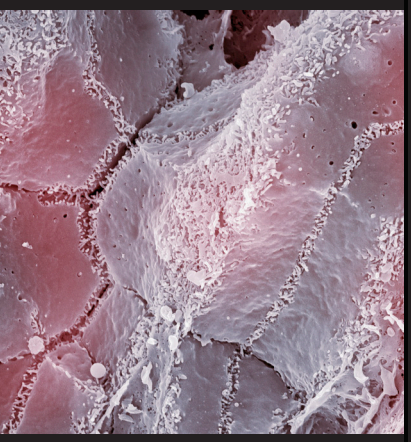
Volume 23 Spring 2012

MURJ

Massachusetts Institute of Technology
Undergraduate Research Journal

Cover Story: Implantable tissue-engineered liver p. 23



MURJ

Massachusetts Institute of Technology
Undergraduate Research Journal

Contents

UROP Summaries

Reports

Linn Hobbs
MIT Professor
Departments of Material Science and Nuclear Engineering
President, MIT Chapter of Sigma Xi

Massachusetts Institute of Technology
77 Massachusetts Avenue, Building 13-4054
Cambridge, Massachusetts 02139–4307
hobbs@mit.edu

May 2012

Dear MIT Community,

Forty-three years ago, MIT launched an exciting new endeavor, the Undergraduate Research Opportunities Program (UROP)—at the urging of (then) Associate Provost (later President) Paul Gray, prompted by the vision and support of Edward H. Land, founder of Polaroid, and under the initial zealous direction of the late Margaret MacVicar (later professor and subsequently Dean of Undergraduate Education). It was an inspired foundation: after all, at MIT, "Research ' ' Us." Today, three-quarters of MIT undergraduates take advantage of these research opportunities and experience the frustrations and elations of joining with other undergraduates, graduate students, post-docs and faculty in the pursuit of real-life research projects.

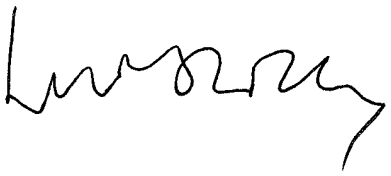
Scientific curiosity and technological investigation figure surprisingly early in the half-million year history of human endeavor, but in the United States a watershed date is 1886, when the Society of Sigma Xi was founded at Cornell, as an honor society for those pursuing scientific study (which, by definition, has always involved research, because that is how this system of "knowing" about our world functions). The new society paralleled Phi Beta Kappa, whose criteria then excluded those pursuing science and engineering disciplines. It was quickly apparent to the founding chapter that what set apart their scientifically active membership was a strong shared sense of companionship in pursuing their research endeavors—a sense of community still apparent today in the research groups our UROPers join. Indeed, the Greek motto adopted by the fledging organization, Spoudos Xynones, roughly translates as "Companions in zealous research." Graduate students at MIT, almost all of whom are pursing research degrees, have effective support structures (like the Graduate School Council, GSC) for socializing their research experiences. Yet, our thousands of undergraduate researchers do not, despite the striking commonalities in their fledgling forays into the research enterprise. The MIT Undergraduate Research Journal (MURJ) has for many years provided one of the few ways for a limited number of our undergraduate investigators to share at least the fruits of their research experiences with the whole MIT community.

Linn Hobbs
MIT Professor
Departments of Material Science and Nuclear Engineering
President, MIT Chapter of Sigma Xi

Massachusetts Institute of Technology
77 Massachusetts Avenue, Building 13-4054
Cambridge, Massachusetts 02139–4307
hobbs@mit.edu

Sigma Xi, the Scientific Research Society, has since grown to embrace more than 100,000 research professionals in science and technology in more than 500 chapters and club throughout North America and other parts of the world, wherever serous research is pursued, and publishes the wonderfully informative journal American Scientist. The MIT chapter has traditionally elected to membership undergraduate seniors and graduate students completing their research careers at MIT, a practice that provides little opportunity to promote a sense of community in just that part of our MIT community who are first learning about the excitement of research endeavor. This lacuna is particularly evident in the communities of our underrepresented minority students, who may have seldom encountered role models for research careers and, without peer support, may feel reluctant to approach faculty members for a UROP project. So, Sigma Xi at MIT has decided to change its modus and instead focus its membership recruitment, chapter activities and governance structure on MIT undergraduates, where they properly belong. An integral part of this effort is an agreement between Sigma Xi and MURJ to join forces, in a way that lets MURJ become the house organ for an active organization of undergraduate researchers and allied to a prominent national professional organization and publisher, through whom MURJ can exercise a deserved national leadership role in undergraduate research publication. This is an exciting alliance, and one that we hope will strengthen both the zeal and companionship that accompany the UROP experience, for many more of our undergraduate investigators.

Sincerely,



Linn Hobbs
Professor of Materials Science
Professor of Nuclear Engineering
President, MIT Chapter of Sigma Xi

Massachusetts Institute of Technology

UNDERGRADUATE RESEARCH JOURNAL
Volume 23, Spring 2012

Printed at Universal Millenium

PSB 07-02-0097

MURJ Staff
MIT Undergraduate Research Journal

Massachusetts Institute of Technology
murj.mit.edu

May, 2012

Dear MIT community,

We are thrilled to present to you the 23rd issue of the MIT undergraduate research journal. As newly appointed editors-in-chief, it has been an honor to work alongside such dedicated and intelligent staff to be able to present this issue to you today. The outstanding work of the undergraduate researchers in this issue remind us of their unlimited potential but also the necessity of a medium through which to communicate their work.

The topics in this issue range from a look at Biomimetic Liver Scaffold with Intrinsic Microvasculature, featured on the cover of this issue to a reexamination of the “Southwest Effect", and even include an interview with the Nobel prize winning scientist Peter Diamond. As the new editors-in-chief, it is our desire to lead MURJ to the forefront of student research by publishing engaging research articles but also featuring the talents of our staff writers through articles on interesting and controversial topics in research.

Additionally, we are honored to collaborate with Sigma Xi in order to further the betterment of undergraduate research. Research is a field that is only possible through collaboration and this alliance will allow us to better serve the undergraduate research population. The guidance that we have received from Dr. Hobbs has been indispensable and we look forward to the partnership to help guide future

Massachusetts Institute of Technology

UNDERGRADUATE RESEARCH JOURNAL
Volume 23, Spring 2012

PSB 07-02-0097

issues of MURJ.

We would like to thank all of the student authors and contributors for their work and hope you enjoy this issue of MURJ.

Best,

Elizabeth Bearrick (Co-Editor-in-Chief)

Ana Lyons (Co-Editor-in-Chief)

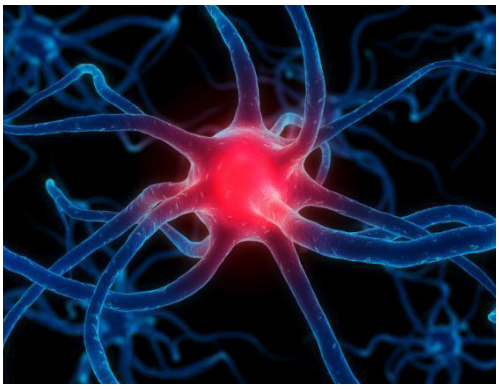
Sarine Shahmirian (Co-Editor-in-Chief)

Science News In Review

NEUROSCIENCE

High fat diets and nerve cell production

Are high fat diets necessarily bad for you? A study undertaken by researchers at the Johns Hopkins University School of Medicine suggests that high fat diets may affect the production of nerve cells in the brain, which may in turn be sending signals for weight gain.



Nerve cells conduct electrical signals, information sent to and from the brain that direct physical and biochemical functions.
Credit: http://medicalpicturesinfo.com/wp-content/uploads/2011/09/Nerve-Cell-4.jpg

Mice that were fed a fatty diet showed increased rates of nerve cell production in the median eminence, an area of the brain that is important in collecting hormones secreted by the hypothalamus before they are sent to the anterior pituitary. Mice on the high fat diet also showed increased weight gain and decreased levels of activity.

Seth Blackshaw, a neuroscientist from Johns Hopkins, and his team of researchers then proceeded to shut off nerve cell production in the mice by targeting the brain with a laser. The mice in which nerve cell production was halted but were still fed a high fat diet showed increased levels of activity and did not gain weight as quickly as the ones who were still producing nerve cells. This suggests that the newly formed nerve cells have an effect on weight gain. It is still unknown whether a similar observation can be made in humans, but further study could help in the fight against obesity.

—J. Sanchez

Source: http://www.sciencenews.org/view/generic/id/339458/title/Fatty_diet_leads_to_fat-loving_brain_cells

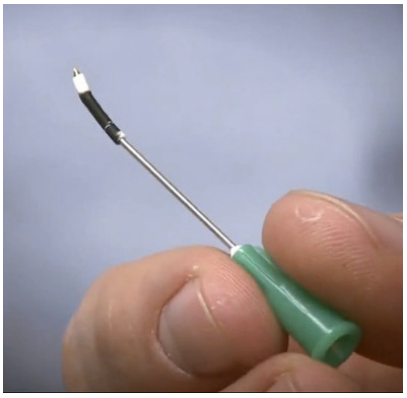
HEALTH

LSD may have useful therapeutic benefits

According to studies published in the late 1960's and early 1970's, lysergic acid diethylamide (LSD), an infamous psychedelic drug, can potentially be utilized as a novel therapy for alcoholism. Compiled by Norwegian scientists Teri Krebs and Pål-Ørjan Johansen, results from these studies show that, of the 536 participants in the clinical trials, 59% of people receiving LSD treatment reported lower levels of alcoholism compared to 38% of patients on placebo.

Before political pressures against such drugs ended their work, psychiatrists in the 1950's would prescribe psychedelics, drugs that alter cognition and perception, in order to treat a range of medical diseases, including schizophrenia and alcoholism. Today, modern researchers are now considering using this previously documented effect of certain illicit drugs to treat a variety of conditions, including post-traumatic stress disorder, drug and alcohol dependency, and smoking addiction.

The exact mechanism of how these illicit drugs work is currently unknown. It is possible that psychedelics may act like serotonin, a neurotransmitter linked to mood and pleasure, binding to the same receptor sites. However, what happens downstream of binding can vary greatly, suggesting that much can also be learned by studying behavioral responses. In fact, current work suggests that psychedelics work at both a psychological and biological level.



Capsules that, when embedded in your skin, would release medication appropriate to any sickness you may have.
Credit: : http://tech.li/wp-content/uploads/2012/02/Biocapsule.jpg

—C. Huang

Source: http://www.nature.com/news/lsd-helps-to-treat-alcoholism-1.10200

NASA Biocapsules

The NASA Biocapsule is like a doctor implanted under your skin, able to diagnose and treat effectively. A seemingly insignificant and tiny little bundle of carbon, these nanotubes essentially provide healthcare for astronauts, a miraculous breakthrough that could potentially save millions of lives.

So how does it all work? A number of NASA Biocapsules are implanted under the astronaut's

Chemicals in the environment as instigators of weight gain

The popularly accepted energy balance model of "calories in, calories out" may not be enough to explain weight gain or weight loss. New research suggests that there are industrial chemicals that may have an effect on the body's metabolism, changing how it responds to caloric intake.

Pollutants, such as pesticides are commonly used for organism control and sanitation.
Credit: http://digitaljournal.com/img/8/9/9/i/4/5/7/0/Pesticide.jpg

University of California, Irvine's Professor Bruce Blumberg's work studies the effect of organic pollutants on the body's metabolism. These pollutants involved in the manufacture of plastics, and used as fungicides, pesticides, slimicides, wood preservatives, and marine antifouling agents, are coined "obesogens" and may cause animals to have bigger and more fat cells.

One particular study showed that animals treated with these chemicals gained significantly more weight than the control animals, even when fed the same diet. Another study conducted at Princeton University showed found that a rats with a diet containing high fructose corn syrup gained more weight than rats drinking sugar water. However, on the other hand, another study published in The American Journal of Clinical Nutrition suggests that only the net number of calories consumed that matters in terms of weight gain.

There is a predominant belief that the wide range of substances in our food supply does have an effect on weight gain. A review of the literature published in The Obesity Epidemic states that although there has been an increase in obesity, there is no concrete evidence that people are eating more food. Additionally, although the amount of calories consumed by people of different races, and socioeconomic classes is relatively constant, obesity tends to be higher among the poor, African Americans, and Latinos.

This research suggests that there is another mechanism causing weight gain; the author refers to the possibility of environmental factors including obesogens. However, this is just one possible explanation, and the science behind weight gain is not fully understood.

—C. Huang

Sources: http://www.theatlantic.com/health/archive/2012/03/whats-really-making-us-fat/254087/; http://www.ajcn.org/content/early/2012/01/17/ajcn.111.026328

skin, which would then allow the austronaut to deal with problems such as the high levels of radiation that they are exposed to in space. No current known enzyme is capable of breaking down the biocapsules' nanostructures, and because they are inert, they are well-tolerated by the body.

Astronauts aside, the potential benefits from such a biocapsule are staggering. The NASA Biocapsule can also be used to provide doses of insulin to diabetes patients, and to aid cancer treatment. It may also lend a hand in gene therapy and severe allergy treatment.

Despite the biocapsule's potential, it still remains inexpensive and easy to create. With animal and human trials scheduled for the near future, it is thus not a stretch to say that this tiny bundle of nanotubes could change the face of medicine forever.

—V. Li

Source: http://tech.li/2012/02/nasa-biocapsule-medicates-from-the-inside/

COMPUTING

Fast is made even faster

In the era of speed, IBM is not holding back. Researchers at IBM have recently built a prototype of an optical chip that can transfer a terabit of data per second. This chip utilizes a unique design that requires 48 tiny holes to be drilled into a standard CMOS chip. This innovative feature facilitates the movement of light. Termed "Holey Optochip", this new design is faster and more power-efficient than other existing optical chips, and can increase the power of supercomputers.

By nature, optical chips transmit data with light instead of electrons. These chips are used to interconnect supercomputers and are found in some IBM supercomputer systems. Optical technology offers benefits over electrical chip technology, in that optical chips can afford to transfer high-bandwidth data over longer ranges.

The power of optical technology is the prime reason it is used for telecommunications, according to IBM Optical Links Group manager Clint Schow: "I think the number one supercomputer ten years ago had no optics in it whatsoever, and now you're seeing large scale deployments, mostly for rack-to-rack

interconnects within supercomputers. It's making its way deeper into the system and getting closer and closer to the actual processor."

While IBM itself will not be commercially manufacturing these chips, they should be commercially available within a couple of years. With increased transmission speed, these new chips may shape the future for supercomputers.

—R. Kumar

Sources: http://arstechnica.com/business/news/2012/03/holey-chip-ibm-drills-holes-into-optical-chip-for-terabit-per-second-speed.ars

PHYSICS

Ultra-efficient LEDs

Against all odds, MIT physicists have recently built a light-emitting diode (LED) with a 230 percent efficiency, generating 69 picowatts of power when supplied with only 30 picowatts of power.

While this LED seems to violate the principle of conservation of energy, it actually does not. As published in Phys. Rev. Lett., the team working on this project under the supervision of MIT professor Rajeev Ram explains. Using electrical work, this device converts energy from both electrons and heat in the surroundings into photon energy. By having an inverse relationship between its power conversion efficiency and its output power, the LED is capable of further drawing in heat energy to generate even more photons, making it more than 100 percent electrically efficient.

Currently, although the power output is minimal, the concept behind this LED can be widely applied to thermodynamic heat engines, systems that convert heat energy to mechanical work. Furthermore, by also implementing fast and precise electrical control, we would ultimately be able to create efficient low-power electronics, a boon as we work our way towards a greener future.

—R. Kumar

Sources: http://arstechnica.com/business/news/2012/03/holey-chip-ibm-drills-holes-into-optical-chip-for-terabit-per-second-speed.ars

China makes a breakthrough physics discovery

China has again proven its ability for scientific advancement as particle physics has finally become a more popular field of study in this nation.

Daya Bay Nuclear Power Plant in Shenzhen, China
Credit: :http://www.hk-phy.org/energy/power/nuclear_phy/images/daya_bay.jpg

A group of researchers in China at the Daya Bay Nuclear Power Plant have recently discovered the third "mixing angle", a critical component for determining the introversion on the three various types of neutrinos. Oscillating particles called neutrinos are vital aspects of the subatomic world, and come in three different flavors: electron neutrinos, muon neutrinos and tau neutrinos. Electron neutrinos are derived from nuclear reactions, while muons surface from the decay of pion particles, subatomic particles that are important in understanding the strong nuclear force. Tau neutrinos have been observed in particle collision in accelerator labs.

Previously, through an array of experiments involving underground detectors, particle accelerators and reactors, physicists had measured all but one of the parameters in a theoretical scheme to illustrate neutrino oscillations. The collected data suggested there are two mass differences among the three different neutrinos and three abstract "mixing angles" that depict how much of one type of neutrino mixes into another.

Now, nearly 240 physicists working with the Daya Bay Reactor Neutrino Experiment at the Daya Bay Nuclear Power Plant and two peripheral plants in Da Peng, China have measured the last mixing angle, termed θ13. The team recently presented its results in Beijing at the Institute of High Energy Physics of the Chinese Academy of Sciences. The Daya Bay group discovered a non-zero value for θ13, reassuring physicists that neutrino research will be extensive for at least another decade. Some scientists have even affirmed that this discovery is arguably the greatest physics discovery to come from China.

—R. Kumar

Sources: http://news.sciencemag.org/sciencenow/2012/03/physicists-in-china-nail-a-key.html?ref=hp

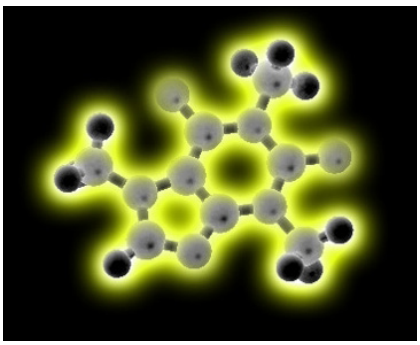
CHEMICAL ENGINEERING

Fluorescent Chemical Detectors

MIT researchers have now found a method to make small-molecule detection even more sensitive. By combining fluorescent molecules

with a metal-organic framework (MOF), researchers led by Dr. Mircea Dinca, Assistant Professor of Chemistry, succeeded in triggering fluorescence upon detection of a target molecule. This method of detection achieves much greater sensitivity compared to the conventional quenching approach.

MOF structures are extremely porous, leading to high surface area to mass ratios. Research by the Dinca group focused on binding chromophores, which are a type of fluorescent molecule, to the MOF structure. Subsequently, these chromophores are fluorescently activated when they form clumps, and attaching these to the scaffold



Metal-organic frameworks (MOFs) provide open spaces where molecules can settle, thereby increasing fluorescent intensity.
Credit: http://img.mit.edu/newsoffice/images/article_images/20111213171809-1.jpg

structure created by the MOF allows for adequate space and available binding sites to which the target molecules can attach and cause activation of the chromophores.

One of the advantages of this fluorescence activation approach is that the fluorescence intensity, which is directly related to the number of target molecules bound by the MOF, can be more easily quantified. While the intensity can be an indicator of other factors, it will allow for more information to be obtained in a single reading, as a color change will indicate the presence of a target molecule, such as a toxin or pathogen.

—J. Lee

Sources: http://web.mit.edu/newsoffice/2011/fluorescent-chemical-detectors-1214.html

MEDICINE

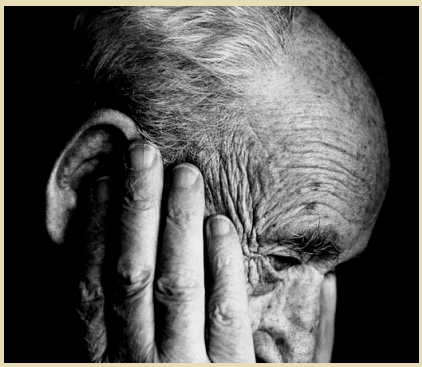
New device can help diagnose sickle cell disease

Sickle cell anemia has been shown to result from a single mutation in the hemoglobin protein, causing red blood cells to form a sickle shape that can more easily result in blood clots and blockage of blood flow. Despite extensive studies aiming to illuminate the molecular mechanisms behind the disease, scientists still cannot explain why the severity of symptoms varies significantly among individuals, with some requiring simple medications and others requiring major medical interventions.

MIT professor Sangeeta Bhatia and collaborators at Harvard University, Massachusetts General Hospital, and Brigham and Women's Hospital have now developed a simple device that can measure the vaso-occlusive risk of blood samples: how rapidly sickle cells begin to block or slow blood flow after being triggered by deoxygenation.

Reversing Alzheimer's

Alzheimer's disease, a neurological illness and a highly common form of dementia, currently affects 5.4 million Americans, and the number of victims is projected to continue to increase. Previously, MIT neuroscientists led by Dr. Li-Huei Tsai, currently the MIT Picower Professor of Neuroscience, found that the ability to create new memories is directly affected by an enzyme known as HDAC2. Recently, they found that inhibiting HDAC2 could reverse symptoms of Alzheimer's in mice.



Alzheimer's disease is a debilitating neurological illness with serious social repercussions, affecting up to 5.1 million Americans.
Credit: http://www.doctortipster.com/wp-content/uploads/2012/03/alzheimer11.jpg

Gene expression can be inhibited when the DNA is tightly coiled, usually around histones, ultimately forming a structure known as chromatin. Histone deacetylases (HDACs) are a family of enzymes that regulate gene expression by modifying histones via deacetylation. HDAC2 represses expression of the genes responsible for learning and memory.

Tsai's research team found that, for Alzheimer's patients, HDAC2 is present in excessive levels in the hippocampus, an area of the brain that is essential in the creation of new memories. Especially affected were genes that influence the brain's control over neural connections based on stimuli. When HDAC2 activity was inhibited, normal expression of these genes returned and the mice returned to normal cognitive activity levels.

Researchers hope that drugs inhibiting HDAC2 will be able to help Alzheimer's patients, but Tsai says, "an approved drug would probably take at least 10 years." (MIT News Office)

—J. Lee

Sources: http://web.mit.edu/newsoffice/2012/alzheimers-hdac2-inhibitors-0301.html

This device has been proven to be helpful in predicting whether a patient is affected by a more severe form of the disease that will commonly result in multiple organ failure and death. This prediction cannot be made by any currently available devices measuring other blood properties such as red or white blood cell concentration. Furthermore, this device can be used to quantify the efficacy of a drug in reducing vaso-occlusive risk, and thereby in treating sickle cell disease. The researchers hope to continue developing this technology into a reliable diagnostic and drug development tool.

—C. Chen

Sources: http://web.mit.edu/newsoffice/2012/sickle-cell-blood-flow-0301.html

The Bridge Project

On March 6, 2012, the David H. Koch Institute for Integrative Cancer Research at MIT and the Dana-Farber/Harvard Cancer Center launched one of the largest cancer research collaborations ever seen. The collaboration aims to bring cancer biologists, clinicians, and bioengineers from both institutes "to gain a more sophisticated understanding of the underlying biology that's driving these diseases... and to design fresh approaches for how we might intervene.", as David Livingston,deputy director of Dana-Farber/Harvard Cancer Center, phrases.

The Bridge Project, as it is called, will focus on the prognosis of pancreatic cancer and glioblastoma, which are currently one of the least-understood and most difficult types of cancers to cure. The Bridge Project is receiving immense financial support from individuals such as Arthur Gelb and Thomas Peterson; and non-profit cancer research organizations such as The Lustgarten Foundation and the National Brain Tumor Society, and is currently funding four research teams, members of which include Robert S. Langer (MIT), J. Christopher Love (MIT), Jeffrey W. Clark (MGH), and Keith L. Ligon (Dana-Farber). Ambitiously, the Bridge Project aims to raise $50m in the next 5 years and make hallmark advances in cancer treatment.

THE BRIDGE PROJECT

The Bridge Project will bridge the gap between biologists, physicians, and engineers studying cancer.

Credit: http://ki.mit.edu/files/ki/img/134/mainimage/bridge-art-blue.jpg?1299173238

—P. Paul

Sources: http://ki.mit.edu/approach/partnerships/bridge

MURJ Features

Bridging the Gap Between the Sciences and the Humanities

Professor Michael Scott Cuthbert, a leader in the emerging field of computational musicology, talks to MURJ about combining science and art in his research.

BY ANNE HUANG

Michael Scott Cuthbert, an Associate Professor of Music, Homer A. Burnell Career Development Professor at MIT, and a self-described "music geek," is one of the leaders in the field of computational musicology. Computational musicology, a relatively new area of research, is the study of music with computational modeling and simulation. Professor Cuthbert and his research team are developing music21, an open-source toolkit for computer-aided musical analysis. Professor Cuthbert also uses quantitative techniques to test hypotheses and analyze music, combining the sciences and the humanities to solve problems in musicology.

Professor Michael Scott Cuthbert, a leader in the field of computational musicology.

Professor Cuthbert fell in love with musicology and medieval music as a freshman at Harvard University. He realized that two modern-day recordings of the same piece from medieval times could sound entirely different, even though the musicians would play from the same medieval manuscript. The recordings sounded different because there were no written indications about the intended tempo, the dynamics, or even the instruments that should be used for a particular piece. Problems such as these intrigued Professor Cuthbert, and he became interested in solving questions in musicology.

As his interest in musicology deepened, Professor Cuthbert became fascinated with using computer programs to solve problems in musicology. While in music graduate school at Harvard University, Professor Cuthbert took several classes that further developed his interest in computational musicology. In one class, Professor Cuthbert and his peers were analyzing the order of songs in Gregorian chant inventories. The songs in each inventory were sorted by key, but songs of the same key could be sorted differently between inventories. In other words, if two inventories had the same four songs in a particular key, these four songs could be in different orders. Professor Cuthbert and his peers were arguing back and forth about the order of songs in several Gregorian chant inventories when Professor Cuthbert thought, "Why don't we put everything in a computer program and run the correlation coefficient between the order in the first and second inventories?" Using a computer program to analyze the order of songs quickly and definitively determined whether songs in different inventories were in the same order or not.

In another graduate class on West African drumming at Harvard, Professor Cuthbert used computer programs to study the theory of rhythm. He studied the work of Professor Willie Anku, a renowned music theorist who had very interesting ways of looking at rhythm. However, Professor Anku never wrote down the rules he used to analyze the theory and rhythm of music. To find Professor Anku's rules, Professor Cuthbert used a computer program to extract the rules from Professor Anku's analyses of different pieces. Professor Cuthbert was inspired by the first spam filters developed by the National Bureau of Economic Research. In 2001, when spam messages began clogging up email inboxes, the programmers at the National Bureau of Economic Research devised a spam filter that scored messages based on what characteristics they had.

For example, if a message contained the word "Viagra," it was given a score of three points. If a message contained a dollar sign followed by six or more digits, it was given a score of two points. A message with a total score over a certain number of points was deemed spam and then filtered out of the inbox. Using a "modified spam filter," Professor Cuthbert was able to classify Western African rhythms and recreate Professor Anku's analyses. Based on his experiences in graduate school, Professor Cuthbert soon understood that many problems in musicology could easily be resolved using quantitative thinking and computational techniques.

"...many problems in musicology could easily be resolved using quantitative thinking and computational techniques."

Late in graduate school, Professor Cuthbert began developing music21, a toolkit for computer-aided musical analysis. He was inspired by a musical analysis toolkit called Humdrum, developed by Dr. David Huron at Ohio State University in the 1990s. Humdrum was one of the first software that allowed musicologists to answer research questions using computational techniques. For instance, Humdrum could answer questions such as, "In pieces by Stravinsky, are dissonances more common in strong metric positions than in weak ones?" However, Humdrum had a "high learning curve" and was difficult to use, so few musicologists implemented it in their research. Professor Cuthbert wanted to develop a set of computer tools that could quantitatively analyze musical questions like Humdrum but was also easy enough for musicologists to learn and use. And so, the idea behind music21 was born.

Professor Cuthbert began writing code for music21 on his own during graduate school, but he soon realized that it would take a very long time without any collaborators. He thought that there must be other computational musicologists working on projects similar to music21, but he could not find anyone seriously developing software like his. A few years after receiving his Ph.D. from Harvard in 2006, Professor Cuthbert was invited to be a faculty member at MIT. He began sharing the idea behind music21 with undergraduates who were interested in combining music and computer science. Some undergraduates would even submit small programs to help expand music21. Professor Cuthbert began a UROP program so undergraduates could help further develop music21.

The UROP students involved in Professor Cuthbert's research have worked on several projects related to music21 that are breakthroughs in the development of computer-assisted musical analysis. Jose Cabal-Ugaz, a Course 21E major (Bachelor of Science in Humanities and Engineering, joint major in Music and Computer Science) who graduated in February 2012, developed a program called fbRealizer that can provide all the possible realizations, or solutions, of a given figured bass. Figured bass is a type of musical improvisation that has been used since the 1600s. The musician is given a bass line and associated figures, which tell the musician what chords to play and when to play them. Based on the bass line and the figures, the musician can improvise the higher notes and the melody. For any given figured bass, there exist many possible realizations. If we are given an eight-note figured bass line and each note has ten different combinations of high notes that can be associated with it, there are 108 possible realizations of a single, short figured bass line. Realizing each of the 100,000,000 solutions by hand is impossible, but fbRealizer can show and play all the solutions to a given figured bass within minutes. fbRealizer also has the ability to apply common rules which limit how notes may move from one chord to another, and it can even recognize special chords and resolve them properly. fbRealizer has many practical applications, especially in music theory instruction and restoration of historical music works that have been damaged.

Jose has also developed a free, all-purpose Braille Music Transcriber (BMT) that can transcribe standard music notation into Braille music notation. Braille music notation is complex and difficult to master (Figure 1). Unfortunately, this makes it difficult to find people who can translate music into Braille, because they need extensive training before they can begin transcribing music. The Braille Music Transcription Manual, which has detailed explanations on how to translate music into Braille, is a 500-page behemoth used for the certification of Braille music transcribers. To become an official transcriber, one needs to complete and submit all the exercises in the manual. As of 2005, there were only about 40 official Braille music transcribers in the world. There are transcription softwares online that can transcribe music into Braille, but it is very expensive. To provide the visually impaired with better access to musical scores, Jose began developing BMT in July 2011. While there is still much to be done before BMT can become an official Braille music transcriber,

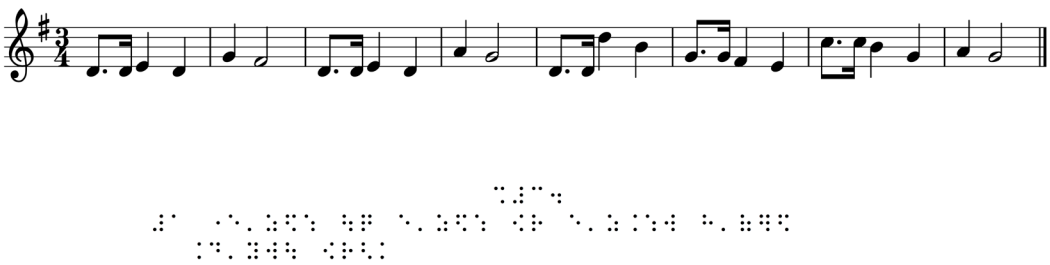


Figure 1 | The "Happy Birthday" song in standard music notation and in Braille music notation. *Jose's work on Braille music notation may lead to faster and more easily accessible Braille music transcriptsion in the future.*

Jose's work with BMT may one day lead to fast and easily accessible Braille music transcriptions.

Beth Hadley ('15) is a UROP student currently working with Professor Cuthbert. To date, she has contributed to several projects. Her first project used music21 to analyze the harmonies of more modern, popular music. She acquired music files online, parsed through them using existing music21 tools, and extracted information about the root and bass motion of the harmonies present in each piece. Beth's analysis found some interesting differences in harmonic progressions and root motion between the music of the 1950s-1960s and the music after the 1980s. In both eras, the most common root motions were the perfect fourth interval, the perfect fifth interval, and the whole step interval. However, post-1980s music had a greater spectrum of motion when compared to music of the 1950s-1960s. Specifically, root motion by a half step interval (also known as a semitone) increased to 8% in post-1980s music compared with 3% in music from the 1950s-1960s. Likewise, the predicted motion of fourth and fifth intervals decreased, providing other types of root motion to increase. In essence, Beth proved that as time progresses, popular music incorporates a greater variety of chord progressions. Beth's work is an example of research that can help musicologists run feature extraction analysis on unknown pieces. Feature extraction uses what is already known about certain trends in music, such as the distribution of root motion, to predict something unknown about music. For example, a musicologist can predict what era a piece was written in by analyzing its root motion and comparing it to Beth's data.

Currently, Beth and Lars Johnson ('15), another UROP student, are developing the music21 Theory Analyzer. This package provides musical analysis tools to identify common counterpoint errors. Counterpoint is the relationship between two or more voices that are harmonically interdependent but independent in melodic contour and rhythm. Counterpoint was most highly developed during the Baroque period of Western music (1600-1750). At its height, counterpoint had many strict rules. Chords had to resolve in particular ways, and the progressions of certain intervals between voices were prohibited. Today, writing music that obeys these counterpoint rules is still one of the essential skills that all students studying music theory must learn. The music21 Theory Analyzer has the potential to transform the way students and teachers approach music theory education. If a teacher gives his students an exercise in composing counterpoint, the teacher can use the music21 Theory Analyzer to identify any counterpoint errors in the students' exercises. It can then display the results directly to the student or email the teacher with the results, coupled with an annotated version of the students' assignments. Not only will the music21 Theory Analyzer make it easier for music theory teachers to grade assignments, it will also be a great educational tool to help students learn to compose music according to strict counterpoint rules. Beth and Lars hope to have a working prototype of the music21 Theory Analyzer soon, so music theory students can begin testing it.

Apart from music21, Professor Cuthbert has also used computational techniques to analyze other aspects of musicology. In 2010, Professor Cuthbert published a controversial article called "Tipping the Iceberg: Missing Italian Polyphony from the Age of Schism." For many years, musicologists believed that only 1% of the music written during medieval times has survived today. The analogy commonly used was that the medieval music that has withstood the test of time is only "the tip of a submerged iceberg" of all the other work that had been lost. However, no one had ever tried to study or quantify how much medieval music has survived today. Professor Cuthbert used a biological technique called "capture-recapture" and basic statistics to estimate the percentage of medieval music that has survived. Capture-recapture is often used by ecologists to estimate the size of a population. If one wants to know

the number of deer in a forest, one can capture a set number of deer and tag them. After some time, one can capture the same number of deer again and see how many of them have tags. If most of the recaptured deer have tags, the deer population is relatively small. On the other hand, if most of the recaptured deer do not have tags, the deer population is relatively large. Professor Cuthbert applied the idea of capture-recapture to medieval music by looking at medieval manuscripts and seeing how many of the songs were the same. He found that even if one compares manuscripts found in different areas, most of the songs are the same, implying that many of pieces written in medieval times have survived today. Using basic statistics, he calculated that roughly half of medieval music survived to the present day.

One of Professor Cuthbert's greatest challenges is convincing humanists that quantitative analysis should be used to answer questions in musicology and getting the scientists and engineers interested in musicology. For instance, Professor Cuthbert found it difficult to publish his article, "Tipping the Iceberg," because of the gap between the sciences and the humanities. When he first submitted his article, the journal reviewing it sent his article to a musicologist and a statistician. Both reviewers rejected his article. The musicologist said that while he could not follow the math, Professor Cuthbert's conclusions contradicted the long-standing assumption that 99% of medieval music has been lost. Since his conclusions were "so obviously wrong," there must have been an error. The statistician rejected Professor Cuthbert's article because the math was so basic—just plugging numbers into known equations—that he thought the article was not publishable. Professor Cuthbert wants to persuade the humanists that "quantitative skills are not terribly difficult to learn." He also wants to get scientists and engineers interested in musicology. "Right now, the problem is that although the engineers have the ability to solve problems in musicology, they are not interested in the answers," Professor Cuthbert explains. "We need them to see that these are worthwhile tasks because the answers on the humanist side can be transformative."

Professor Cuthbert believes that "the humanities are having a 'moneyball moment,'" referring to the book by Michael Lewis and the critically acclaimed movie starring Brad Pitt. In the late 1990s, baseball was transformed by the use of statistical analysis. Teams with tight budgets, like the Oakland Athletics, used statistics to judge a player's performance, giving them a great edge in scouting and finding undervalued players. Likewise, says Professor Cuthbert, the field of musicology can be revolutionized by using quantitative skills, like statistical and mathematical analysis, to test many unproven hypotheses. He stresses the importance of both quantitative and traditional musical skills. While the computational skills are important, traditional music training is still essential to musicology. Professor Cuthbert encourages MIT undergraduates who are interested in the humanities and the sciences to join the field of computational musicology. Students at MIT, who value a rigorous quantitative training and do not neglect the humanities and the social sciences are "poised to be power players," contends Professor Cuthbert. If you are interested in learning more about music21, Professor Cuthbert and his research team encourage you to visit their website, mit.edu/music21.

"It is like using a Rosetta Stone for unlocking possibilities in the humanities."

Professor Cuthbert praises the use of computational techniques in musicology. "You can use quantitative analysis to figure out things that you would not think you would be able to figure out," he says. "It is like using a Rosetta Stone for unlocking possibilities in the humanities." He loves computational musicology, because it combines nearly all of his diverse interests. "I can't think of too much that I like to do that I haven't found a way to use in musicology," he muses. "Well, I suppose baseball." Professor Cuthbert's UROP students are equally excited about the future of computational musicology and music21. Jose observes, "The role of computational musicology tools provided by music21 is to master our current definition of music and to explore the myriad possibilities that lie beyond. The Mozart of the future will be the engineer who can use these tools to his or her advantage." Beth agrees that computational analysis is essential for the future of musicology: "Any field of research should use the most technologically advanced tools to reach conclusions quickly, and although it is impossible to predict the impact of music21, I am confident it has and will continue to transform the way music is studied and understood worldwide. The possibilities are endless, which is the virtue of using computers to analyze music!" With motivated people like Professor Cuthbert and his research time bridging the gap between the sciences and the humanities, computational musicology has a bright future. ■

Of Mice and Men: The Chemistry of Smell Sensing

Recent studies on mouse olfaction receptors have shown that metals play a central role in mammalian odor sensing, a discovery that may have ramifications for scientists' understanding of ligand-receptor interactions.

BY EBAA F. AL-OBEIDI

The intersection between Main Street and Windsor Street, just seven minutes from MIT, is the sweetest spot in Cambridge – literally. If you walk down Massachusetts Avenue and turn right onto Windsor, you will be charmed by the unmistakable aroma of mint chocolate wafting from the factory buildings on your left. Delving into a bit of Cambridge history, you might be amused to discover that those factory buildings are the property of the Tootsie Roll Industries, and are the site where 15 million Junior Mints have been crafted daily since 1949. The setup is a somewhat less glamorous affair than Willy Wonka's Chocolate Factory, but it still delights us with all the sweet smells. But how exactly do our noses sense smell? This question has gone largely unanswered until fairly recently, as new strides in chemoreception have been made that shed light on some of the longtime mysteries of odor sensing.

Satellite image of the intersection between Main Street and Windsor Street, where the Junior Mint factory still operates.
Credit: Google Maps: Neighborhood Four, Cambridge, MA

It is no secret that the human sense of smell is easily surpassed by that of other species, yet even we are capable of detecting nearly 10,000 distinct scents. This vast diversity is, in part, due to the fact that three percent of our genome is dedicated to smell-sensing genes alone, which translates to about 1,000 olfactory genes.

The structure of the nose is very much tailored to its function. The roof of the nasal passage measures approximately 2.5 cm2 and is coated with a layer of mucous. Beneath the mucous reside olfactory receptors which are capable of detecting thousands of different odors and transmitting the information to the olfactory bulbs in the back of the nose. These bulbs contain sensors which are hard wired through synapses—junctions between nerve cells—directly to the brain. Intriguingly, the part of the brain which receives the olfactory messages – the limbic system – is associated with feelings and memories. This reveals that, of all our senses, the sense of smell is most intimately connected with the brain and our emotions.

The mucous that covers the olfactory sensors plays a critical role in smell sensing; it acts as a solvent layer into which vaporized odor molecules dissolve. Indeed, our noses are akin to gas chromatographs (GC), which are instruments used to analyze mixtures of compounds. GCs contain a column coated with a thin layer of liquid (the stationary phase) which helps to partition the components of the mixture between the mobile phase and the stationary phase, ultimately separating the mixture into its component parts to be detected by the detector. The olfactory sensors behave in much the same way since they are wired to receptors which will distinguish between smells and transmit them to the brain. The mucous also acts as a protective layer for the tissue because it contains powerful antibacterial enzymes which act as the body's first line of defense against invading species.

The last decade has witnessed new advances in understanding the human sense of smell with the help of mouse models. Among the inorganic chemistry community, it is generally believed that if a volatile compound can coordinate efficiently with a metal ion, then that compound can be smelled strongly. Recently studies confirm this by showing that copper ions are involved in capturing the smell of methyl thiol molecules. Thiols are sulfur-containing compounds, which are one of the strongest smelling families of organic compounds. These studies show that the metal copper allows mice to detect particularly stinky thiols, confirming that smell receptors are really metallo-receptors[5]. This lends credence to the long-held suspicions that metals may help humans and animals smell certain volatile chemicals. Although these studies described here have shown proof that metals play a role in odor sensing in mice, it remains to be seen whether the dependence can be extended to humans, and that is the direction that research is now heading after this recent discovery.

In summary, the sense of smell is arguably our most primal faculty, and, until relatively recently, one of the least well understood. New breakthroughs using mouse models reveal that metals such as copper play a crucial role in odor sensing, possibly forcing scientists to "rethink traditional models of how ligands activate biological receptors."[5] ■

References

1. Buck, L., & Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell, 65, 175-187.

2. Mombaerts, P. (1999). Seven-transmembrane proteins as odorant and chemosensory receptors. Science, 286, 707-711.

3. Rakow, N. A., & Suslick, K. S. (2000). A colorimetric sensor array for odour visualization. Nature, 406, 710-714.

4. Wang, J., Luthey-Schulten, Z., & Suslick, K. S. (2003). Is the olfactory receptor a metalloprotein?. Proc. Natl. Acad. Sci., 100, 3035-3039.

5. Wolf, L. (2012). Metals' role in odor sensing. Chemical & Engineering News, 90(7), 9.

Nobel Spotlight: Professor Peter Diamond MIT Department of Economics

This issue's Nobel Spotlight features Professor Peter Diamond, 2010 Nobel Prize winner in Economic Sciences.

BY PAIGE FINKELSTEIN & JAMIE KANG

Professor Peter Diamond, 2010 Nobel Prize winner in Economic Sciences
Credit: http://www.belowyourmeans.com/wp-content/uploads/2011/06/peterdiamond.jpg

MURJ: Can you give us a short summary of your Nobel Prize work?

Diamond: The picture that you learn about in your basic economics classes is that there is demand, supply, and a price that clears the market. However, the mindset is not pairwise trading; instead of buying goods from individuals, you are trading with "the market." It's an abstraction that goes back to Adam Smith, and probably even earlier than that. For many purposes, that model works very well, but there are also many purposes for which it does not work well. I started developing models in somewhat different contexts. In the retail market, the labor market, and the housing market, the process from whom you are going to buy from or whom you are going sell to are actually rather different. I actually started with the model of the retail market. The critical new ingredient was that it takes resources—time or money—to learn about your options. In standard theory, there is a set of goods out there and you can buy what you like. If somebody charges too high of a price, they don't sell anything because everyone knows the prices elsewhere. My starting point was then: What if you have to visit a store to find out what the price is? Now, mind this was written in the late '60s, before the Internet. If there is a better price out there, you now have to leave that store and go to another store. Now what kind of price setting would firms do in that kind of setting? Does it look like the typical supply and demand picture that we are used to seeing? The answer is no, it doesn't.

So that work was published in '71. I then spent the next decade trying to apply that same kind of theory around the labor market. For another decade I wrote a lot of follow up stuff, coming up with other empirical implications as well.

MURJ: What is the coolest part about winning a Nobel Prize?

Diamond: The biggest pleasure is sharing the joy with your friends and family and colleagues. There

were moments where there was a sense of people sharing the pleasure with me—and obviously it's a great pleasure. During the week in Stockholm, each laureate gets a certain number of places at the banquet, and I had a combination that was half family and half colleagues that were good friends. It's not a moment; it's an overall experience.

MURJ: What is the coolest part about being a professor?

Diamond: High school was high school. I went to college, loved it, and basically I never left. I love the intellectual environment and learning new things—I'm curious about all kinds of things. A university, in particular, is a great place to enjoy the intellectual life.

MURJ: We heard that you went to Yale as an undergraduate and you studied mathematics, not economics. How did you make the transition?

Diamond: Before I started, I thought I wanted to study electrical engineering. It sounds silly, but I knew there was employment out there. I took the beginning required classes, such as Mechanical Drawing, and some other mechanics classes, but the class I liked the most was Calculus. I had never taken calculus before because it typically wasn't taught in high school then, except in elite areas, and I had come from an ordinary, suburban public high school. During some point of freshman year, I took a step back from the presumption I had and asked myself, "What do I want to major in?" The class I liked the best, and the class I did the best in was the math class. So I decided to become a math major.

My second year, I ended up taking Introductory Economics because of breadth requirements at Yale. I thought it was pretty good, and I got pretty friendly with the teacher, so when the next year I had to select another elective, he convinced me that I would really enjoy taking Intermediate Micro and Macro in the honors section, even though I wasn't an economics major. I took it, and I did like it.

At the time, there were a number of us who weren't math majors and weren't sure if we wanted to go on in math, so we took graduate level Mathematical Economics at Yale. I really liked that class. It was more the kind of math I enjoyed. At this point I was unsure about what I wanted to do, so I applied to both math and economics graduate programs. I started off at MIT in the graduate math department, but I had already finished half of the first year's program, so I could take economics courses and put off deciding which one I liked better. By the end of the first semester, it was clear to me that I really preferred economics, so I switched.

MURJ: How did you transition into becoming a professor here?

Diamond: I actually did not become a professor at MIT immediately. MIT has a requirement that you get your Ph.D. somewhere else. So I taught at Berkeley from '63 to '66. Afterwards, I got an offer to teach at MIT, and although I loved my time at Berkeley, the MIT offer was irresistible.

MURJ: Do you prefer to study a specific aspect or theory in economics?

Diamond: I've always worked in multiple areas of economics almost all of the time, including when I was doing my Nobel Prize work.

"I love the intellectual environment and learning new things—I'm curious about all kinds of things. A university, in particular, is a great place to enjoy the intellectual life."

I've done work on social security, taxes, growth models, role of public debt, etc. I move around a lot, and it's connected to teaching. I would teach different things, so I had to get on top of some literature. I find teaching very stimulating for research. There is just something about having a set of very bright people in front of you who will spot anything that you say wrong. You have to try and teach not just what the literature is saying, but also how to address the question.

MURJ: What is your current research? Do you work with any undergraduate students?

Diamond: I have actually retired from MIT, because I am Emeritus. I don't teach anymore, but I do give the occasional guest lecture. In the past I have worked with undergraduates, but not often, because the kind of work I do required more background than an undergraduate had. I did work with graduate students regularly, though currently I am not. Currently I'm working on two issues. The first is unemployment, and how to evaluate the literature addressing

it. On the backburner, I'm also focusing on basic research in the capital market and how to regulate big banks. The unemployment thing is a little more pressing though.

MURJ: Regarding the national debt, how do you feel about debt increasing more under Obama than Bush?

Diamond: Well, debt started increasing because of tax cuts. Then, when you go into a serious recession, tax revenues go way down without changes in tax rates and government spending for things like unemployment insurance and welfare goes up. So Bush, in my mind, had bad policy. We were cutting taxes way more than we could afford, and we weren't paying attention to what we spent on. There was a lot of spending—some good and some not so good. He introduced prescription drug coverage, which was long overdue, but without providing any financing. So, he ran up a big increase in debt because of his chosen policies. Then, when the recession hit in Bush years, he ran up a lot more debt. Some of it was just the response of the economy; some of it was the appropriate choice to try to stimulate spending to make the economy's fall not so big. Obama continued and expanded the fiscal stimulus approach, and he inherited the ongoing huge drop in tax revenue because of the state of the economy.

MURJ: With that said, do you believe the Federal Reserve Board is doing a good job in trying to stimulate the economy again?

Diamond: The Fed has done a terrific job at preventing something as bad as the Great Depression. If the Fed hadn't done the job as it did, things would have been a whole lot worse. The early fiscal stimulus, while it didn't do what the Obama Administration said it would do, was an enormous help. Though the stimulus was a big contributor to the deficit, the economy would have been a whole lot worse without it. The problem now is that we have come out of the recession a bit, but unemployment is still too high. So we need more fiscal stimulus, but that obviously isn't happening with this Congress.

MURJ: Are you a Republican or Democrat?

Diamond: I have been a registered Democrat for a long time.

MURJ: Do you believe that Romney, with a business background, would be of help? Or do you think that could make things worse?

Diamond: Romney, compared to the other Republican candidates, looks a lot better. When compared to the Obama administration, it's a red herring, because any president is going to pick a set of advisors that will be both economists and people with business backgrounds. Obama has had terrific advisors. What they faced was really unprecedented. They didn't get it all right, but they got a lot of it right. As a Democrat, I like the policies of the advisors advising Obama more than those advising Romney. Romney, without a political background, would have been a disaster. It would be like bringing a businessman into run a university. Dealing with students and faculty is different than dealing with people who work for you.

MURJ: And now for the fun questions. Do you have a favorite movie?

Diamond: Can I name more than one? I'm thinking of the movies I see from time to time again. Hunt for Red October, Men in Black, Princess Bride, and Midnight Run.

MURJ: Favorite music?

Diamond: I like classical music... and a little jazz.

MURJ: Favorite restaurant?

Diamond: I'm not a foodie, so I care more if it's a nice place to sit and talk. When it comes to food, I'll eat anything that isn't still alive on the plate.

MURJ: Which newspaper do you follow?

Diamond: I get daily delivery of The Global and The Times. I start with the sports, and then I move onto the news.

MURJ: Out of curiosity, was Ben Bernanke really one of your students?

Diamond: Well, he was a graduate student when I was professor. I don't think I had him in class, but I did chat with him a few times about his thesis, though I wasn't his advisor. He was mainly focused on macro when I was focused on other things.

To read more about Professor Diamond, please visit: http://www.nobelprize.org/nobel_prizes/economics/laureates/2010/diamond.html; http://economics.mit.edu/faculty/pdiamond ■

MURJ UROP Summaries

In vitro evaluation of a biomimetic liver scaffold with intrinsic microvasculature[1]

Jesse Kirkpatrick[2], Thomas Cervantes[3], Katherine Kulig[4], Cathryn Sundback[5], Joseph Vacanti[6]

[1,3,4,5,6]Massachusetts General Hospital, The Laboratory for Tissue Engineering & Organ Fabrication, Boston, MA

[2]Student Contributor, Class of 2015, MIT

Introduction

The clinical need for a tissue-engineered liver is due to a severe shortage of donor organs for patients waiting for a transplant. Currently, there are over 16,000 patients on the liver transplant waiting list, and more than 1500 patients die each year while waiting for transplant.1 Tissue engineering offers a possible alternative to donor organ transplantation, but no tissue-engineered liver has yet been described.

One of the greatest challenges in tissue engineering is to rapidly perfuse engineered tissues upon implantation, particularly in solid organs with high metabolic activity. Hepatocytes, the parenchymal cells of the liver, have very high metabolic activity, and blood must pass within a few hundred micrometers of each cell to sustain function.2

Our research group is in the preliminary stages of developing a tissue-engineered liver with intrinsic microvasculature. Currently, the device is a poly(dimethylsiloxane) (PDMS) scaffold embedded with biomimetic microfluidic channels resembling native liver vascular architecture (**Figure 1**). The PDMS layers are fabricated using soft lithographic techniques.3 The microfluidic channels are covered with a thin (~10 μm) membrane, which allows for maximum diffusion of nutrients and oxygen between fluid and hepatocytes.

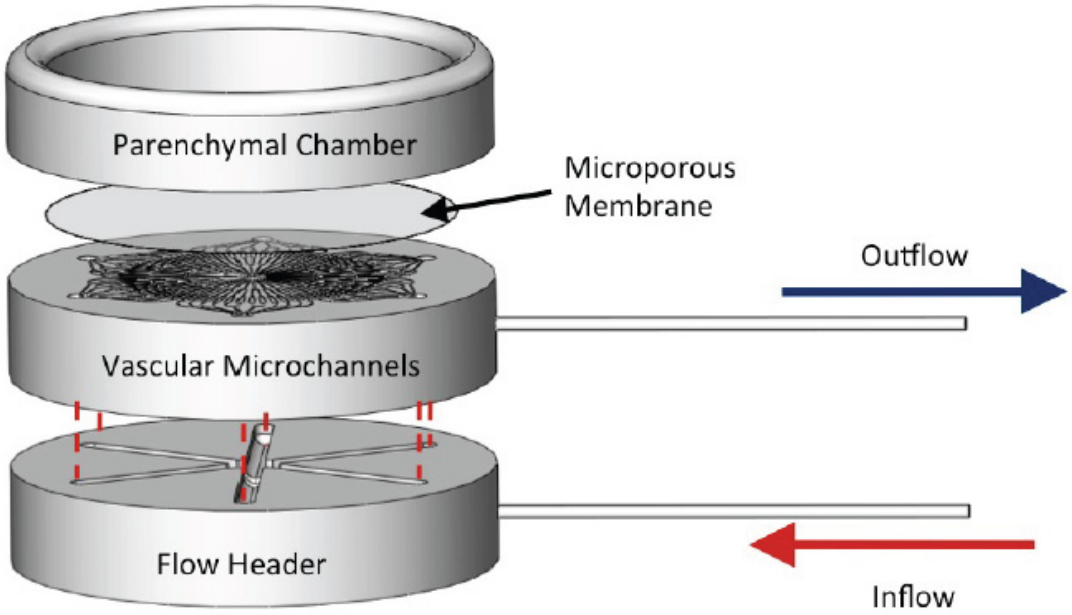


Figure 1. Diagram of liver device fabrication. The header layer (flow header) binds to a vascular layer (vascular microchannels), which is covered by a microporous polycarbonate membrane. A parenchymal chamber houses the cells and seals the culture from atmospheric pressure. Inflow and outflow of cells are indicated.

This device has the potential to be developed into an implantable, fully vascularized tissue-engineered liver.

Polycarbonate Membrane

A polycarbonate membrane was selected to cover the PDMS vascular layer of the device. Porous polycarbonate (.6μm) was chosen because of its ability to support gas and nutrient exchange. However, because polycarbonate does not share functional groups with PDMS, conventional "plasma bonding" methods were infeasible. Therefore, other options were explored for bonding these dissimilar substrates. Our first attempt was a "glue-stamp" method, bonding the membrane to the PDMS with silicone glue.4 This method failed because of low bond strength between PDMS and polycarbonate, and the glue clogged the vascular channels. A chemical, 3-aminopropyltriethoxysilane (APTES), was tested which has been shown to covalently bond PDMS and polycarbonate.5 APTES successfully bonded the vascular PDMS layer and the polycarbonate membrane without plugging the microvascular channels. Subsequent flow tests demonstrated leakage-free bonding at infusion rates of 200 μL/hr for 48 hours.

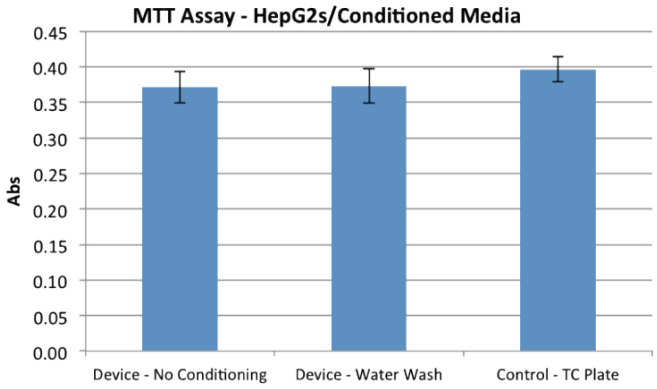


Figure 2. MTT Assay after 24 hours in vitro culture. Higher absorbance (Abs) values indicate greater enzymatic activity of cells in culture. Here, no significant difference in absorbance was appreciated between HepG2 hepatocytes cultured in conditioned media and those cultured in control media. Error bars are indicated as shown.

Toxicity

Few reports have described the use of APTES in cell culture, so a conditioned media study was performed to evaluate toxicity. Media was conditioned for 24 hours on: APTES-soaked polycarbonate, APTES-soaked and water-washed polycarbonate, and a control plate. HepG2 human hepatocytes were cultured in each type of medium for 24 hours. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays examined enzymatic activity, as a measure of cellular viability. All samples expressed comparable absorbance levels (**Figure 2**), indicating that APTES does not inhibit hepatocyte function in vitro.

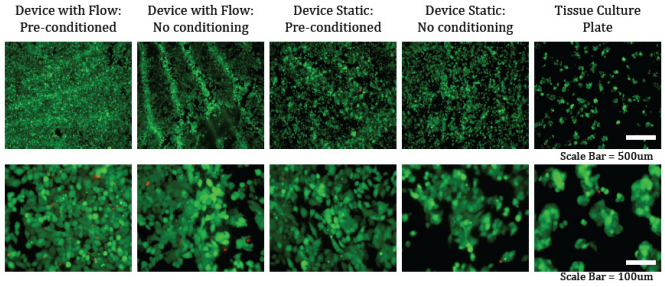


Figure 3. Live/Dead staining of HepG2 hepatocytes after 48 hours *in vitro* culture. Hepatocytes exposed to media flow through the microfluidic channels of the device exhibited improved morphology over hepatocytes cultured in a standard tissue culture plate and hepatocytes cultured on static devices.

Live/Dead Testing

HepG2 hepatocytes were seeded and cultured on polycarbonate membranes (.6 μm) in devices to assess cell attachment and proliferation. After 24 hours, flow was introduced into some devices through the microfluidic networks while others were left static without flow.

Live/dead staining after 48 hours of in vitro culture showed that the majority of hepatocytes survived when seeded directly onto the polycarbonate membrane. Furthermore, hepatocytes in devices with flow exhibited better morphology than hepatocytes in static devices.

Conclusions and Future Plans

Our research group has established the design principles for a biomimetic, microfluidic device, successfully bonded a polycarbonate membrane to PDMS, and demonstrated cellular viability after 48 hours of in vitro culture. Remarkably, hepatocytes exposed to fluid flow exhibited better morphology after 48 hours than hepatocytes cultured in static devices. Further experimentation is needed to confirm this result. Finally, the conditioned media study confirmed biocompatibility of APTES in vitro.

The next step will be to determine the ideal membrane pore size by measuring albumin output of hepatocytes

cultured on membranes of different pore sizes. In the future, we will assess liver zonation, a phenomenon in which hepatocytes closer to the device inlet (mimicking the portal blood vessels) uptake more oxygen than downstream hepatocytes and develop specialized function.6 This physiologic phenomenon is one of the motivations for employing biomimetic vasculature that mimics the engineered organ's blood vessel architecture. Other biological assays will be performed to confirm sustained function in vitro.

Currently, this device could be used as a bioreactor to simulate drug-induced liver injury. Future studies will include incorporation of biodegradable materials, as a next step toward an implantable, tissue-engineered liver.

Acknowledgements

I would like to acknowledge my mentor in the Laboratory of Tissue Engineering and Organ Fabrication, Tom Cervantes, for taking his time to train me to be a scientist. I also want to thank Katherine Kulig, a master of cell biology, who taught me the art and science of cell work. I would finally like to thank the laboratory directors, Cathryn Sundback, ScD, and Joseph P. Vacanti, MD, for providing me the opportunity to work on this exciting and inspirational project.

References

1. United States Department of Health and Human Services. (2012). Removal Reasons by Year (Data file). Retrieved from http://optn.transplant.hrsa.gov/latestData/rptData.asp
2. Hoganson D.M, Pryor II H.I., Spool I.D., Burns O.H., Gilmore J.R. and Vacanti J.P. (2010). Principles of Biomimetic Vascular Network Design Applied to a Tissue-Engineered Liver Scaffold. Tissue Engineering, 16(5), 1469–1477
3. McDonald J.C., Duffy D.C., Anderson J.R., Chiu D.T., Wu H., Schueller O.J.A, Whitesides, G.M. (2000). Fabrication of microfluidic systems in poly(dimethylsiloxane). Electrophoresis, 21(1), 27-40
4. Chueh B.H., Huh D., Kyrtsos C.R., Houssin T., Futai N., Takayama S. (2007). Leakage-free bonding of porous membranes into layered microfluidic array systems. Analytical Chemistry, 79(9), 3504-3508
5. Sunkara V., Park D.K., Hwang H., Chantiwas R., Soper S.A., and Cho Y.K. (2011). Simple room temperaturebonding of thermoplastics and poly(dimethylsiloxane). Lab on a Chip, 11(5), 962-965
6. Allen J.W., Khetani S.R., Bhatia S.N. (2005). In vitro zonation and toxicity in a hepatocyte bioreactor. Toxicological Sciences. 84(1), 110–119

Examining DNA damage, signaling, and repair at the single molecule level[1]

Sarine Shahmirian[2]

[1]Department of Chemical Engineering, MIT

[2]Student Contributor, Class of 2014, Department of Electrical Engineering & Computer Science, MIT

Drugs that target chromatin-altering enzymes are a new line of chemotherapy. HDAC inhibitors have demonstrated promise in clinical trials, however the mechanism of action and the downstream targets of these drugs is unknown. While it is generally presumed that these drugs alter chromatin structure, thereby altering gene expression which results in differentiation and apoptosis of tumor cells, it is unclear that this is the case. In addition, spontaneous activation of the DNA damage response has been observed in cells treated with HDAC inhibitors. Dissecting the global transcriptional changes caused by these drugs away from the physical alterations in chromatin structure caused by these drugs is difficult if not impossible in cell culture and in vivo systems. As such it is unclear whether activation of the DNA damage response in HDACi-treated cells is due to structural changes in chromatin structure, resulting in increased accessibility to DNA damage signaling machinery.

To determine the role of HDACi in the activation of the DNA damage response, experiments were carried out focusing on the single molecule level in vitro. The kinetics of recruitment of DNA damage signaling molecules to damaged DNA at the single molecule level were measured in real time using magnetic tweezers and slit-like nanochannels. The machinery was fashioned to permit buffer exchange with cell lysates. Active nuclear lysates were robustly purified from DNA damaged cells, and it was found that epigenetically modified chromatin from HDAC-inhibitor treated cells showed significantly enhanced gH2AX loading. Future studies will examine the effect of HDACi on the kinetics of DNA replication at a single genetic locus.

References

1. Balducci, A.G., Tang, J., & Doyle, P.S. (2008). Electrophoretic stretching of DNA molecules in cross-slot nanoslit channels. Macromolecules, 41:9914-9918.
2. Ladoux, B., Quivy, J., Doyle, P., du Roure, O., Almouzni, G., & Viovy, J. (2000). Fast kinetics of chromatin assembly revealed by single-molecule videomicroscopy and scanning force microscopy. Proceedings of the National Academy of Sciences, 97(26):14251-14256.
3. Trahan, D.W. & Doyle, P.S. (2011). Simulating the relaxation of stretched DNA in slitlike confinement. Macromolecules, 44:383-392.

The "Southwest Effect" revisited: an empirical analysis of the effects of Southwest Airlines and JetBlue Airways on incumbent airlines from 1993 to 2009[1]

Steve Wu[2]

[1]Department of Economics, MIT

[2]Student Contributor, Class of 2011, Department of Economics, Department of Mathematics, MIT

The Airline Deregulation Act of 1978 dramatically altered the competitive landscape of the US airline industry, ushering an era of head-to-head competition between low-cost carriers (LCCs) and legacy carriers. In particular, the continued expansion of Southwest Airlines, the most profitable player in the LCC space today, has become a principal driving force behind the growth of LCCs and the ubiquity of low fares across routes in general. Unlike legacy carriers which utilize a hub-and-spoke network and operate with a variety of different aircrafts, LCCs operate within a point-to-point network, allowing them to implement considerable flexibility in routes flown, operate with lower costs, and expand into newer hubs. The impacts of LCCs have been well documented in the empirical literature. For example, the number of passengers flying LCCs more than doubled from 1997 to 2007 and LCCs entered a total of 598 routes from 1997 to 2007 (Tan, 2010). From 1993 to 2004, Southwest alone nearly tripled its revenues from $2.3 to $6.5 billion (Goolsbee and Syverson, 2008). Notable contributions include Morrison (1998) and Dresner, Lin and Windle (1996), which used cross-sectional data to analyze the impact of Southwest Airlines on consumer welfare and savings. More recent studies such as Goolsbee and Syverson (2008) have attempted to focus more on the threat of entry, rather than actual entry itself of a particular LCC entrant. Within the airline industry literature, entry threat captures the scenario in which a particular LCC has begun operations in two endpoints of a route, but before flying the actual route itself.

This project extended upon Goolsbee and Syverson's (2008) study by examining incumbent airline prices as a result of entry threat and actual competition from both Southwest Airlines and JetBlue Airways from 1993 to 2009. The data used in this project originated from the Bureau of Transportation Statistics' Airline Origin and Destination Survey (DB1B) database which provides a 10% distribution of airfares per quarter. I used Lexis-Nexis, corporate news releases, and business press releases to identify and verify sample routes that were threatened and entered by either Southwest or JetBlue. Once identified, these routes were marked from the broader sample set with the use of binary variables, similar to a standard event study. I incorporated a panel ordinary-least-squares regression model with fixed effects that examined discrete shifts in incumbent airline fares surrounding the quarters of Southwest or JetBlue threat and entry.

Consistent with the empirical results presented by Goolsbee and Syverson (2008), this project found that

incumbents significantly reduced fares when threatened by Southwest. Controlling for market concentration, airport control costs, and seasonality, and selecting only routes flown by legacy carriers, fares dropped 10.8% during the initial threat quarter and ultimately dropped 23% after Southwest's actual route entry. Roughly, 35-40% of price drops can be attributed to Southwest threat alone (simply operating from two endpoint hubs without flying between the two endpoints). Similarly, incumbents also reacted preemptively to the threat of JetBlue, albeit less aggressively, with fares dropping 4.4% upon initial threat and ultimately declining by 14% upon actual entry. Additional sensitivity analysis revealed that legacy incumbents cut fares more aggressively than low-cost incumbents and routes dominated by low-cost carriers and those connected between hubs with substantial air traffic saw greater drops in fares upon Southwest's actual entry.

The application of empirical models to the airline industry and the findings presented in this project have powerful policy implications. If the impact of Southwest is as beneficial to the average traveler as the research suggests, policies should be designed to promote low-cost presence in markets with high congestion and equally high fares. The potential future roadmap for empirical work on the threat of low-cost carriers and for the powerful policy implications that lie ahead is one that should garner considerable attention from both academics and industry practitioners alike.

References

1. Dresner Martin, Chris Lin and Robert Windle. The Impact of Low-Cost Carriers on Airport and Route Competition. Journal of Transport Economics and Policy. 1996. Vol. 30(3), 309-328.
2. Goolsbee, Austan and Chad Syverson. How Do Incumbents Respond to the Threat of Entry? Evidence from the Major Airlines. The Quarterly Journal of Economics. 2008. Vol. 123(4), 1-37.
3. Morrison, Steven A. Actual, Adjacent, and Potential Competition: Estimating the Full Effect of Southwest Airlines. Journal of Transport Economics and Policy. 2001. Vol 35, 239-256.
4. Tan, Kerry. Incumbent Response to Entry by Low-Cost Carriers in the US Airline Industry. Ohio State University Working Paper. 2010. 1-33.

Quantification of protein carbonylation[1]

Nayoon Kim[2]

[1]Department of Chemistry, MIT

[2]Student Contributor, Class of 2012, Department of Biology, MIT

Many epidemiological studies indicate correlation between protein carbonyls and diseases, such as heart disease, lung disease, neurodegenerative disorders, and inflammatory bowel disease. In a mouse model of human inflammatory bowel disease, inflammatory cells, such as phagocytes, release reactive oxygen and nitrogen species1. These reactive chemicals travel throughout the body and react with proteins and DNA to carbonylate them. These carbonylated proteins are potential biomarkers for these diseases.

A major pathway for protein carbonylation begins by lipid peroxidation of polyunsaturated fatty acids (PUFAs) by enzymes, such as lipooxygenases (LOXs) and cyclooxygenases (COXs) or through ROS-mediated action. These hydroperoxides form a variety of decomposition compounds, including 4,5-epoxy-2-decenal (EDE), 4-hydroperoxy-2(E)-nonenal (HPNE), 4-hydroxy-2-nonenal (HNE), 4-oxo-2-nonenal (ONE), and 9,12-dioxo-10-dodecenoic acid (DODE). These proteins readily carbonylate proteins. Current methods to quantify protein carbonylation utilize mass spectrometry, Western blot, and proteomics2-6, but these methods measure only the proteins that are quantified using specific decomposition products, time-inefficient, and costly. Therefore, the purpose of this project is to develop new time-efficient, cost-effective methods to quantify global protein carbonylation.

In this study, ONE and HNE are used to create carbonylated protein samples. Two approaches have been proposed to quantify protein carbonylation. In the first approach, N-(Aminooxyacetyl)-N'-biotinylhydrazine (Aldehyde Reactive Probe, ARP) will react with protein to label biotin at carbonyl site. Then the ARP-reacted proteins will undergo purification procedures. The biotinylated proteins will bind to the protein immobilization plates. After the removal of unbound proteins by washing, streptavidin conjugated with horseradish peroxidase (HRP) will be added. Biotin and streptavidin are known to interact strongly with high specificity, and these enzyme-labeled streptavidin form complexes with the proteins previously bound to the plate. After another washing step, the enzyme bound complex bound to the plate will bind to tetramethylbenzidine (TMB) chromogenic substrate. The absorbance will be read at the absorbance of 450 nm.

In the second approach, the carbonylated, but not biotinyalted proteins will bind to the protein immobilization plates. After the removal of unbound proteins by washing, primary DODE antibody that binds to carbonyls formed using DODE, will be added. After the removal of unreacted primary DODE antibody by washing, secondary DODE antibody conjugated with HRP, will be added. These secondary antibodies conjugated with HRP form complexes with the proteins previously bound to the plate. After another washing step, the enzyme bound complex bound to the plate will bind to TMB substrate. The absorbance will be read at the absorbance at 450 nm. For both of these methods, the amounts will be quantified by the standard curve constructed from the standards. After these methods are optimized, both of these methods will be used to crosscheck protein carbonylation in clinical samples.

References

1. Dedon, P.C., & Tannenbaum, S.R. (2004). Reactive nitrogen species in the chemical biology of inflammation. Archives of Biochemistry and Biophysics, 432, 12-22.
2. Slade, P.G., Williams, M.V., Brahmbhatt, V., Dash, A., Wishnok, J.S., & Tannenbaum, S.R. (2010). Proteins modified by the lipid peroxidation aldehyde

9,12-dioxo-10(E)-dodecenoic acid in MCF7 breast cancer cells. Chemical Research in Toxicology, 23(3), 557-567.

3. Chung, W., Miranda, C.L., & Maier, C.S. (2008). Detection of carbonyl-modified proteins in interfibrillar rat mitochondria using N'-aminooxy methylcarbonylhydrazino-D-biotin as an aldehyde/ keto-reactive probe in combination with Western blot analysis and tandem mass spectroscopy. Electrophoresis, 29, 1317-1324.
4. Williams, M.V., Lee, S.H., & Blair, I.A. (2005). Liquid chromatography/mass spectrometry analysis of bifunctional electrophiles and DNA adducts from vitamin C mediated decomposition of 15-hydroperoxyeicosatetraenoic acid. Rapid Communications in Mass Spectrometry, 19, 849-858.
5. Williams, M.V., Wishnok, J.S., & Tannenbaum, S.R. (2007). Covalent adducts arising from the decomposition products of lipid hydroperoxides in the presence of cytochrome C. Chemical Research in Toxicology, 20(5), 767-775.
6. Zhu, X.,Tang, X., Anderson, V.E., & Sayre, L.M. Mass spectrometric characterization of protein modification by the products of nonenzymatic oxidation of linoleic acid. Chemical Research in Toxicology, 22(8), 1386-1397.

MURJ Reports

Identification of selective inputs to striosomes of the striatum[1]

Dordaneh Sugano[2]

[1]McGovern Institute, Department of Brain and Cognitive Sciences, MIT

[2]Student Contributor, Class of 2012, Department of Biology, MIT

The striatum, particularly the compartments known as "striosomes," is known to be a key brain region necessary for the formation of habits and stereotypies. Microcircuits consisting of anatomical connections between functionally distinct brain regions are integral to understanding how habits and stereotypies are manifested. In order to elucidate the anatomical basis of these microcircuits, I traced the afferent projections from two segregated brain sites known to project to the striatum, and potentially striosomes: the paraventricular nucleus of the thalamus (PVT) and the infralimbic cortex (IL). This was done by injecting the GFP-expressing anterograde tracer virus AAV5 (regulated by a CAMPKII promoter) into the PVT or the IL of rat brains, a novel method to trace these pathways. I observed that the projections from the IL were striosome-heavy (confirmed with further methods to highlight striosomes biochemically), unlike the PVT, which projected uniformly to the entire striatum. Interestingly, I found that afferents from both injection sites innervated the striatum, amygdala, and either IL or PVT (for PVT or IL injection, respectively) suggesting the existence of a microcircuit that has a role in determining which inputs could form, induce, and/or modulate a habit or stereotypye.

Introduction

Habit is a defining aspect of behavior. A habit is a series of actions or thoughts that is triggered by the brain in response to a certain stimulus (Graybiel, 2008). Similar to the habit is the stereotypy, which is a more rigid, repetitive behavior, such as hand wringing or marching in place (Muthugovindan & Singer, 2009). In habits and stereotopies, the brain integrates sensory information from both the environment and internal/body cues and processes the information to yield a behaviorally relevant action. Important information can enter the brain via many channels, for instance eyes (vision), ears (hearing), spinal cord/brainstem (internal and postural information) and so on. This information is then processed by many stages of brain evaluation: first by subcortical structures, such as the thalamus, and then by cortical structures, before being directed to an action or output (Pennartz, Berke, Graybiel, et al., 2009).

The basal ganglia are a set of subcortical interconnected and functionally related brain nuclei which play a major role in habit formation. They are responsible for integrating sensorimotor, associative, and limbic information from other brain regions and determining which behaviors are most useful when translated into action (Bolam, 2000). When habits are formed, there is a loss in the decision making process that leads to an action or thought so that assessment of a particular behavior prior to action (evaluation-bound circuit) is bypassed and processed directly by the motor circuit, the basal ganglia (Tanimura, King, Williams, et al., 2011). It is easy to see how habitual behavior is relevant in situations in which automaticity is useful- for example,

routinely checking to make sure the stove is off before you leave the house.

Dysfunctions involving habit formation are seen in many neurological disorders such as OCD and autism, as well as in addiction (Aliane et al., 2009, Graybiel, 2008). In order to better study these disorders, we must first understand the neural circuits behind them. Rodents are an excellent model for studying habits and stereotypies (e.g. whisker grooming) as the circuits and behaviors involved are well preserved across species.

The striatum plays a vital role in habit formation and in the function of the basal ganglia itself. The striatum is the largest nucleus of the basal ganglia, and it receives almost all the information for the basal ganglia from other brain structures (Bolam et al., 1999). A large proportion of he input to the basal ganglia is derived from the cerebral cortex, which is involved in conscious decision making (Bolam et al., 1999). This input is known to affect the motor output of the basal ganglia (Ridley, 1994). The striatum also receives a large input from the thalamus, which is responsible for receiving sensorimotor information (Bolam et al., 1999). The striatum can be divided into two intermingled but separate compartments called "striosomes" and "matrix" with distinct biochemical make-up and discrete afferent inputs. For instance, striosomes can be identified based on their relatively high density of μ-opioid receptors (MOR-1), in the comparison to the matrix that can be identified, amongst other things, by the high density of cholinergic interneurons (Miura et al., 2007).

The interplay between striosomes and matrix has been shown to affect habit formation. It has been hypothesized, for example, that habit formation results in long-term inhibition of the matrix, resulting in activation of the striosomes (Canales, 2005 and Aliane et al., 2011). In fact, activation of the striosomes is directly correlated with the induction of repetitive behavior. It has been shown in the rat that inhibiting the striosomes decreases the presence of stereotypies, which can be induced by treatment with cocaine and methamphetamine (Aliane et al., 2009 and Horner et al., 2010). Therefore, I hypothesize that I can induce stereotypies, which are more easily quantified than habits, in rats by selective stimulation of the striosomes, thereby providing an excellent behavioral model. However, as the striosomes consist of several, irregular patches, it is impossible to stimulate them in their entirety merely by the insertion of an electrode. Thus, an alternative strategy would be to stimulate regions of the brain which project directly to the striosomes, such as the thalamus or cortex.

Previous research has shown that the paraventricular thalamic nuclei (PVT) project to the striosomes in cats (Ragsdale and Graybiel, 1991). The PVT is a midline thalamic nucleus, involved in arousal and attention, which communicates a great deal with the limbic forebrain (Morgane, Galler, and Mokler, 2005). The limbic forebrain, which includes the infralimbic cortex (IL), processes emotions and motivations, and the interplay between the limbic forebrain and the PVT influences learning and memory (Morgane, Galler, and Mokler, 2005). This is very interesting, as inactivation of the IL has been shown to counteract habitual behaviors in rats (Coutureau and Killcross, 2003) and preliminary evidence suggests that the IL projections to the striatum may be striosome specific.

This study hopes to demonstrate that the PVT-striosome and IL-striosome pathways exist in the rat, as there is much more precedent regarding electrophysiological experimentation in rats than in cats. Although there have been several studies concerning afferent projections to the striosomes in the rat using fluorogold or Phaseolus vulgaris methods (Morgane, Galler, and Mokler, 2005; Vertes and Hoover, 2008; Moga, Weiss, and Moore, 1995; Hurley, Herbert, Moga, et al., 1991; Sesack, Deutch, Roth, et al., 1989; Vertes, 2002), this study intends to use this viral method to further verify this pathway and the PVT-striosome pathway. I hypothesize that there is a circuit, involved in habit formation, that links the PVT, IL, and striosomes, and that the hijacking of this circuit could lead to the unfavorable habitual behavior seen in disorders such as autism and OCD.

Materials and Methods

Animals

All experimental procedures were carried out according to MIT animal care guidelines on adult male Sprague-Dawley rats, 250-450 kg (Charles River, Margate, UK). Rats were housed in groups of two with access to food and water ad libitum. All experiments were performed in accordance with the Massachusetts Institute of Technology's Committee on Animal Care policy.

Tracer Virus

Anterogradely traveling GFP-expressing adeno-associated virus 5 (AAV5, UNC Vector Core), was injected into the rat brains. GFP and channelrhodopsin were expressed under the CAMKII promoter, which is inserted into the host cell genome. The CAMKII promoter is only activated by glutamatergic cells, and thus the GFP and channelrhodopsin will be selectively expressed in glutamatergic cells. The majority of cells that project out of the PVT and the IL are glutamatergic.

Viral Injection

Viral injections were performed while animals were under deep general anesthesia. Anesthesia was induced with isoflurane (IsoFlo, Abbot) by inhalation and a mixture of ketamine and xylazine i.p. (Ketaset 100 mg/kg, Fort Dodge and TranquiVed 10 mg/kg, Vedco, respectively) and maintained thereafter with ketamine i.p.. Anesthesia levels were assessed by testing the pedal withdrawal reflex or response to gentle corneal stimulation, and additional ketamine was injected if necessary. Corneal drying was prevented with application of Puralube ointment (Dechra).

The fur covering the skull was shaved off and the rat was placed in a stereotaxic frame (David Kopf Instruments). An incision was made in the skin over the center of the skull and the skin clamped apart to expose the surface of the skull. The soft tissue covering the skull was removed. A needle was positioned directly above Bregma reference point (zero position) and the stereotaxic coordinates noted. The needle was then placed, using coordinates derived from the atlas of (Paxinos and Watson, 1986) directly above the desired injection sites (**Figure 1**). A hole was made in the skull using a hand-held electric dental drill (Moto-Tool 380, Dremel) and any bone chips removed with tweezers. A NanoFil microsyringe (WPI) was positioned at the desired injection site and virus was injected (0.1-0.5 µL) using a Quintessential Stereotaxic Injector (Stoelting) over a period of approximately 30 minutes. The skin was sutured closed and disinfected with Neosporin (Johnson & Johnson). Animals were returned to their home cages for recovery and the virus was allowed to replicate over a period of 2-4 months.

Perfusion Fixation and Sectioning

Animals were deeply anesthetized with a lethal dose of Euthasol (pentobarbital, 200 mg/kg, Virbac). They were then perfused via the ascending aorta with about 100 mL PB (0.1 M, pH 7.4) followed by about 250 mL of fixative solution (4% paraformaldehyde in 0.1M PB). The brain was immediately removed from the skull and stored in the fixative solution for 2 hours, then replaced with glycerol sinking solution (25% glycerol in 0.1 PB with sodium azide). Coronal sections (40 µm) were cut using a cryomicrotome (American Optical) and maintained in 0.01 M PB with 0.1% Sodium Azide.

Immunohistochemistry and light microscopy

Sections were rinsed in PBS-Tx (0.2% Triton X-100, Sigma in 0.01 M PBS) then incubated in 3% H_2O_2 in PBS-Tx. Non-specific binding was blocked with 10% Normal Goat Serum in PBS-Tx. Sections were then incubated with a polyclonal rabbit anti-GFP primary antibody (1:2000, A 11122, Invitrogen), followed by Biotinylated goat anti-rabbit IgG (Vector). Sections were washed with PBS-Tx between steps. The secondary antibody was visualized by an ABC-DAB-Ni peroxidase method (Peroxidase Substrate Kit, Vector). Sections were mounted onto glass slides using a PB-gel-azide solution (0.1 M PB, 0.1% Sodium Azide, 0.08% gel) and left at room temperature until dry. The sections were then dehydrated through a series of dilutions of ethanol (70% v/v, 95% v/v, and 100% v/v for 2 minutes each) and then of xylene (2-3 rounds of 100% v/v for 5-12 minutes). Coverslips were mounted onto the slides using the Eukitt

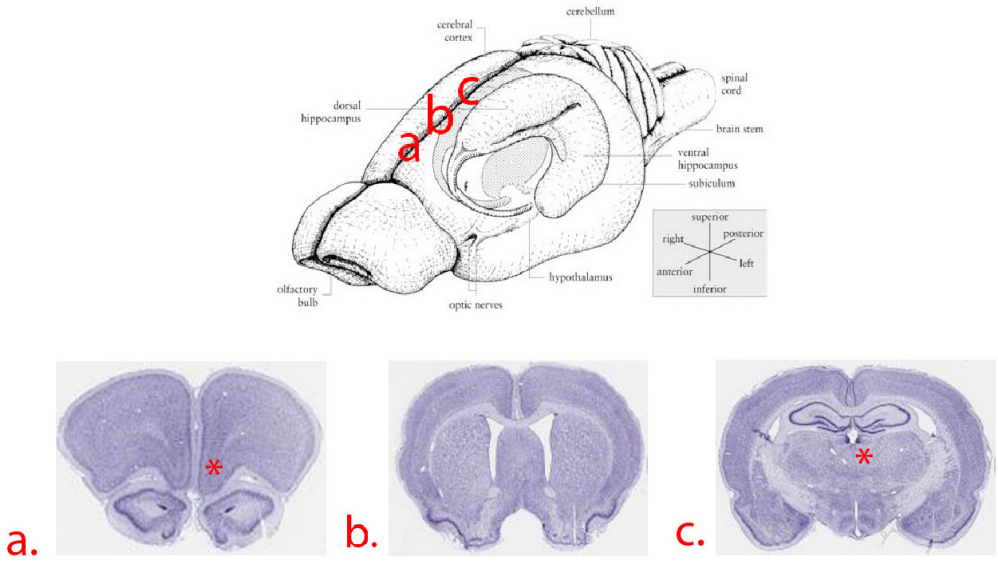


Figure 1. Illustration of a rat brain, showing major structures (Cheung and Cardinal, 2005). This study focuses on the following regions of the brain. (a)-(c) are coronal sections showing the rat brain at the level of (a) IL, (b) striatum, (c) PVT (Jones, E. G., BrainMaps.org). (*) indicates putative injection site.

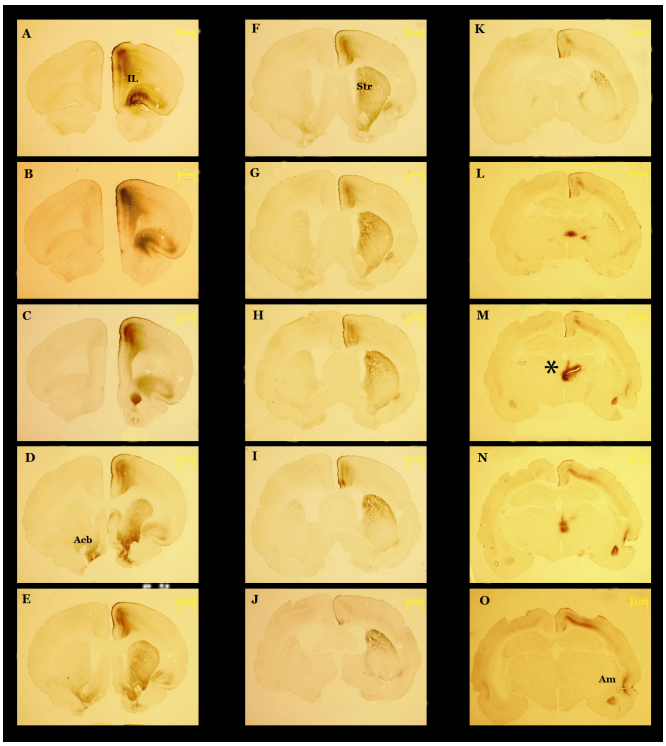


Figure 2. Progression of serial coronal sections of rat brain after injection of GFP-expressing anterograde virus into paraventricular nucleus of the thalamus. (A) is the most rostral section moving back to the most caudal (O). GFP was revealed with an ABC/DAB method. * indicates putative injection site, PVT. Acb, nucleus accumbens; IL, infralimbic cortex; Am, amygdala; Str, striatum. The projections shown in this figure are representative of all three successful injections. Scale bar = 2.0 mm.

mounting medium (Calibrated Instruments, Inc.). The sections were examined under a light microscope.

Immunohistochemistry and Fluorescent Microscopy

Sections were rinsed with PBS-Tx. They were then incubated with rabbit anti-MOR-1 (24216, Immunostar) and the antibody was detected with Alexa Fluor 568 goat anti-rabbit IgG (Vector). Sections were mounted onto glass slides using the PB-gel-azide solution and left at room temperature until dry. Coverslips were mounted onto the slides using Vectashield mounting medium (Vector). The sections were examined under an Olympus DP70 microscope.

Images from fluorescent microscopy were captured using Olympus DP70 Imaging software and manipulated with Adobe Photoshop and Image J.

Results

In order to determine the specific origin of inputs to the compartments of the striatum, I injected an anterograde viral tracer into two brain areas which putatively project to the striosomes: the PVT and the IL (**Figure 1**). I found that the PVT and the IL project to essentially the same brain areas; however, the PVT, unlike the IL, does not project to the striosomes.

Thalamic (PVT) injections

Anterograde tracer virus was injected into the PVT of five rats and visualized with both immunohistochemical (ABC/DAB) light microscopy and fluorescent methods. Of the five injections, two failed due to limited spread and uptake and the results were discarded from analysis. In the three successful injections, immunohistochemical methods revealed that the virus (dark brown reaction product) was localized to cell bodies in the PVT (**Figure 2m**). However, in most cases the virus at the injection site exceeded the borders of the PVT and thus it is possible that virus was taken up by cells in surrounding thalamic nuclei. The injections visualized under fluorescent microscopy (that is, the GFP expressed without additional immunohistochemistry) were concomitant with those visualized with immunohistochemistry methods.

Of the three successful injections into the PVT, two were bilateral (that is, expressed in the PVT of both hemispheres) and one injection was confined to one hemisphere (**Figure 2**). The projections themselves were easily visualized with both methods as the virus caused the cells it infected to express GFP throughout the entirety of the cell body, the dendrites, and the axon. In the single hemisphere injection, projections

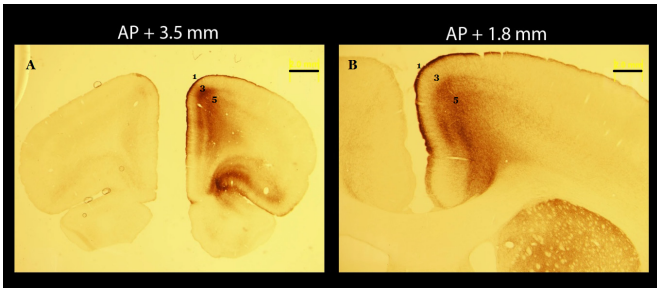


Figure 3. Coronal section of cortex demonstrating layers innervated by virally injected paraventricular nucleus. Viral GFP was revealed with an ABC/DAB method. Labels indicate cortical layers 1, 3, and 5 of the same brain. These regions show a high concentration of labeled projections. This figure is representative of all three successful injections. Scale bar is 2.0 mm.

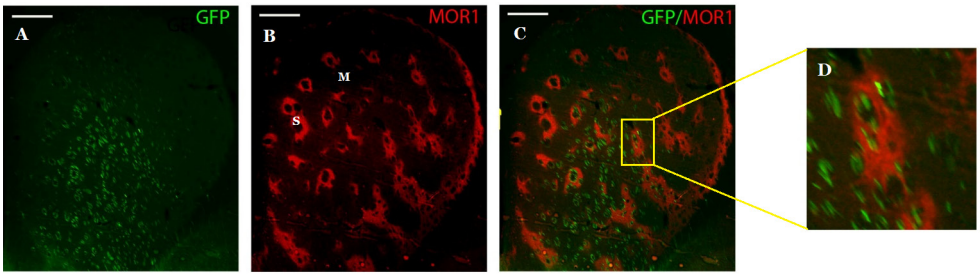


Figure 4. A single coronal section through the striatum showing GFP-PVT projections (A) and MOR-1 (B). Panel C shows merge of (A) and (B) and the lack of yellow shows that there is no correlation between the GFP (green) and the MOR-1 (red) protein. (D) Zoom of the colocalization in the box. (S) striosomes, (M) matrix. This figure is representative of all three successful injections. Scale bar = 150 μm.

(PVT cell axons) were found in the striatum, the cortex, the amygdala and the nucleus accumbens (**Figure 2**). The projections to the striatum, the cortex, and the amygdala were unilateral and ipsilateral (**Figure 2**). The projections to the nucleus accumbens, another nucleus of the basal ganglia, were bilateral. I found that the afferent projections to the cortex terminated specifically in layers 1, 3, and 5 (**Figure 3**). In addition, a single labeled cell body (not an axon originating from the PVT) was found in the amygdala of one brain section (data not shown).

The PVT projections to the striatum were fairly widespread (**Figure 2g**). Although there were no darker patches that would indicate that the PVT projections were striosome-specific, in order to test this striosome-specificity, striatal sections were processed for an opioid receptor (MOR-1) protein, a biochemical marker for striosomes (**Figure 4**). At this magnification, there was no colocalization of the GFP-projections and the striosome marker, suggesting that PVT projections were not striosomal (**Figure 4d**). In addition, the projections in the striatum appeared to be mostly fibers of passage (that is, not forming boutons and synapses in the striatum). However, inspection under higher magnification (X 100) revealed the presence of some terminal boutons and, therefore, likely synapses (**Figure 5**). At the higher magnification, I found that the GFP-containing axons and boutons (axon terminals) did invade the striosomes, but the projection was not selective, with axons and boutons also present in the matrix (**Figure 5**).

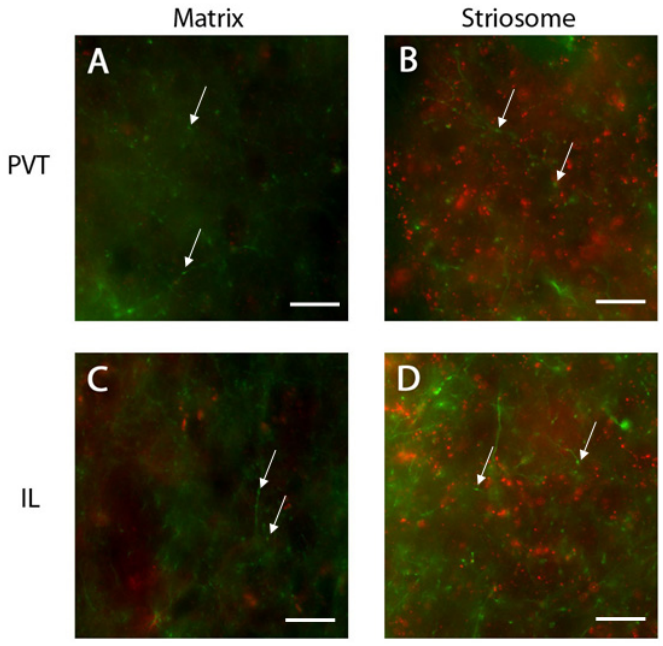


Figure 5. Fluorescent images show the presence of boutons in the striatum (striosomes and matrix) from both the PVT and the IL. The GFP-expressing neurons are shown in green and the MOR-1 expressing striosomes in red. Arrows indicate examples of boutons. Note that there are boutons in areas with and without red. Scale bar = 15 μm.

Cortical (IL) injections

Three cortical injections were performed with the anterograde tracer virus in order to determine the projections of the infralimbic cortex (IL), specifically whether the IL projects to the striosomes. All three cortical injections were localized to the infralimbic region (**Figure 6a**), although the viral spread at the injection site exceeded the boundaries of the IL. This resulted in widespread labeling as was seen in the thalamic injections. The projection pattern of the cortical injections is very similar to that of the thalamic injections: labeling was found in the striatum, the PVT, the amygdala, and the nucleus accumbens (**Figure 6**).

The IL projections were mostly confined to a column on the medial wall of the striatum (**Figure 6f**). The IL projection to the striatum was observed to have a "patchy" pattern that is distinctly striosomal. To further verify that these patches were striosomes, the striatal sections were processed for the MOR-1 protein (striosome dense), and fluorescent light microscopy revealed colocalization of afferent fibers from the cortex with this striosome marker (**Figure 7**). Under a higher magnification (100x), I found that the projections had terminal boutons, likely synapses, in not only the striosomes but also the matrix (**Figure 4**). Many of the afferent axons and fibers from

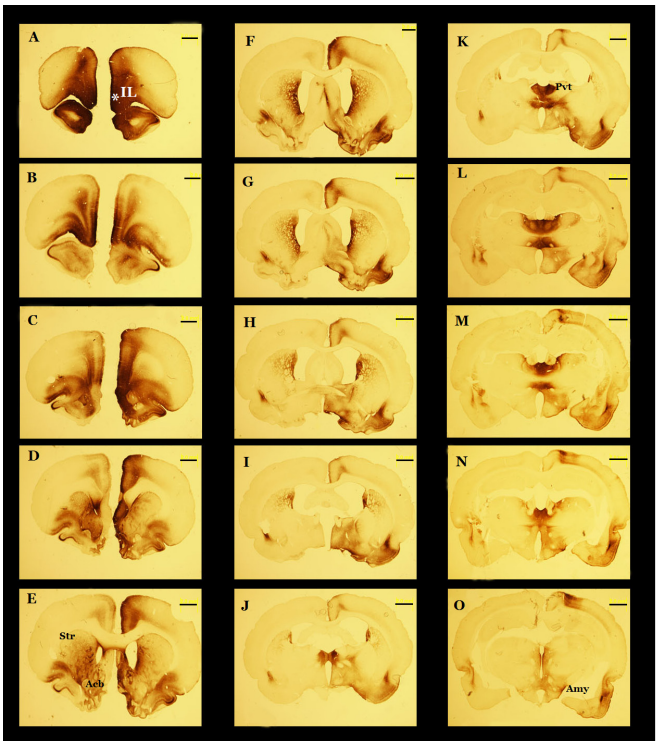


Figure 6. Progression of serial coronal sections of rat brain after injection of GFP-expressing anterograde virus into the infralimbic region of the cortex. (A) is the most rostral section moving back to the most caudal (O). GFP was revealed with an ABC/DAB method. (*) indicates putative injection site (IL); Acb, nucleus accumbens; Amy, amygdala; Pvt, Paraventricular Nucleus; Str, striastum. This figure is representative of all three injections. Scale bar = 2.0 mm.

the cortex appeared to be fibers of passage. The fibers of passage did not seem to correspond with the striosomes–the thicker, "green" fibers of passage in **Figure 7c** do not localize with the "red" striosomes. This indicates that although the IL does project to the striosomes and the matrix, it also has many projections that traverse the striatum in a non-striosome-matrix-specific manner and terminate outside of the striatum.

Discussion

Using a novel virus I determined the afferent projections of the PVT and the IL to the striatum and revealed the presence of a circuit potentially important to habit formation. I found that the PVT and IL project to the striatum, nucleus accumbens and amygdala, and furthermore, the IL and the PVT project to each other. At the level of the striatum, the IL projections were largely striosome-selective, whereas the PVT projections were not compartment specific. From this data, I hypothesize that the IL and the PVT, not only functionally influence the same brain areas separately, but also that they have a connection that allows them to work together.

Efferent Projections of the PVT and the IL

I found that the PVT projects to the striatum, IL, nucleus accumbens, and amygdala in the rat. This is in concordance with previous research using other anterograde tracers (Vertes and Hoover, 2008; Moga, Weiss, and Moore, 1995; Groenewegen, 1988). My observations indicate that the projections from the PVT to the striatum were uniform across compartments (both striosome and matrix) in the striatum, and not striosome-selective. This is contrary to research which has found the PVT projections to be striosome-specific in the striatum of cats (Ragsdale and Graybiel, 1991) and in the ventral striatum of rats (Berendse, Voorn, and Kortschot et al., 1988). It is possible that the differences between our results and previous research are a result of the spread of the virus into thalamic nuclei that project to the matrix, thus obscuring any selectivity we might have seen with localized PVT injections. It is also noteworthy that the previous studies used alternative markers for striosomes, as well as different tracers.

I found that the IL projects to the striatum, PVT, nucleus accumbens, and amygdala in the rat, which is supported by previous studies (Hurley, Herbert, Moga, et al., 1991; Sesack, Deutch, Roth, et al., 1989; Vertes, 2002). The IL was found to project to the same areas as the PVT, with the notable exception that the IL projections to the striatum are striosome-selective.

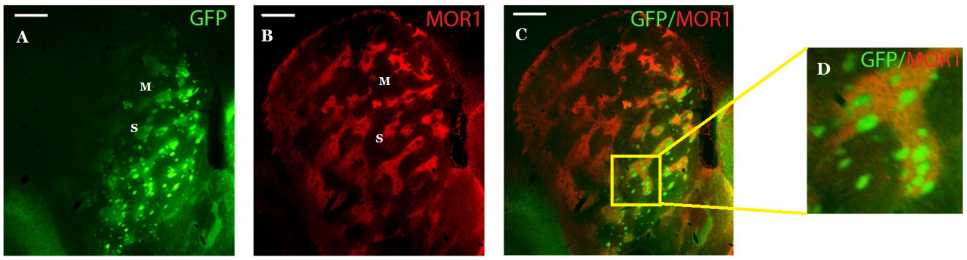


Figure 7. A single coronal section through the striatum showing GFP-IL projections (A) and MOR-1 (B). Panel C shows merge of (A) and (B) the yellow shows that there is a correlation between the GFP (green) and the MOR-1 (red) protein. (S) striatum, (M) matrix. (D) Zoom of the colocalization in the box. This figure is representative of all three successful injections. Scale bar = 150μm.

Some projections were also found in the matrix. To my knowledge, there is no previous research indicating that IL projections are striosome-selective; however, other areas of the prefrontal cortex, including cortical layers Vb and VI, have been shown to project to striosomes (Eblen and Graybiel, 1995; Kincaid and Wilson, 1996). It is possible that the matrix projections from my data originate, not from the IL itself, but from adjacent areas which were labeled with excess viral tracer at the injection site, and that the IL projections are, in fact, specific to only the striosomes.

Methological Considerations

The injection sites were greater than the boundaries of both the PVT and the IL. As a result, it is probable that neurons outside of the intended injection sites expressed the viral tracer. In future experiments, I would use a smaller bolus of virus to limit spread. The paratenial nucleus (PT) of the thalamus, which is adjacent to the PVT, projects more densely to the medial prefrontal cortex than the PVT (Vertes and Hoover, 2008). Perhaps my injection site includes the PT, and thus it is possible that some of the projections to the prefrontal cortex originate more from the PT rather than the PVT. The injection sites in the cortex covered a large portion of the medial prefrontal cortex. The virus appeared to spread more in the injection sites in the cortex than in those in the thalamus, although the amount of virus injected was roughly the same. This could be a result of differing compositions of cells and white matter patterns in the cortex and the thalamus. Axons from the infralimbic cortex have been known to project to neurons in layers III and V, which could explain the wider spread of the injection site in the cortex in relation to the thalamus as well as the fact that the projections at the IL injection site segregate mostly to layers I, III, and V (Hurley, Herbert, Moga et al., 1991).

The accuracy of this novel viral technique is supported previous findings in rats concerning the projections of both the PVT and the IL using a non-viral tracer (Morgane, Galler, and Mokler, 2005; Vertes and Hoover, 2008; Moga, Weiss, and Moore, 1995; Hurley, Herbert, Moga, et al., 1991; Sesack, Deutch, Roth, et al., 1989; Vertes, 2002). However, previous studies also find projections in additional sites. PVT projections have been discovered in the bed nucleus of the stria terminalis, the perirhinal cortex, and the ventral subiculum, while IL projections have been found in several thalamic nuclei, the hypothalamus, and the bed nucleus of the stria terminalis (Vertes and Hoover, 2008; Moga, Weiss, and Moore, 1995; Groenewegen, 1988; Hurley, Herbert, Moga, et al., 1991; Sesack, Deutch, Roth, et al., 1989; Vertes, 2002). There appears to be some variation in the efferent projection sites enumerated in different studies, but projections are consistently found in the striatum and the amygdala.

The discovery of a single labeled cell body in the amygdala in one the thalamic injections is truly an anomaly, indicating that the virus may have crossed the synapse of one axon projecting to the amygdala. If true, this would introduce a caveat into the use of the virus as a functional tracer. However, as there was only one cell body found labeled outside of the injection site, it would appear that such occurrences are very rare.

Functional Implications

Habit formation is a complex process (Graybiel, 2008), and the ability of the PVT, IL, and striatum to communicate would be, I hypothesize, vital to this process. My data supports the existence of a functional circuit between these structures and, thus, both the IL and the PVT could regulate the role of the striatum in habit formation. It is known that inactivation of the IL can reverse the effects of habit formation in rats, indicating how vital this region is to habit formation and maintenance (Coutureau and Killcross, 2003). In my hypothesis, the PVT determines which inputs are significant enough to create or induce a habit in the IL, in part through communication with the IL. Indeed, previous work suggests that the PVT acts on the limbic forebrain to choose certain courses of action over others (Vertes and Hoover, 2008), and while my data supports this, it also indicates that the limbic forebrain can influence the PVT.

Both the PVT and the prefrontal cortex are connected by another factor: stress. Stress is most closely linked to the amygdala, to which both sites project, and is known to cause associated changes in both the prefrontal cortex and the amygdala. The amygdala may regulate the role of the prefrontal cortex in learning and memory (Roozendaal, McEwen, and Chattarji, 2009). It has also been hypothesized that the PVT may modulate amygdalar processing of stress through inhibition (Roozendaal, McEwen, and Chattarji, 2009). The relationship of this circuit to stress reinforces what is already known about the huge influence of stress on habit formation (Graybiel, 2008). Regulation of the amygdala in response to stressors is known to increase learning, and learning is the first stage of habit formation. It is possible that the PVT can sort through stressful inputs and determine which are relevant, modulating the amygdala to recognize these as important, increasing learning and memory for events related to these inputs. The PVT would then pass the relevant inputs along to the IL resulting in induction or creation of a stress-related habit and also connecting many habits to stressful inputs. This would explain why

many habits and stereotypies are more pronounced in stressful situations (Graybiel, 2008).

Future Experimentation

One enormous advantage of my study is that it paves the way for future optogenetic studies involving channelrhodopsin or halorhodopsin. The same virus, expressing light sensitive calcium or chloride channels, can be injected into specific regions brain, resulting in expression of these channels in cells of that region. Therefore, small sections of the brain can be stimulated or inhibited in vivo, in a matter of milliseconds. As the striosomes are distributed seemingly randomly throughout the striatum, it would be nearly impossible to stimulate or inhibit all of them selectively. However, it would be possible to influence their behavior by modulating a site which projects exclusively to striosomes. If future research indicates that the infralimbic cortex is such a site, it will be very useful for optogenetic experiments.

Summary

In conclusion, the prefrontal cortex and the paraventricular nucleus appear to be linked in a circuit that has great implications for habit formation and stereotypies. My data demonstrates that the IL projections are striosome-selective, while the PVT projections do not appear to be striosome or matrix-selective. Therefore, the IL would be an excellent candidate for selective stimulation of striosomes with a virus containing channelrhodopsin. My next goal is to use optogenetics to evaluate the role of the striosomes in habits and stereotypies.

Acknowledgements

Many thanks to my direct supervisor and mentor, Dr. Carolyn J. Lacey and to my PI, Dr. Ann Graybiel.

References

1. Aliane, V., Pérez, S., Bohren, Y., Deniau, J. M., and Kemel M. L. (2011) Key role of striatal cholinergic interneurons in processes leading to arrest of motor stereotypies. Brain: a journal of neurology, 134 (Pt. 1), 110-118.
2. Aliane, V., Pérez, S., Nieoullon, A., Deniau, J. M., Kemel, M.L. (2009) Cocaine-induced stereotypy is linked to an imbalance between the medial prefrontal and sensorimotor circuits of the basal ganglia. European Journal of Neuroscience, 30(7), 1269-1279.
3. Bolam, J. P., Hanley, J. J., Booth, P. A. C., and Bevan, M. D. (2000) Synaptic organisation of the basal ganglia. Journal of Anatomy, 196, 527-542.
4. Canales, JJ. (2005) Stimulant-induced adaptations in neostriatal matrix and striosome systems: transiting from instrumental responding to habitual behavior in drug addiction. Neurobiology of Learning and Memory, 83(2), 93-103.
5. Crittenden, J. R. & Graybiel, A. M. (2011) Basal ganglia disorders associated with imbalances in the striatal striosome and matrix compartments. Frontiers in Neuroanatomy, 5, article 59.
6. Coutureau, E., Killcross, S. (2003). Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. Behavioural Brain research, 146(1-2), 167-74.
7. Eblen, F., and Graybiel, A. M. (1995) Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey. Journal of Neuroscience, (9):5999-6013.
8. Fujiyama, F., Sohn, J., Nakano, T., Furuta, T., Nakamura, K. C., Matsuda, W., and Kaneko, T. (2010) Exclusive and common targets of neostriatofugal projections of rat striosome neurons: a single neuron-tracing study using a viral vector. European Journal of Neuroscience, 33, 668-677.
9. Ferguson, S.M., Sandygren, N.A., and Neumaier, J.F. (2009) Pairing mild stress with increased serotonin-1B receptor expression in the nucleus accumbens increases susceptibility to amphetamine. European Journal of Neuroscience, 30(8), 1576-1584.
10. Gerfen, C. R. (1987) Synaptic organization of the striatum. Journal of Electron Microscopy Technique, 10, 265-281.
11. Graybiel, A. M. (2008) Habits, Rituals, and the Evaluative Brain. Annual Reviews in Neuroscience, 31, 359-387.
12. Groenewegen, H. J. (1988) Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to mediodorsal-prefrontal topography. Neuroscience, 24, 397– 431.
13. Horner, K. A., Noble, E. S., and Gilbert, Y.E. (2010) Methamphetamine-induced stereotypy correlates negatively with patch-enhanced prodynorphin and arc mRNA expression in the rat caudate putamen: the role of mu opioid receptor activation. Pharmacology, Biochemistry, and Behavior, 4, 410-421.

14. Hurley, K. M., Herbert, H., Moga, M. M., and Saper, C. B. (1991) Efferent projections of the infralimbic cortex of the rat. Journal of Comparative Neurology, 308: 249-276.
15. Ji, N. Y., Capone, G. T., Kaufmann, W. E. (2011) Autism spectrum disorder in Down syndrome: cluster analysis of Aberrant Behaviour Checklist data supports diagnosis. Journal of intellectual disability research, j.1365-2788.2011.01465.
16. Kincaid, A. E., and Wilson, C.J. (1996) Corticostriatal innervation of the patch and matrix in the rat neostriatum. Journal of Comparative Neurology, 374(4):578-92.
17. Lanovaz, M. J. (2011) Towards a comprehensive model of stereotypy: integrating operant and neurobiological interpretations. Research in developmental disabilities, 32 (2), 447-455.
18. Liu B, Ouyang L, Mu S, Zhu Y, Li K, Zhan M, Liu Z, Jia Y, and Lei W. (2011) The morphological characteristics of corticostriatal and thalamostriatal neurons and their intrastriatal terminals in rats. Surgical and radiologic anatomy, s00276-011-0823-9.
19. Miura, M., Saino-Saito, S., Masuda, M., Kobayashi, K., and Aosaki T. (2007) Compartment-specific modulation of GABAergic synaptic transmission by mu-opioid receptor in the mouse striatum with green fluorescent protein-expressing dopamine islands. Journal of Neuroscience, 27(36), 9721-9728.
20. Moga, M.M., Weiss, R.P., and Moore, R.Y. (1995) Efferent projections of the paraventricular thalamic nucleus in the rats. Journal of Comparative Neurology, 359: 221–238
21. Morgane, P.J., Galler, J.R., and Mokler, D.J. (2005) A review of systems and networks of the limbic forebrain/limbic midbrain. Progress in Neurobiology, 75(2):143-6.
22. Muthugovindan, Deivasumathy and Singer , Harvey. (2009) Motor stereotypy disorders. Current Opinion in Neurology, 22, 131–136 .
23. Pennartz, C. M., Berke, J.D., Graybiel, A.M., Ito, R., Lansink, C.S., van der Meer, M., Redish, A.D., Smith, K.S., and Voorn, P. (2009) Corticostriatal Interactions during Learning, Memory Processing, and Decision Making. The Journal of Neuroscience, 29(41): 12831-12838.
24. Ragsdale, C.W. and Graybiel, A.M. (1991) Compartmental Organization of the Thalamostriatal Connection in the Cat. The Journal of Comparative Neurology, 311, 134-167.
25. Ridley, R.M. (1994) The psychology of perservative and stereotyped behaviour. Progress in neurobiology, 44(2), 221-231.
26. Roozendaal, B., McEwen, B.S., and Chattarji, S. (2009) Stress, memory, and the amygdala. Neuroscience, 10:423-432
27. Sesack, S.R., Deutch, A.Y., Roth, R.H., and Bunney, B.S. (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. Journal of Comparative Neurology, 290(2):213-4
28. Takahashi, C., Ohata, H., Shibasaki, T. (2011) Corticotropin-releasing factor (CRF) receptor subtypes in mediating neuronal activation of brain areas involved in responses to intracerebroventricular CRF and stress in rats. Peptides, Epub.
29. Tanimura Y., King M. A., Williams D. K., and Lewis M. H. (2011) Development of repetitive behavior in a mouse model: roles of indirect and striosomal basal ganglia pathway. International journal of developmental neuroscience, 29 (4), 461-467.
30. Tran, L and Greenwood-Van Meerveld, B. (2011) Lateralized amygdala activation: Importance in the regulation of anxiety and pain behavior. Physiology & Behavior, 105(2), 371-375.
31. Vertes, R. P. (2002) Analysis of projections from the medial prefrontal cortex to the thalamus in the rat, with emphasis on the nucleus reuniens. Journal of Comparative Neurology, 442: 163-187).
32. Vertes, R.P. and Hoover, W.B. (2008) Projections of the paraventricular and paratenial nuclei of the dorsal midline thalamus in the rat. Journal of Comparative Neurology, 508(2), 212-237.
33. Weiner, D. J. I. (1997) The connections of the primate subthalamic nucleus: indirect pathways and the open-interconnected scheme of basal ganglia-thalamocortical circuitry. Brain Research Reviews, 23, 62-78.