantly modify the urinary excretion of albumin, suggesting that at the time of analysis, the urinary excretion of albumin was already at its maximum, and therefore not further increased by ETS exposure.

Several recent epidemiological studies have demonstrated that consumption of tobacco products accelerates the progression of chronic kidney disease, both in diabetic and non-diabetics.[8,9,10,11] The mechanisms involved, however, are not well understood. We have recently demonstrated that human mesangial cells are endowed with nicotine receptors,[19] and that nicotine worsens renal injury in rat model of glomerulonephritis.[4] Our current studies now demonstrate that ETS exposure results in significant mesangial expansion associated with increased expression of the extracellular matrix protein fibronectin and TGF-β, a pro-fibrotic cytokine that plays a major role in the pathogenesis of chronic kidney disease, including diabetic nephropathy. Importantly, our studies were performed with ETS exposures that resulted in levels of the stable nicotine metabolite cotinine in the range observed in the plasma of smokers.[22]

Our studies also suggest that TGF-β plays an important role as a mediator of the deleterious effects of smoking in the progression of diabetic nephropathy. Whether or not the observed increase in TGF-β is the result of a direct effect of compounds present in tobacco on the expression of this cytokine, however, remains to be determined and is the focus of additional studies in our laboratory.

Based on our previous studies,[19] we hypothesize that nicotine, a compound present in large amounts in tobacco smoke, may be mediating in large part the deleterious effects of ETS on the progression of diabetic nephropathy. However, we also recognize that other biologically active compounds present in tobacco smoke may also be playing an important role on these effects. In summary, our studies demonstrate the effects of ETS on the progression of diabetic nephropathy, unveil some of the mechanisms involved, and may result in the development of novel strategies in the treatment and prevention of diabetic nephropathy in smokers

References

1. Gerberding JL. Diabetes - At a Glance. In: U.S. Department of Health and Human Services CfDCaP, ed. Atlanta: U.S. Department of Health and Human Services; 2007.

2. Prevention CfDCa. National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2007. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2008.

3. Valmadrid C, Klein R, Moss S, Klein B. The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. *Arch Intern Med.* 2000;160(8):1093-1100.

4. Zelmanovitz T, Gerchman F, Balthazar A, Thomazelli F, Matos J, Canani L. Diabetic nephropathy. *Diabetol Metab Syndr.* 2009;1(1):10.

5. Ling B, Matsunaga H, Ma H, Eaton D. Role of growth factors in mesangial cell ion channel regulation. *Kidney Int.* 1995;48(4):1158-1166.

6. Pohlers D, Brenmoehl J, Löffler I, Müller C, Leipner C, Schultze-Mosgau S, Stallmach A, Kinne R, Wolf G. TGF-beta and fibrosis in different organs - molecular pathway imprints. *Biochim Biophys Acta.* 2009;1792(8):746-756.

7. Sawicki P, Didjurgeit U, Mühlhauser I, Bender R, Heinemann L, Berger M. Smoking is associated with progression of diabetic nephropathy. *Diabetes Care.* 1994;17(2):126-131.

8. Ejerblad E, Fored C, Lindblad P, Fryzek J, Dickman P, Elinder C, McLaughlin J, Nyrén O. Association between smoking and chronic renal failure in a nationwide population-based case-control study. *J Am Soc Nephrol.* 2004;15(8):2178-2185.

9. Stengel B, Couchoud C, Cénée S, Hémon D. Age, blood pressure and smoking effects on chronic renal failure in primary glomerular nephropathies. *Kidney Int.* 2000;57(6):2519-2526.

10. Chapman A, Johnson A, Gabow P, Schrier R. Overt proteinuria and microalbuminuria in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol.* 1994;5(6):1349-1354.

11. Ward M, Studenski S. Clinical prognostic factors in lupus nephritis. The importance of hypertension and smoking. *Arch Intern Med.* 1992;152(10):2082-2088.

12. Perry HJ, Miller J, Fornoff J, Baty J, Sambhi M, Rutan G, Moskowitz D, Carmody S. Early predictors of 15-year end-stage renal disease in hypertensive patients. *Hypertension.* 1995;25(4 Pt 1):587-594.

13. Nuyts G, Van Vlem E, Thys J, De Leersnijder D, D'Haese P, Elseviers M, De Broe M. New occupational risk factors for chronic renal failure. *Lancet.* 1995;346(8966):7-11.

14. Steenland N, Thun M, Ferguson C, Port F. Occupational and other exposures associated with male end-stage renal disease: a case/control study. *Am J Public Health.* 1990;80(2):153-157.

15. Hovind P, Rossing P, Tarnow L, Parving H. Smoking and progression of diabetic nephropathy in type 1 diabetes. *Diabetes Care.* 2003;26(3):911-916.

16. Carmona R. The Health Consequences of Involuntary Exposure to Tobacco Smoke: A Report of the Surgeon General. In: U.S. Department of Health and Human Services CfDCaP, Coordinating Center for Health Promotion, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, ed. Atlanta, GA: U.S. Department of Health and Human Services; 2006:iii.

17. Prevention CfDCa. Smoking-Attributable Mortality, Years of Potential Life Lost, and Productivity Losses—United States, 2000–2004. In: U.S. Department of Health and Human Services CfDCaP, Coordinating Center for Health Promotion, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, ed. Atlanta: Centers for Disease Control and Prevention; 2008.

18. Chen C, Chow D, Chiamvimonvat N, Glatter K, Li N, He Y, Pinkerton K, Bonham A. Short-term secondhand smoke exposure decreases heart rate variability and increases arrhythmia susceptibility in mice. *Am J Physiol Heart Circ Physiol.* 2008;295(2):H632-639.

19. Jaimes E, Tian R, Raij L. Nicotine: the link between cigarette smoking and the progression of renal injury? *Am J Physiol Heart Circ Physiol.* 2007;292(1):H76-82.

20. Elliot S, Karl M, Berho M, Xia X, Pereria-Simon S, Espinosa-Heidmann D, Striker G. Smoking induces glomerulosclerosis in aging estrogen-deficient mice through cross-talk between TGF-beta1 and IGF-I signaling pathways. *J Am Soc Nephrol.* 2006;17(12):3315-3324.

21. Sharma K, McCue P, Dunn S. Diabetic kidney disease in the db/db mouse. *Am J Physiol Renal Physiol.* 2003;284(6):F1138-1144.

22. Seccareccia F, Zuccaro P, Pacifici R, Meli P, Pannozzo F, Freeman K, Santaquilani A, Giampaoli S. Serum cotinine as a marker of environmental tobacco smoke exposure in epidemiological studies: the experience of the MATISS project. *Eur J Epidemiol.* 2003;18(6):487-492.

Evaluating Sex Ratios of Hawksbill Hatchlings on St. Croix, U.S. Virgin Islands

Courtney Conklin[1], T. Wibbels[1], J.E. Layton[1],
[1]Biology Department, University of Alabama at Birmingham

Abstract

Like all sea turtles, the hawksbill (Eretmochelys imbricata) has temperature-dependent sex determination (TSD) in which the temperature during the middle third of egg incubation period determines the sex of the hatchlings. TSD can produce a wide range of sex ratios, and those sex ratios can impact the ecology and conservation of sea turtles. Buck Island (U.S. Virgin Islands) is a natural nesting beach for hawksbill sea turtles and thus represents an ecological model for examining natural sex ratios produced from TSD. Further, Buck Island is a major nesting site for the hawksbill, so the sex ratio is of conservational importance since it affects the recovery of this endangered species. The purpose of this study was 1) to evaluate nest temperatures in order to estimate hatchling sex ratios and 2) to verify sex ratios via the histology of hatchings found dead in the hawksbill nests on Buck Island during the 2007 nesting season. The average temperatures of the middle third of incubation were used to predict the sex ratios produced by each nest. Analysis of the nest temperature data suggest an overall strong female-bias with 25 female-biased nests, one male-biased nest and one nest with a pivotal temperature yielding a 1:1 sex ratio. Histological analysis also suggested a female-bias with approximately 92% of hatchlings identified as female and approximately 8% identified as male.

Introduction

Many reptiles and all sea turtles display temperature-dependent sex determination (TSD) (reviewed by Wibbels, 2003). Sea turtles have a MF (male/female) pattern meaning warmer incubation temperatures produce females while cooler incubation temperatures produce males. (Mrosovsky, 1994). Previous studies suggest that TSD can produce a wide variety of sex ratios and those sex ratios could significantly impact the reproductive ecology and conservation of endangered sea turtle populations. Therefore it is important to evaluate and monitor hatchling sex ratios produced from nesting beaches (Mrosovsky, 1994; Wibbels, 2003). The current study investigates hatchling sex ratios of the hawksbill sea turtle. Hawksbills typically inhabit coral reef environments in tropical regions of the Atlantic and Pacific oceans (Meylan and Donnely, 1999). Hawksbill turtles are an endangered species in large part due to their carapace, which is a popular world trade item because of its thick and ornate shell scutes (Carr, 1952; Meylan and Donnely, 1999). A previous study of TSD in hawksbills in Antigua revealed that temperatures of approximately 28.5°C and below will produce 100% males and above approx. 30.3°C yields 100% females and the estimated pivotal temperature of the hawksbill is approx. 29.2°C (Mrosovsky et al., 1992).

The current study evaluates hatchling sex ratio being produced by hawksbill sea turtles on Buck Island, which is located several km off of St. Croix in the U.S. Virgin Islands. Buck Island is a major nesting beach for hawksbills in the Caribbean and consists of an undeveloped island with several natural nesting beaches. Thus, this study investigates naturally occurring sex ratios on an undeveloped and uninhabited island. Hawksbills are well known for nesting in various types of habitat. There are several distinct nesting beaches on Buck Island (Figure 1). The current study evaluates nest temperatures and hatchling sex ratios from each of the four beaches and in three different beach zones (open beach, seaward vegetation, and beach forest) within each beach.

Methods and Materials

This study was conducted during the 2007 hawksbill nesting season on Buck Island, U.S. Virgin Islands. As indicated above, Buck Island is a major nesting beach for hawksbill sea turtle in the Caribbean, and is an uninhabited and undeveloped island. Incubation temperature in hawksbill nests were recorded using HOBO data loggers from Onset Computer Corporation (Poccassett, MA). The data loggers have a precision of approximately +/- 0.3° Celsius and are calibrated in a laboratory incubator to ensure their accuracy. Data loggers were programmed using Hoboware Pro software to record temperature at 1 hour intervals for the entire duration of incubation. The data loggers were placed in the center of the egg mass in each nest by National Park Service (NPS) while the female turtle was nesting. Some nests were relocated by NPS to other areas on the nesting beach for this study. These nests were in danger of being flooded because of their original location on the nesting beach. Nest temperatures were examined from nests on four different beaches on Buck Island: South Shore (SS), Turtle Bay (TB), West Beach (WB), and North Shore (NS). Nests were monitored by NPS and once the hatchlings emerged, the data loggers were collected and sent back to UAB for data analysis. For these data, incubation durations were estimated and the middle third of incubation was identified because this is when temperature sensitive period (TSP) of TSD occurs. The maximum, minimum and average middle third temperatures were calculated. The average temperature during the middle third was used to determine the sex ratio of the hatchlings. The temperatures were used to predict sex ratios based on a previous study done on incubation

temperatures of the hawksbill in the Caribbean which showed that temperatures below approximately 28.5°C would produce all male, above approx. 30.3°C produced all females and a pivotal temperature of approx. 29.2°C (Mrosovsky et al., 1992). Accordingly, nests with average middle third temperatures of 28.5°C or higher were expected to yield 100% male hatchlings, 30.3°C or higher temperatures were expected to yield 100% female and nests with average middle third temperatures of 29.2°C were expected to yield a 1:1 sex ratio. Average middle third temperatures between pivotal temperature, 29.2°C, and 28.5°C were expected to generate male-biased nests, and average temperatures between pivotal and 30.3°C were expected to generate female-biased nests. Nests were located at different beaches on Buck Island and the type of beach zone was noted for each nest. Location was recorded to see if it had any effect on incubation temperatures.

In addition to temperature analysis, sex ratio information was obtained through the histological evaluation of hatchings that were found dead in the nest after all live hatchlings had emerged. In the field, dead hatchlings were collected, partially dissected, and then preserved in buffered formalin to protect the tissue and prevent decay. In the laboratory, the kidney/gonad complex was dissected from each hatchling and then processed with standard paraffin histological techniques (Humason, 1972). The structure of the gonad was examined under a compound microscope to evaluate the development of the cortical and medullary regions in order to verify if the tissue was an ovary or testis (Yntema and Mrosovsky, 1980; Wibbels, 2003).

Results

Temperatures were examined in a total of 27 nests. Average temperatures during the middle third of incubation, along with the predicted sex ratio for each nest are shown in Table 1 for all nests examined during the 2007 nesting season. Examples of nest temperature throughout the incubation period are shown for each of the four beaches, north shore, west beach, south shore, and turtle bay, in Figures 2-6 respectively.

A total of 399 hawksbill hatchlings were examined via histology in this study from the 2007 nesting season on Buck Island. Table 2 shows a summary of the results. Of the hatchlings examined approximately 49.6% were verified, but the tissues from approximately 50.4% of the hatchlings were too decomposed to determine their sex. Of the hatchlings whose sex was verified histologically, 92% were female and 8% were male (Table 2). The only beaches with nests that produced several male hatchlings were TT1 and TT4 (Table 1, 2 & Figure 2, 5). Table 1 indicates a 100% female bias for TT4 nests; however, histological analysis showed some males hatchlings were produced there despite the warmer incubation temperatures. The male hatchlings from nests on beach TT1/North Shore were also in beach forest zones indicating that these may be cooler locations than open beach and seaward vegetation zones (Table 1, Figure 2). Average incubation temperatures and the overall predicted sex ratio can be seen in Table 1.

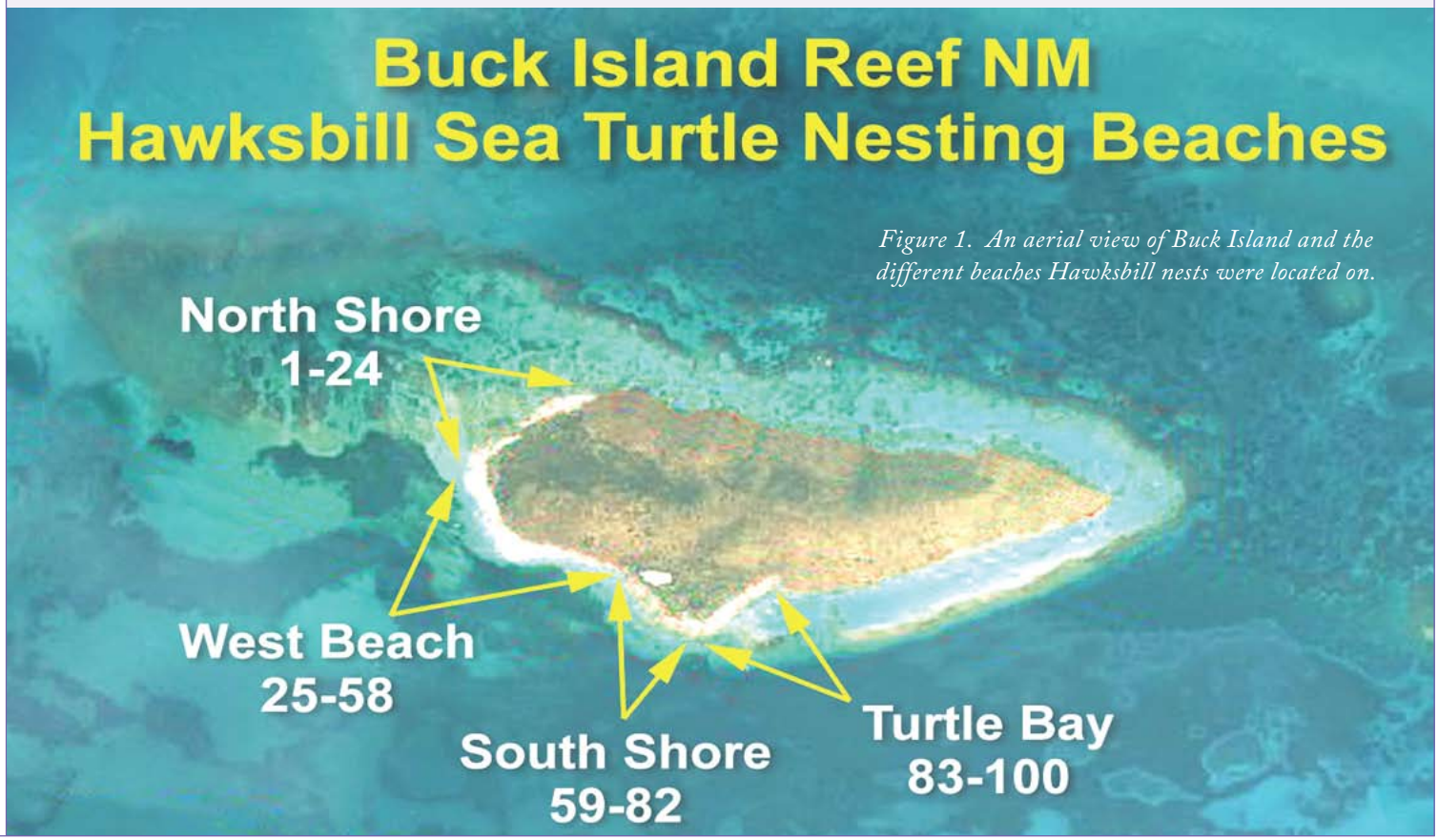


Figure 1. An aerial view of Buck Island and the different beaches Hawksbill nests were located on.

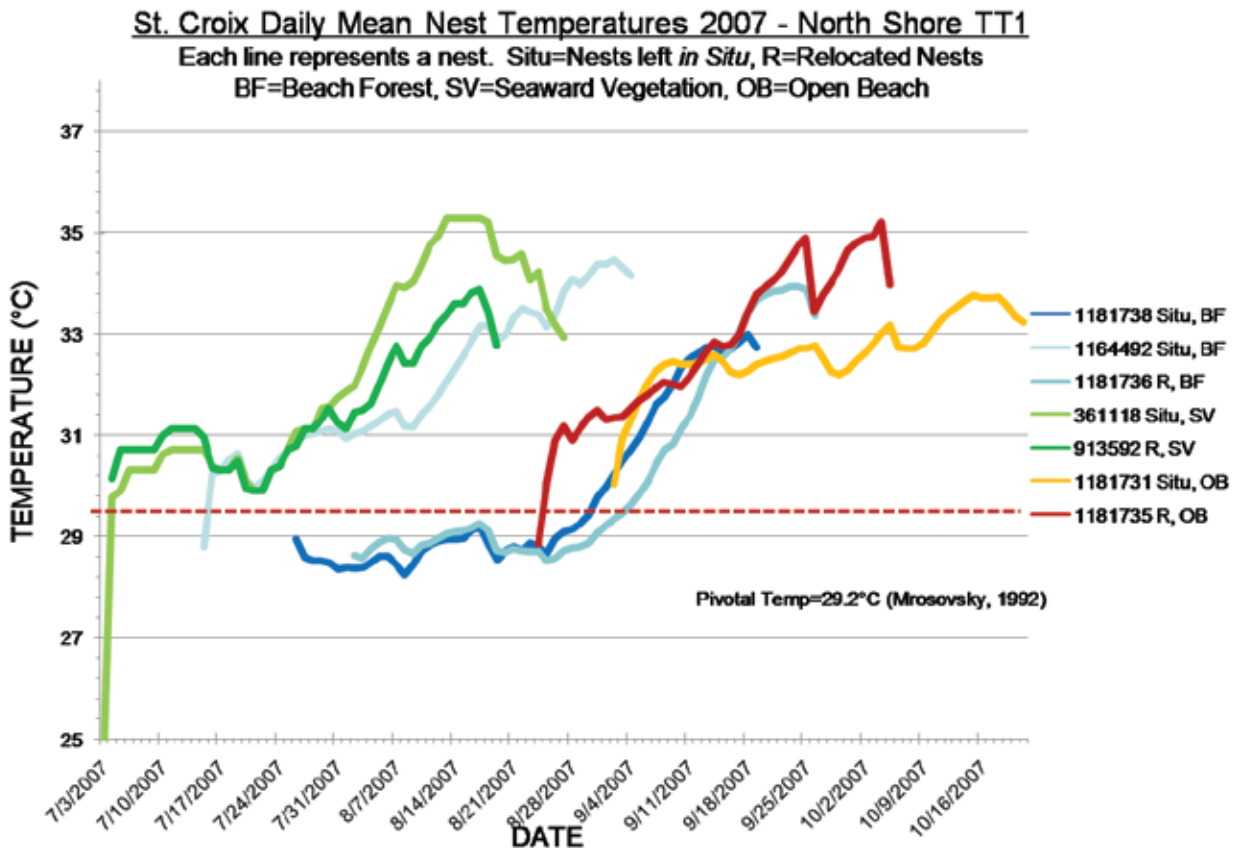


Figure 2. Nests located on the North Shore. This chart shows average incubation temperatures and predicted sex ratios. The pivotal temperature is marked by the red, dashed line.

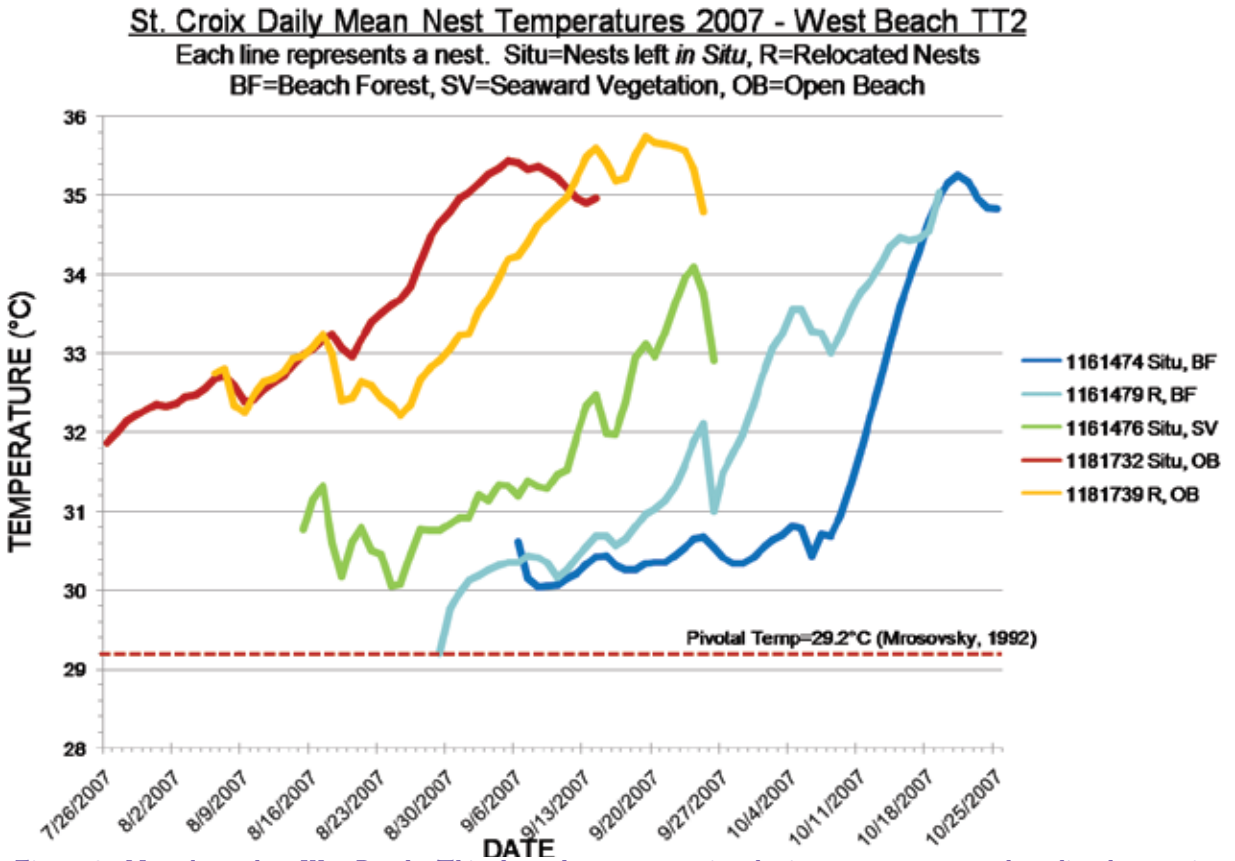


Figure 3. Nests located on West Beach. This chart shows average incubation temperatures and predicted sex ratios. Nests located in open beach areas had higher overall incubation temperatures than other nest areas.

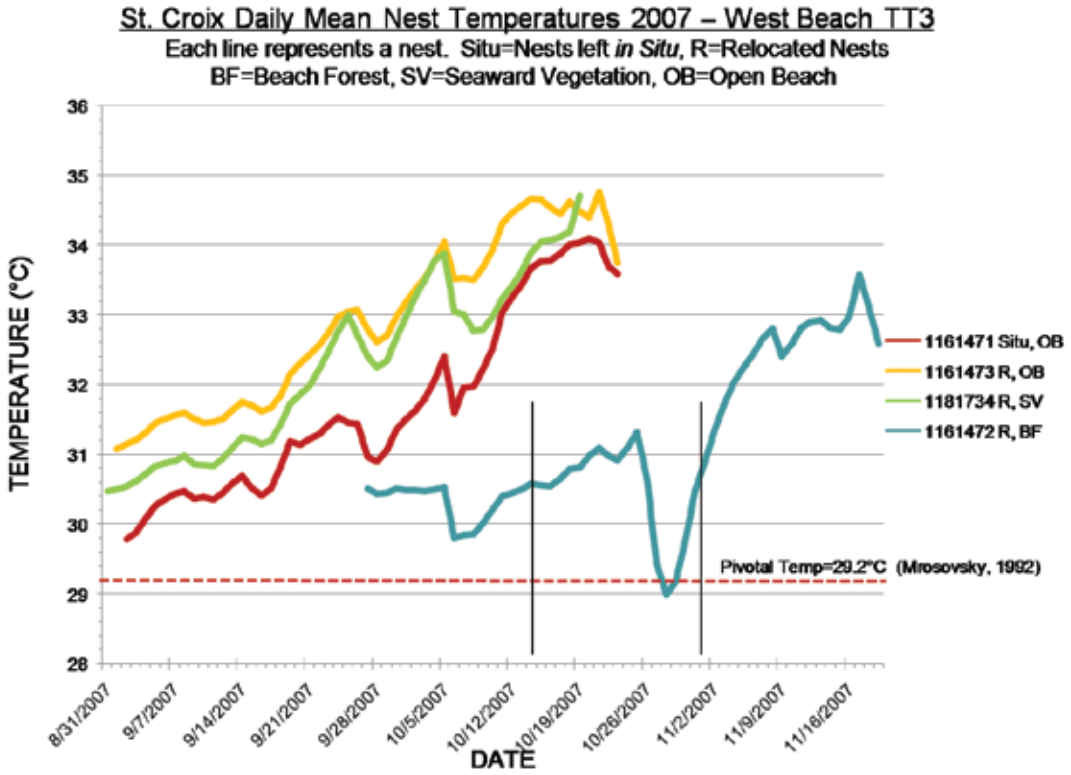


Figure 4. Nests located on West Beach. This chart shows average incubation temperatures and predicted sex ratios. The drop in temperatures between October 26-30 for 1161472 is most likely due to the early stages of hurricane Noel and significant rainfall. This is an example of weather affecting middle third incubation temperatures.

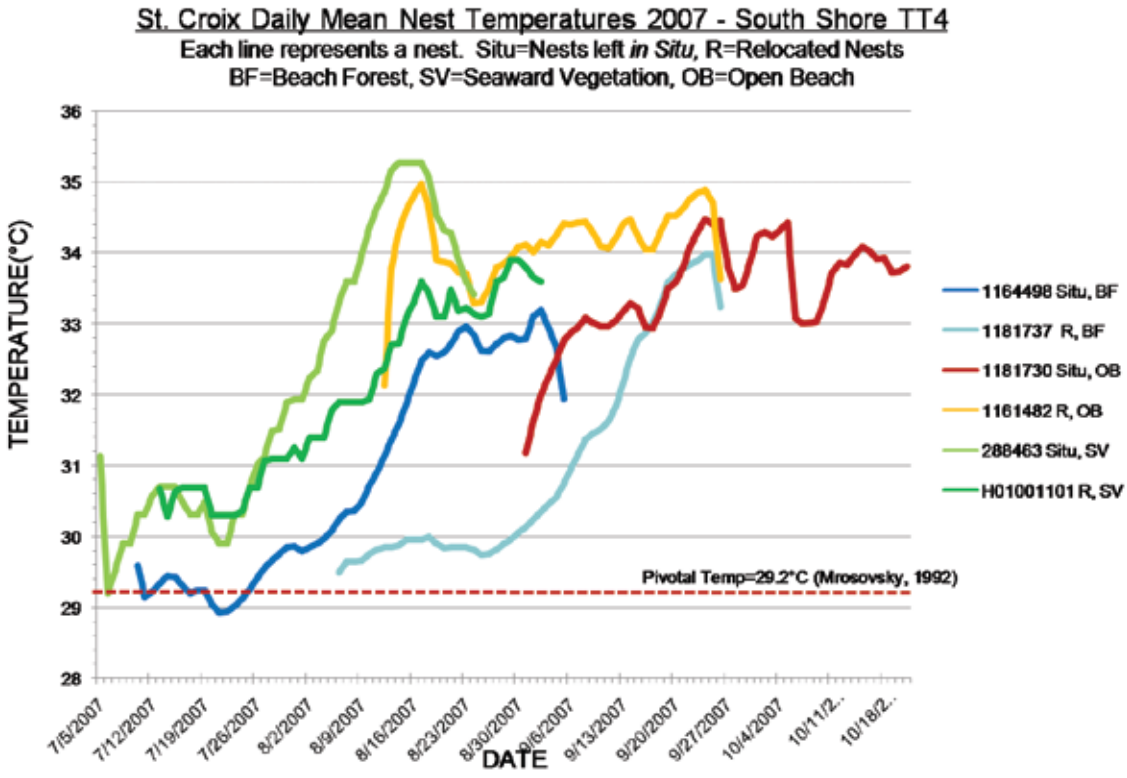


Figure 5. Nests located on South Shore. This chart shows average incubation temperatures and predicted sex ratios. Nests located in open beach areas had higher overall incubation temperatures than other nest areas.

TT#	Incubation Beach	Original Beach	Relocated Beach Zone	SN#	Incubation Duration	TSP Mid 1/3 Dates	Mean Temp	Predicted Sex Ratios
TT1	NS	NS	*in situ*, BF	1181738	56	8/14-9/1	**29.04**	Male Bias (very close to piv temp)
TT1	NS	NS	*in situ*, BF	1164492	52	8/1-8/18	**31.76**	100% Female
TT1	NS	NS	*in situ*, BF	1164499	Est. 51	7/31-8/16	**32.17**	100% Female
TT1	NS	WB	Reloc., BF	1181736/ 1161736	56	8/21-9/8	**29.20**	Unbiased (piv temp)
TT1	NS	NS	*in situ*, SV	361118	56	7/22-8/9	**31.94**	100% Female
TT1	NS	NS	Reloc., SV	913592	47	7/20-8/4	**30.87**	100% Female
TT1	NS	NS	*in situ*, OB	1181731	50	9/19-10/5	**32.58**	100% Female
TT1	NS	NS	Reloc., OB	1181735	43	9/7-9/21	**32.77**	100% Female
TT2	WB	WB	*in situ*, BF	1161474	50	9/23-10/9	**30.60**	100% Female
TT2	WB	NS	Reloc., BF	1161479	52	9/15-10/2	**31.50**	100% Female
TT2	WB	WB	*in situ*, SV	1161476	43	8/29-9/12	**31.23**	100% Female
TT2	WB	WB	*in situ*, OB	1181732	Est. 51	8/12-8/28	**33.32**	100% Female
TT2	WB	NS	Reloc., OB	1181739	51	8/23-9/8	**33.29**	100% Female
TT3	WB	WB	*in situ*, OB	1161471	52	9/19-10/6	**31.45**	100% Female
TT3	WB	NS	Reloc., OB	1161473	53	9/19-10/6	**32.99**	100% Female
TT3	WB	NS	Reloc., SV	1181734	50	9/17-10/3	**32.41**	100% Female
TT3	WB	TB	Reloc., BF	1161472	54	10/15-11/1	**30.51**	100% Female
TT4	SS	SS	*in situ*, BF	1164498	58	7/29-8/17	**30.64**	100% Female
TT4	SS	TB	Reloc., BF	1161737/ 1181737	52	8/23-9/9	**30.35**	100% Female
TT4	SS	SS	*in situ*, OB	1181730	Est. 52	9/17-10/4	**33.92**	100% Female
TT4	SS	TB	Reloc., OB	1161482	46	8/27-9/11	**34.15**	100% Female
TT4	SS	SS	*in situ*, SV	288463	51	7/22-8/7	**31.72**	100% Female
TT4	SS	NS	Reloc., SV	H100101	Est. 52	7/30-8/16	**31.97**	100% Female
TT5	TB	TB	Reloc., BF	1164500	54	8/20-9/6	**30.37**	100% Female
TT5	TB	TB	*in situ*, SV	1181729	55	8/19-9/6	**32.02**	100% Female
TT5	TB	TB	Reloc., SV	889243	49	8/9-8/25	**30.44**	100% Female
TT5	TB	TB	Reloc., OB	1164496	Est. 53	8/3-8/20	**33.74**	100% Female
						Overall Avg.	**31.74**	

Table 1. The highlighted data shows average mean temperatures during the middle third of incubation produced by the nests and the predicted sex ratio outcomes. The table indicates the beach zone and island location that the original nests (in situ) were located and where relocated nests were. TT1 represents North Shore, TT2 and TT3=West Beach, TT4=South Shore, and TT5=Turtle Bay.

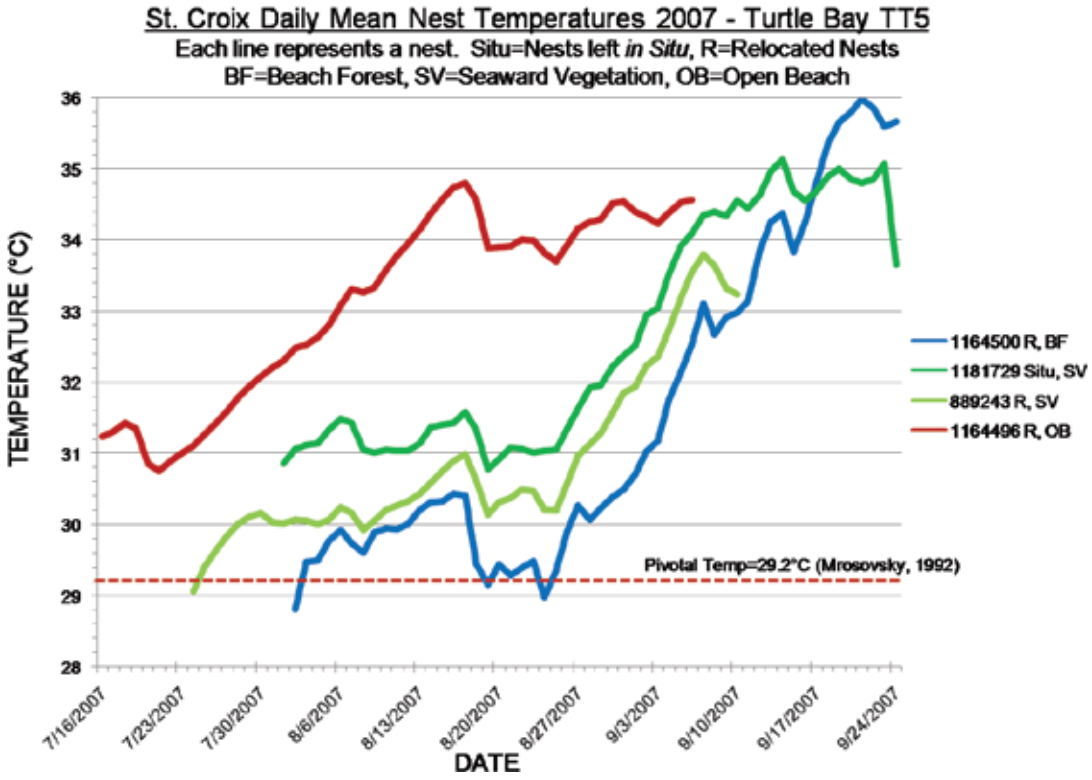


Figure 6. Nests located on Turtle Bay. This chart shows average incubation temperatures and predicted sex ratios. Nests located in open beach areas had higher overall incubation temperatures than the other nest areas.

TT#	Total Hatchlings	Female	Male	Undetermined
TT1	92	49	5	38
TT2	109	41	1	67
TT3	52	25	1	26
TT4	78	28	8	42
TT5	68	40	0	28
Total	**399**	**183**	**15**	**201**

Table 2. This table shows the total number of hatchlings for each beach and the number of female, male or undetermined hatchlings. Undetermined indicates tissues that were too decomposed or degraded to identify. A strong female biased ratio can be seen.

Discussion

Most nests located on the North Shore/TT1 position were predicted to produce 100% female, although one nest was predicted to produce a male-biased and one nest was predicted to produce a 1:1 sex ratio. All of those nests were located in beach forest zone and three were in situ nests while the nest with a pivotal temperature had been relocated to BF from West Beach (Figure 2). All of the nests from the TT2, TT3, TT4 and TT5 locations were predicted to produce 100% females. Collectively, the data indicate that incubation temperatures are relatively warm and well above pivotal temperature in the majority of the nests examined. This clearly suggests an overall female bias (Table 1). Further, the location of the nests on the beach may affect incubation temperatures and thus sex ratios. For example, nests located in the more wooded, less open area on the North Shore may be cooler due to shading. The data indicate warmer overall temperatures for open beach areas, followed by slightly less warm seaward vegetation temperatures and beach forest areas having coolest overall temperatures for TT3, TT4, and TT5 beaches (Figures 4-6). No apparent trend could be seen for relocated nests. The results also provide examples of how weather events may play key roles in controlling nest incubation temperatures. For example, Hurricane Noel passed nearby Buck Island when it was a tropical depression in late October. Although the weather did not change the outcome of the sex ratio of hatchlings, incubation temperatures were cooler during that time period (Table 2, Figure 4). As seen in data logger 1161472 (Figure 4), a storm caused a decrease in nest temperature of approx. 3°C during the latter part of TSP, however this did not decrease below the estimated pivotal temperature of 29.2°C therefore that nest still had a female biased outcome.

In summary, these results reveal a female biased hatchling sex ratio on Buck Island for the 2007 nesting season. This is consistent with a previous study done on the island (Wibbels et al., 1999). The results also suggest that factors such as weather, location of a specific nesting beach, and location of a nest on a nesting beach may influence incubation temperatures (Table 1). The findings of this study are being forwarded to the U.S. National Park Service in St. Croix and will be used in the development of the management strategy for this endangered sea turtle.

References

Carr, A. 1952. Handbook of Turtle: Turtle of the United States and Canada. Cornel University Press.

Godfrey M.H., D'Amato, A.F., Marocvaldi, M.A., Mrosovsky, N., 1999. Pivotal temperature and predicted sex ratios for hawksbill turtles from Brazil. Canadian Journal of Zoology 77, 1465- 1473.

Humason, GL. 1972. Animal Tissue Techniques. W.H. Freeman and Co., San Francisco, Ca.

Meylan, A., Donnely, M. 1999. Status justification of listing the hawksbill turtle (*Eretmochelys imbricate*) as critically endangered on the 1996 IUCN Red List. Chelonian Conservation and Biology 3:200-224.

Mrosovsky, N., Bass, A., Corliss, L.A., Richardson, J.I., and Richardson, T.H. 1992. Pivotal and beach temperatures for hawksbill turtles nesting in Antigua. Can. J. Zool. 70: 1920-1925.

Mrosovsky, N. 1994. Sex ratios of sea turtles. J. Exp. Zool. 270, 16-27.

National Park Service Photo.

Wibbels, T., Z. Hillis-Starr, B. Phillips. 1999. Female-biased Sex Ratios of Hatchling Hawksbill Sea Turtles from a Caribbean Nesting Beach. In: Journal of Herpetology, Volume 33, No. 1. Society for the Study of Amphibians and Reptiles. Pp 142-144.

Wibbels, T. 2003. Critical Approaches to Sex Determination in Sea Turtles. In: The Biology of Sea Turtles, Volume II. P. Lutz, J. Musick, J. Wyneken (eds), CRC Press. Pp 103-134.

Yntema, C.L., Mrosovsky, N. 1980. Sexual differentiation in hatchling loggerheads (*Caretta caretta*) as a function of temperature. Herpetologica, 36, 33-36.

Spatial Analysis of Environmental Factors Related to Lyme Disease in Alabama Using NASA Earth Observation Systems

NASA Marshall Space Flight Center - DEVELOP National Program at Laboratory for Global Health Observation
The University of Alabama at Birmingham

Nathan Renneboog, Team Leader; Emily G. Capilouto, Stephen L. Firsing III, M.P.A., M.A., Kyle Levy, M.S., Marilyn McAllister, M.S., USA Lt. Col. Retired; Kathryn Roa, M.D., Shveta Setia, B.D.S., Lili Xie, M.D., Jeff Luvall, Ph.D., NASA Science Advisor; Donna Burnett, Ph.D., UAB Research Advisor

Abstract

Lyme disease accounts for more than 95% of vector-borne diseases in the US, but controversy surrounds its presence in Alabama. Barriers to a consensus on its geographical distribution include underreporting, diagnostic difficulties, and human travel. The objective of our project is to present the probability of Borrelia transmission in various areas of Birmingham, Alabama by demonstrating that the presence of the chain of infection (infectious agent: Borrelia burgdorferi; reservoir: white-footed mouse; vector: Ixodes spp. of ticks; and susceptible host: white-tailed deer, dogs or humans) translates to infection risk. NASA Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) and Quickbird satellite imagery was used to make Land use Land cover (LULC), Normalized Difference Vegetation Index (NDVI) and Soil Moisture classifications to identify the likely tick habitats. The maps highlight high risk zones for contracting the disease. Other uses of the maps include identifying factors that increase the probability of presence of Lyme disease in the Birmingham area as well as showing locations of areas that may not be high risk areas but have vectors present. Knowledge and recognition of these locations are vital to the prevention, early recognition and, if contracted, treatment of Lyme Disease. The final output of this project will be the engagement of stakeholders in an attempt to increase community awareness of the disease and recommend several primary and secondary prevention strategies.

Introduction

Lyme disease (LD) accounts for more than 95% of vector-borne diseases in US.[1,2,3,4,5] It is caused by a tick bite and the causative organism is *Borrelia burgdorferi*. [6] Due to the complex pathogenesis of Lyme disease, it is often difficult to recognize, diagnose, and treat. Currently, controversy exists surrounding the possibility that Lyme disease could be contracted in the state of Alabama.[7] Early localized infection is identified by the appearance of a characteristic red rash called erythema migrans, which presents itself in 80% of patients on the site of the tick bite; fever, fatigue, and headache also indicate early disseminated infection. [8] Infection may also be characterized by meningeal irritation or cardiac involvement. If left untreated, approximately 60% of patients develop late, persistent infection which manifests as frank arthritis. [9] Early treatment can lead to quick and complete recovery, thus avoiding any long-term problems. [10] Standard treatment for LD involves the administration of antibiotic regiments and pain relievers in order to treat the infection and provide symptomatic relief. [11] A subgroup of treated LD patients continues to have subjective symptoms such as musculoskeletal pain, neurocognitive difficulties or fatigue. Chronic LD or post-LD syndrome presents complications similar to those of chronic fatigue syndrome or fibromyalgia. [12] Delayed treatment of LD may result in a longer convalescent period.

The chain of infection is a model used to understand the infection process, that is, how the pathogen is transmitted by a vector to a host, causing a disease. In examining the Lyme disease chain of infection, the presence of the pathogen (*B. burgdorferi*), vectors (ticks: *Ixodes scapularis*), and hosts (i.e. deer, mice, mammals, birds and humans) must be identified. For Lyme disease to occur, these components must be in close proximity, while each of these components are influenced by other biotic and abiotic factors. For example, *B. burgdorferi* is dependent on the biotic environment of its vector and hosts. The vectors are highly influenced by local temperature and humidity. The reservoir hosts may be attracted by an environment that provides food and shelter.

Characterization of tick-borne disease foci may be done in three levels. The microscale level considers factors that determine individual tick survival, such as litter layer, where the larvae undergo diapause. The mesoscale level includes factors that determine the interaction between the parasites, hosts, and vectors. This includes wooded areas where humans are at risk of infection. The macroscale level examines factors such as climate, vegetation, and land cover. [13]

Borrelia burgdorferi is a bacterium of the spirochete class and is the causative agent of Lyme disease. It is found predominantly in North America but is also present in Europe. It was named after Willy Burgdorfer who first isolated the bacterium in 1982. *B. burgdorferi* is present in Alabama as proven by the presence of serum antibodies to the spirochete in deer and rodents. [14] This spirochete has not been identified to proliferate in an environment outside of a vertebrate or invertebrate host. It is maintained in a natural cycle of infection by ticks. The ticks acquire and transmit

the bacteria during blood meals from a wide range of vertebrate animals including small mammals, lizards, and birds. The causative agent resides in the ticks and reservoir animals (enzootic transmission cycle); transovarial transmission is rarely observed. [15]

Ticks (Acari: Ixodida) are blood-feeding ectoparasites second only to mosquitoes in importance as vectors of human diseases. Their importance as vectors of animal diseases is also widely recognized. Worldwide, ticks parasite wild and domestic vertebrates except fishes. [16] *Ixodes scapularis* is recognized as the principal vector of the spirochetes to humans in the northeastern region of the United States of America. [17] It has also been identified in *Amblyomma americanum* in Alabama. [18] Larval ticks typically hatch uninfected by *B. burgdorferi* but may acquire an infection when feeding from a reservoir host.[19] They are non-selective in their choice of hosts and get their blood meal from different species of mammals, birds and lizards. Rodents such as eastern chipmunks (*Tamias striatus*) and the white-footed mice are common hosts.[20] Nymphs get their blood meal from white-footed mice (*Peromyscus leucopus*), other rodents and small mammals. Some types of birds have also been identified as intermediate hosts.[21] Serologic surveys of antibodies to *B. burgdorferi* done in several states, including Alabama, evidenced bacterium prevalence to be 35.7% and 27.3% in 56 *P. leucopus* and *535 P. gossypinus* (cotton mice), respectively. [22] The white-tailed deer (*Odocoileus virginianus*) is the principal blood meal source for the adult tick.[23,24] Deer perpetuate high tick populations yet do not support tick–pathogen transmission. A study conducted in New Jersey observed that a decline in deer population led to an increase in tick density. The ticks began feeding on rodents when deer were missing, thus leading to a potential tick borne disease hot spot. [25]

Ticks are vulnerable to dessication, requiring temperatures between -10 to 35°C and a constant humidity no lower than 80%.[26,27,28] Climatic factors that reduce relative humidity (i.e. low precipitation) may decrease tick abundance. [29] Areas with dense vegetation cover and/or decaying vegetation help maintain relative humidity above 80% at the base of vegetation, which supports tick populations.[30,31,32,33,34] Habitats with moist soils are generally preferred by ticks.[35,36,37,38] B. burgdorferi is transmitted by ticks which feed on infected wildlife reservoir hosts, particularly rodents and birds. Both tick species are indiscriminate in their choice of host and will feed on humans; therefore ticks can transmit pathogens from wildlife to humans. [39]

Ticks are mostly found in areas which support these microclimate and host requirements, including deciduous woodlands, particularly those with oak and beech trees, which harbor significant numbers of large animals such as deer. However, ticks may also survive in coniferous forests as long as there is sufficient ground litter and moist climate. [40] Ecological factors such as vegetation, soil, topography and climate directly impact ticks' survival. One study found that deciduous, dry/mesic and dry forests, fertile soils such as alfisols, and sandy and loamy/sand soil texture were positively associated with tick presence. [41] Studies also prove that soil type and texture influences type of overlying vegetation and the extent of drainage, respectively. Soil texture influences soil moisture regardless of precipitation. Dry and/or mesic forests have vegetation such as oaks and jack pines that prefer well-drained, sandy soils. These trees also provide a canopy layer for the underlying vegetation. [42]

Typical hosts include White-footed mice and white-tailed deer. White-footed mice are terrestrial and live in temperate regions. They live in a wide variety of habitats but are said to be most abundant in warm, dry forests and brush lands at middle elevations. Mice found in these habitats are omnivorous. Diet can vary seasonally and geographically and may include seeds, berries, nuts, insects, grains, etc. A study on the effects of season and habitat on mice infestation found that relative density of *I. scapularis* larvae and prevalence of nymphs on mice did not differ significantly among varying forest types (bottomland, field-forest, and upland).[43] However, the total number of mice and ticks found was higher in the bottomland forest. Further, larvae density was higher in mid-late summer and prevalence of nymphal infestation was highest in early summer. [44] White-tailed deer live in a variety of terrestrial habitats but generally prefer open woodland areas. They often also reside on the fringes of urban areas and farming country. They often enter human inhabited areas and feast on flowers and grass. White-tailed deer feed on a variety of vegetation such as twigs, leaves, bark, shrubs, fruits, nuts, and lichens. They also feed on conifers in winter when other foods are scarce. [45]

Transmission of *B. burgdorferi* to humans or domestic animals (then to humans) can occur if there is sufficient opportunity for exposure. Exposure depends on demographic patterns, socioeconomic status, and recreational and occupational activities. [46] Outdoor work has been found to be positively associated with LD risk. [47] Outdoor recreation such as camping, fishing, golfing, camping and hunting also was found to increase risk. Additionally, residents living in forested areas were more likely than those living in non-forested areas to have LD in Baltimore County, Maryland. [48]

Lyme Disease in Alabama

The first LD case in Alabama was reported in 1986 in a Lee County resident, who was bitten by a tick during a camping trip in Cleburne County. [49] The first case of Lyme disease reported in the state of Alabama was by Dr. Mullen at Auburn University in 1986. A 42-year-old woman developed the characteristic skin lesion, erythema chronic migrans, after a tick bite in Choccolocca Wildlife Management Area, Cleburne County. The case was confirmed through serological testing by CDC, Atlanta, GA.[50] In 2007, the Centers of Disease Control and Prevention (CDC) reported a total of 238 LD cases in the state of Alabama from the years 1986-2007 (see figure 1). [51] In 1988, the Alabama Department of Public Health (ADPH) requested physicians and laboratories to begin reporting LD cases to calculate the incidence and

prevalence rates. [52] Despite underreporting, diagnostic difficulties, and other social complication factors, LD cases are still prevalent in Alabama. Case patients are defined as Alabama residents reported to the ADPH and subsequently confirmed according to CDC case definition. [53]

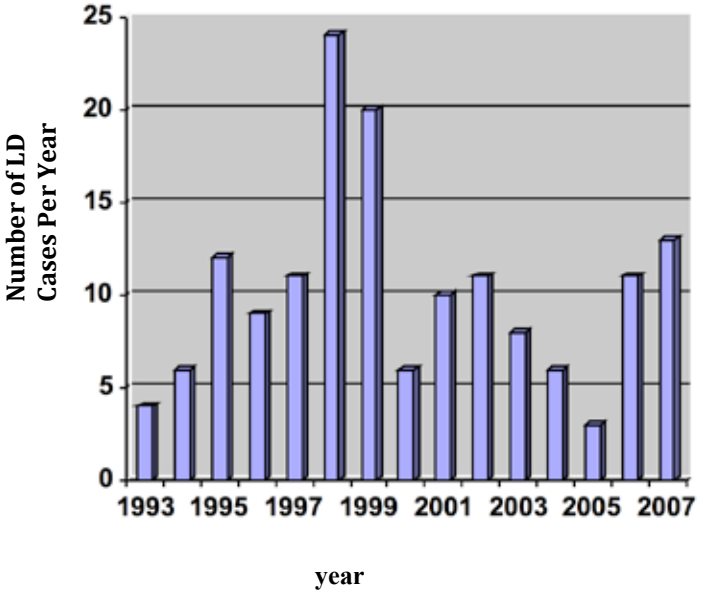


Figure 1: Number of LD cases in Alabama from 1993-2007 reported from CDC. [54]

A subsequent study was conducted in the hunting season (winter) of 1988-89 and 1989-90 to collect ticks from white-tailed deer in Alabama (see table 1 below). [55] Four species of ticks were collected from 537 white tailed deer. The most common tick was *Ixodes scalpularis,* which infested 54% of deer and accounted for 57% of all ticks collected. With the exception of 2 nymphal ticks, all ticks were adults. Over 80% of the deer examined at the Barbour County wildlife Management Area were infested with this species; whereas, none of the deer examined at the Choccolocco Wildlife management Area in Cleburne County were infested. This variation in prevalence of different areas may be due to habitat types and management practices, which influence the availability of rodents and other small mammals serving as hosts for the immature tick stages. The second most common tick, *Dermacentor albipictus* (1,253 specimens) infested 15% of the deer. *Amblyomrna americanum* (315 specimens) infested 24% of the deer and was the only species collected from deer at all sampling locations. *Amblyomma maculatum* was an infrequent parasite (five specimens) and infested only 1% of the deer; this tick species was only recorded during the 1989-90 season. [56]

Number of White-tailed deer surveyed	Percentage of deer infested with *Ixodes Scapoularis*	Number of Adult *Ixodes Scapoularis* ticks	Number of Nymph *Ixodes Scapoularis* ticks	Number of *B.Burgdoferi* infected ticks	Percentage of infected ticks
243	54%	1023	1	12	1.2%

Table 1: B. Burgdoferi *infection in* Ixodes Scapoularis *from White-tailed deer in Alabama.* [57]

Hosts	Ticks
White-tailed deer	81.8% adult
Small mammals	97.4% numphs

Table 2: Ault and nymph Ixodes Scapoularis prevalence for hosts in Alabama.[58]

A further study was conducted at the site of known transmission of *B. burgdorferi* in east central AL (8 sites in Lee County) chosen based on proximity to confirmed human cases of Lyme disease. [59] Sites were characterized by abundant leaf litter and late successional vegetation dominated by oaks, beech, sweetgum and hickory. The study's objectives were to determine species of ticks present at these sites, their host associations and species of ticks and small mammals naturally infected with *B. burgdorferi*. A total of 859 ticks were recovered from hosts and by using the dragging method. Hosts included but were not limited to small mammals, reptiles and white-tailed deer. Cotton-mice were the most abundant small mammal hosts and accounted for 85.4% of all species trapped. Of ticks recovered from small mammals, 97.4% were in their larval stages. 81.8% of the ticks recovered from white-tailed deer were adults (see table 2 above). *Amblyomma americanum* (33.4%) and *I. scapularis* (20%) accounted for 53.4% of ticks collected. Nearly half (49.1%) were examined for *B. burgdorferi* using direct and indirect fluorescent antibody assays. Spirochetes were detected in four nymphal and two adult *A. americanum* ticks recovered from white-tailed deer (4% of all *A. americanum* tested); three larval and two adult *I. scapularis* were recovered from the cotton-mice and white-tailed deer respectively. (3% of *I. scapularis* tested). Thus, it has been proposed that cotton mice and white tailed deer were common hosts for larval and adult *I. Scapularis* respectively in Alabama. [60]

It is not clear if *A. americanum* is a potential vector for *B. burgdorferi*, although a patient bit by *A. americanum* tick develops the characteristic red rash suggesting Lyme disease. The causative agent of Lyme disease has not been cultured from skin biopsy specimens from those patients and diagnostic serum antibodies usually present upon infection have not been found. [61] A DNA amplification technique produced *B. lonestari* sequences from both the skin of the patient and the *A. americanum* tick attached to it. Thus, *B. lonestari* is a probable cause of erythema migrans in humans. This condition is usually referred to as Southern Tick Associated Rash Illness (STARI) and seen in the South-eastern U.S.[62]

The presence of Lyme disease in the state of Alabama is not universally accepted. The purpose of this study is to identify the presence of the

organisms involved in the chain of infection for LD in Alabama and, by establishing this chain of infection, demonstrate the possibility of contracting LD in the state of Alabama. In Alabama the primary vector of LD is *Ixodes scapularis* (black legged tick). This tick has a life span of two years and has three feeding stages: larvae, nymph and adult.[63,64] The tick acquires the bacteria during the larval stage when it gets its first blood meal. The bacteria reside in the gut of the tick and the bacteria are transmitted to the host during nymphal feeding.[65,66] Nymphs usually parasitize small mammals including cotton mice. The proportion of nymphs infected with *B. burgdorferi* is substantially higher than adults of the same cohort. Because of their smaller size, nymphs are less likely to be detected on hosts, including humans, increasing the probability of transmission of *B. burgdorferi* resulting in LD. Most human cases of LD are seen during the spring and summer months when both tick activity and human outdoor activity is greatest. [67] The preferred host for adult ticks is the white-tailed deer. Deer play a major role in the transportation of ticks and tick population sustainability, although adult ticks do not infect the deer with *B. burgdorferi*. [68]

The goals of this project are to answer the following questions: 1) Can the presence of the chain of infection for LD be established in the state of Alabama? 2) Using NASA Earth Observing System data, can environmental factors necessary to support tick population be identified in Central Alabama? Based on the results of the study, the information will be used to raise public awareness of the areas where vectors of the disease can be found. NASA ASTER and Quickbird satellite imagery was analyzed to identify Land use/Land cover (LULC) and Normalized Difference Vegetation Index (NDVI) unsupervised classifications in order to distinguish likely tick habitats. Awareness of LD presence in Alabama aims to improve prevention, early detection and early treatment of the disease.

Methods

A Land use/Land cover (LULC) classification provides an enhanced awareness of the environment's affect on disease vectors. This study generated unsupervised classifications in ER Mapper 7.1™, using three August ASTER images and one March Quickbird image, all of which were classified separately. All images were analyzed separately to avoid misclassification since vegetation can differ between varying days and years. Unsupervised classification was used to cluster pixels in a dataset based on spectral reflectance without any user-defined training classes using the minimum spectral distance formula.[41] Analyses were conducted in ER Mapper 7.1™ using an ISODATA clustering algorithm (ISOCLASS) on ASTER data from August 4, 2004 and August 21, 2001 and Quickbird imagery from March 30, 2004. Classification of the ASTER imagery used bands 1, 2 and 3, corresponding to the visible and near-infrared parts of the electromagnetic spectrum. The Quickbird classification was run using the 2nd, 3rd, and 4th bands, which also correspond to the visible and near-infrared parts of the electromagnetic spectrum. Once classes were labeled and identified, they were combined and recoded with land cover being placed into seven categories: 1) dense vegetation/shade/water, 2) vegetation, 3) low vegetation/grass, 4) grass/golf courses/artificial grass, 5) urban vegetation, 6) road/highways and 7) urban. By narrowing the unsupervised classifications into distinct categories, the major land cover types indicating likely tick habitats could be monitored on a large scale.

Vegetation indices measure and provide an indication of the condition of vegetation and soil, including their chlorophyll and moisture content. [69] A vegetation index was applied to the satellite imagery using the Normalized Difference Vegetation Index (NDVI) formula (see Appendix, Fig. 1). The NDVI measures the condition of vegetation within a given image by applying an algorithm to each pixel, assigning a value between -1 and 1 to each pixel using a ratio of the visible red and near-infrared bands of the electromagnetic spectrum. The pixels with values around 0.5 to 0.8 indicate dense vegetation, while water and clouds have negative NDVI values. For the three ASTER images the 1st, 2nd, and 3rd bands were used to make the NDVI with the 3rd and 2nd bands representing the visible red and near-infrared parts of the electromagnetic spectrum, respectively. For the Quickbird the 2nd, 3rd and 4th bands were used since the 3rd and 4th correspond to the visible and near–infrared bands, respectively. [70]

The vegetation index was applied and saved as a raster dataset so the NDVI could then be classified using the ISODATA clustering algorithm. Classes representing the different levels of vegetation: 1) dense vegetation/shade, 2) moderately dense vegetation, 3) vegetation, 4) low vegetation, 5) grass, 6) graveyards, 7) asphalt, 8) road/urban, 9) clay/gravel, 10) swamp and 11) urban were assigned by comparing the NDVI classification with a corresponding Google Earth Pro™ image.

Soil moisture analyses provide a representation of soil moisture levels in the first few inches of the ground. Soil moisture is read in multiple parts of the electromagnetic spectrum but is most strongly reflected in the mid- and thermal infrared bands of the electromagnetic spectrum. Soil moisture was assessed in the three ASTER images using a ratio algorithm of the 14th thermal infrared and 10th mid-infrared bands.[71,72] After applying the algorithm the images were all classified using the ISODATA clustering algorithm from ER Mapper 7.1.™ Google Earth Pro™ was then used to identify each class of soil moisture.

Three ASTER images were combined in a mosaic using the "Image display and mosaic wizard" in ER Mapper 7.1.™ A mosaic was made for the LULC, NDVI and soil moisture classification after they were analyzed. The four DigitalGlobe Quickbird images were combined using the same ER Mapper 7.1™ mosaic wizard. The mosaic was then analyzed with LULC and NDVI algorithms.

Results

A literature review showed the relationship between dense vegetation, high soil moisture and Black legged tick populations. ASTER imagery was used to make NDVI, LULC and soil moisture classifications. Vegetation maps were produced by applying the NDVI algorithm to the imagery and then classifying the image to get a representation of the different levels of vegetation (see appendix, Figure 2). Google Earth Pro™ was successful at identifying these categories. A similar methodology, using a ratio algorithm followed by a classification, produced a soil moisture map of the Central Alabama area (see appendix, figure 4). This map was also compared to high resolution Google Earth Pro™ imagery to identify each soil moisture level. The spectral signature (represented by a number) was also used to identify each level of soil moisture. The same methodology produced an ASTER Land use Land cover classification of Central Alabama (see appendix, figure 3). Quickbird imagery produced a more precise Land cover and vegetation representation of the downtown Birmingham area and Central Jefferson County (see appendix, figures 5 and 6 respectively).

Discussion

It was determined that the chain of infection for LD is present in the state of Alabama. The pathogen, vector and host form the chain of infection. For LD, the ticks are the vectors, *Borrelia burgdorferi* is the pathogen and hosts range from small mammals, reptiles to large mammals. *Ixodes scapularis* is the proposed vector for LD. It has been found infected with *Borrelia burgdorferi*, the causative organism. The preferred hosts for larval and nymphal stage are small mammals, especially cotton mice in Alabama. White tailed deer are the preferred host for adult ticks. [73] The tick activity is greatest during later spring and summer. [74] This seasonality is explained by a corresponding peak in seasonal nymphal *Ixodes scapularis* activity. [75] This is also the time when human activity is greatest. Exposure in the periresidential setting proximal to a nature preserve and the presence of deer as well as outdoor workers are at increased risk. [76,77] CDC has classified AL as a low risk area. However, in studies done in Maryland and Connecticut where the disease is endemic, it was found that despite the high number of cases reported, underreporting is still an issue. [78,79] Therefore it has been suggested that states with low incidence may have the same underreporting problem. [80]

LD is a systemic infection. It infects all systems of the body. If not treated in early stages, it can be disabling. The non-specific symptoms and low level of suspicion on the part of clinicians, can lead to delayed diagnosis or misdiagnosis, thus leading to economic, physical and psychological burden on the patient. Furthermore, there is controversy surrounding chronic Lyme disease syndrome as a legitimate medical condition.

Classifications of three ASTER NDVI images produced a digital representation of the vegetation density patterns in Central Alabama. It identified the ideal levels of vegetation necessary to maintain tick populations. The classification of all soil moisture ASTER images identified the presence of high soil moisture areas in Central Alabama. Although both environmental variables are known to support and maintain tick populations, black legged ticks thrive in areas containing both variables. Using visual analysis, areas containing both variables were identified and labeled as likely tick habitats and, assuming Lyme disease is present, risk zones.

The Quickbird analysis produced LULC and NDVI maps but did not produce significant results because the Quickbird imagery was taken in March 2005. The vegetation was therefore not a strong and dense as it would have been in August. The NDVI classification is therefore not completely trustworthy. For the same reason, the LULC results are not accurate.

The findings in this study are subject to several limitations. An unknown portion of all Lyme diseases cases are reported, which leads to underestimation in Alabama because of misdiagnosis, underreported, or some other social issues. There is difficulty in establishing a definitive diagnosis for LD because not all practitioners perform the serological test for confirmation prior to treatment so some treat for LD after identification of erythema migrans. Also, cases are reported based on residence state, not exposure states. Since Lyme Disease is transmitted by tick, without tick data, it would be inappropriate to create a statistics predictive model to directly link environmental factors to number of Lyme Disease cases in Alabama.

One of the initial goals of the project was to use GIS software to show the proportion of risk for the contraction of Lyme disease across the state of Alabama. Due to a limited number of studies involving Lyme disease in the state of Alabama, the amount and the type of data was also limited. The data that was deemed most comprehensive of the available data sets and consequently used in the project was composed of the number of ticks found by county, in the state of Alabama. The data was representative of presence but cannot be used to determine absence. It was pooled from a collection of many sources. Due to the fact that there was no set procedure for the collection of data, many sets were randomly collected and did not include attribute or spatial data such as the coordinates, using GIS capabilities was not practical in this stage of the project. Since the tick population data only indicated presence, the data could not be used to test the correlations between the environmental variables and the tick populations.

Community Outreach

Primary and secondary prevention methods have been established by the CDC to reduce the risk of contracting LD.[81,82] Primary prevention aims at reducing the risk of tick exposure while secondary prevention targets the disease's development to decrease the severity of symptoms upon contracting LD. [83] The CDC suggests that reducing exposure to ticks is the best defense against LD. Additional recommendations are wearing protective clothing

and applying tick repellants such as DEET to skin or Permethrin to clothing.[84,85] Checking oneself for ticks and proper removal behavior is effective because ticks typically feed for 36-48 hours prior to transmission of bacteria. [86] For proper removal, tweezers or fingers can be used to extract the tick-mouth parts from the host's skin. Removal in this manner insures that the contents of the tick mid gut (where the bacteria resides) are not regurgitated and transmitted into the host. [87] Secondary preventive methods include administration of antibiotics and pain killers as soon as early signs of disease, like the characteristic red rash, manifest. [88] If the condition becomes chronic, then administering medications providing symptomatic relief is the treatment of choice.[89,90]

Studies examining public health disease prevention interventions conclude that the effectiveness of any intervention is determined by its theoretical efficacy and level of engagement by the target population. [91] In the future the team will conduct a pilot study in Central Alabama to assess the risk perception and barriers to prevention. Based on the result, the team will frame a campaign message tailored to the needs (perception/barriers) of the people of Central Alabama. HBM will be used to draft the message in an effort to spread awareness effectively and efficiently. [92] Previous studies on LD prevention campaigns suggest the implementation of Health Belief Model (HBM) constructs, i.e., perceived susceptibility, severity, benefits, barriers, cues to action, and self-efficacy.[93]

Acknowledgements
Sarah Parcak, PhD, Assistant Professor, UAB School of Social and Behavioral Sciences; Director, Laboratory for Global Health Observations

Jonathan Gleason, DEVELOP National Program, NASA Langley Research Center

Karen Allsbrook, DEVELOP Financial Manager, Science Systems and Applications, Inc., NASA Langley Research Center

Tracey Silcox, DEVELOP National Program, NASA Langley Research Center

Kartikey Acharya, Resident at the University of Arkansas for Medical Sciences

Appendix

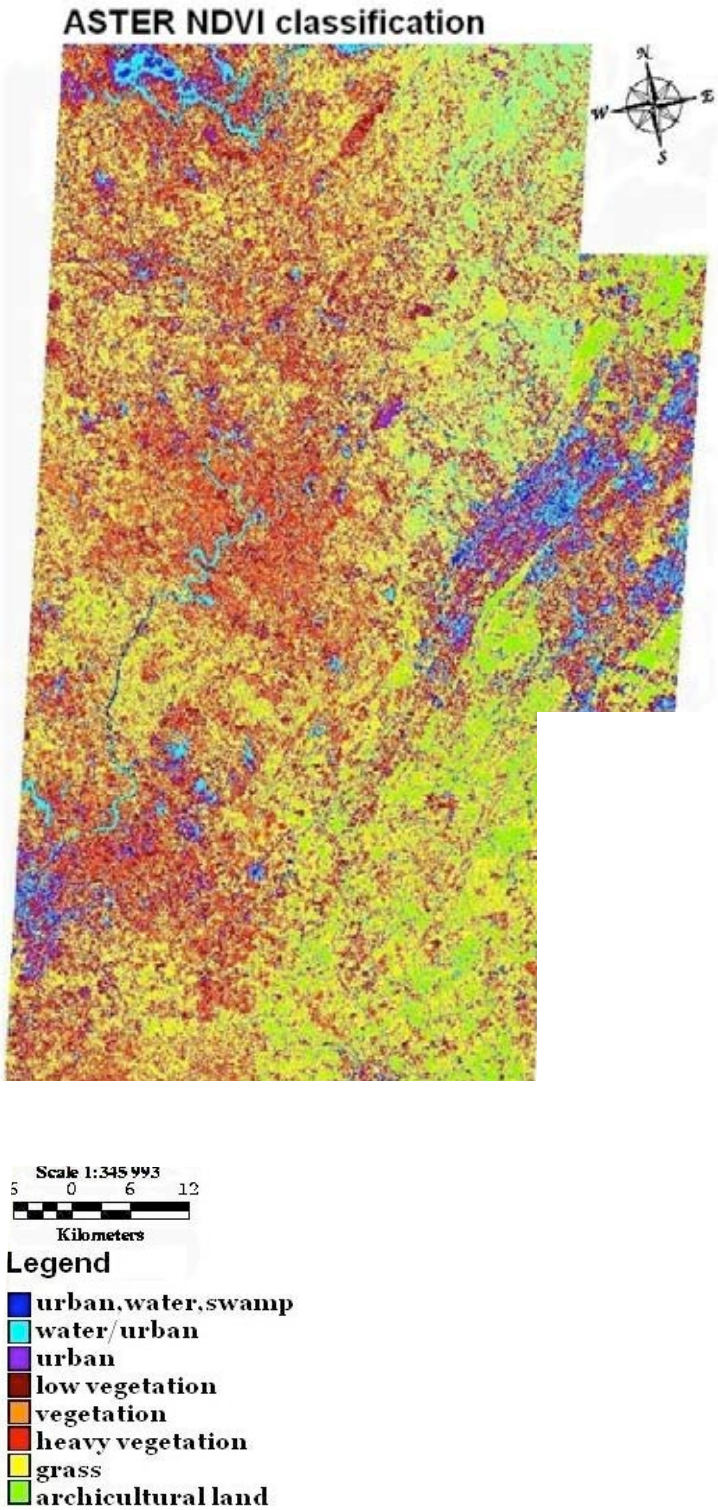


Figure 2: Unsupervised Normalized Difference Vegetation Index classification mosaic of two August 2001 and one August 2004 ASTER images showing levels of vegetation in Central Alabama.

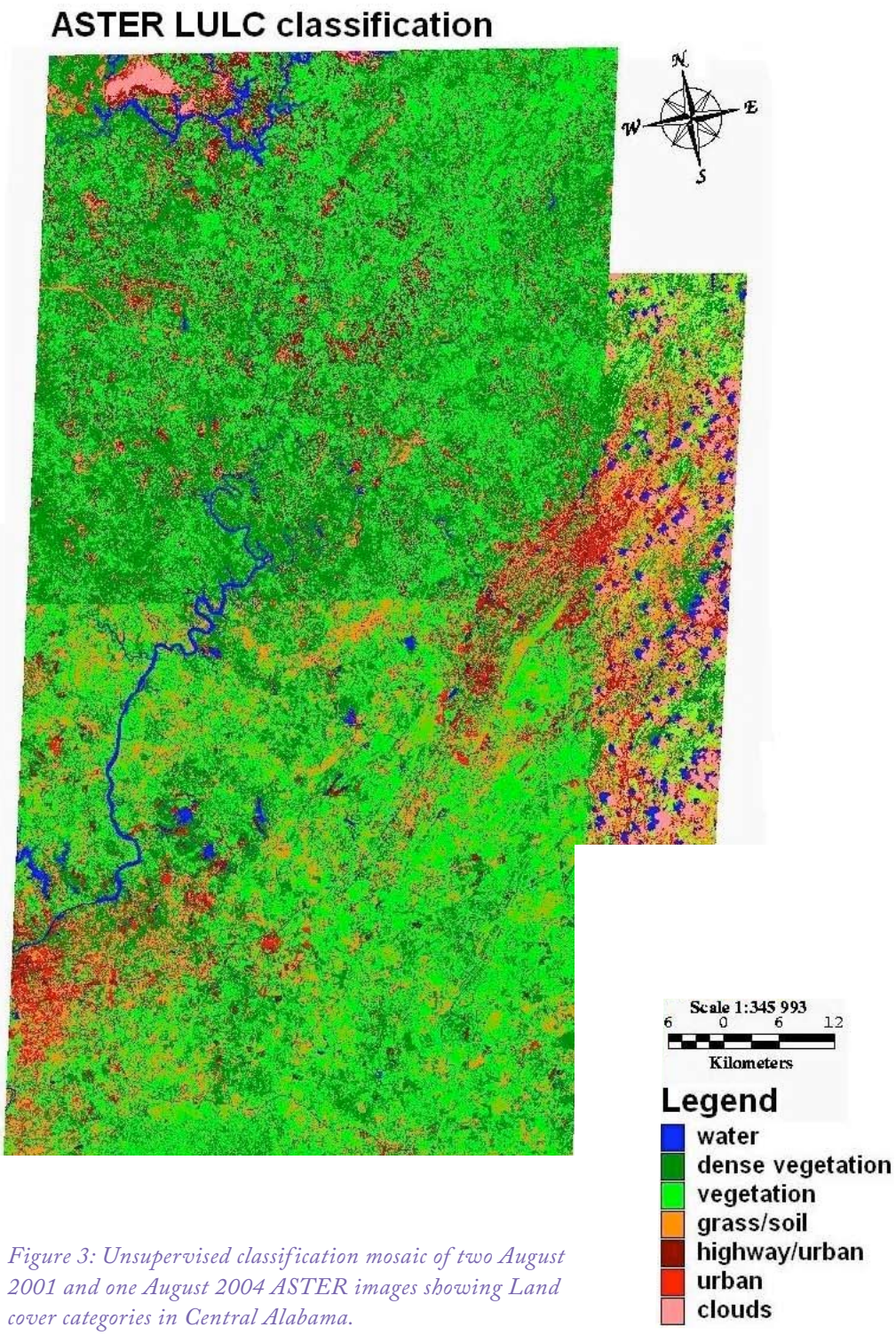


Figure 3: Unsupervised classification mosaic of two August 2001 and one August 2004 ASTER images showing Land cover categories in Central Alabama.

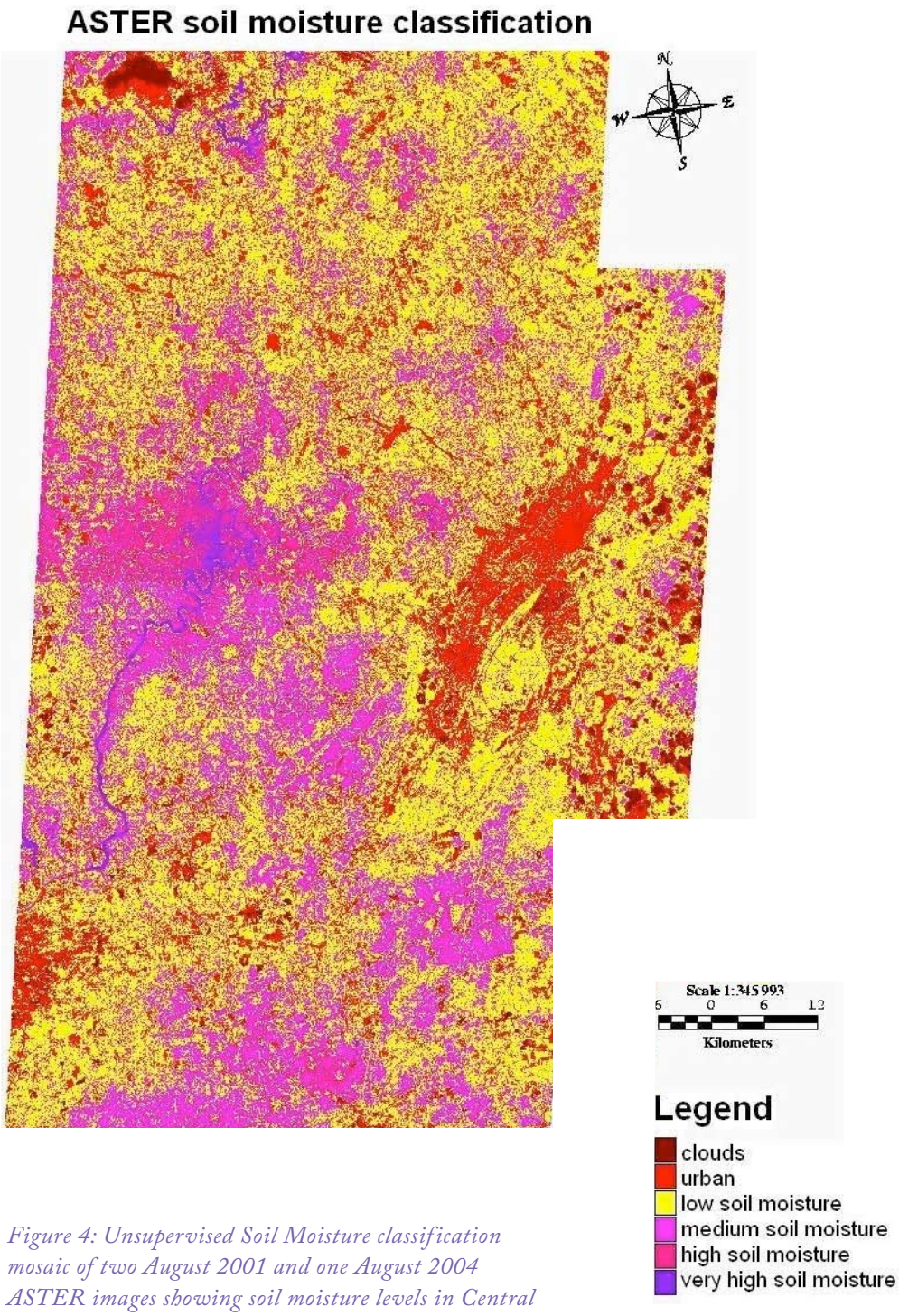


Figure 4: Unsupervised Soil Moisture classification mosaic of two August 2001 and one August 2004 ASTER images showing soil moisture levels in Central Alabama.

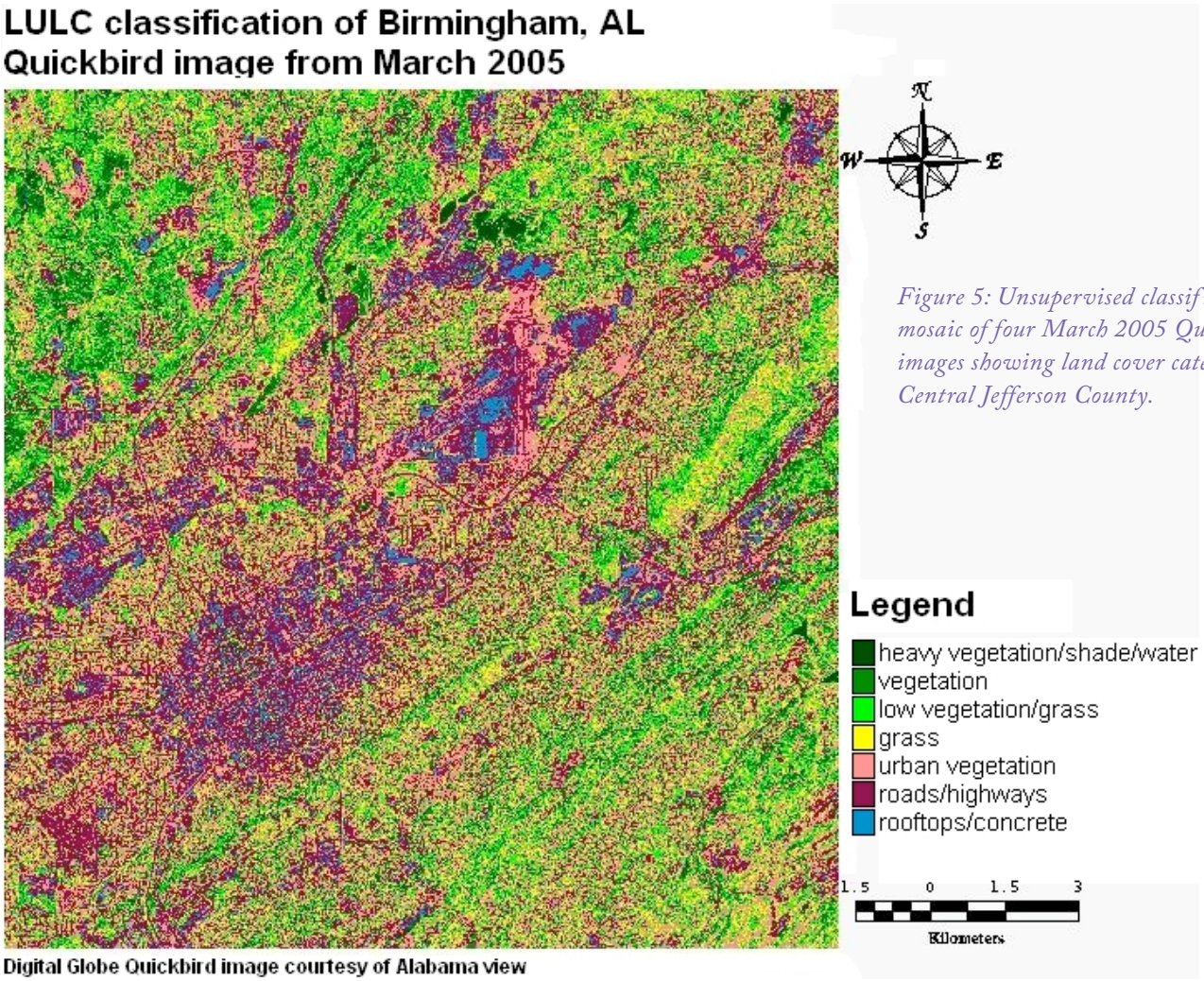


Figure 5: Unsupervised classification mosaic of four March 2005 Quickbird images showing land cover categories in Central Jefferson County.

References

1. Occupational Safety and Health Administration, United States Department of Labor. Safety and Health Information Bulletins: Potential for *Occupational Exposure to Lyme Disease. Occupational Safety and Health Administration.* Available at http://www.osha.gov/dts/shib/shib021103.html. SHIB 02-11. Accessed November 23, 2009.

2. Wormser GP, Nadelman RB, et al. Practice guidelines for the treatment of Lyme Disease. *Clin Infect Dis.* 2000; 31(Suppl 1);S1-14.

3. Orloski KA, Hayes EB, et al. Surveillance for Lyme Disease - United States, 1992-1998. *MMWR.* 2000;49(SS03); 1-11.

4. Lyme Disease-United States 1999. *MMWR.* 2001;50(10);181-5.

5. United States Department of Labor. Available at http://www.osha.gov/dts/shib/shib021103.html

6. Centers for Disease Control and Prevention (CDC). Lyme disease-United States, 2000. *MMWR* 2002;51:29-31.

7. The National Science Foundation. Available at http://www.nsf.gov/news/news_images.jsp?cntn_id=115714&org=NSF

8. Tibbles CD, Edlow JA. Does this patient have erythema migrans? *JAMA.* June 20, 2007; 297(23): 2617-27.

9. Lyme disease Clinical Presentation, The Johns Hopkins Arthritis Center. Available at: http://www.hopkins-arthritis.org/arthritis-info/lyme-disease/clinical-presentation.html. Accessed Nov. 25, 2009.

10. CDC Lyme Disease Transmission Web page. Available at http://www.cdc.gov/ncidod/dvbid/lyme/ld_humandisease_treatment.htm. Accessed Nov. 25, 2009.

11. Lyme Disease Transmission Web page. Available at http://www.cdc.gov/ncidod/dvbid/lyme/ld_humandisease_treatment.htm. Accessed Nov. 25, 2009.

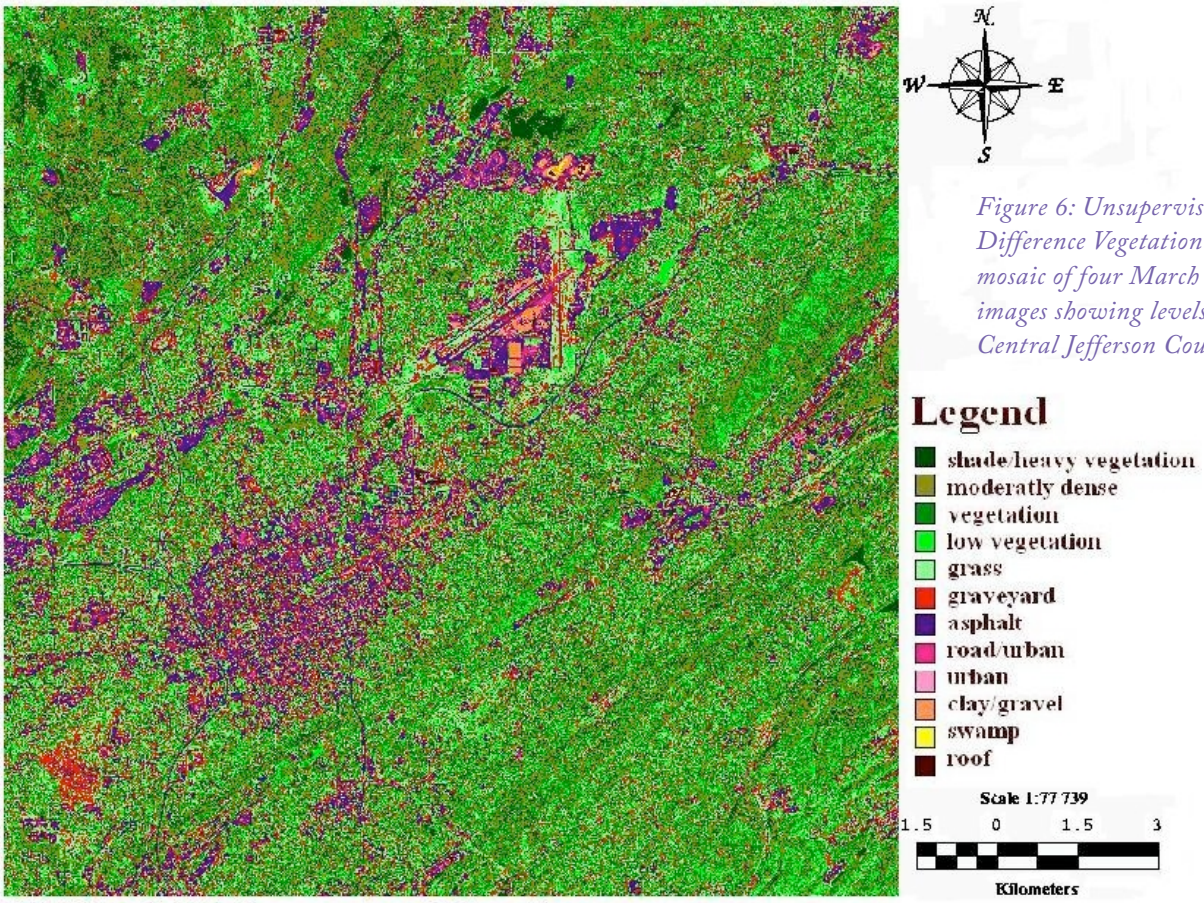


Figure 6: Unsupervised Normalized Difference Vegetation Index classification mosaic of four March 2005 Quickbird images showing levels of vegetation in Central Jefferson County.

Vegetation Index	Formula
NDVI	NIR-VIR/NIR+VIR

Figure 7: Vegetation Index Formulae

12. Fibromyalga Symptoms, Available at: http://www.fibromyalgia-symptoms.org/fibromyalgia_lyme.html . Nov. 25, 2009

13. Guerra, M, Walker E, et al. Predicting the risk of Lyme disease: Habitat suitability for Ixodes scapularis in the north central United States. *Emerg Infect Dis.* 2002; 8(3):289-297.

14. Luckhart S, Mullen GR, Durden A, et al. Borrelia sp. in ticks recovered from white-tailed deer in Alabama. *J Wildlife Dis.* 1992;28:449-452.

15. Oliver JH. Lyme Borreliosis in the Southern United States: A Review. *J Parasitol.* 1996; 82 (6): 926-935.

16. Piesman J, Donahue JG, et al. Transovarially aquired Lyme disease spirochetes (Borrelia burgdorferi) in field-collected larval Ixodes dammini (Acari:Ixodidae). *J Med Entomol.* 1986;23:219.

17. CDC Lyme Disease Transmission Web Page. Available at http://www.cdc.gov/ncidod/dvbid/Lyme/ld_transmission.htm. Accessed Nov. 3, 2009.

18. Luckhart S, Mullen GR, Durden A, et al. Borrelia sp. in ticks recovered from white-tailed deer in Alabama. *J Wildlife Dis.* 1992;28:449-452.

19. Goodwin BJ, Ostfeld RS, Schauber EM. Spatiotemporal Variation in a Lyme Disease host and Vector: Black-Legged Ticks on White-Footed Mice. *Vector-Borne & Zoonotic Dis.* 2001; 1:129-138.

20. Magnarelli LA, Oliver JH, et. al. Antibodies to Borrelia burgdorferi in rodents in the eastern and southern United States. *J Clin Microbiol.* 1992; 30:1449-1452.

21. Humair PF. Birds and Borrelia. *International Journal of Medical Microbiology.* Jun. 2, 1991; 30:70-74

22. Magnarelli LA, Oliver JH, et. al. Antibodies to Borrelia burgdorferi in rodents in the eastern and southern United States. *J of Clin Microbiol* 1992; 30:1449-1452.

23. Burgdorfer W. Vector/host relationships of the Lyme disease spirochete, Borrelia burgdorferi. *Rheum Dis Clin North Am.* 1989; 15:775-787.

24. CDC Lyme Disease Transmission Web page. Available at http://www.cdc.gov/ncidod/dvbid/lyme/ld_statistics.htm. Accessed Nov. 25, 2009.

25. Perkins SE, Cattadori IM, et al. Localized deer absence leads to tick amplification. *Ecology.* 2006;87(8):1981-1986.

26. Guerra, M, Walker E, et al. Predicting the risk of Lyme disease: Habitat suitability for Ixodes scapularis in the north central United States. *Emerg Infect Dis.* 2002; 8(3):289-297.

27. Estrada-Pena A. Increasing habitat suitability in the United States for the Tick that Transmits Lyme disease: A remote sensing approach. *Environ Health Perspect.* 2002; 10(7):635-640.

28. Eisen RJ, Lane RS, et al. Spatial patterns of Lyme disease risk in California based on disease incidence data and modeling of vector-tick exposure. *Am J Trop. Med. Hyg.* 2006; 75(4):669-676.

29. Gray JS. The ecology of ticks transmitting Lyme borreliosis. *Exp Appl Acarol.* 1998; 22: 249–258.

30. Guerra M, Walker E, et al. Predicting the risk of Lyme disease: Habitat suitability for Ixodes scapularis in the north central United States. *Emerg Infect Dis.* 2002; 8(3): 289-297.

31. Killilea M, Swei A, et al. Spatial dynamics of Lyme disease: A review. *EcoHealth.* 2008;5: 167-195.

32. Estrada-Pena A. Increasing habitat suitability in the United States for the Tick that Transmits Lyme disease: A remote sensing approach. *Environ Health Perspect.* 2002;10(7): 635-640.

33. Eisen RJ, Lane RS, et al. Spatial patterns of Lyme disease risk in California based on disease incidence data and modeling of vector-tick exposure. *Am J Trop. Med Hyg.* 2006; 75(4):669-676.

34. Lubelczyk CB, Elias SP, et al. Habitat associations of Ixodes scapularies (Acari: Ixodidae) in Maine. *Environ Entomol.* 2005;33(4): 900-906.

35. Guerra M, Walker E, et al. Predicting the risk of Lyme disease: Habitat suitability for Ixodes scapularis in the north central United States. *Emerg Infect Dis.* 2002;8(3):289-297.

36. Killilea M, Swei A, et al. Spatial dynamics of Lyme disease: A review. *EcoHealth.* 2008; 5:167-195.

37. Lubelczyk CB, Elias SP, et al. Habitat associations of Ixodes scapularies (Acari: Ixodidae) in Maine. *Environ Entomol.* 2005;33(4):900-906.

38. Rodgers SE, Zolnik CP, Mather TN. Duration of exposure to suboptimal atmospheric moisture affects nymphal black-legged tick survival. *J Med Entomol.* 2007;44(2):372-375.

39. CDC Lyme Disease Transmission Web Page. Available at http://www.cdc.gov/ncidod/dvbid/Lyme/ld_transmission.htm. Accessed Nov. 3, 2009.

40. Guerra M, Walker E, et al. Predicting the risk of Lyme disease: Habitat suitability for Ixodes scapularis in the north central United States. *Emerg Infect Dis.* 2002;8(3):289-297.

41. Mannelli A, Kitron U, et al. Influence of Season and Habitat on Ixodes scapularis Infestation on White-footed Mice in Northwestern Illinois. *J Parasitol.* 1994;80(6); 1038-1043.

42. Mannelli A, Kitron U, et al. Influence of Season and Habitat on Ixodes scapularis Infestation on White-footed Mice in Northwestern Illinois. *J Parasitol.* 1994. 80(6); 1038-1043.

43. Mannelli A, Kitron U, et al. Influence of Season and Habitat on Ixodes scapularis Infestation on White-footed Mice in Northwestern Illinois. *J Parasitol.* 1994. 80(6); 1038-1043.

44. Mannelli A, Kitron U, et al. Influence of Season and Habitat on Ixodes scapularis Infestation on White-footed Mice in Northwestern Illinois. *J Parasitol.* 1994. 80(6); 1038-1043.

45. The National Science Foundation. Deer: Quickfacts. Available at http://www.nsf.gov/news/special_reports/animals/textonly/deer_quickfacts.jsp

46. Pennsylvania Department of Health. Available at http://www.dsf.health.state.pa.us/health/cwp/view.asp?a=171&Q=230464

→ *top of page 68*

47. Glass GE, Schwartz BS, et al. Environmental risk factors for Lyme disease identified with geographic information systems. *Am J Public Health.* 1995; 85:944-948.

48. Bronstein JS, Skelly DK, et al. Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. *Oecologia.* 2005;146: 469–475.

49. Luckhart S, Mullen GR, Durden A, et al. Borrelia sp. in ticks recovered from white-tailed deer in Alabama. *J Wildlife Dis.* 1992;28:449-452.

50. Mullen GR, Piesman J. Serologically substantiated case of Lyme disease and potential tick vectors in Alabama. *Ala J Med Sci.* 1987 Jul;24(3):306-7.

51. CDC Lyme Disease Transmission Web page. Available at http://www.cdc.gov/ncidod/dvbid/lyme/ld_statistics.htm. Accessed Nov. 25, 2009.

52. Luckhart S, Mullen GR, Wright, C. Etiologic agent of Lyme Disease, Borrelia burgdorferi, detected in ticks (Acari:Ixodidae) collected at a focus in Alabama. *J. Med Entomol.* 1993; 28:652-657.

53. Centers for Disease Control and Prevention (CDC): Lyme Disease Cases Reported to CDC by State Health Departments, 1982-1997. Available at http://www.cdc.gov/ncidod/dvbid/ld82_97.pdf. Accessed Nov. 3, 2009.

54. Centers for Disease Control and Prevention (CDC): Lyme Disease Cases Reported to CDC by State Health Departments, 1982-1997. Available at http://www.cdc.gov/ncidod/dvbid/ld82_97.pdf. Accessed Nov. 3, 2009.

55. Durden LA, Luckhart S, et al. Tick infestations of white-tailed deer in Alabama. *J Wildl Dis.* 1991 Oct;27(4):606-14.

56. Durden LA, Luckhart S, et al. Tick infestations of white-tailed deer in Alabama. *J Wildl Dis.* 1991 Oct;27(4):606-14.

57. Durden LA, Luckhart S, et al. Tick infestations of white-tailed deer in Alabama. *J Wildl Dis.* 1991 Oct;27(4):606-14.

58. Durden LA, Luckhart S, et al. Tick infestations of white-tailed deer in Alabama. *J Wildl Dis.* 1991 Oct;27(4):606-14.

59. Luckhart S, Mullen GR, Wright JC. Etiologic agent of Lyme disease, *Borrelia burgdorferi,* detected in ticks (Acari: Ixodidae) collected at a focus in Alabama. *J Med Entomol.* 1991 Sep;28(5):652-7.

60. Luckhart S, Mullen GR, Wright JC. Etiologic agent of Lyme disease, *Borrelia burgdorferi,* detected in ticks (Acari: Ixodidae) collected at a focus in Alabama. *J Med Entomol.* 1991 Sep;28(5):652-7.

61. James AM, Liveris D, et al. Borrelia lonestari infection after a bite by an Amblyomma americanum tick. *J Infect Dis.* 2001 Jun 15;183(12):1810-4.

62. James AM, Liveris D, et al. Borrelia lonestari infection after a bite by an Amblyomma americanum tick. *J Infect Dis.* 2001 Jun 15;183(12):1810-4.

63. Hayes EB, Piesman J. How can we prevent Lyme disease? *N Engl J Med.* June 12, 2003; 348: 2424-30.

64. CDC Lyme Disease Transmission Web Page. Available at http://www.cdc.gov/ncidod/dvbid/Lyme/ld_transmission.htm. Accessed Nov. 3, 2009.

65. CDC Lyme Disease Transmission Web Page. Available at http://www.cdc.gov/ncidod/dvbid/Lyme/ld_transmission.htm. Accessed Nov. 3, 2009.

66. Pal U, Ynag X, Chen M, et al. OspC fascilitates *Borrelia Burgdorferi* invasion of Ixodes scapularis salivary glands. *Journal of Clinical Investigation.* January 15, 2004; 130(2): 220-230.

67. CDC Lyme Disease Transmission Web Page. Available at http://www.cdc.gov/ncidod/dvbid/Lyme/ld_transmission.htm. Accessed Nov. 3, 2009.

68. CDC Lyme Disease Transmission Web Page. Available at http://www.cdc.gov/ncidod/dvbid/Lyme/ld_transmission.htm. Accessed Nov. 3, 2009.

69. USGS (United States Geological Survey). Greenness of the Conterminous U.S. Available at http://ivm.cr.usgs.gov/whatndvi.php . Accessed Nov. 23, 2009.

70. USGS (United States Geological Survey). Greenness of the Conterminous U.S. Available at http://ivm.cr.usgs.gov/whatndvi.php . Accessed Nov. 23, 2009.

71. Shih SF, Jordan JD. Use of Landsat Thermal-IR Data and GIS in Soil Moisture Assessment. *J Irrig Drain Engrg.* 1993;119(5):868-879

72. Shih SF, Jordan JD. Landsat mid-infrared data and GIS in regional surface soil-moisture assessment. *J Am Water Resour As.* 2007; 28(4):713-719.

73. Durden LA, Luckhart S, et al. Tick infestations of white-tailed deer in Alabama. *J Wildl Dis.* 1991 Oct;27(4):606-14.

74. CDC Lyme Disease Transmission Web Page. Available at http://www.cdc.gov/ncidod/dvbid/Lyme/ld_transmission.htm. Accessed Nov. 3, 2009.

75. Guerra M, Walker E, et al. Predicting the Risk of Lyme Disease: Habitat Suitability for Ixodes scapularis in the North Central United States. *Emerg Infect Diseases* 2002;8(3):289-297.

76. Lastavica CC, Wilson ML, et al. Rapid emergence of a focal epidemic of Lyme disease in coastal Massachusetts. *N Engl J Med* 1989;320:133-7.

77. Schwartz BS, Goldstein MD. Lyme disease in outdoor workers: risk factors, preventive measures, and tick removal methods. *Am J Epidemiol* 199O;131:877-85.

78. Coyle BS, Strickland GT, et al. The public health impact of Lyme disease in Maryland. *J Infect Dis.* 1996 May;173(5):1260-2.

79. Meek JI, Roberts CL, et al. Underreporting of Lyme disease by Connecticut physicians. *J Public Health Manag Pract.* 1996; 2(4):61-5.

80. Young JD. Underreporting of Lyme disease. *N Engl Journ Med.* 1998;338(22):1629.

81. Hayes EB, Piesman J. How can we prevent Lyme disease? *N Engl J Med.* 2003;348:2424-30.

82. Lyme Disease Prevention and Control. Available at http://www.cdc.gov/ncidod/dvbid/lyme/ld_prevent.htm. Accessed Nov. 3, 2009.

83. Corapi K, Whit, M, et al. Strategies for primary and secondary prevention of Lyme disease. *Nat Clin Pract Rheum.* 2007;3(1):20-25.

84. Hayes EB, Piesman J. How can we prevent Lyme disease? *N Engl J Med.* June 12, 2003; 348: 2424-30.

85. Lyme Disease Prevention and Control. Available at http://www.cdc.gov/ncidod/dvbid/lyme/ld_prevent.htm. Accessed Nov. 3, 2009.

86. Piesman J, Dolan MC. Protection against Lyme disease spirochete transmission provided by prompt removal of nymphal *Ixodes scapularis* (Acari: Ixodeae). *J Med Entomol.* 2002;39: 509-521.

87. Corapi, K, White, M, et al. Strategies for primary and secondary prevention of Lyme disease. *Nat Clin Pract Rheum.* 2007;3(1):20-25.

88. Luckhart S, Mullen GR, Wright JC. Etiologic agent of Lyme disease, Borrelia burgdorferi, detected in ticks (Acari: Ixodidae) collected at a focus in Alabama. *J Med Entomol.* 1991 Sep;28(5):652-7.

89. Hayes EB, Piesman J. How can we prevent Lyme disease? *N Engl J Med.* 2003;348:2424-30.

90. Centers for Disease Control and Prevention. Lyme Disease Prevention and Control. Available at http://www.cdc.gov/ncidod/dvbid/lyme/ld_prevent.htm

91. CDC Theory at a Glance: A Guide for Health Promotion Practice. NIH Publication No. 05-3896. Sept. 2005:12. Available at http://www.cancer.gov/PDF/481f5d53-63df-41bc-bfaf-5aa48ee1da4d/TAAG3.pdf. Accessed Nov. 3, 2009.

92. Becker MH. The Health Belief Model and Personal Health Behavior. *Health Educ Monogr.* 1974;2:324-473

93. Becker MH. The Health Belief Model and Personal Health Behavior. *Health Educ Monogr.* 1974;2:324-473

Single Walled Carbon Nanotubes as a Regenerative Substrate in Spinal Cord Injury

Jose A. Roman[1], Robert C. Haddon[3], Vladimir Parpura[2], Candace L. Floyd[1]
[1]Department of Physical Medicine and Rehabilitation, University of Alabama at Birmingham, Birmingham, USA
[2]Department of Neurobiology, University of Alabama at Birmingham, Birmingham, USA
[3]Department of Chemistry, University of California at Riverside, Riverside, USA

Corresponding author: Candace L. Floyd, Ph.D., Department of Physical Medicine and Rehabilitation, Center for Glial Biology in Medicine, University of Alabama at Birmingham

Abstract:
Traumatic spinal cord injury (SCI) induces tissue damage and results in the formation of a cavity that inhibits axonal regrowth. Filling this cavity with a growth-permissive substrate would likely promote regeneration and repair. Single walled carbon nanotubes grafted with polyethylene glycol (SWNT-PEG) have been shown to increase the length of neuronal processes in vitro and promote growth of neurons in vivo. We hypothesized that an administration SWNT-PEG administered after an SCI contusion injury will promote regeneration of axons into the lesion cavity and functional recovery of the hind limbs. To evaluate this hypothesis, complete transection spinal cord injury was induced at the T9 vertebrae. One week after transection, the epicenter of the lesion was injected with 25µL of either vehicle (saline), 1.0 µg/mL, 10.0 µg/mL, or 100.0 µg/mL of SWNT-PEG. Behavior analysis was conducted before injury, before treatment, and once every seven days for 28 days after treatment. At 28-days post-injection the rats were euthanized and spinal tissue was extracted. Immunohistochemistry was used to detect the area of the cyst, the thickness of the glial scar, and axonal morphology. We found that post-SCI administration of SWNT-PEG increases neurofilament-positive fibers in the lesions and does not increase reactive gliosis. Additionally, post-SCI administration of SWNT-PEG improved hind limb locomotor recovery without inducing allodynia and hyperalgesia. These data suggest that SWNT-PEG may be an effective substrate to promote axonal repair and regeneration after SCI.

Introduction

A hallmark of traumatic spinal cord injury (SCI) is the development of a cystic cavity at the epicenter of the lesion, which is a barrier to subsequent axonal regrowth, regeneration, and functional recovery (Silver et al., 2004). This barrier or reactive gliosis is comprised of astrocytes that serve as both protection against further damage and a blockage against further axonal regrowth and regeneration. We hypothesize that adding a growth permissive substrate to this cavity could promote axonal growth and functional recovery. Single walled carbon nanotubes (SWNTs) are comprised of cylindrical graphene sheets that can range in size from 0.4 nm to 100 nm in diameter and 1 µm to hundreds of µms in length. They can easily be modified to alter biological characteristics such as solubility and polarity depending on their application. Carbon nanotubes (CNTs) have many intrinsic characteristics that make them ideal for engineering capabilities such as small size, high elasticity, high strength, stability, and conductivity. However, CNTs can also be used in a biological context and modified to interact with biological compounds (Malarkey et al., 2007). Previous studies have shown that the attachment of copolymer grafts (functionalization) such as polyethylene glycol (PEG) increases neurite outgrowth and branching *in vitro* (Ni et al., 2005). Although SWNTs have been shown *in vitro* to help stimulate neural regeneration, no *in vivo* experiment has been done to test the regenerative properties of SWNT-PEG in a living organism. Consequently, we evaluated the effect of SWNT-PEG administration on regeneration and repair and functional recovery after SCI in adult rats. The central hypothesis of this study was that administration of SWNT-PEG, either acutely or one week after a spinal cord injury, will promote axonal regeneration/ repair and functional recovery of hind limb locomotion in a rat model.

Materials and Methods

Experimental Groups and Method of Spinal Cord Injury

All surgical protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Fifty-eight adult female Sprague-Dawley rats (250-300g, 2 months old) received a complete transection SCI induced by severing the spinal cord at the T9 vertebral level with a MicroFeather ophthalmic scalpel (Feather Safety Razor Co., Japan). For the acute administration (immediately after SCI) experiment, animal were randomly assigned to the following experimental groups: 1) uninjured control (n=6), 2) complete transection SCI (n=8), 3) complete transection SCI with post-SCI administration of 10 µg/mL of SWNT-PEG in a volume of 25µL (n=5) or 4) complete transection SCI with post-SCI administration of 10 µg/mL of SWNT-PEG in a volume 50 µL (n=5). For the delayed administration experiment, animal received a treatment one week following injury with 25 µL of either vehicle (saline, n=8), 1.0 µg/mL (n=8), 10.0 µg/mL (n=9), or 100.0 µg/mL (n=9) of the SWNT-PEG solution stereotaxically injected into the lesion site epicenter. For all experiments, Matristem tissue sealant (Acell®, Inc. Vet, Columbia, MD) was placed over the transected dura after administration of SWNTs to seal the dura and prevent overflow of SWNT-containing solution into surrounding tissue.

Behavioral Outcome measures

Locomotor and sensory function was assessed weekly after the injection of the SWNTs (post-SCI days 14-35) using the Basso Beattie Bresnahan (BBB) open-field locomotor test, the Noldus Catwalk™ gait analysis, sensitivity to Von Frey filaments and Tail Flick analysis. Hind limb function was assessed with the BBB locomotor test (Basso et al., 1995) pre-injury, and once each week for the following thirty-five days. For this test, animals were placed in a 1.2m diameter metal, smooth-surfaced activity chamber for 4 minutes, and hind-limb movement was scored by two trained investigators who were naïve to the treatment of the animal. All discrepancies in scores were resolved by discussion between the raters at the conclusion of the test and scored to the deficit. Scores were generated for each hind limb and averaged. The BBB test is a gross hind limb locomotor test based on a scale from 0 (no hind limb movement) to 21(normal hind limb movement) that is scored objectively between two trained investigators who are naïve to the treatment. On post-SCI day five prior to administration of SWNT-PEG, in order to standardize the injury among animals, animals with a BBB score greater than 1 were excluded from subsequent analysis. Finer kinematic analysis is computed using The Catwalk™ gait analysis (Hamers et al., 2006). Animals traverse a glass walkway and a high speed camera digitally records the paw prints in real time. The gait is then analyzed, frame by frame, with the assistance of integrated software. One week preceding the injury, the animals were introduced to the procedure and a baseline result was obtained one day pre-injury, and then subsequent analysis was conducted once every week following the injury for thirty-five days. Mechanical allodynia (hypersensitivity to an originally non-noxious stimulus) was measured by determining the animal's sensitivity to von Frey filaments (Chaplan et al., 1994). By applying a stimulus force of 2 g, 4g, 6g, 8g, 15g, 26g, or 60g, the plantar surface of the hind paw was evaluated for a positive response- sensation by lifting the paw. The lowest filament receiving at least 50% positive response of five trials (at least three positive responses) was recorded as the conclusive sensitivity level. Only animals with plantar placement in the BBB test on test day were evaluated Thermal latency was determined by applying radiant heat to the rat's tail. Two tail-flick latencies were obtained per each weekly session. Tail Flick analysis was used to determine the sensitivity to a noxious thermal stimulus when applied to the tail with reaction time and hyperalgesia–an originally noxious stimulus is now more painful (Deakin et al., 1978).

Histological Outcome measures

Spinal cord tissue preparation: At thirty-five days post-SCI, animals were euthanized with an overdose of pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) and perfused with 4% paraformaldehyde (PFA). The spinal cord tissue was post-fixed for 24 hours at 4°C in 4% PFA and then cryoprotected in increasing concentrations of sucrose (10-30%) for 48 hours at 4°C. Spinal cords were then frozen over dry ice and then blocked into 5 mm longitudinal sections (2.5 mm rostrally and caudally from the epicenter). Tissue is then embedded in OCT-Compound (Tissue-Tek, Fisher Scientific, Pittsburg, PA, USA) and stored at -80°C. Serial random sections (30µm) were sliced on a cryostat (Global Medical Instrumentation, Inc., Ramsey, MN, USA), and placed on 1%-gelatin coated slides and stored at -20°C until further analysis.

Glial fibrillary acid protein (GFAP) immunohistochemistry: Sections were encircled with a hydrophobic barrier (ImmEdge™, Vector Laboratories, Burlingame, CA, USA) and then dried overnight at room temperature. Slides were rehydrated through graded alcohols, and blocked with an endogenous peroxidase treatment with 0.5% H2O2. Sections were then washed and non-specific reactivity was blocked with 3% goat serum + 0.3% Triton X + 3% bovine serum albumin + 0.1M phosphate buffered saline (PBS) at 37°C for one hour. Tissue sections were rinsed and then incubated in rabbit polyclonal anti-GFAP antibody (1:4000, Z0334, Dako, Carpentaria, CA, USA) for 25 hours at 4°C. Afterwards, the sections were washed in PBS and incubated in Alexa Fluor® 488 goat anti-rabbit secondary antibody (1:4000, A-11008, Invitrogen Co., Carlsbad, CA, USA) at 4°C for 24 hours. Sections were then rinsed, dried overnight, and cover-slipped with Permount mounting media (Fischer Scientific, Pittsburg, PA).

Neurofilament heavy immunohistochemistry: Sections were encircled with a hydrophobic barrier (ImmEdge™, Vector Laboratories, Burlingame, CA, USA) and then dried overnight at room temperature. Slides were rehydrated through graded alcohols, and blocked with an endogenous peroxidase treatment with 0.5% H2O2. Sections were then washed and non-specific reactivity was blocked with 3% goat serum + 0.3% Triton X + 3% bovine serum albumin + 0.1M phosphate buffered saline (PBS) at 37°C for one hour. Tissue sections were rinsed and then incubated in mouse monoclonal to 200 kD Neurofilament Heavy antibody (1:1000, ab24572, Abcam, Cambridge, MA, USA) for 25 hours at 4°C. Afterwards, the sections were washed in PBS and incubated in Alexa Fluor® 488 goat anti-mouse secondary antibody (1:4000, A-11001, Invitrogen Co., Carlsbad, CA, USA) at 4°C for 24 hours. Sections were then rinsed, dried overnight, and cover-slipped with Permount mounting media (Fischer Scientific, Pittsburg, PA).

Stereological quantification

GFAP: Stereological estimates of reactive gliosis rostrally, at the epicenter, and caudally were quantified using relative fluorescence intensity. Relative fluorescence intensity was determined by using a 10x objective on an Olympus BX51 microscope with a motorized stage. The sample size per section was 1mm x 2.5 mm in area. Relative fluorescence intensity per section was computed using StereoInvestigator (MicroBrightField Inc., Colchester, VT). Outlining of the serial sections was conducted by a single investigator naïve to the treatment group.

Neurofilament: Stereological estimates of heavy chain neurofila-

ment positive fibers rostrally and caudally were quantified using the optical fractionator method (West et al., 1991). Neurofilament fiber (NF) counts were performed under a 20x objective on an Olympus BX51 microscope with a motorized stage. NF were counted using StereoInvestigator (MicroBrightField Inc., Colchester, VT). The counting frame was started at a randomized first section and then the inclusion criteria included a NF of minimum length of 5 μm, inclusion in the boundaries set by StereoInvestigator, and in order to minimize error due to irregular slicing NF location within 1 μm of the set z-axis. Outlining of the serial sections was conducted by a single investigator naïve to the treatment group. The total approximation by optical fractionator and the second coefficient of error were calculated as estimates and a value of 0.1 for the second coefficient of error was accepted.

Statistical Analysis

Data are shown as mean ±standard error of the mean and were analyzed using SigmaPlot® (v11, Systat Software Inc., San Jose, CA, USA). Data were analyzed using the independent two-sample student's t-test. Statistical significance was set at $p<0.05$.

Results

Acute administration of SWNT-PEG promotes axonal repair and regeneration without inducing gliosis

In the first experiment, we evaluated acute post-SCI administration of SWNT-PEG on markers of axonal repair. The tissue of animals that received either a laminectomy, complete transection and no treatment with SWNT-PEG, or treatment with 10 μg/mL of SWNT-PEG at 25 μL or 50 μL had immunohistochemistry analysis conducted on serial sections with an antibody against heavy neurofilament (NF). Figure 1 shows a representative micrograph of a longitudinal section of rat spinal cord at the epicenter of the lesion processed with immunohistochemistry for neurofilament (panel A). Stereological quantification of neurofilament positive fibers showed that administration of 25 or 50 μL of 10 μg/mL SWNT-PEG induced a significant increase (indicated by +) in neurofilament positive fibers compared to the non-treated, injured animals (SCI + Sealant). Non-treated animals also showed a significant decrease in NF-positive fibers (indicated by *) compared to uninjured animals (LAM). These data suggest that treatment with SWNT-PEG promotes axonal repair and regrowth into the cavity after a spinal cord injury.

In a second set of serial sections, immunohistochemistry analysis with an antibody against glial fibrillary acidic protein (GFAP) was conducted. Figure 2 shows a representative micrograph depicting a longitudinal section of rat spinal cord at the epicenter of the lesion processed with immunohistochemistry for GFAP (panel A). Quantification of GFAP-reactivity with relative fluorescence intensity showed that treatment with SWNT-PEG induced GFAP relative fluorescence intensity values similar to that of the non-treated and non-injured groups, suggesting that SWNT-PEG administration acutely post-SCI does not induce gliosis.

Delayed administration of SWNT-PEG promotes functional recovery of hind limb locomotion

After determining that acute SWNT-PEG promoted axonal repair and regrowth, we evaluated a more clinically relevant delayed administration with treatment one week post-injury. Treatment of 25 μL with either saline, 1.0 μg/mL, 10.0 μg/mL, or 100.0 μg/mL was administered one week post-injury. Behavior was evaluated once a week for twenty-eight days after SWNT-administration (35 days after SCI). The BBB test was performed weekly for thirty-five days post-injury. As shown in figure 3, animals receiving SWNT-PEG at either 1.0, 10.0, or 100.0 μg/mL had improved BBB scores as compared to animals that received no treatment (0μg/mL). This suggests that delayed post-SCI administration of SWNT-PEG caused increased recovery of locomotor function compared to animals that received no treatment. Figure 4 shows the results of the Catwalk™ kinematic analysis of fore to hind limb coordination as evaluated using the regularity index. The regularity index indicates the percentage of steps that are placed in a regular, coordinated step pattern. Animals that received the 100mg/mL treatment of SWNT-PEG showed a trend toward greater fore to hind limb coordination, but this did not reach statistical significance. Hind paw print area, which indicates the contact area of the plantar surface of the paw in stepping whereby larger print areas indicate more plantar contact, was also analyzed using the Catwalk™ kinematic system. As shown in Figure 5, animals that received the 100 μg/mL treatment showed a trend toward improvement in maximum paw print area at day 35 day post-injury. These data suggest that the 100mg/mL concentration of SWNT-PEG may improve hind-limb kinematics, an indicator of improved hind limb locomotor recovery.

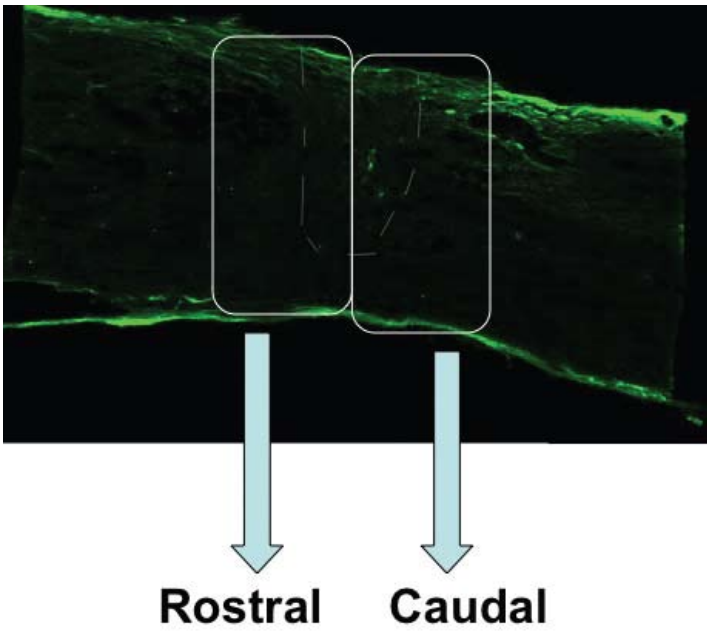
Post-SCI administration of SWNT-PEG does not induce neuropathic pain

Allodynia was evaluated using von Frey filaments. Figure 6 shows the quantification of the sensitivity to a stimulus using the von Frey filaments. No decrease in mechanical threshold over pre-injury assessment was observed suggesting no allodynia was induced. Figure 7 shows the latency of a response due to a thermal stimulus in the tail flick assay. After SCI, reaction time to the thermal stimulus decreased, suggesting that SCI caused hyperalgesia. Administration of SWNTs after SCI did not alter the response to thermal stimulus which suggests no induction of neuropathic pain below the level of the SCI lesion.

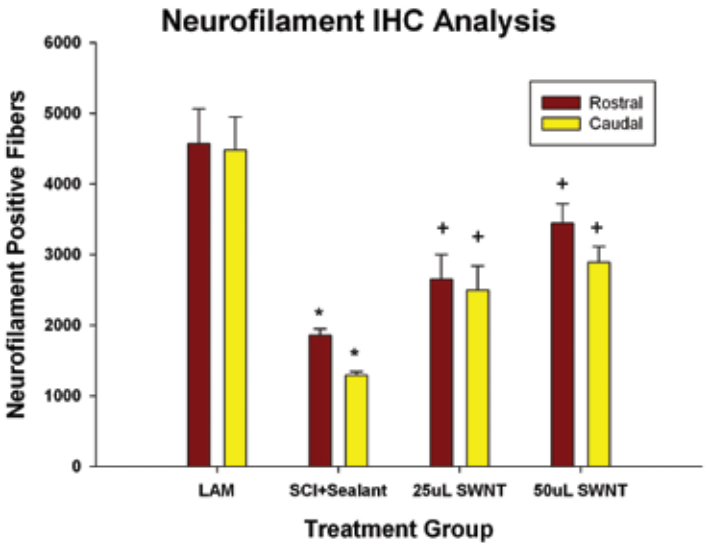
Discussion

In this study, we sought to evaluate a growth permissive substrate that would enhance the regrowth of damaged axons after injury. We first report that acute post-SCI administration of 10.0 μg/mL of SWNT-PEG at both volumes (25 μL and 50 μL) was shown to have statistically more neurofilament growth compared to the injured, non-treated group suggesting that SWNT-PEG increases axonal regrowth. Another factor to determine whether SWNT-PEG could be an effective treatment for spinal cord in-

Figure 1: SWNT-PEG animal showed increase in neurofilament-positive fibers.

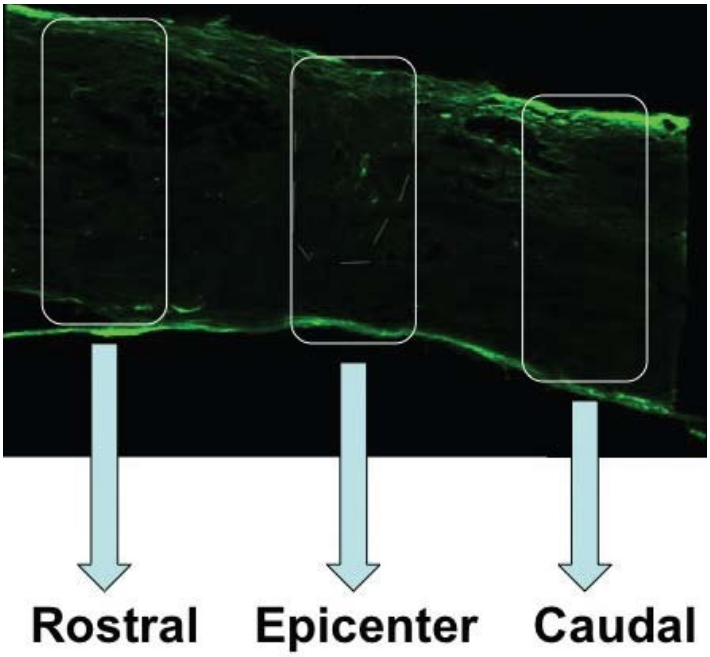


Panel A: Representative transverse section of rat spinal cord at the epicenter of the lesion indicating regions of interest wherein neruofilament positive fibers were quantified. Regions of interest 1mm rostral (red) and caudal (yellow) to the lesion site were quantified using stereological techniques to determine numbers of NF-positive fibers among all four treatment groups of SWNT-PEG.

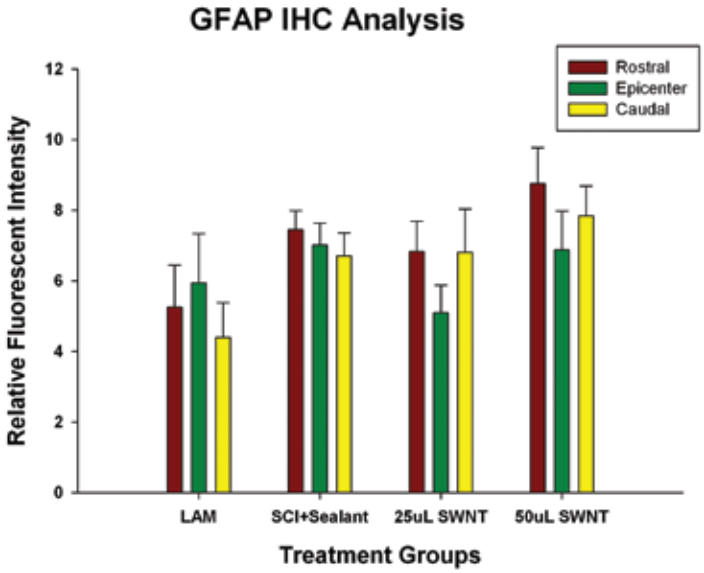


*Panel B: Stereological quantification of NF-positive fibers was conducted using optical fractionator. SCI caused a significant reduction in the number of NF-fibers (SCI + Sealant) as compared to the uninjured animals (LAM), indicated by *. Post-SCI administration of SWNT-PEG significantly increased the numbers of NF-positive fibers, indicated by + (n=5-8 per group).*

Figure 2: SWNT-PEG does not induce reactive gliosis.



Panel A: Representative transverse section of rat spinal cord at the epicenter of the lesion indicating regions of interest wherein GFAP immunoreactivity was quantified. Sections 1mm rostral (red), epicenter (green), and caudal (yellow) to the lesion site were quantified by measuring the relative fluorescent intensity in regions of interest (1mm x 2.5mm) among all four treatment groups of SWNT-PEG.



Panel B: Quantification of GFAP-immunoreactivity indicated that no volume of SWNT-PEG altered relative fluorescence intensity which suggests that treatment with SWNT-PEG did not increase reactive gliosis (n=5-8 per group).

→ top of page 74

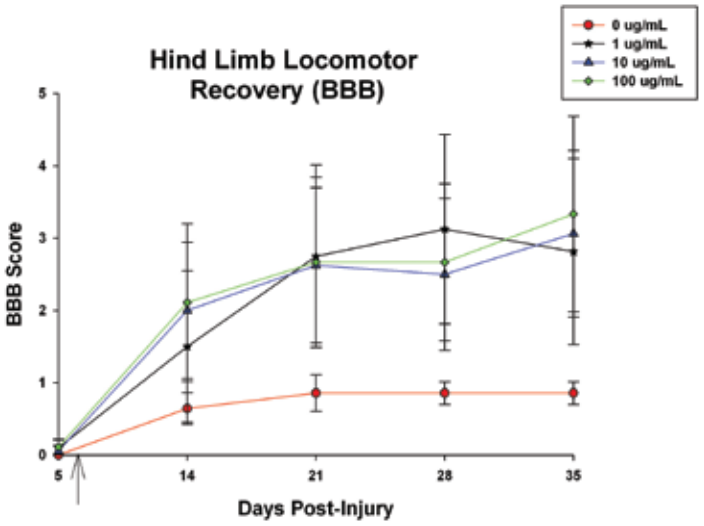


Figure 3: SWNT-PEG treated animals showed improved recovery of hind limb locomotion in the open field.

Hind limb locomotor function was evaluated in the open field and scored using the BBB test. Post-SCI evaluation was conducted once prior to treatment (day 5) and weekly after SWNT-PEG administration (days 14, 21, 28, 35). Arrow indicates time of SWNT-PEG administration (post-SCI day 7). Animals receiving SWNT-PEG were found to regain locomotor function compared to animals that received no treatment (0 ug/mL), suggesting that treatment with SWNT-PEG promotes hind limb recovery (n=8-9 per group).

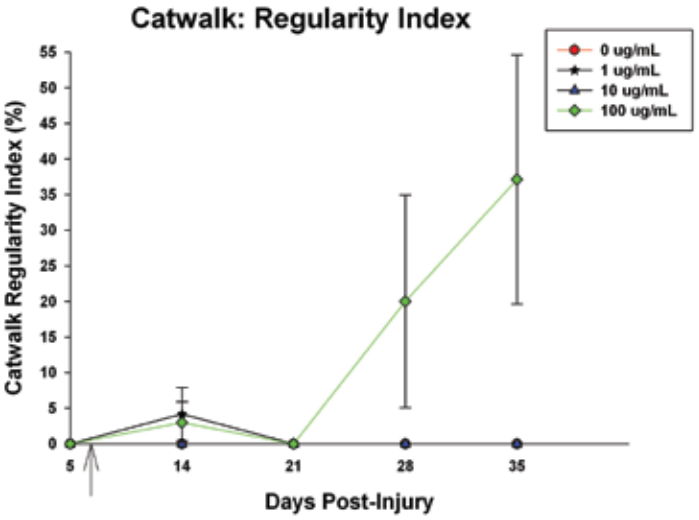


Figure 4: Post-SCI administration with 100 µg/mL of SWNT-PEG improved locomotor coordination in the Catwalk task.

The Catwalk™ gait analysis and integrated software analyzed fine kinematic paw placements and computes various measures such as regularity index. Kinematic analysis of fore to hind limb coordination was evaluated using the regularity index. The regularity index indicates the percentage of steps that are placed in a regular, coordinated step pattern. Animals that received the 100 µg/mL treatment showed a slight improvement in regularity index at 28 and 35 days post-injury, suggesting that treatment with 100 µg/mL of SWNT-PEG slightly improves locomotor coordination (n=8-9 per group).

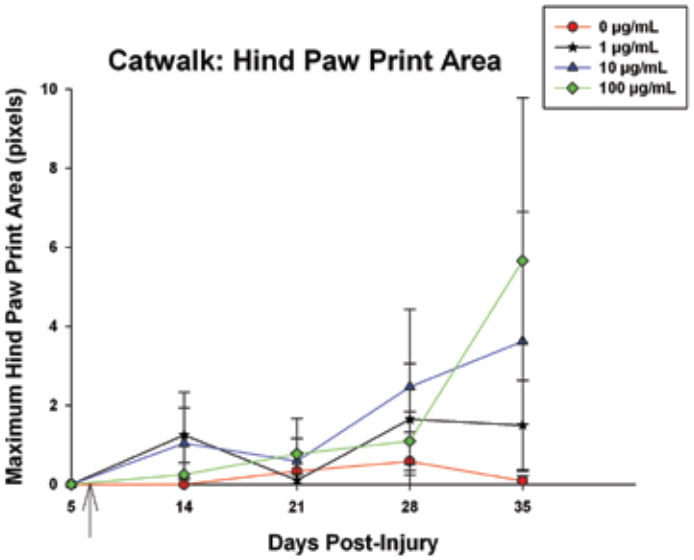


Figure 5: Post-SCI administration of SWNT-PEG produced a trend toward increased plantar placement of the hind paw in the Catwalk task.

The Catwalk™ gait analysis and integrated software analyzed fine kinematic paw placements and computes various measures such as hind paw print area. Kinematic analysis of maximal hind paw print area was conducted prior to SCI (data not shown), after SCI, and post-SCI administration of SWNT-PEG (indicated by arrow). The hind paw print area indicates the contact area of the plantar surface of the paw in stepping and larger print areas indicate more plantar contact. Animals that received the 100 µg/mL treatment showed a trend toward improvement in maximum paw print area at day 35 day post-injury, suggesting that treatment with 100 µg/mL of SWNT-PEG slightly improves plantar placement of the hind paw (n=8-9 per group).

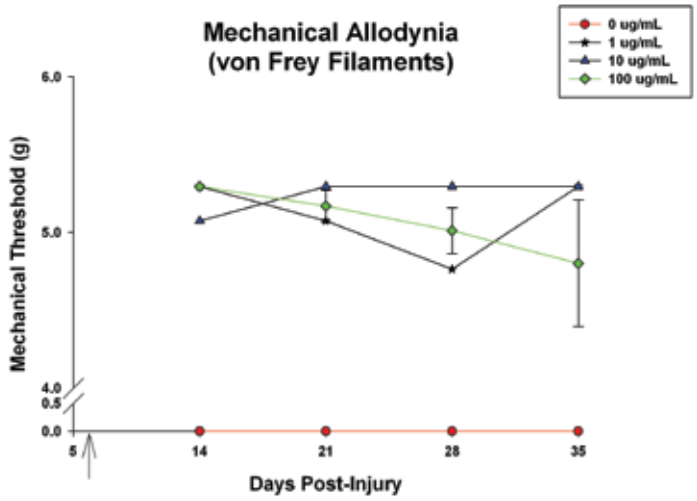


Figure 6: (bottom of previous page) SWNT-PEG treated animals showed recovery of sensation but no allodynia.

A non-noxious light touch of the plantar surface of the paw with von Frey filaments was used to assess hind limb sensation and mechanical allodynia. The withdrawal threshold in response to mechanical stimulation was quantified. Only animals with plantar placement on test day were evaluated. Pre-SCI values are indicated on day 0 and the post-SCI administration time (day 7) of SWNTs is indicated with the arrow. Rats treated with SWNT-PEG responded to a mechanical stimulus similar to the original response prior to injury (day 0) and greater than rats than received vehicle. No decrease in mechanical threshold over pre-injury assessment was observed suggesting no allodynia was induced (n=1-2 per group).

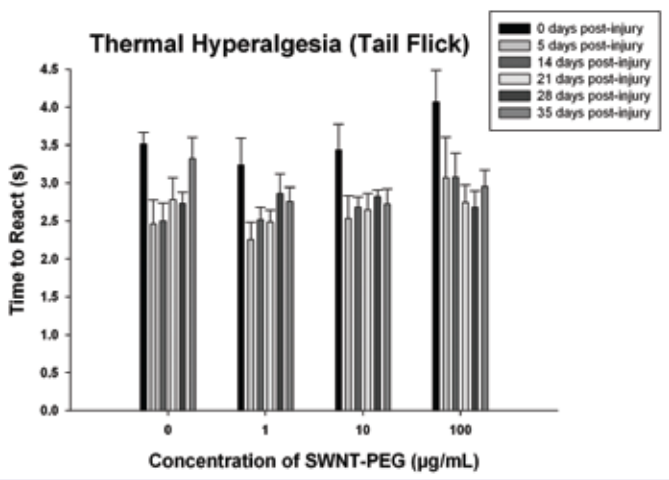


Figure 7: Post-SCI SWNT-PEG administration did not alter reaction to a noxious stimulus, hyperalgesia.

Sensitivity to a noxious thermal stimulus applied to the tail was assessed with reaction time. The latency to move the tail out of the heated light was measured before (day 0) and after SCI (days 14-35) and then weekly after administration of SWNT-PEG (post-SCI days 7, 14, 21, 28 and 35). After SCI, reaction time to the thermal stimulus decreased, suggesting that SCI caused hyperalgesia. Administration of SWNTs after SCI did not alter the response to thermal stimulus (n=8-9 per group).

jury is a change in reactive gliosis levels since this is a key element in scar formation and can limit the permissiveness of the graft (Nieto-Sampedro, 1999). We report that acute post-SCI administration of SWNT-PEG did not induce reactive gliosis.

After determining that administration with 10 µg/mL of SWNT-PEG was effective in increasing neurofilament positive fibers and did not cause an increase reactive gliosis, a more clinically relevant administration time point of one week was evaluated. Weekly behavioral analysis was conducted using the BBB test, Catwalk™ kinematic analysis, sensation with the von Frey filaments, and Tail-Flick assay. We found that very little functional recovery of hind limb locomotion occurred in animals treated with saline; however, all three SWNT-PEG treatment groups showed slightly better improvement. Although the increase in BBB score from a score less than 1 (control group) to a score of 3 (SWNT-PEG treated groups) is modest, this represents functional recovery from no observable movement in the hind limb to extensive movement of two joints of the hind limb, most often the hip and knee. These data suggest that SWNT-PEG improves hind limb locomotor recovery post-spinal cord injury, albeit modestly. In further kinematic analysis using the Catwalk™ gait analysis, we found that a treatment with 100 µg/mL SWNT-PEG induced a trend toward an increase in regularity index and hind paw print area in days twenty-eight and thirty-five days post-injury. This suggests that a treatment with 100 µg/mL of SWNT-PEG may improve hind limb locomotor recovery and may be the best dose for further exploration. In addition, because SWNT-PEG had not been tested *in vivo* after SCI, pain needed to be assessed before and after injury and treatment with SWNT-PEG. We evaluated the mechanical threshold using von Frey filaments before and after injury and treatment and there was no significant difference suggesting that treatment with SWNT-PEG does not induce mechanical allodynia. An important limitation of these data evaluating sensory response is that very few animals were included in the analysis as the test requires the ability to plantar place the hind paw. Since most animals did not regain a level of hind limb function that was sufficient for plantar placement, only a fraction of the animals used for other tests were evaluated. We also assessed the latency to move the tail after application of thermal stimulus before and after injury and treatment as well. We report that all four groups show the same trend of a decrease in latency immediately after injury and a gradual increase in latency following treatment. This data suggest that treatment with SWNT-PEG does not induce hyperalgesia.

In conclusion when taken together, these data indicate that SWNT-PEG, especially at a concentration of 100 µg/mL, improves axonal repair and regeneration into the lesion cavity and induces modest functional recovery, which suggests that this may be an effective substrate to promote axonal repair and regeneration after SCI. Future studies will include: analysis of tissue sparing and regeneration using immunohistochemistry on animals treated one week post-injury and evaluation of the administration of SWNT-PEG into a chronic SCI in a rat model. Additionally, the addition of growth factors and/or growth supporting cells may improve the extent of axonal repair and regeneration as well as functional recovery which makes it possible that SWNT-PEG may be an excellent candidate for a combinational therapy approach to repairing the injured spinal cord.

Acknowledgements

This study was supported by the UAB BioMatrix Engineering and Regenerative Medicine (BERM) Center Pilot Grant, the Center for Glial Biology in Medicine, and the Department of Physical Medicine and Rehabilitation. This study was also partially supported by the Ronald E. McNair Post-Baccalaureate

Achievement Program and the Wachovia Scholars Foundation Fund. Special thanks to Tracy Niedzielko for assistance with behavioral analysis.

Reference List

Basso, D. M., M. S. Beattie, and J. C. Bresnahan (1995). "A sensitive and reliable locomotor rating scale for open field testing in rats." J. Neurotrauma 12: 1-21.

Chaplan, S. R., et al (1994). "Quantitative assessment of tactile allodynia in the rat paw." J. Neurosci. Methods 53: 55-63.

Deakin, J. F. and J. O. Dostrovsky (1978). "Involvement of the periaqueductal grey matter and spinal 5-hydroxytryptaminergic pathways in morphine analgesia: effects of lesions and 5-hydroxytryptamine depletion." Br. J. Pharmacol. 63: 159-165.

Hamers, F. P., G. C. Koopmans, and E. A. Joosten (2006). "CatWalk-assisted gait analysis in the assessment of spinal cord injury." J. Neurotrauma 23: 537-548.

Malarkey, E. B. and V. Parpura (2007). "Applications of carbon nanotubes in neurobiology." Neurodegener. Dis. 4: 292-299.

Ni, Y., et al (2005). "Chemically functionalized water soluble single-walled carbon nanotubes modulate neurite outgrowth." J. Nanosci. Nanotech. 5: 1707-1712.

Nieto-Sampedro, M (1999). "Neurite outgrowth inhibitors in gliotic tissue." Adv. Exp. Med. Biol. 468: 207-224.

Silver, J. and J. H. Miller (2004). "Regeneration beyond the glial scar." Nat. Rev. Neurosci. 5: 146-156.

West, M. J., L. Slomianka, and H. J. Gundersen (1991). "Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator." Anat. Rec. 231: 482-497.

faculty interview: chemistry

An Interview with Dr. Jacqueline Nikles: Assistant Professor and UAB's Organic Chemistry Program Coordinator

Andrew Buie

While at UAB, undergraduates tend to associate specific courses with certain professors. Organic chemistry is no exception. When it comes time for a student to begin the organic chemistry sequence, one name stands out above the rest, Dr. Jacqueline Nikles.

Born and raised in Uniontown, Ohio, Nikles attended Marietta College, a small, private 4-year college in Marietta, OH. Marietta had an ACS approved chemistry tract with a degree requiring undergraduate research. While pursuing her undergraduate degree, Nikles did a research project on organometallic complexes. She also participated in experience for undergraduates (REU) at the University of Toledo during the summer between her junior and senior year. From her lab experience she realized her passion for chemical research, and she decided to earn a doctorate in chemistry. She attended Case Western Reserve in Cleveland, OH and did research on effect of micelles on the reduction of ketones. She earned her Ph.D. in physical organic chemistry in 1985. After completing her Ph.D. she did a post doc in the chemistry department at Rutgers University in New Brunswick, NJ. She did research on the effect of micelles on oscillating chemical reactions. She also did research with the medical school on the use of micelles to bind antibiotics to the surface of prosthetic devices.

Dr. Nikles and her family moved to Tuscaloosa in 1990 and

began working at UAB in June 2001 as assistant professor and coordinator of the organic chemistry program. She has been responsible for developing the organic chemistry curriculum, which includes both the lectures and the laboratories. Since her arrival, she has introduced a recitation section as a means of enhancing student learning and has also created an Honors Organic Chemistry course for qualified undergraduates that includes both lecture and lab.

I had the pleasure of being enrolled in Dr. Nikles' Honors Organic Chemistry lab. Centered on guided inquiry, this lab section allowed me to choose my own research project, giving me a sense of freedom. The students learn the skills needed to do research in organic chemistry. The capstone experience in the lab allows the students to choose a target molecule and propose a viable synthesis. Each group is charged with the task of ordering the proper chemicals and developing an experimental procedure that fits within the time constraints given. By devising such a project, students are allowed to get a feel for life in a research lab where one starts with nothing but a long-range goal and must devise a pathway to achieve that goal on their own with minimal instruction.

Dr. Nikles' husband, the other Dr. Nikles, is a chemistry professor at the University of Alabama. They collaborate on research focusing on targeted drug delivery using polymeric micelles. One of the options for cancer treatment is chemotherapy. However, the toxic drugs kill both normal and cancerous cells leaving a patient with side effects such as hair loss, weight loss, and a compromised immune system. Dr. Nikles and her husband are working to design a water-soluble package with targeting sensors on the surface of the micelles. These targeting micelles will deliver the drugs specifically to the cancer cells and will allow for the drug to be turned on and off at those particular sites. David Curiel in the Gene Therapy Center at UAB, along with some co-workers, have teamed up with Nikles and her husband on this innovative research project. Undergraduates also play an active part in the processes of the research. On several instances, their research has been brought to UAB to use certain equipment not available elsewhere.

The importance of participating in research is something that Dr. Nikles tries to instill in undergraduates considering research as a career option. "Research is not always glamorous, and sometimes things don't always work," says Nikles. She stresses the fact that students sometimes get the wrong idea about research when subjected to regular chemistry course labs where experiments are always designed to work. Research is about exploring the unknown. "There could be a period of 6 months in a graduate school lab where no results are obtained," she says, "and research experience as an undergraduate can help prepare you for these obstacles. One should be able to adjust and then subsequently readjust when the time comes for it." She warns students against taking the 5-year commitment plunge that is graduate school without having experienced firsthand how research relates to them. Dr. Nikles truly believes that students have a multitude of opportunities at UAB to determine if research is a future career option. "One can find plenty of things for undergraduates to do besides washing dishes," she points out. Overall, Dr. Nikles is pleased with the progress the UAB chemistry department has made in getting students involved in research. "Since Dr. Graves' arrival as Chair of this department, we have really stepped up the push to get students involved in labs earlier than junior year. Some are even starting as freshmen to gain experience in the laboratory." With this initiative, Nikles has high hopes for the future of undergraduate chemistry students.

As she continues to mold the organic chemistry program at UAB, Dr. Nikles will continue to relay the importance of undergraduate research to her students. Her educational research has yielded a successful program where undergraduates can get a taste of graduate school life, not to mention a love for working in the laboratory and the desire to pursue research as a career.

paper: chemistry

Microcrystal Analysis of Cocaine Hydrochloride and Added Adulterants

Altovise Broaden, Hannah Nelson, University of Alabama at Birmingham, Department of Justice Sciences

Abstract:

Microcrystalline tests were performed on samples of cocaine with 10, 20, and 50% adulterant. The tests were first performed with aqueous solutions of the reagents. The microcrystal tests were then done on powders to more closely simulate actual drug testing. Specific trends in the changes of the crystal morphology could be identified, making it possible to determine both the identity and the concentration of the adulterant.

Introduction:

Cocaine submitted into evidence at trials is usually between 60-70% pure. Caffeine, sugar, lidocaine, and procaine are commonly added to increase the bulk and the street value (1). Levamisole, a cancer drug and also a pig dewormer, is one of the most recent adulterants seen in cocaine submitted to crime labs. Because the identity of the adulterant is generally unknown, extraction for analysis in the crime lab can be difficult and time consuming. Without prior purification, microcrystal tests are complicated by alteration of the crystal formation due to the reaction of adulterants with the reagents (2). The objective of this project was to see if the trends in the changes of the crystal morphology could be linked to particular adulterants and their concentrations in the cocaine samples.

Microcrystal tests are highly developed chemical precipitation tests that use specific reagents and a polarizing light microscope to document the characteristic crystal formation of an unknown substance (3,4). These tests are specific and sensitive enough to detect even small quantities of a substance such as cocaine and eliminate false positive results that may result with simple color tests. In the analysis of controlled substances, microcrystal tests are a presumptive test used for screening unknown powders quickly. A positive microcrystal test for cocaine is followed by two confirmatory tests, generally, gas chromatography and mass spectroscopy.

One presumptive test for controlled substances uses gold chloride as a reagent to form a distinctive crystal morphology specific to the drug in question. Cocaine and gold chloride form cocaine hydroaurate characterized by cross-shaped crystals with branches perpendicular to the axes (2). However, the microcrystalline test depends on having a relatively pure sample of the illicit drug. The presence of impurities will affect the shape or morphology of the crystal making a definite identification difficult.

A study done by Swaitko *et al.* used three color tests (Wagner's, marquis, and cobalt thiocyanate) and two microcrystal tests to determine specificity for cocaine. They tested 20 drugs using the color tests. The color tests could not differentiate between cocaine and nine other compounds. With the gold chloride the microcrystal test, there were no false positives for any of the compounds examined (4).

A second study by Weilbo and Tebbett combined the microcrystal test and Fourier transformed infrared (FTIR). They used 19 reagents and determined that gold chloride was sensitive enough to detect micrograms of cocaine and is suitable for FTIR. The microcrystal test is quick, sensitive to a specific drug, and excellent for screening use (2).

Materials and Methods:

Stock solutions (2.50 mg/ml) were made for cocaine, sugar, and levamisole. Pure samples of each drug were then tested by adding 10 µL of the stock solution to a glass microscope slide followed by 10 µL of 5% gold chloride and 10 µL 20% acetic acid. The above procedure was then used on cocaine/adulterant mixtures with 10%, 20%, or 50% of sugar or levamisole added. For the preparation of 10% samples, 1 µL of adulterant/ 9 µL cocaine, for 20% samples, 2 µL adulterant /8 µL cocaine, and for 50% samples, 5 µL adulterant /5 µL cocaine. Each sample was repeated ten times.

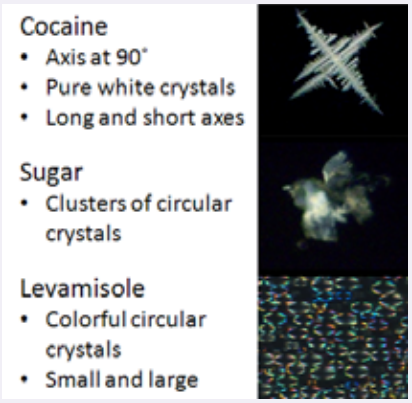


Figure 1. Crystal habit of cocaine, sugar, and levamisole

The microcrystal tests were then performed on solid samples with the adulterant in the same concentrations of 10, 20 and 50% concentrations. The mixtures were ground with a mortar and pestle. Then 10 µL of 5% gold chloride and 10 µL 20% acetic acid was added to a 2-4 mg of the solid mixture and crystal formation was observed.

Results:

The crystals formed from pure solutions of cocaine, sugar, and levamisole are shown in Figure 1. The characteristic crystal morphology of cocaine is two perpendicular axes with branching. The sugar crystals are circular, and the levamisole forms

small circular crystals with an X in the center.

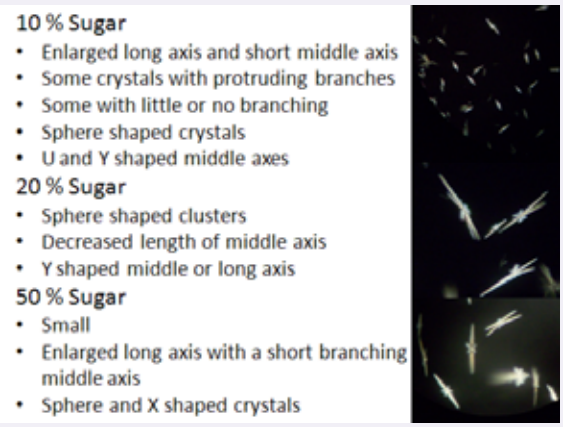


Figure 2. Crystal habit of cocaine with 10%, 20%, and 50% Sugar

The first adulterant studied was sugar (Figure 2). As increasing amounts of sugar were added to cocaine, the resulting crystals began to have increased branching on the long and middle axes. With 10% sugar, one axis was elongated, the branching became three dimensional, and the middle axis was deformed into either a Y or U shape. At 20%, small number of sphere shaped crystals appeared and the long axis began to deform. At 50%, the same trends were observed and the crystals were smaller.

At 10% levamisole, the cocaine crystals had varied shapes composed of multiple branching of different lengths. The middle axis took on an X or Y shape. Most of the crystals were spherical or shaped like an asterisk. With 20% levamisole, the branching increased on one or both axes. Sphere and asterisk shapes formed with protruding branches (Figure 3).

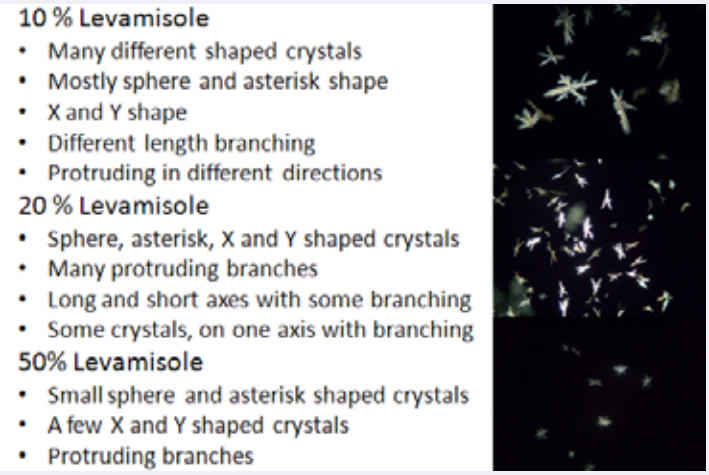


Figure 3. Crystal habit of cocaine with 10%, 20%, and 50% Levamisole

While the initial tests were performed on aqueous solutions of the cocaine and adulterants, in a forensic crime lab, the microcrystal tests are performed on street drugs that are in powder form. The microcrystal tests were repeated with powder samples to more closely emulate the actual tests done in the lab. Similar changes were observed in the powder samples (Figure 4).

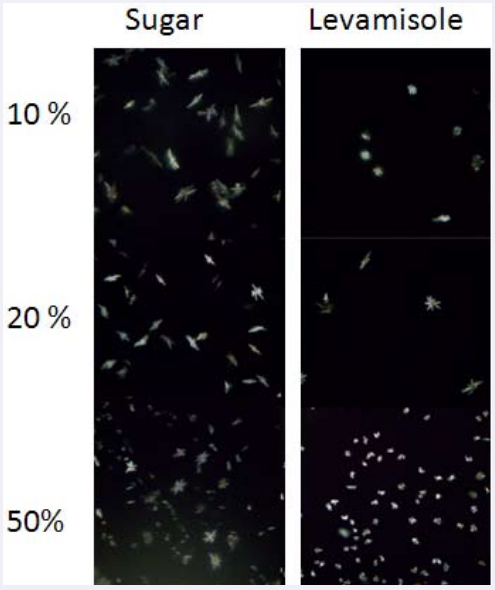


Figure 4. Crystal habit of powder samples of cocaine with levamisole and sugar

Conclusions:

The results indicate that the cocaine crystal morphology changes if an adulterant is present and the characteristics of the changes can be correlated to the type and amount of adulterant present in the sample. Sugar, as the adulterant, can be identified by X and Y-shaped middle axis and the concentration by the amount of branching on both axes, size, and spheres. Levamisole as the adulterant can be identified by variation of the crystal morphology and the concentration by degradation of the crystals and the amount of sphere, asterisk, and X-shaped crystals.

References

1. López-Artíguez, M., Cameán, A., and Repetto, M., (1995) Unequivocal Identification of Several Common Adulterants and Diluents in Street Samples of Cocaine by Infrared Spectroscopy, *Journal of Forensic Sciences,* 602-10.

2. Wielbo, D., and Tebbett, I.R., (1991). The Use of Microcrystal Tests in Conjunction with Fourier Transform Infra Red Spectroscopy for the Rapid Identification of Street Drugs. *Journal of Forensic Sciences,* 37 (4), 1134-1148.

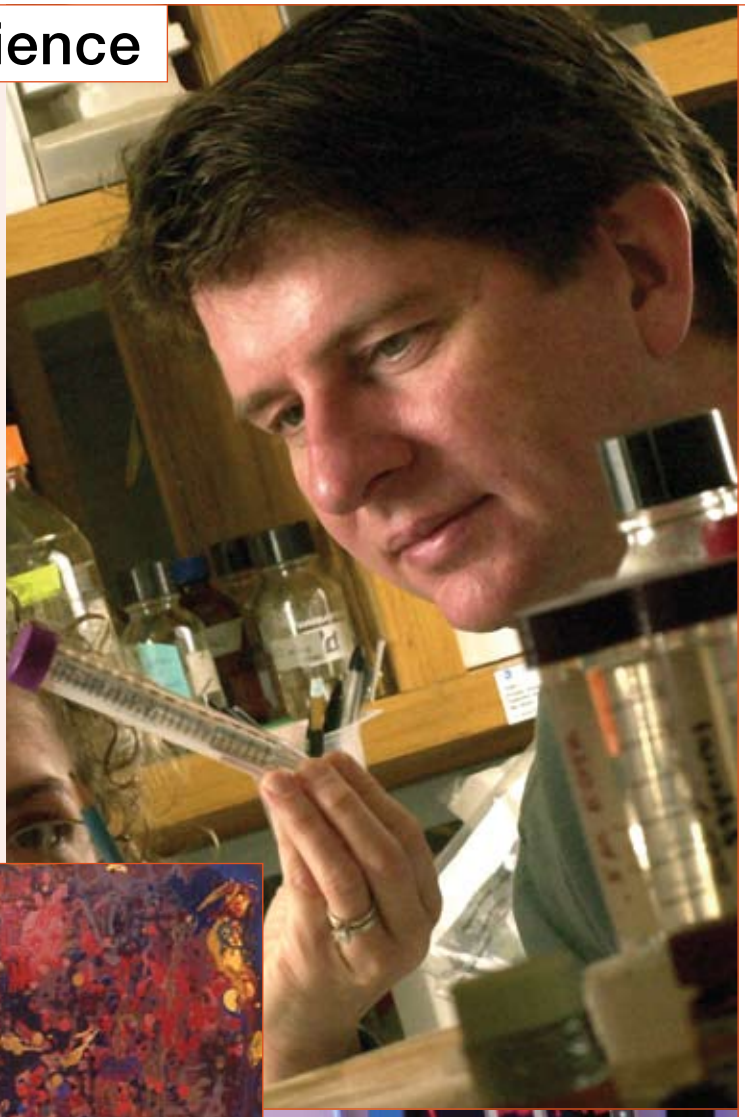
3. Fulton, C.C. (1969). *Modern Microcrystal Test for Drugs.* New York: Wiley Interscience.

4. Saferstein, R. (1982). *Forensic Science Handbook.* Englewood Cliffs: Prentice Hall.Swiatko, J., De Forest, P.R., and Zedeck, M.S. (2003). Further Studies on Spot Tests and Microcrystal Tests for Identification of Cocaine. *Journal of Forensic Sciences.* 48 (3), 581-585.

faculty interview: neuroscience

A Quest to Find a Cure for Alzheimer's Disease: *An Interview with J. David Sweatt, PhD*

Atbin Doroodchi

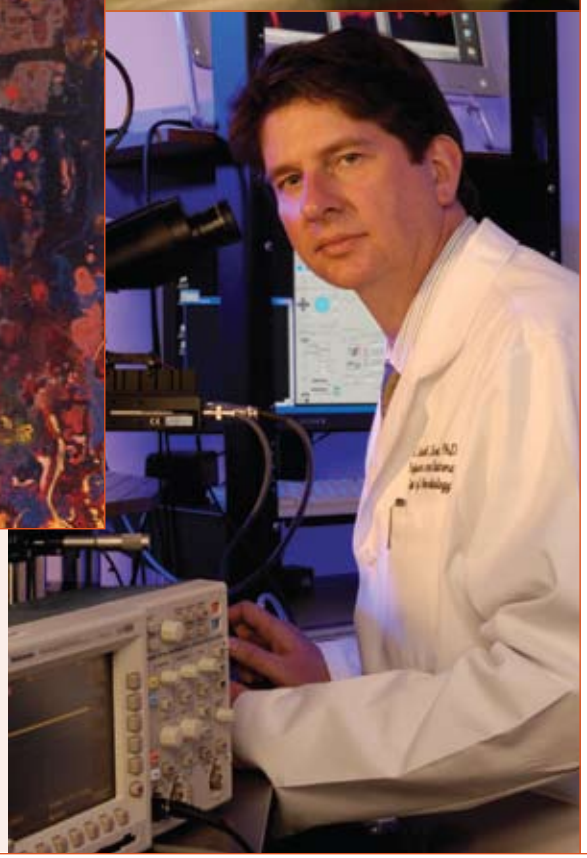


The day I went to interview Dr. Sweatt, my adrenaline content reached the maximum level. First, I couldn't believe I was given such an opportunity to interview a world renowned neuroscientist. It was like a dream come true for me. As I was walking down University Blvd, I looked at my watch and realized that I would be late, if I maintained the same pace. I started running like a hunted fugitive. As I arrived at Dr. Sweatt's office, I began worrying that I was underdressed with my casual, mildly rushed appearance. However, Dr. Sweatt's energetic greeting made me feel calm.

Dr. Sweatt is an internationally renowned neuroscientist at UAB. He joined the university three years ago as the chairs of the Department of Neurobiology and the Evelyn McKnight Brain Institute. Sweatt was recently featured in a program on PBS regarding his cutting-edge research on Alzheimer's disease. Outside of the laboratory, he is also an artist. However, Sweatt's research still shines through, as he enjoys drawing abstract paintings of neurons. A number of his paintings are currently on display in the Evelyn McKnight Brain Institute.

One of Dr. Sweatt's paintings

Dr. Sweatt, an Alabama native, began his illustrious career as an undergraduate Chemistry major and medical school-bound student at the University of South Alabama. To gain an edge over the competition for medical school, he started conducting research in Dr. Gene Palmer's drug synthesis and discovery laboratory at the University of South Alabama. Getting into a lab as an undergraduate was a struggle for Dr. Sweatt. Though he frequently heard "we don't have any positions available for undergraduates," he remained persistent. After continually approaching Dr. Palmer, he finally found a position and gained exposure to an entirely new career path.



While working in the lab, Sweatt realized that he was more interested in being a scientist rather than a doctor. It did not take long for him to reorient his focus and discontinue the pre-medicine track. To Dr. Sweatt, "being a scientist was much

cooler than being a doctor. I'd rather be in a lab with passionate scientists and discover interesting scientific information." After obtaining his B.S., he pursued graduate studies at Vanderbilt University and received his Ph.D. in Pharmacology. Sweatt's thesis was focused on "the mechanisms of chemical signaling." Basically, he was a "biochemist who was interested in pharmacology".

During our conversation, Dr. Sweatt mentioned an interesting fact about scientific research: the field makes the most boring topic in the textbook far more interesting. "Back in graduate school, lipids were the most boring materials to me, because my only exposure to them was through textbooks. However, when I did my Ph.D. thesis actually working on them in the laboratory, my opinion changed about them, and they became fascinating to me."

Looking beyond graduate school, Dr. Sweatt realized that his post-doc would ultimately determine his job. Therefore, he reasoned that his post-doc must be on "the most interesting subject in the world." Dr. Sweatt sat down and started asking himself, "What is the most interesting subject in the world?" To him and many others, understanding the mechanism of learning and memory clearly stood above the rest.

For his post-doc, Dr. Sweatt moved from the Deep South to Columbia University. He started working under the direction of Eric Kandel, M.D., who later went on to win the Noble Prize in Physiology or Medicine. Dr. Sweatt's lab work at Columbia was his first real exposure to neuroscience. Though an expert today, he admits that before Kandel's lab, he "never took a course on neuroscience before." However, Sweatt's dedication and intelligence propelled him to a top position as one of the most successful neuroscientists at Columbia University.

Following his post-doc, he received a job offer from Baylor College of Medicine, where he was subsequently appointed Director of the Neuroscience Program. After 17 years at Baylor, he returned to his home state and joined UAB as the Chairs of the Department of Neurobiology and Evelyn McKnight Brain Institute. While native to Alabama, Sweatt contends that the reason for moving to UAB was the strong and growing focus on neuroscience.

Currently Dr. Sweatt works on understanding the molecular and cellular basis of memory formation and learning. Another goal of his lab, albeit an audacious one, is to find a cure for Alzheimer's disease. Finding a cure for this mysterious memory loss is Dr. Sweatt's personal Holy Grail. He lost his mother to Alzheimer's disease, but is using the unfortunate event to fuel a personal quest to discover the elusive cure. He told me that "many scientists choose their research interests at least partly based on their personal experience, for example if they lost a loved one to a particular disorder."

...choose a topic that is interesting, whether it be probing the mechanisms of immune system or understanding the mechanisms of memory. Above all, "aggressively" pursue your interests and research.

Keeping his struggles to find a research lab as an undergraduate in mind, Dr. Sweatt has made a strong effort to change attitudes at UAB. As a result, he and the Department of Psychology established the Neuroscience Program at UAB. "The Neuroscience Program at UAB is one of the best of its kind in the nation, in which students receive the same quality education as they would receive at Harvard, Stanford and other elite colleges." In the Neuroscience Program, students will conduct research during their last two years of their undergraduate career, making them standout candidates for professional and graduate schools.

His advice to undergraduates who want to pursue research is to choose a topic that is interesting, whether it be probing the mechanisms of immune system or understanding the mechanisms of memory. Above all, "aggressively" pursue your interests and research.

Cognitive Abilities and Cortical Activity: a functional MRI Investigation of Figurative Language Comprehension

Sandhya L. Kumar & Rajesh K. Kana
Department of Psychology, University of Alabama at Birmingham

Abstract

Understanding the meaning of figurative language has been thought to be a challenging cognitive process, since it requires integrating what is said and what is implied. This process also depends on the salience of the figure of speech and sometimes a literal utterance also can be demanding, where a set of words string together to elicit a definite meaning. The main objective of this fMRI study was to identify the influence of cognitive abilities, specifically intelligence, on the brain activity associated with language processing, and to integrate the results with current neural models of language comprehension. Twenty-five healthy adults (divided into average and above-average groups based on IQ) read sentences, in the fMRI scanner, involving idiomatic phrases and control sentences presented visually in a blocked design format, and answered simple comprehension questions. Imaging data were acquired from a Siemens 3.0 Tesla scanner at the UAB Civitan International Research Center. The above average IQ group showed greater activation in the left orbitofrontal cortex and in the right medial prefrontal cortex while processing idiomatic sentences than literal sentences. The average IQ group showed greater activation in several right hemisphere areas, including the right inferior frontal gyrus, right superior parietal lobule, and right superior temporal gyrus. Overall, the results suggest that the average IQ group is facing more difficulty and are recruiting more right hemisphere regions while comprehending language.

Introduction

Traditionally, it has been thought that comprehension of figurative language is a challenging process, because it involves recognizing literal meaning, understanding the speaker's intentions, and integrating word meaning with context. However, some studies have challenged this idea and suggested that the comprehension of literal language may also be demanding since it involves deciphering a novel thought from a series of unique phrases. The main objective of this functional MRI study was to investigate the relationship between cognitive abilities, such as intelligence and brain functioning in the context of comprehending figurative speech, such as an idiom. Idioms are typically described as frozen phrases whose meanings are stipulated directly in a mental lexicon, and the speaker's meaning cannot be derived from an analysis of the words' typical meanings. Since idiom phrases are stored as single units with solitary meanings, sometimes they may be more easily accessible and comprehendible (Swinney and Cutler, 1979). Additionally, according to the Graded Salience Hypothesis (GHS), while comprehending figurative speech, the salient meaning is always initially accessed. The salient meaning is the meaning encoded in the mental lexicon, which can be either the literal meaning, or the contextually appropriate meaning, or both depending on the context (Giora, 2004). Salience is determined by frequency, conventionality, familiarity and prototypicality. Therefore, comprehending a familiar idiom phrase may activate less than comprehending a novel literal sentence. Taking into consideration the above mentioned hypotheses of language comprehension, we investigated the following questions: a) How does the brain process more salient figurative speeches? and b) What is the relationship between cognitive abilities, such as intelligence and cortical activity in the context of figurative language processing?

Methods

Twenty-five healthy right-handed adults (age range: 18-30 years, mean IQ: 110), with no history of psychiatric disorders, participated in this fMRI study. Data from these participants were assigned to two groups (average IQ: <115; and above average IQ: >115) based on their verbal IQ scores measured by the Kauffman Brief Intelligence Test, which assesses crystallized intelligence based on performance of verbal knowledge tasks and riddles. Sixteen participants were assigned to the Average IQ group (mean IQ: 102.5 ±7.1, 10 female, 6 male) and nine participants were assigned to the Above Average IQ group (mean IQ: 123 ±9.7, 3 female, 6 male). Participants were selected from the pool of Psych 101 students at UAB who received credit for research participation. Participants read literal or idiomatic utterances (presented visually) in the fMRI scanner and answered a comprehension question that followed each sentence by button press for "yes" or "no." The stimuli were presented in a blocked design format with 3 blocks for each experimental condition (each block had 5 sentences). The literal and idiom blocks were alternated in presentation. Examples of stimuli are included below. Five 24- second fixation periods during which participants fixated on a centered asterisk without performing any task provided a baseline measure of brain activation. Presentation of conditions was counterbalanced across all participants. The fMRI data were acquired from the Siemens 3.0 Tesla Allegra Scanner located at the UAB Civitan International Research Center (TR= 1000ms; TE=30 ms; 17 slices were acquired in an oblique-axial plane in an interleaved fashion). The fMRI data were analyzed by using Statistical Parametric Mapping (SPM2) (Wellcome Department of Cognitive Neurology, London). Images were motion corrected, realigned, normalized and smoothed in preprocessing. The preprocessed data were analyzed further using General Linear Model.

Example Idiom Condition Stimuli and Corresponding Equivalent Literal Condition Stimuli

Idiom: Once the teacher started speaking in the class, the students were all ears to her.
Literal: Once the teacher started speaking in the class, the students all paid attention.
Idiom: To understand and enjoy poetry, you need to read between the lines.
Literal: To understand and enjoy poetry, you need to be able to understand the intent of the author.

Results

Overall, both average and above average IQ participants activated language regions, such as the left inferior frontal gyrus and the left posterior superior temporal sulcus while comprehending idiomatic as well as literal sentences. However, there were statistically reliable group differences in brain activation while comprehending idiomatic utterances. Participants in the above average group showed significantly greater activation in the left orbitofrontal cortex (OFC) and in the right medial prefrontal cortex (MPFC) while processing idiomatic sentences (e.g. Tom is a great tour guide. He knows the city like the back of his hand) (see figure 1). The medial prefrontal cortex has been implicated in coherence processes in language comprehension for establishing the pragmatic connection (Ferstl and von Cramon, 2002). The above average IQ group may be making this connection more effectively than the average IQ group.

On the other hand, the average IQ group showed reliably greater activation than the above average group in areas, such as the right inferior frontal gyrus (pars triangularis and pars opercularis), bilateral middle frontal gyri, right superior parietal lobule, and right superior temporal gyrus (see figure 1). It should be noted here that the average IQ participants are recruiting mostly right hemisphere language regions for comprehending idiomatic utterances. A common pattern in language comprehension is to rely primarily on left hemisphere regions but incorporate bilateral regions in the right hemisphere when there is a high cognitive load.

When comprehension of literal sentences was contrasted between groups, the above average IQ group did not show significantly greater activation than the average group. However, the average group showed reliably greater activation in the left middle frontal gyrus and several right hemisphere sites, including the inferior frontal gyrus (pars triangularis and pars opercularis), middle and superior frontal gyri, precuneus, and superior parietal lobule (see figure 2). The average IQ participants seem to recruit several different regions in both hemispheres, especially the right, while comprehending both literal and idiomatic utterances.

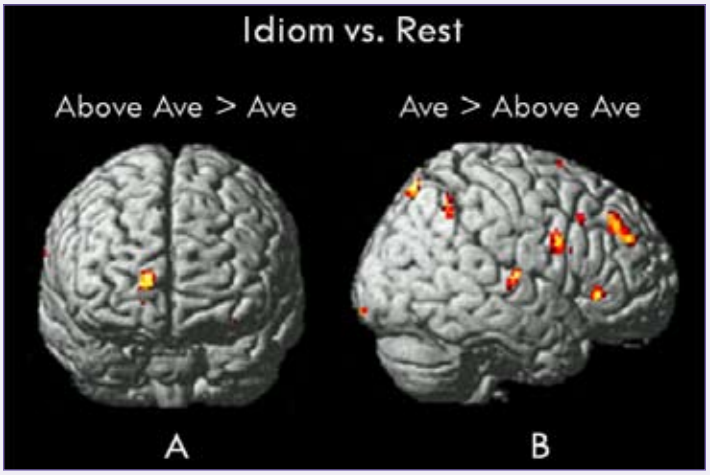


Figure 1: Group difference in brain activation while comprehending idiomatic utterances: A) increased activation in the above average group in medial prefrontal cortex, relative to the average group, and B) Increased activation in the average group in right frontal, temporal and parietal regions, relative to the above average group ($p < 0.005$).

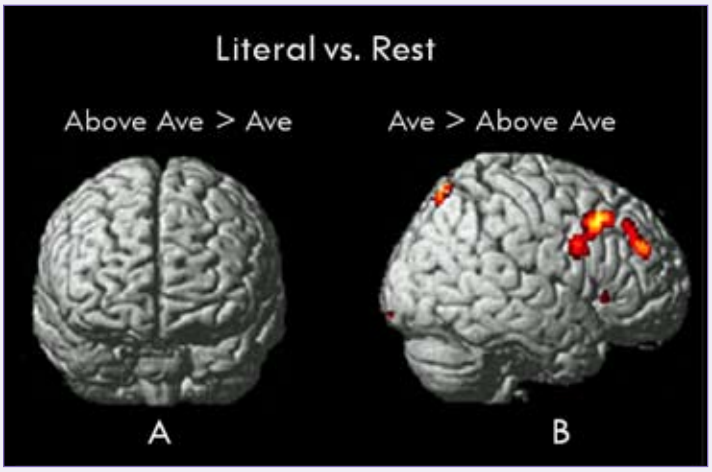


Figure 2: Group difference in brain activation while comprehending literal utterances: A) the above average group does not show greater activation, relative to the average group, and B) Increased activation in the average group in right frontal, and parietal regions, relative to the above average group ($p < 0.005$).

Discussion

The main finding is that the average IQ participants had more distributed activation and recruited right hemisphere regions significantly greater than the above average IQ participants while comprehending literal as well as idiomatic utterances. Considering the GSH hypothesis, this might indicate that both literal and idiomatic utterances may be less salient and posed more difficulty for the average IQ participants. This is especially evident in the recruitment of the right hemisphere homologues of Broca's (pars opercularis and pars triangularis) and Wernicke's (superior temporal gyrus) areas during both idiom and literal processing in the average IQ group. According to Mashal, Faust, Hender, and Jung-Beeman, (2008), specific right hemisphere regions, including the right precuneus, right

middle frontal gyrus, and right superior temporal gyrus, are involved in determining the non-salient meaning of idiom phrases. A recent study showed that activation in the right hemisphere homologue of Broca's area was correlated with higher behavioral accuracy in a language comprehension task (van Ettinger-Veenstra et al., 2009). Although the average IQ group performed as well as the above average IQ group in both idiom and literal utterance, this might be accomplished through a neural route that relies heavily on the right hemisphere.

On the other hand, the above average IQ participants showed greater activation, relative to average in the medial prefrontal cortex, only while processing idioms. MPFC activation has been found in the context of pragmatic comprehension, i.e., plausibility judgment (Bottini et al., 1994), reasoning (Goel, Gold, Kapur, and Sylvian, 1997), coherence judgment (Ferstl and Cramon, 2002), and self-referential processing (Gusnard, Akbudak, Shulman, and Raichle, 2001). In light of these previous studies, Jung-Beeman (2005) suggested the role of the MPFC in detecting, maintaining, or building coherent natural language representations. The medial prefrontal cortex also has a prominent role in regulating the cross talk between perisylvian language areas and that the coherence of activation in these areas is higher when a choice between 2 competing hypotheses is required, such as in the case of idiomatic sentences. The medial prefrontal area might mediate the inhibition of the alternative interpretation in favor of the correct one (Lauro, Tettamanti, Cappa, and Papagno, 2008).

Overall, the above average IQ participants may be processing figurative language through the typical neural route that emphasizes coherence and pragmatic integration. However, the participants in the average group may be accomplishing this through a network of areas distributed across both hemispheres. The resources needed for complex information processing may be easily accessible in the higher IQ group, whereas the average IQ groups may have to work harder to accomplish such tasks.

References

Bottini, G., Corcoran, R., Sterzi, R., Paulesu, E., Schenone, P., Scarpa, P., Frackowiak, SJ., & Frith, D. (1994). The role of the right hemisphere in the interpretation of figurative aspects of language: A positron emission tomography activation study. *Brain.*117(6). 1241-1253.

Ferstl, E.C. & von Cramon D.Y. (2002). What Does the Frontomedian Cortex Contribute to Language Processing: Coherence or Theory of Mind? *NeuroImage,* 17(3). 1599-1612.

Giora, R. (2004). On the Graded Salience Hypothesis. *Intercultural Pragmatics.* 1(1). 113-123.

Goel, V., Gold, B., Kapur, S., & Sylvian, H. (1997). The seats of reason? An imaging study of deductive and inductive reasoning. *NeuroReport.* 8(5). 1305-1310.

Gusnard, D.A., Akbudak, E., Shulman, G.L., & Raichle, M.E. (2001). Medial prefrontal cortex and self-referential mental activity: Relation to a default mode of brain function. *Proceedings of the National Academy of Sciences of the United States of America.* 98(7). 4259-4264.

Jung-Beeman, M. (2005). Bilateral brain processes for comprehending natural language. *Trends in Cognitive Sciences.* 9(11). 512-518.

Lauro, L.J.R., Tettamanti, M., Cappa, S.F., and Papagno, C. (2008). Idiom Comprehension: a prefrontal task? *Cerebral Cortex.* 18: 162-170.

Mashal, N., Faust, M., Hender., & Jung-Beeman, M. (2008). Hemispheric differences in processing the literal interpretation of idioms: Converging evidence from behavioral and fMRI studies. *Cortex.* 1-13.

Swinney, D. & Cutler, A. (1979).The Access and Processing of Idiomatic Expressions. *Journal of Verbal Learning and Verbal Behavior,* 18. 535-534.

Van Ettinger-Veenstra, H., Ragnehed, M., Hallgren, M., Karlsson, T., Landtblom, A. M., Lundberg, P. & Engstrom, M. (2009). Right-hemispheric brain activation correlates to language performance. *Neuroimage,* [Epub ahead of print].

Acknowledgements

The authors would like to thank Heather Wadsworth, Mark Pennick, Kathy Pearson, and Hrishikesh Deshpande for their help with data collection, data processing, and comments on an earlier version of the manuscript.

Obese Women with Greater Impulsivity Show Reduced Executive Function Brain Activation During Delay Discounting

Felix Kishinevsky, Dr. Rosalyn Weller

Abstract

Obesity is a serious public health issue. Obesity may be accompanied by abnormalities in executive function circuitry related to inhibitory control or impulsivity. A useful task for studying impulsivity is the delay discounting (DD) of money task, in which an individual chooses between immediate and delayed, but greater, amounts of money. Selecting mostly immediate choices is related to increased impulsivity. Studies using functional magnetic resonance imaging (fMRI) have shown that difficult vs. easy choices on the DD task produce more activation in executive function circuitry. We report results of an fMRI study in obese women that examined modulation of executive function brain circuitry by trial difficulty. We hypothesized that obese women who were more impulsive on DD would show less activation of their executive function system on difficult choices compared to less impulsive obese women. Obese (Body Mass Index or BMI > 30) women (n = 60) first completed a standard version of a DD task on a PC in the lab. An individualized fMRI version of the DD task was made for each magnet-eligible participant (n = 21) such that half the trials were difficult and half were easy. The task was presented in an event-related design using a Siemens Allegra 3 Tesla head-only magnet. Task difficulty was analyzed based on relative number of immediate choices and on reaction time. Based on useful fMRI data obtained from 12 individuals, we found that obese women who made more impulsive choices on the DD task had less activation of executive function regions of the brain when making difficult vs. easy choices than women who showed less impulsivity. Our results suggest that impulsivity, a possible risk factor for obesity, may stem from hypoactivation of brain regions mediating executive functions. Knowledge of brain structures that are working less effectively in obese individuals could inform drug or behavioral treatments for obesity.

Introduction

The increasing prevalence of obesity and the fact that is a major contributing risk factor for a variety of illnesses has pushed the issue of obesity to the forefront of healthcare concerns, with the CDC ranking obesity as the number one health threat facing America (Mokdad, Marks, Stroup, & Gerberding, 2004). Obesity currently ranks as the second leading cause of preventable death in the United States, killing 400,000 individuals annually at a cost of $122.9 billion (Mokdad et al., 2004; NIH: National Institute of Diabetes, 2008). Given that obesity has a complex and multifaceted etiology, any research into potential risk factors for the development of obesity is important (Baskin, Ard, Franklin, & Allison, 2005; Ogden et al., 2006; Wyatt, Winters, & Dubbert, 2006). Suboptimal decision making, resulting in greater impulsivity, may be one of the risk factors for developing obesity.

Current neurobiological models have suggested that drug addiction is accompanied by abnormalities in the brain circuits involving the executive function system (Goldstein & Volkow, 2002; Jentsch & Taylor, 1999), specifically involving brain areas mediating decision making or impulsive behavior. In an addicted state, the saliency of the substance of abuse (alcohol, tobacco, or illicit substance) is able to overcome inhibitory control. Without this inhibitory effect to stop the drive to seek drugs, a positive feedback system is established, where consumption of the addictive substance leads to increased saliency, increased activation of the reward pathway, a larger subjective feeling of reward and finally the need to continuously consume the addictive substance (Volkow, Fowler, & Wang, 2004).

While this model is best applied to addiction, some obese people may display a similar decrease in effective use of executive circuitry to exert inhibitory control of their appetite (Volkow & Li, 2005; Volkow, Wang, Fowler, & Telang, 2008; Wang, Volkow, Thomas, & Fowler, 2004). Cognitive impairments have been found in obese as compared to healthy weight adults, specifically with regard to executive function and the decision making process (Cournot et al., 2006; Cserjési Luminet, Poncelet, & Lénárd, 2009; Elias, Elias, Sullivan, Wolf, & D'Agostino, 2003; Li, Dai, Jackson, & Zhang, 2008). While performing the Iowa Gambling Task, a useful task for assessing the decision making process, obese individuals or those with a higher Body Mass Index or BMI made more bad decisions than those with a lower BMI (Davis, Levitan, Muglia, Bewell, & Kennedy, 2004; Pignatti et al., 2006). Obese women have been shown to have deficits on a measure of inhibitory control, the Stop-Signal Task, and obese children tend to behave more impulsively than their normal-weight counterparts (Braet, Claus, Verbeken, & Van Vlierberghe, 2007; Nederkoorn, Braet, Van Eijs, Tanghe, & Jansen, 2006; Sigal & Adler, 1976).

Delay-discounting (DD), a particularly useful task for tapping into impulsivity (e.g., Reimers, Maylor, Stewart, & Chater, 2009), is the extent to which an individual will discount the value of a future reward as a function of the delay to it. The more distant a future reward is, the more its subjective value decreases in comparison to a smaller yet immediate reward. Initially, most people would choose a large amount of money over a small amount of money. However, if a delay to the larger amount of money is introduced and gradually increased; at some point some individuals

will prefer the smaller reward. The individual's subjective value of the larger amount of money has decreased with the introduction of a delay; an individual's indifference point is the point at which a larger, delayed reward and a smaller, immediate reward have the same subjective value for an individual (Bickel, Odum, & Madden, 1999; Kirby, Petry, & Bickel, 1999; Mazur, 1987).

A measure of DD is obtained by determining multiple indifference points, using them to plot a curve and from that extrapolating a single value (k). The hyperbolic function that is used to describe DD (Madden, Begotka, Raiff, & Kastern, 2003) is:

$$V = A / (1 + kD)$$

V is the present value of the delayed reward A at a delay of D and k is a free parameter that determines the discount rate. A higher k indicates a steeper slope of the hyperbolic curve and a higher rate of discounting. An individual with a high k discounts the future more steeply than one with a lower k and can be thought of as being more impulsive; thus k can also be considered as an impulsiveness parameter (Madden et al., 2003). Another way of characterizing DD is to plot subjective value vs. delay and determine the area under the curve, which has been suggested as a theoretically neutral measure of discounting (Myerson, Green, & Warusawitharana, 2001). While the DD task can be administered using a variety of rewards, money is used most often, sometimes with real monetary rewards (Johnson & Bickel, 2002).

Research has shown that there are established links between high alcohol consumption, smoking, opioid dependence, various other substance use disorders, gambling, having ADHD, and higher rates of DD (Barkley, Edwards, Laneri, Fletcher, & Metevia, 2001; Bickel & Marsch, 2001; Bickel et al., 1999; Kirby et al., 1999; Madden, Petry, Badger, & Bickel, 1997; Mitchell, 1999; Monterosso, Ehrman, Napier, O'Brien, & Childress, 2001; Vuchinich & Simpson, 1998). For example, Vuchinich and Simpson (1998) found that college students who were identified as heavy drinkers had significantly higher discount rates, meaning that they were more impulsive, than light drinkers in hypothetical DD studies. Reynolds et al. (2004) found that adult smokers discounted more and were more impulsive than non-smokers. Kirby et al. (1999) found that heroin addicts discounted more steeply than control patients, and their discount rates were positively correlated with impulsivity. Previous research has also found that obese women display higher rates of impulsivity on the DD task than non-obese women, and that those with higher BMI's show greater impulsivity on a DD task (Reimers et al., 2009; Weller, Cook, Avsar, & Cox, 2008).

Executive function is usually attributed to areas including the prefrontal cortex (PFC), anterior cingulate cortex (ACC), superior parietal cortex (SPC), and inferior parietal cortex (IPC) (Gazzaniga, Ivry, & Mangun, 2002; Seeley et al., 2007). Several studies have been conducted to show a link between obesity and a deficit in these brain regions, as seen in those with substance abuse disorders (Volkow & Wise, 2005). Hypoactivation of the right PFC in obese individuals has been proposed as an explanation for overall poorer cognitive control of food intake (Alonso-Alonso & Pascual-Leone, 2007). Individuals with a high BMI are more likely to have smaller brain volumes and structural brain abnormalities in executive function areas such as the middle frontal gyrus (MFG) (Alkan et al., 2008; Gazdzinski, Kornak, Weiner, & Meyerhoff, 2008; Gunstad et al., 2008; Pannacciulli et al., 2006; Taki et al., 2007). As BMI increases, in otherwise healthy adults, a negative correlation between PFC and cingulate gyrus glucose metabolism emerges.

Functional Magnetic Resonance Imaging (fMRI) combines the high resolution anatomical images of an MRI with a way of indirectly measuring neural activity. Neural functional activity is measured using the "Blood Oxygen Level Dependent" (BOLD) effect, which correlates metabolic activity with neural activity. Activity in neurons causes increased blood flow which results in an increase in oxygenated (vs. deoxygenated) blood to the area of increased metabolic activity; the MRI scanner is able to detect deoxygenated blood due to its slightly magnetic nature. fMRI is a very useful neuroimaging tool due to its noninvasive nature and its very good spatial and fairly good temporal resolution.

Previous findings have established the validity of using fMRI analysis to examine the neural pathways that are involved in delay discounting and decision making. More difficult choices on the DD task produce greater brain activation in executive function structures in the prefrontal and parietal cortex than easier choices (McClure, Laibson, Lowenstein, & Cohen, 2004). Additional DD fMRI studies in those with a substance abuse disorder have suggested abnormal executive function activation when making difficult choices. Sober alcoholics showed reduced lateral orbitofrontal cortex (OFC) activation, whereas methamphetamine-dependent subjects showed lower activation in the ACC (Boettiger et al., 2007; Hoffman et al., 2008; Monterosso et al., 2007).

The present study is the first fMRI study of obese individuals using an executive function task. We hypothesized that obese women with higher impulsivity, as indexed by the DD parameter k, would show less activation of executive function circuitry during more difficult choices on the DD task than those with less impulsivity.

Methods

Participants

This research was approved by the UAB Institutional Review Board for Human Use. Participants were recruited using fliers posted around the UAB campus and in the *UAB Reporter*. Participants were obese (BMI > 30 kg/m^2) right-handed women between the ages of 18-50, without history of an addictive disorder or current use of psychoactive medication. Additional exclusionary criteria were being a cigarette smoker, pregnant or nursing, having a chronic health condition such as diabetes or past history of a serious medical condition, having a Shipley score < 85, show-

ing evidence of Axis I psychopathology or history of psychosis or active depression, having vision not correctable with contacts or the plastic eyeglass lenses available at the magnet (± 7 diopters), having ferromagnetic material in the body, history of loss of consciousness greater than 5 min., being claustrophobic, and exceeding the Siemens magnet bore (22.5" upper body width, 57-58" upper body girth) limits, which corresponded to weighing more than about 260 pounds.

Lab Session

Participants were screened in our lab prior to participating in the fMRI part of the study. While in the lab, participants had their height and weight measured to compute their BMI and completed questionnaires assessing IQ (estimated using the Shipley Institute of Living Scale, found to predict WAIS full scale IQ scores; Zachary, Paulson, & Gorsuch, 1985) and household income. Participants also completed an Eating Disorder Scale (EDDS) in order to screen for any eating disorders (Stice, Telch, & Rizvi, 2000).

A modified 27-choice version of the DD of money task (Kirby et al., 1999; Monterosso et al., 2007) was presented on a PC running Eprime v.1.2 software (Psychology Software Tools, Inc.). Choices were made using a button response pad similar to the one used in the magnet. The task consisted of 96 real choices (e.g., $20 now vs. $54 in 94 days) and 12 control choices (e.g., $0 now vs. $0 now). Control trials were needed to serve as sensorimotor controls for the fMRI version of the task, for future fMRI data analyses, and were given in the lab version simply to familiarize subjects with the procedure. The lab version of the task spanned a wide range of k's, in order to allow us to determine an individual k for each subject. Each trial was 11 sec. long, beginning with a fixation cross (+) presented for 2, 4, or 6 sec., followed by the two choices presented on the left or right side of the monitor (Fig. 1).

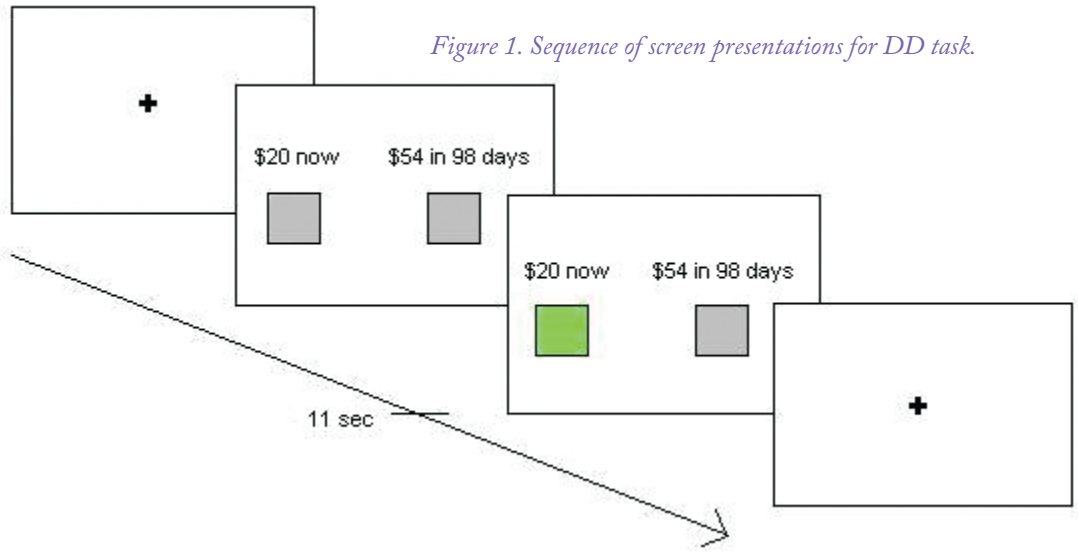


Figure 1. Sequence of screen presentations for DD task.

When the subject pushed the button for the left or right choice, the gray box under the choice turned green.

The DD task used was a real money task, and so, in addition to receiving money just for attending the lab session, each participant subsequently received half (lab session) or all (magnet session) of one of her choices , randomly selected, after the specified interval. Monetary choices ranged from $1 to $86, so the participant could receive between $.50 to $43 immediately or at a delay of 1 to 116 days (following procedures of Kable & Glimcher, 2007; McClure et al., 2004).

We used nonlinear regression to estimate each participant's k, with more impulsivity resulting in a higher k. This calculated k was then used to develop an individualized version of the DD task that would be used in the magnet session for that participant. Subjects were rejected if it became evident that they did not understand the task, were not paying attention, or resorted to using a rule to as opposed to assessing each trial independently during the lab session.

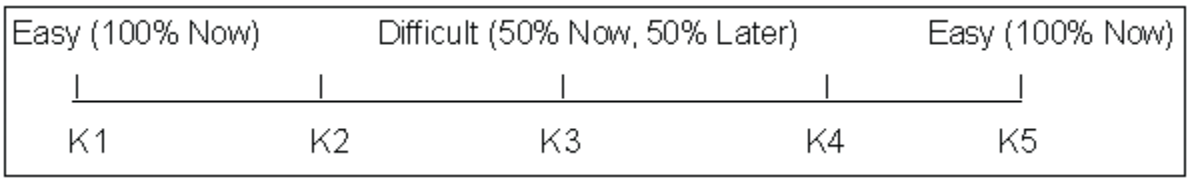
Magnet Session

Exclusionary criteria for participants subsequently used in the fMRI part of the study were that they did not make consistent choices during the DD lab task, their k was too low (> 0.002) or too high (< .05) for an individualized task to be developed for them, their Shipley IQ was < 85, or they had attributes that would prevent them from being safely scanned in the magnet. All women were scanned while in the follicular phase of their menstrual cycle.

An individualized version of the DD task was created for each participant's fMRI session consisting of 160 trials: 120 trials of five k categories and 40 control choices, divided into four blocks. The task was designed to ensure that there would be enough of each trial type; e.g., difficult, easy, immediate choice, later choice, for the fMRI data analysis. Across all of the available DD tasks, monetary amounts range from $0.37 - $78 for Now choices and from $29 - $86 for Later choices. Delays ranged from 1 - 116 days. The 120 real trials comprised five implied k (Imp-k) values. For all trials at a given Imp-k value, the Now and Later choices would have equal subjective values for a person whose value of k matched the Imp-k value (based on the equation Subjective Value = Later Value / [1+k*Delay]; Mazur, 1987). For each magnet participant, the Imp-k values spanned a range from well below to well above that person's lab k. The lowest and

highest Imp-k (K1 and K5) values represented trials intended to be easy for that person (large differences in subjective value between the Now and Later choices), and the intermediate Imp-k values (K2 and K4) represented trials intended to be more difficult (similar subjective values of the Now and Later choices). There was also a central Imp-k value (K3) that represented the most difficult trials (Fig 2).

Figure 2. Task difficulty on a k scale.



See text for details.

Task difficulty was measured using two measures: the percent of now choices when compared to later choices of equal subjective value (% Now), and reaction time or latency to button push. Some choices were easy, defined as being far from the subject's k, and others were difficult, defined as being close to the subject's k. The lowest and highest k categories represented easy trials, and the intermediate k categories represented difficult trials. Reaction time was defined as the period between the presentation of the two choices and the button push. A longer reaction time was considered an indication of a more difficult decision.

BOLD-fMRI data were collected from each participant using a Siemens Allegra 3 Tesla head-only magnet, IFIS visual display system, and MR-compatible response buttons. Functional MRI images were acquired using a single-shot T2*-weighted gradient-echo EPI pulse sequence. We used a TE of 30 ms, TR of 2.2 s, and a 70º flip angle for 30 axial-oblique slices 4 mm thick.

Obtaining useful data from a subject was dependent on her making consistent choices between her lab and magnet tasks and throughout her magnet task. If a subject's choices could be graphed and result in a hyperbolic curve with minimal interruption, then she was classified as consistent. If a participant appeared to have changed her level of impulsivity such that the individualized DD task was inappropriate for her (a "shifter"), then after the first block of trials, we paused the session and switched tasks, allowing us to choose a task that more accurately reflected the subject's k and would provide us with more useful choices. If a participant was later determined to have used a rule to answer all of the trials or was not paying attention, then her data were deemed unusable.

The fMRI data analysis was performed using the SPM5 software package (Wellcome Dept. Imaging Neuroscience, London, UK) run within Matlab (version 7.3; Mathworks, Inc.). Before the fMRI data could be analyzed, slice timing correction was applied as well as spatial preprocessing. The EPI images were first corrected for motion and then spatially transformed (normalized) into standard Montreal Neurological Institute (MNI) space, using a customized algorithm (Friston, Holmes, & Worsley, 1995). This transformation was then applied to the corresponding functional images, which were re-sliced into 2x2x2 mm resolution in MNI space. Finally, the images were spatially smoothed using a 6 mm FWHM Gaussian filter (Stoeckel, Weller, Cook III, Twieg, Knowlton, & Cox, 2008). Data sets in which movement before correction was greater than 2 mm in translational movement or 2˚ in rotational movement were excluded from analysis. The decision point used in the fMRI analysis for each trial was set at 500 ms before the button push.

fMRI data were analyzed using a Regions of Interest (ROI) approach, with ROI's being executive system structures that were expected to be activated more by difficult choices: gyral or lobular subdivisions of prefrontal (inferior frontal gyrus, middle frontal gyrus, superior frontal gyrus) and posterior parietal cortex (inferior parietal lobule, superior parietal lobule), anterior cingulate cortex, ACC, and lateral (Lat) orbitofrontal cortex (Boettiger et al., 2007; Hoffman et al., 2008; Monterosso et al., 2007). For the event-related analysis, trials were sorted into the k categories and these were used for the contrasts, Difficult > Easy choices. One analysis was used to validate that our DD task was tapping into executive function areas that were assumed to be more activated by greater mental effort. The other analysis was used to establish a correlation between executive function activation and impulsivity. Specifically, could we predict neural activation based on levels of k? Significance thresholds were $p < .001$, for the straight analysis of activation during difficult trials and $p < .05$ for the correlational analysis, with a minimum cluster size of 7 contiguous voxels.

A two-stage procedure was used for the statistical analysis of a mixed-effects design. At the first level, activation was computed for each individual subject on difficult > easy trials; difficulty was defined in terms of %Now, with trials closer to 50% now being more difficult, or in terms of reaction time (RT) activation, with longer reaction times being indicative of more difficult trials. For each voxel, SPM5 computed the contrast weights based upon the correlation between activation and reaction time. At the second level, these contrast weights were then entered into a random effects analysis to combine results at the group level. The correlation analysis was done using a conservative split-half analysis, in order to avoid strongly biased initial estimates of relationships between fMRI activation and log k (Vul, Harris, Winkielman, & Pashler, 2009; the log of k was used because the distribution of k was severely skewed, which is typical). First, areas of interest were defined based on negative correlations between difficulty-related

activity and ln k on half of the runs (e.g., runs 1 and 3). Then a standard correlation between log k and %Now activation was calculated to determine if a relationship existed between mean activity and impulsivity. Group data from runs 1 and 3 were used to identify a voxel within each ROI for which the difficult > easy contrast estimates showed the largest negative correlation with ln k. Using the MarsBar toolbox within SPM5, a sphere with a radius of 6 mm was constructed around the peak voxel within each ROI. The second level analysis of the correlation examined the difficult > easy contrast on runs 2 and 4, and then used MarsBar to calculate the average contrast estimate within each sphere for each subject. Correlations were then computed between these activations and ln k.

Results

Of the 21 participants run in the magnet, useful data using reaction time were obtained for n = 12 and for the %Now analysis, n = 10. Data from the other two participants were not used because of a lack of variability. Data from the other participants were not used either because these individuals were classified as "shifters" before correction procedures were implemented (n = 6), provided inconsistent responses that did not yield useful data (n = 1), appeared to use a shortcut or rule (n = 1), or had too much head movement (n = 1).

Table 1. Demographic Data

	Weight (lbs)	BMI (kg/m²)	Ethnicity
Magnet Session	197.38 ± 61.68	35.73 ± 3.97	5 AA (42%); 7 CA (58%)

n = 12, African American (AA), Caucasian American (CA).

Our first goal was to validate the use of our DD task by demonstrating that more difficult trials did produce greater brain activation in executive function areas, as had been found previously. We found that many executive brain areas did show greater activation on difficult vs. easy trials (Table 2). We also found that reaction time and %Now were convergent measures of task difficulty, in that analyses with both measures showed that more difficult choices produced greater activation in executive function brain areas than easy choices (Table 2). Reaction times for control trials, which served as sensorimotor controls, were on average one second. Reaction times for actual trials ranged from 2-4 seconds across subjects, with harder decisions have longer reaction times. We found greater activation on difficult choices as defined by %Now in the inferior (Inf.) frontal gyrus (Fig. 3A), Lat. OFC (Fig. 3B), middle (Mid.) frontal gyrus (Fig. 3C), anterior cingulate gyrus (Fig. 3D), and the inferior and superior parietal lobules. We found greater activation on difficult choices as defined by longer reaction times in the inferior and middle frontal gyri and the Lat. OFC.

Our other and main goal of the study was to show that level of impulsivity could predict executive system neural activation. Table 3 and Figures 4 and 5 shows brain areas that showed significantly less activation on difficult vs. easy trials, as defined by %Now and RT, associated with increasing impulsivity, presented as ln k, or a negative correlation (r). Higher k predicted less activation in the inferior and superior frontal gyri, anterior cingulate gyrus, and superior parietal lobule.

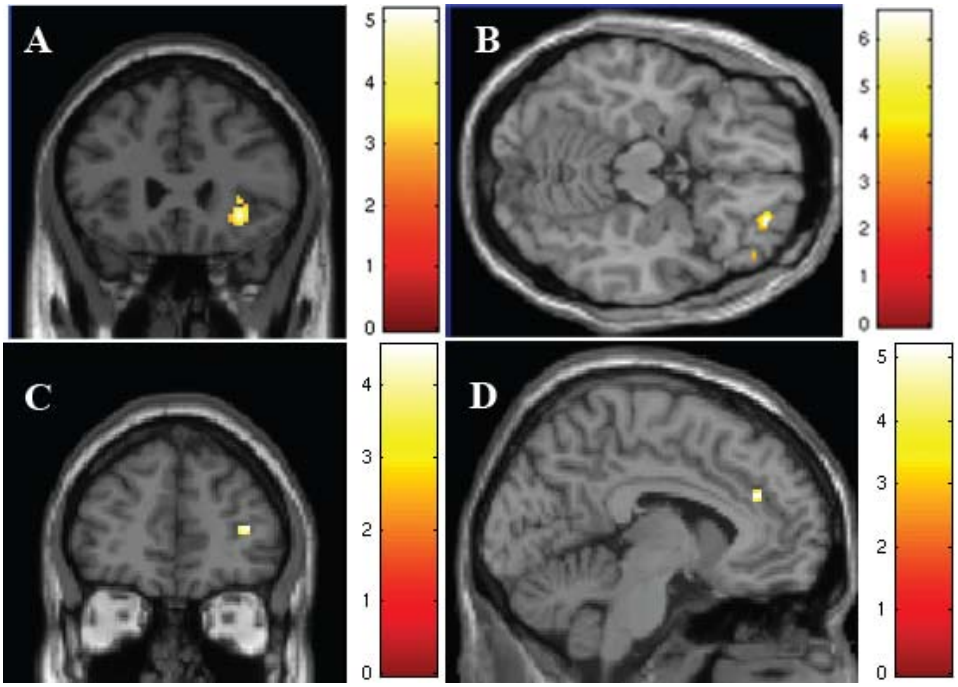


Figure 3 (left). Activation on difficult > easy contrasts.

(A) right inferior frontal gyrus, coronal view; (B) right lateral orbitofrontal cortex, axial view; (C) right middle frontal gyrus, coronal view; (D) left anterior cingulate cortex, sagittal view. Scale bar represents t-values.

Discussion

fMRI data collected to date from 12 obese women showed that our modified version of the delay-discounting of money task produced greater activation in executive function areas of the brain such as the inferior frontal gyrus and anterior cingulate cortex during difficult trials, compared to easier trials, mirroring the results of other comparable studies in normal or addicted individuals (Boettiger et al., 2007; Mc-

Difficulty Metric	ROI	Hem.	Cluster	t	x	y	z
% Now	Mid. Frontal Gyrus	L	7	4.17	-24	30	38
		R	11	4.55	38	46	10
	Lat. Orbitofrontal Cortex	L	22	4.81	-36	32	-8
		R	29	6.59	28	48	-16
	Ant. Cingulate Gyrus	L	9	5.2	-6	38	26
		R	7	4.08	14	22	28
	Inf. Parietal Lobule	L	16	5.84	-56	-38	36
		R	8	8.69	48	-30	32
	Sup. Parietal Lobule	L	7	4.27	-24	-74	46
		R	11	5.03	20	-72	44
RT	Inf. Frontal Gyrus	L	31	4.69	-32	20	-4
		R	83	5.5	32	26	-6
	Mid. Frontal Gyrus	L	15	4.73	-48	18	36
	Lat. Orbitofrontal Cortex	L	18	7.47	-40	48	-14

Table 2. ROIs with significantly greater activation on difficult > easy contrasts as defined by %Now and RT.

ROI, region of interest; Hem., hemisphere; x, y and z, Montreal Neurological Institute coordinates relative to the anterior commissure (AC) or AC-PC (posterior c) line; Inf., inferior; Med., medial; Sup., superior; Ant., Anterior; Lat., lateral. p <.001, at least 7 contiguous voxels. T- values based on t-tests

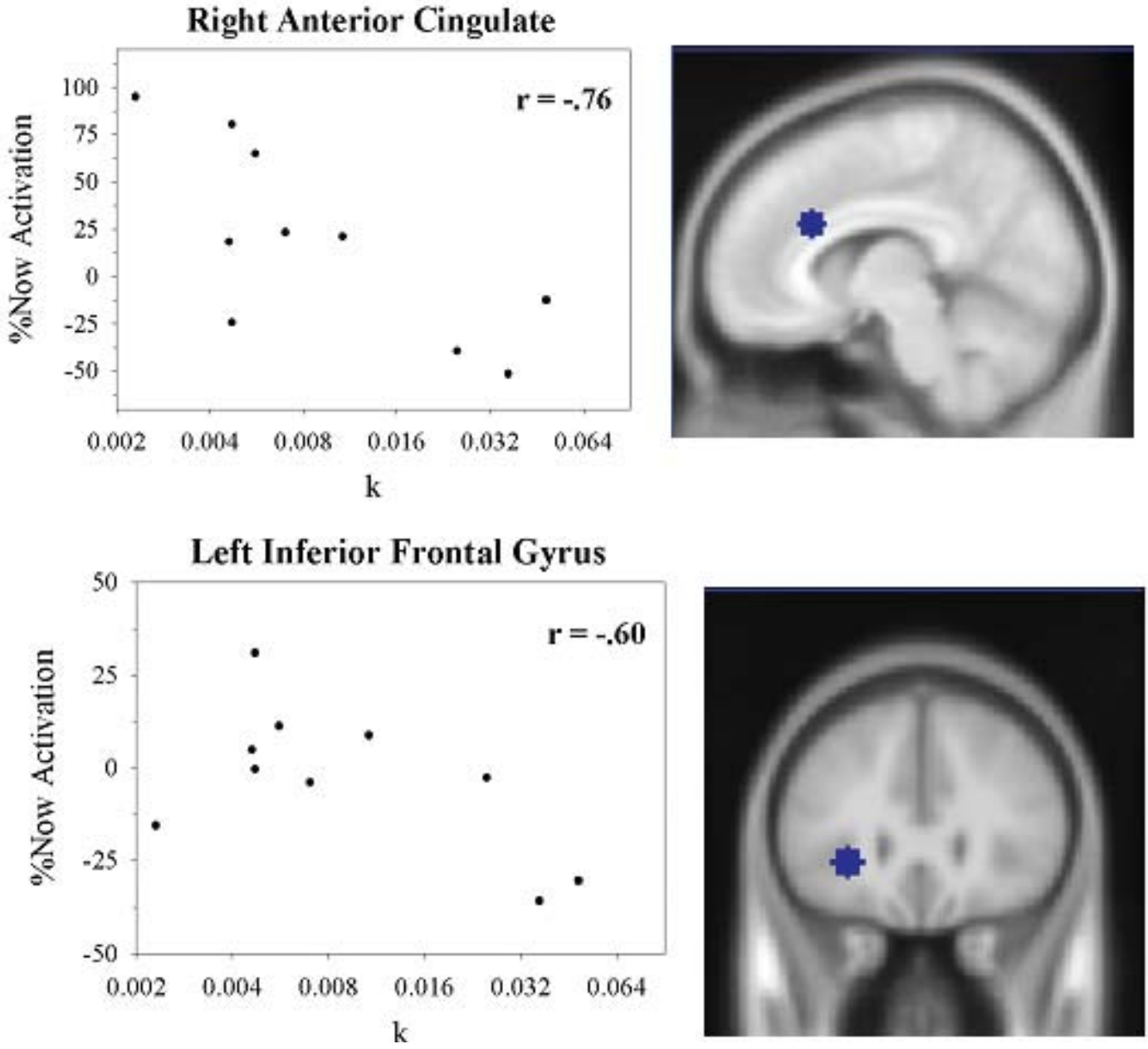
Difficulty Metric	ROI	Hem.	r	x	y	z
%Now	Inf. Frontal Gyrus	L	-0.59	-30	28	-2
	Sup. Frontal Gyrus	R	-0.56	28	46	30
	Ant. Cingulate Gyrus	R	-0.76	8	24	26
RT	Sup. Parietal Lobule	R	-0.59	24	-78	40

Conventions as in Table 1.

Table 3. ROIs with a significant correlation between task difficulty and impulsivity, difficult > easy vs. ln (k).

Conventions as in Table 2. Correlations computed using a split half analysis. p < .05, at least 7 contiguous voxels.

Figure 4. Difficult > easy contrast estimates plotted vs. k on a log scale and the corresponding brain ROI.

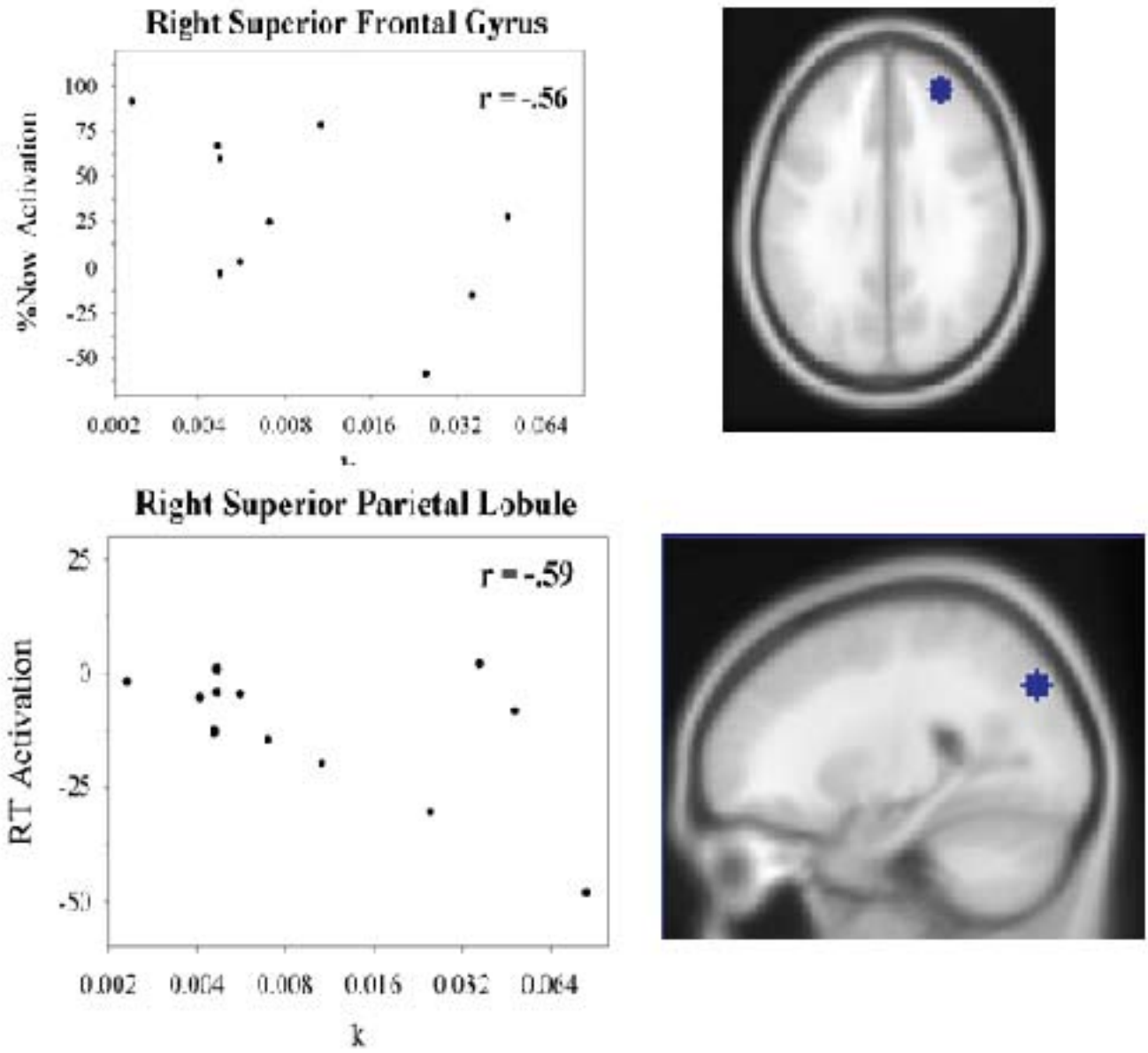


The graphs on the left illustrate the relationship or correlation between impulsivity (ln k) and activation on difficult vs. easy trials as defined by %Now for the brain ROI indicated, with the corresponding MR images on the right highlighting the MarsBar-created ROI used for the split-half analysis. Top right, sagittal view; bottom right, coronal view.

Clure et al., 2004; Monterosso et al., 2001). The two measures of task difficulty, reaction time and %Now, provided convergent results with regard to executive function activation. As a reminder, the delayed-discounting task has been shown to tap into impulsivity, and other research has established the relationship between greater impulsivity on the delayed-discounting task and higher BMI (Reimers et al., 2009; Weller et al., 2008). We also found, as hypothesized, greater impulsivity in obese women, as assessed by the discount parameter k on the delay-discounting task, was associated with less activation in executive system regions during difficult vs. easy choices.

Efficient executive control involves many processes, including planning, monitoring and correcting errors, multitasking, deciding between alternatives, and inhibition (Gazzaniga et al., 2002). A restatement of our results about deciding between alternatives, perhaps by using inhibitory control, is that less impulsive obese women showed greater activation of executive function cortex

Figure 5. Difficult > easy contrast estimates plotted vs. k on a log scale and the corresponding brain ROI.



Conventions as in Figure 4, except that the top right MarsBar-created ROI is shown on an axial view and the bottom ROI on a sagittal view.

during difficult vs. easy trials. One implication of this result is that self control utilizes executive function neural circuitry to a greater extent than does "giving in". This is exactly the result found in a recent fMRI study by Hare and colleagues (Hare, Camerer, & Rangel, 2009), who studied dieters or "self-controllers" vs. "non-self-controllers". When the self-controllers had to decide between choosing (for later consumption) tasty but unhealthy foods or neutral foods, they had greater activation in dorsolateral pre-frontal cortex, in particular, the left inferior frontal gyrus, during successful than failed self-control trials. The IFG was one of the ROI's we found to be less activated during difficult trials in the more impulsive obese women.

Inhibition is crucial in human behavioral control since it allows us to suppress automatic, impulsive, or routine behavior, and therefore avoid errors. However, when an error is made, detecting that error helps us to adapt our behavior appropriately so as to avoid further errors (Simões-Franklin, Hester, Shpaner, Foxe, & Garavan, 2009). The inferior frontal gyrus (IFG) has been implicated in inhibitory control, and is especially activated under conditions

of increased response competition. While some studies have suggested that it is mainly involved in inhibition of motor responses, others have shown that the IFG is also involved in non-motor inhibition and serves as a general purpose inhibition mechanism (Chambers et al., 2007; Swick, Ashley, & Turken, 2008). This is consistent with our findings of decreased activation in the right IFG during difficult vs. easy trials correlated with increased impulsiveness.

Another of the key areas associated with executive function is the anterior cingulate cortex (ACC). Amongst its many suggested roles, the anterior cingulate functions in inhibitory control over motivation (Allman, Hakeem, & Watson, 2002). It helps an individual recognize an error committed and to adapt and reduce future errors. Our results showed that difficult vs. easy trials produced greater activation of the left ACC, and increased impulsiveness was correlated with decreased activation in the left ACC during difficult trials.

Less efficient use of executive system regions may lead to decisions being driven more by other systems (i.e., reward), resulting in relatively more choices for immediate compared to delayed rewards, or more impulsive choices. Within the context of obesity, if executive function areas are functioning less efficiently, this could have a significant impact on diet and lifestyle choices, thereby increasing the risk of obesity. Knowledge of brain structures that are working less effectively in obese individuals could inform drug or behavioral treatments for obesity.

Acknowledgements
I would like to thank Kathy Avsar for making the button response pad used for the DD lab task, Luke Stoeckel and Matt Eddinger for helping run subjects in the lab and magnet sessions, Donna Murdaugh for running subjects and data analysis, Dr. Jim Cox, and the Civitan International Research Center for use of their facility and equipment. Support provided by a UAB/NSF ADVANCE grant to R. Weller and GCRC Grant #M01-RR00032.

Works Cited

Alkan, A., Sahin, I., Keskin, L., Cikim, A. S., Karakas, H. M., Sigirci, A., Erdem, G. (2008). Diffusion-weighted imaging features of brain in obesity. *Magnetic Resonance Imaging, 26*(4), 446-450.

Allman, J., Hakeem, A., & Watson, K. (2002). Two phylogenetic specializations in the human brain. *Neuroscientist, 8*(4), 335-346.

Alonso-Alonso, M., & Pascual-Leone, A. (2007). The right brain hypothesis for obesity. *JAMA, 297*(16), 1819-1822.

Barkley, R. A., Edwards, G., Laneri, M., Fletcher, K., & Metevia, L. (2001). Executive functioning, temporal discounting, and sense of time in adolescents with attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD). *Journal of Abnormal Child Psychology, 29*(6), 541-556.

Baskin, M. L., Ard, J., Franklin, F., & Allison, D. B. (2005). Prevalence of obesity in the United States. *Obesity Reviews, 6*(1), 5-7.

Bickel, W. K., & Marsch, L. A. (2001). Toward a behavioral economic understanding of drug dependence: delay discounting processes. *Addiction, 96*(1), 73-86.

Bickel, W. K., Odum, A. L., & Madden, G. J. (1999). Impulsivity and cigarette smoking: delay discounting in current, never, and ex-smokers. *Psychopharmacology, 146*(4), 447-454.

Boettiger, C. A., Mitchell, J. M., Tavares, V. C., Robertson, M., Joslyn, G., D'Esposito, M., Fields, H. (2007). Immediate reward bias in humans: fronto-parietal networks and a role for the catechol-o-methyltransferase 158Val/Val genotype. *J. Neurosci., 27*(52), 14383-14391.

Braet, C., Claus, L., Verbeken, S., & Van Vlierberghe, L. (2007). Impulsivity in overweight children. *European Child & Adolescent Psychiatry, 16*(8), 473-483.

Chambers, C. D., Bellgrove, M. A., Gould, I. C., English, T., Garavan, H., McNaught, E., Kamke, M, & Mattingley, J. (2007). Dissociable mechanisms of cognitive control in prefrontal and premotor cortex. *J Neurophysiol., 98*(6), 3638-3647.

Cournot, M., Marquie, J. C., Ansiau, D., Martinaud, C., Fonds, H., Ferrieres, J., et al. (2006). Relation between body mass index and cognitive function in healthy middle-aged men and women. *Neurology, 67*(7), 1208-1214.

Cserjési, R. t., Luminet, O. Poncelet, A.S., & Lénárd, L. (2009). Altered executive function in obesity. Exploration of the role of affective states on cognitive abilities. *Appetite, 52*(2), 535-539.

Davis, C., Levitan, R. D., Muglia, P., Bewell, C., & Kennedy, J. L. (2004). Decision making deficits and overeating: a risk model for obesity. *Obesity, 12*(6), 929-935.

Elias, M. F., Elias, P. K., Sullivan, L. M., Wolf, P. A., & D'Agostino, R. B. (2003). Lower cognitive function in the presence of obesity and hypertension: the Framingham heart study. *International Journal of Obesity, 27*(2), 260.

Friston, K., Holmes, A., & Worsley, K. (1995). *Statistical parametric maps in functional imaging: a general linear approach*: Wellcome Department of Imaging Neuroscience.

Gazdzinski, S., Kornak, J., Weiner, M. W., & Meyerhoff, D. J. (2008). Body mass index and magnetic resonance markers of brain integrity in adults. *Annals of Neurology, 63*(5), 652-657.

Gazzaniga, M., Ivry, R., & Mangun, G. (2002). *Cognitive Neuroscience, the Biology of the Mind* (2nd ed.). New York: WW Norton and Co.

Goldstein, R. Z., Volkow, N.D., (2002). Drug addiction and its underlying neurobiological basis: neuroimagine evidence for the involvement of the frontal cortex. *Am. J. Psychiatry*, 159:1642-1652.

Gunstad, J., Paul, R. H., Cohen, R. A., Tate, D. F., Spitznagel, M. B., Grieve, S., et al. (2008). Relationship between body mass index and brain volume in healthy adults. *International Journal of Neuroscience, 118*(11), 1582 - 1593.

Hare, T. A., Camerer, C. F., & Rangel, A. (2009). Self- control in decision making involves modulation of the vmPFC valuation system. *Science, 324*(5927), 646-648.

Hoffman, W., Schwartz, D., Huckans, M., McFarland, B., Meiri, G., Stevens, A., & Mitchell, S. (2008). Cortical activation during delay discounting in abstinent methamphetamine dependent individuals. *Psychopharmacology, 201*(2), 183-193.

Jentsch, D., Taylor, J. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology, 146*, 373-390.

Johnson, C. L., & Bickel, W. K. (2002). Within subject comparisons of real and hypothetical monetary rewards in deayed discounting. *J. Exper. Analysis Behav.,* (77), 129-146.

Kable, J. W., & Glimcher, P. W. (2007). The neural correlates of subjective value during intertemporal choice. *Nat Neurosci., 10*(12), 1625-1633.

Kirby, K., Petry, N., & Bickel, W. (1999). Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. *J. Exp. Psych. General, 128*, 78-87.

Li, Y., Dai, Q., Jackson, J. C., & Zhang, J. (2008). Overweight is associated with decreased cognitive functioning among school-age children and adolescents. *Obesity, 16*(8), 1809-1815.

Madden, G., Begotka, A., Raiff, B., & Kastern, L. (2003). Delay discounting of real and hypothetical rewards. *Exp. Clin. Psychopharmacol., 11*, 129-145.

Madden, G., Petry, N., Badger, G., & Bickel, W. (1997). Impulsive and self control choices in opioid-dependent and non-drug-using control participants: drug and monetary rewards. *J. Exper. Psychol.: Gen* (11), 129-145.

Mazur, J. E. (1987). An adjusting procedure for studying delayed reinforcement *The effect of delay and of intervening events on reinforcement value.* (pp. 55-73): Hillsdale, NJ, England: Lawrence Erlbaum Associates, Inc.

McClure, S., Laibson, D., Lowenstein, G., & Cohen, J. (2004). Separate neural systems value immediate and delayed monetary rewards. *Science* (306), 503-507.

Mitchell, S. H. (1999). Measures of impulsivity in cigarette smokers and non-smokers. *Psychopharmacology, 146*(4), 455.

Mokdad, A. H., Marks, J. S., Stroup, D. F., & Gerberding, J. L. (2004). Actual causes of death in the United States, 2000. *JAMA, 291*(10), 1238-1245.

Monterosso, J., Ehrman, R., Napier, K. L., O'Brien, C. P., & Childress, A. R. (2001). Three decision making tasks in cocaine-dependent patients: do they measure the same construct? *Addiction, 96*(12), 1825-1837.

Monterosso, J. R., Ainslie, G., Xu, J., Cordova, X., Domier, C. P., & London, E. D. (2007). Frontoparietal cortical activity of methamphetamine-dependent and comparison subjects performing a delay discounting task. *Hum. Brain Mapp., 28*(5), 383-393.

Myerson, J., Green, L., & Warusawitharana, M. (2001). Area under the curve as a measure of discounting. *J. Exp. Analysis Behaior* (76), 235-243.

Nederkoorn, C., Braet, C., Van Eijs, Y., Tanghe, A., & Jansen, A. (2006). Why obese children cannot resist food: the role of impulsivity. *Eat Behav., 7*(4), 315-322.

NIH: National Institute of Diabetes, D. a. K. D. (2008). Statistics Related to Overweight and Obesity. 2009, from http://www.win.niddk.nih.gov/statistics/index.htm

Ogden, C. L., Carroll, M. D., Curtin, L. R., McDowell, M. A., Tabak, C. J., & Flegal, K. M. (2006). Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA, 295*(13), 1549-1555.

Pannacciulli, N., Del Parigi, A., Chen, K., Le, D. S., Reiman, E. M., & Tataranni, P. A. (2006). Brain abnormalities in human obesity: a voxel-based morphometric study. *Neuroimage, 31*(4), 1419-1425.

Pignatti, R., Bertella, L., Albani, G., Mauro, A., Molinari, E., & Semenza, C. (2006). Decision making in obesity: a study in using the Gambling Task. *Eat. Weight Disord., 11*(3), 126-132.

Reimers, S., Maylor, E. A., Stewart, N., & Chater, N. (2009). Associations between a one-shot delay discounting measure and age, income, education and real-world impulsive behavior. *Personality and Individual Differences, 47*(8), 973-978.

Reynolds, B., Richards, J.B., Horn, K., & Karraker, K., (2004). Delay discounting and probability discounting as related to cigarette smoking status in adults. *Behav Process., (65),* 35-42.

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., Reiss, A., Greicius, M. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci., 27*(9), 2349-2356.

Sigal, J. J., & Adler, L. (1976). Motivational effects of hunger on time estimation and delay of gratification in obese and nonobese boys. *Journal of Genetic Psychology, 128*(1), 7.

Simões-Franklin, C., Hester, R., Shpaner, M., Foxe, J. J., & Garavan, H. (2009). Executive function and error detection: The effect of motivation on cingulate and ventral striatum activity. *Human Brain Mapping, [*DOI: 10.1002/hbm.20879].

Stice, E., Telch, C.F., Rizvi, S.L., (2000). Development and validation of the eating disorder diagnostic scale: a brief self-report measure of anorexia, bulimia, and binge-eating disorder. Psychol. Assess., 12, 123-131.

Stoeckel, L. E., Weller, R.E., III, Cook III, E. W., Twieg, D. B., Knowlton, R.C., & Cox, J.E. (2008). Widespread reward system activation in obese women in response to pictures of high-calorie foods. *NeuroImage,* (41), 636-647.

Swick, D., Ashley, V., & Turken, A. (2008). Left inferior frontal gyrus is critical for response inhibition. *BMC Neuroscience, 9*(1), 102.

Taki, Y., Kinomura, S., Sato, K., Inoue, K., Goto, R., Okada, K., et al. (2007). Relationship between body mass index and gray matter volume in 1,428 healthy individuals. *Obesity, 16*(1), 119-124.

Volkow, N. D., Fowler, J. S., & Wang, G. J. (2004). The addicted human brain viewed in the light of imaging studies: brain circuits and treatment strategies. *Neuropharmacology, 47 Suppl 1*, 3-13.

Volkow, N. D., & Li, T. K. (2005). Drugs and alcohol: treating and preventing abuse, addiction and their medical consequences. *Pharmacol. Ther., 108*(1), 3-17.

Volkow, N. D., Wang, G. J., Fowler, J. S., & Telang, F. (2008). Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philos. Trans. R. Soc. Lond. B. Biol. Sci., 363*(1507), 3191-3200.

Volkow, N. D., & Wise, R. A. (2005). How can drug addiction help us understand obesity? *Nat. Neurosci., 8*(5), 555-560.

Vuchinich, R. E., & Simpson, C. A. (1998). Hyperbolic temporal discounting in social drinkers and problem drinkers. *Experimental and Clinical Psychopharmacology, 6*(3), 292-305.

Vul, E., Harris, C., Winkielman, P., & Pashler, H. (2009). Puzzlingly high correlations in fMRi studies of emotion, personality, and social cognition. *In Press: Perspect. Psychol. Sci*.

Wang, G., Volkow, N., Thomas, P., & Fowler, J. (2004). Similarity between obesity and drug addiction as assesssed by neurofunctional imaging: a concept review. *J. Addict. Disorders, 23*(3), 39-53.

Weller, R. E., Cook, E. W., 3rd, Avsar, K. B., & Cox, J. E. (2008). Obese women show greater delay discounting than healthy-weight women. *Appetite, 51*(3), 563-569.

Wyatt, S. B., Winters, K. P., & Dubbert, P. M. (2006). Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. *Am. J. Med. Sci., 331*(4), 166-174.

Zachary, R. A., Paulson, M. J., & Gorsuch, R. L. (1985). Estimating WAIS IQ from the Shipley institute of living scale using continuously adjusted age norms. *Journal of Clinical Psychology, 41*(6), 820-831.

faculty interview: physics

Multidisciplinary Approach to Research: *An Interview with Dr. Bradley Newcomer*

Toral Patel

Mostly known as a scientist and professor to many, Dr. Bradley Newcomer, Ph.D. serves many important roles at UAB. Some of his current roles include being a professor in the Department of Clinical and Diagnostic Sciences; an Associate Scientist at Minority Health and Health Disparities Research Center (MHRC), UAB Diabetes Research and Training Center (DRTC), UAB Center for AIDS Research; and the Director of Experiential Learning Scholars Program. It may seem strange that Dr. Newcomer is involved with many centers of research both nationally and internationally, but his passion for magnetic resonance (MR) spectroscopy and motivating scientists is a unifying factor.

Dr. Newcomer began his scientific journey at Juniata College in Huntingdon, PN, where he received his Bachelor's in Physics. He continued his graduate studies at Wright University in Dayton, OH, where he pursued both a Master's in Physics and then a Ph.D. in Biomedical Sciences. As a generalist, Dr. Newcomer was eager to learn how everything works; so he decided to shift gears into Biomedical Sciences where his interests pertained to medical imaging and medical physics. Not only was he able to take medical sciences such as human physiology, anatomy, and cell biology, he was also able to take computer engineering and electrical engineering. Tailoring his classes to better customize to his dissertation and career goals gave him the opportunity to create his own interdisciplinary curriculum.

His research interests stemmed from the "thrill of the chase." Dr. Newcomer enjoys solving and identifying problems, critiquing current understandings, finding the holes and gaps in terms of unanswered questions, and determining a way to solve them. Dr. Newcomer admits that "As soon as I was done with one project, I would hand them onto someone else and begin a new one."

After finishing his doctoral work, Dr. Newcomer came to the University of Alabama at Birmingham to begin his post-doctoral fellowship by working on one of the world's largest MR machines at UAB. After working on MR spectroscopy, he was able to become a Professor at the School Of Health Professions to teach an MR curriculum.

But as a professor, Dr. Newcomer did not halt his curiosity. His current research projects include using photon and 31phosphorous spectroscopy to study lipid metabolism, body composition research, and muscle energetics. Dr. Newcomer uses proton MR spectroscopy to look at intramuscular fat and determine if fat is stored inside the muscle as triglycerides within the mitochondria or outside the cell in the muscle sheaths. This lipid metabolism is analyzed in terms of exercise training, diabetes, and/or obesity. MRI imaging is used to look at muscle mass changes in the elderly and pre and post- intervention to decrease muscle atrophy. He also uses 31phosphorous spectroscopy to analyze muscle's energetic and metabolic efficiencies in different patient populations. His work allows him to collaborate with investigators all over the United States as he assists in the planning and executing of experiments. For example, Dr. Newcomer collaborates with facilities in UT Southwestern in Dallas, TX, to do bed-rest experiments for NASA, studying muscle atrophy, metabolic changes, and mitochondrial function changes due to space flight. He also uses aging models for amino acid supplementation and is involved in mitochondrial myopathy studies.

Both the honors program and his research bring groups of people together with their own specialties to solve wide-scale problems in the real world.

What impact do these projects have on the world of science? Dr. Newcomer states that "In theory, if we really can create these research measurements and move them into clinical rounds, you are now getting to a point where you can non-invasively test individuals for specific anomalies since each patient is different." With these non-invasive tests, investigators are able to individually treat patients with tailored interventions for each underlying cause. He believes that science and medicine are trying to go forward to "individualized medicine" by using MR imaging techniques, proteomics, and genetic screening.

But how does his research affect his position as the director of the new honors program? The Experiential Learning Scholars Program and Dr. Newcomer's research have one commonality: both consist of an interdisciplinary, if not multidisciplinary, approach to solving problems. Both the honors program and his research bring groups of people together with their own specialties to solve wide-scale problems in the real world. The "Strength of UAB is its research facilities," and both Dr. Newcomer and the new program capitalize on this existing strength.

Dr. Newcomer would welcome undergraduate students to do research with him, but with his new position he has decreased his time he spends in the lab. However, with his extensive networking and collaborations around the country and UAB, he is always open to helping students find a position to experience undergraduate research. By viewing himself more as "a mentor and facilitator," Dr. Newcomer has opened up a new way of looking at research by not gearing it himself but acknowledging the teamwork effort. He believes that "In today's world, in today's scientific environment, you cannot do research in a bubble anymore or in your own lab. If you do it on your own, the costs of humans and those working with you is terrible. So it really matters in whether you value people and humanity or people as commodities and what they can bring in."

His selfless attitude is quite evident as he perseveres to excel students and other investigators in their careers and research. I hope that every UAB student can one day find what they enjoy and excel in their careers by helping others just as much as Dr. Newcomer does.

student feature

UAB's 2009 Goldwater Representatives

Shweta Patel

The national, prestigious Barry M. Goldwater Scholarship, founded in 1986, awards research-driven undergraduate students across the United States one- and two-year scholarships of $7,500 per year, covering tuition, fees, books, and room and board. Based on the students' academic accomplishments and their passion for scientific inquiry, 278 Goldwater Scholars were selected from a pool of 1,097 mathematics, science, and engineering students this year. Of these winners, two represented the University of Alabama at Birmingham (UAB): Aaron Neal (Goldwater Scholar) and Luke Stannard (Goldwater Honorable Mention).

Aaron Neal, a member of the Science and Technology Honors Program, is a senior pursuing a Biology major (Molecular concentration) and a Chemistry minor. Career-wise, Neal intends to specialize in infectious diseases, particularly tropical diseases and parasitology, with combined M.D./Ph.D. degrees. Conducting research at a top medical university, treating patients in endemic settings, and teaching at a graduate or medical student level all include Neal's aspirations.

Neal learned about the Goldwater Scholarship his freshman year from the Director of Graduate Fellowships Dr. Nelleke Bak and 2005 Goldwater Scholar Jessica Record. Listening to their advice, he decided to apply his sophomore year. According to Neal, "though I did not advance beyond the UAB nomination round my sophomore year, the following year, I had bolstered my application enough to successfully win the scholarship," demonstrating the strength of perseverance and determination.

UAB's 2009 Goldwater Honorable Mention winner Luke Stannard is a senior pursuing a degree in Mathematics. He is also a member of the University Honors Program. Working with the interface of mathematics and biology, Stannard has modeled the population growth of both the mosquito fish and the arm movement of the Antarctic brittle star. His long-term career goal involves work in designing and applying biomedical techniques.

Luke Stannard *Aaron Neal*

Research-oriented UAB has provided an outlet for competitive and qualified students to apply for the Goldwater Scholarship. In the past, eight UAB students have received this distinction; this year, Aaron Neal and Luke Stannard have added to this growing list of accomplished UAB students. Continuing in their footsteps, many qualified students will follow in the coming years to be Goldwater Scholars, as exemplified by Neal and Stannard.

inquiro staff

Chief Editors

Andrew Buie

Andrew Buie is a junior Chemistry major with a Spanish minor at UAB. He is a member of the Chemistry Fellowship and the University Honors Program. Andrew is a member of Alpha Epsilon Delta and AMSA and is currently the president of SAACS. He is also enrolled in the newly created Spanish for Specific Purposes Certificate program. In Spring 2010, Andrew will become a part of an interdisciplinary research project in the lab of Dr. Peter Prevelige. Upon graduation, Andrew plans on attending medical school at UAB in the fall of 2011.

Shweta Patel

Shweta Patel is a junior Biology major (concentration in Molecular Biology) and a Chemistry and Spanish minor. She is a member of the University Honors Program and the pre-health honor society Alpha Epsilon Delta. Currently, she is researching in the field of Cancer and Aging in Dr. Trygve Tollefsbol's lab for the Honors in Biology program. With a combined MD/PhD degree, her professional career goal is to teach and conduct research at a leading research university where scientific projects and clinical projects complement each other. Outside of academics, Shweta has an unyielding passion for intramural sports.

Staff Writers

Atbin Doroodchi

Atbin is a sophomore Biology and Mathematics double major. Atbin is a member of Science and Technology Honors Program, Math Fast-Track program; Alpha Lambda Delta honors society, Pittman Society, and Golden Key International honors society and is the president of Undergraduate Research Association. Atbin currently works as a student assistant in the Department of Neurology under the direction of Dr. Yuqing Li. He enjoys reading, swimming, cycling and running.

Danuel Laan

Danuel Laan has been a member of Inquiro Editorial Board for two years, and enjoys working with his peers in presenting their work. His scientific interests include cardiac dysfunctions and circadian rhythms. Whether working to increase urban high school student matriculation into college or serving as the first student on the Alabama Rural Health Association Board of Directors, Danuel has proven himself as a leader in the community. Dan was recently accepted to Medical school, and plans to pursue primary medicine as a career.

Natalie Mitchell

Natalie Mitchell is a junior molecular biology major. She is a member of the Science and Technology Honors Program and the Phi Sigma biology honor society. Natalie is currently working on her honors thesis research in the Department of Biology with Dr. Trygve Tollefsbol. In addition, she is a member of the UAB softball team. Following graduation in 2011, Natalie plans to attend medical school.

Matt Morton

Matt Morton is a senior Biomedical Engineering and Pre-medicine major. He is a member of both the Science and Technology Honors Program and the Tau Beta Pi engineering honor society. Currently, he is working on his honors thesis research in the Program in Epidemiology of Immunity and Infection with Dr. Richard Kaslow. After completing his degree in 2010, Matt plans to attend medical school and pursue his interest in either Infectious Diseases or Oncology.

Aaron Neal

Aaron Neal is a senior Science and Technology Honors Program student majoring in Biology. His primary research has been with with Julian Rayner, Ph.D., in the area of malaria vaccine development. In addition to conducting research at UAB and in the Peruvian Amazon, Aaron spent a summer researching MRSA at the Houston Medical Center. He is a 2009 Barry M. Goldwater Scholar and intends to pursue combined M.D./Ph.D. degrees in the area of infectious diseases, specifically parasitology. Aaron is also a teaching assistant for the Department of Chemistry and teaches third grade science classes at EPIC elementary school.

Ashruta Patel

Ashruta Patel is a junior majoring in chemistry with a concentration in Biochemistry and a Biology and Math minor. Ashruta currently works as a student assistant with Dr. Rita Cowell in Psychiatry and Behavior Neurobiology. She recently transferred from the University of Toronto, and hopes to make a decision between graduate school or professional school after completing her term as an undergraduate at UAB.

Toral Patel

Toral Patel is a Junior Biology major with a concentration in Molecular Biology and Chemistry and History minor. She is a member of the Science and Technology Honors Program and chair of its Peer Mentoring Program, as well as a member of the Alpha Epsilon Delta Honor Society and Phi Kappa Phi Honors Society. Her interests include traveling, physiology, and reading. Currently, she is working on her honors thesis in the Atherosclerosis Research Unit with Dr. David Garber. After completing her degree, her professional career goals are to attend medical school and become a translational clinician.

Courtney Sparkman

Courtney Sparkman is a junior majoring in Neuroscience at UAB. This is her second year on the Inquiro editorial review board. She works in Dr. Rita Cowell's lab in the department of Psychiatry and Behavioral Neurobiology. She is in the University Honors Program, Alpha Lambda Delta, Alpha Epsilon Delta, Alpha Omicron Pi sorority, and serves as a UAB Ambassador.

Celeste Stuart

Celeste Stuart is a freshman Biochemistry major. She is co-leader of the Experiential Learning Scholars Program and vice president of Blazer Hall. She is really involved in volunteer projects with ServeComm. In her free time she enjoys traveling, painting, and lap swimming. She plans to specialize in pediatric cardiology because she is passionate about making a difference in children.

Pratik Talati

Pratik Talati is a senior double major in Chemistry and Mathematics. His interests include reading, volunteering, and research. Currently, he works as a research assistant in Dr. Rita Cowell's lab in the Department of Psychiatry and Behavioral Neurobiology. He plans to enroll in a joint MD/PhD program with a PhD specialization in Neuroscience in Fall 2010.

Timmy Wang

Timmy Wang is a junior Biology major with a Chemistry Minor. He is a member of the University Honors Program, Alpha Lambda Delta, and the Early Medical School Acceptance Program. His hobbies are reading, watching movies, and playing tennis. After graduation, he plans on attending medical school.

2010 inquiro submission guidelines

Any student participating in scientific research at UAB is invited to submit a research paper for submission in the 2010 issue of Inquiro. Papers will be subject to student and faculty review.

The deadline for submissions in May 14, 2010; however, students participating in summer research at UAB or another institution are encouraged to submit by August 20, 2010.

Initial submissions should follow these guidelines:

1) 12 point font, double spaced, pages numbered with the author's name appearing in a header on every page (further formatting will be required upon acceptance).

2) Figures, tables, and graphs should be submitted in their original formats in the highest resolution possible as separate files. A .tiff file at 300 dpi is ideal.

3) All research papers should be submitted with the Inquiro Permission to Publish Form.

Staff also invites students to submit research narratives, interviews with faculty members, and science related editorials.

Short Reports: These reports are short papers derived from the text of science posters. Please convert the original poster to a Word document which includes all text, figures, tables, and images from the poster. As above, images should be submitted additionally as a separate file. The suggested length is 2,500 words.

Research Narratives/Other: If students would like to submit editorial or narrative pieces related to scientific research, they may certainly do so. The journal staff will review the article and consider it based on relevance and quality. The suggested length is 900 words.

Anyone who wishes to join the Inquiro staff should fill out the application on our website.

Pleases send submission or questions to sciencejournal.inquiro@gmail.com

For more information or to view previous publications, visit the website at www.uab.edu/undergraduate-research.

**Students retain all rights to their submitted work, except to publish in another undergraduate science journal. Inquiro is an internal university document of the University of Alabama at Birmingham.*

inquiro

acknowledgments

Without the help and support of UAB faculty and staff, the vision of Inquiro could not have been made a reality. Many thanks to the following individuals:

2009 Faculty Reviewers:
Dr. Charles Amsler
Dr. Asim Bej
Dr. Anne Cusic
Dr. Alan Eberhardt
Dr. Gary Gray
Dr. Gregg Janowski
Dr. Mickie Powell
Dr. Rusty Rushton
Dr. Cynthia Ryan
Dr. Mike Sloane
Dr. Alan Sprague
Dr. Trygve Tollefsbol

Dr. Mike Sloane and the University Honors Program
Dr. Diane Tucker and the Science and Technology Honors Program
Dr. Chris Reaves and the Office of Undergraduate Research
Amanda Childress, UAB Printing Solutions (production and printing)

A special thanks to Dr. Lowell Wenger and Dr. Charles Watkins, Dean and Associate Dean of the School of Natural Sciences and Mathematics for their continued support of this endeavor from the very beginning.

The production and publication of this journal was made possible through the funding supplied by the School of Natural Sciences and Mathematics of the University of Alabama at Birmingham.

inquiro staff 2009

inquiro

Volume 3 • 2009

UAB THE UNIVERSITY OF ALABAMA AT BIRMINGHAM