Volume 16 Fall 2007

MURJ Journal

Massachusetts Institute of Technology Undergraduate Research Journal

Redefining the Impossible...

The Biomedical Research Issue

Massachusetts Institute of Technology

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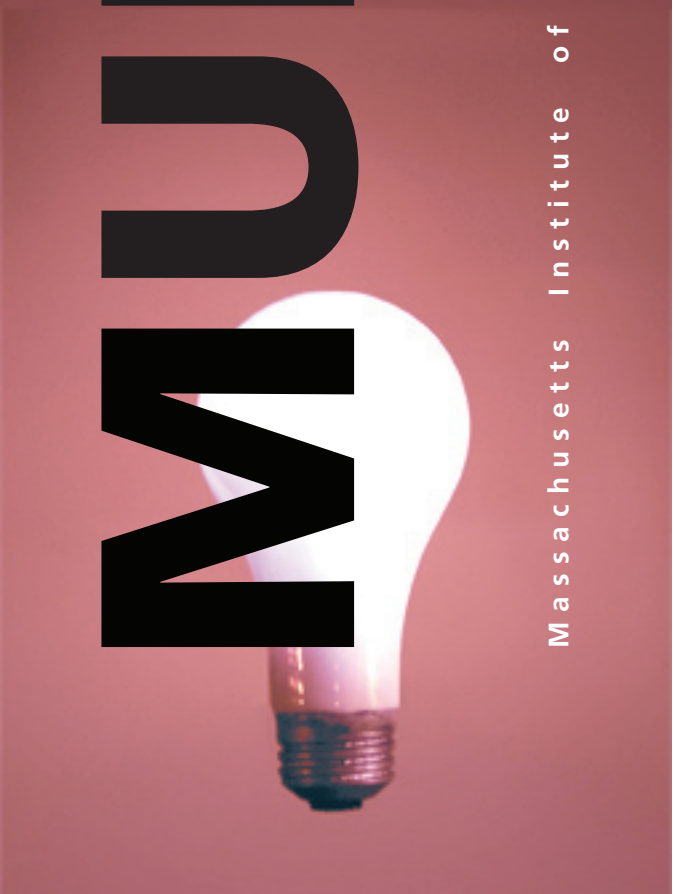

Massachusetts Institute of Technology

UNDERGRADUATE RESEARCH JOURNAL
Volume 16, Fall 2007

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Cover Photo: 'Light bulb,' by Jeff Lieberman, who also built the device. This is not a trick or a photoshop manipulation. The bulb and the casing contain hidden circuitry that uses electromagnetic feedback to levitate the bulb roughly 2.5" from the nearest object, and uses wireless power transfer to beam power from the housing into the bulb itself. Technical details can be found at http://bea.st.

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I am honored to introduce the current issue of the MIT Undergraduate Research Journal (MURJ). This issue focuses on Biomedical Research. This is a very important topic and one in which MIT has made, and will continue to make, enormous contributions.

With its outstanding programs in biology and bioengineering, and its superb Institutes such as the Whitehead, Broad, McGovern, and Picower; its world renowned Center for Cancer Research; and the Harvard-MIT Health Sciences and Technology (HST) program, MIT students are doing incredible cutting edge research in new areas of biomedicine. This current issue of MURJ reflects the breadth and depth of this research. Such topics include prion diseases, new approaches for creating flu vaccines, micropatterning to control cellular organization, effects of neuronal responses due to aging, computational modeling to examine the effects of retinal injury, and developmental regulation during neurogenesis.

Not only have MIT students and faculty made major contributions to basic biomedical research, they have also done a wonderful job translating biomedical discoveries into practical products to help patients. In the past decade, at least 50 biomedical companies were started based on MIT inventions or discoveries. New potentially lifesaving products based on MIT inventions include new drugs based siRNA, nanotechnologies that enable precise targeted delivery to cancer cells, new intelligent medical devices and novel ways to diagnose disease. Biomedical research at MIT—fueled by its outstanding students—is transforming the nation and the world. This issue of MURJ is an exciting contribution to this very important area.

Institute Professor Robert Langer

MIT Science News In Review

[Biomedical Research]

New Mechanism of Gene Control

Whitehead postdoctoral students Stuart Levine and Matthew Guenther discover a new mechanism of gene control.

Although each cell in the human body contains a complete copy of the human genome, only a fraction of the genes within the cell are actually expressed. It was previously believed that DNA expression was controlled by the tight coiling of DNA around proteins known as histones. A new study conducted in the laboratory of MIT Biology Professor and Whitehead Institute Member Richard Young suggests there may be more to the picture.

Although genes are often described in terms of an on/off switch, Whitehead researchers Matthew Guenther and Stuart Levine have discovered that this model may, in fact, be too simplistic for some human genes. Although these genes begin the process of producing a RNA template for protein transcription, the process is never completed. According to Young, "about one-third of our genes, including all the regulators of cell identity, fall into this new class."

Guenther and Levine worked with embryonic stem cells, liver cells and white blood cells in a genetic screen for a particular chemical signal which corresponds to this form of looser DNA packaging. Although they expected to find the landmark chemical in just 30-40 percent of the human genome, they were shocked to discover it in more than 75% of the genes in unspecialized embryonic stem cells and specialized adult cells. Through further experimentation, Guenther and Levine determined that the majority of inactive genes in fact do begin the process of RNA transcription, but without completing it. Many of these genes are also critical to cell developmental pathways, so it is possible that activating these genes might allow a cell to assume completely different properties than it normally exhibits.

"This is a new model for regulation of the developmental regulators," Young says, "It could bring us a step closer to reprogramming cells in a controlled fashion, which has important applications for regenerative medicine."

—A. Chuong

Source: "Team Finds New Mechanism of Gene Control"
http://web.mit.edu/newsoffice/2007/cells-0712.html

Key Protein's Role Elucidated in Tumor Growth

Michael Yaffe, Associate Professor of Biology and Biological Engineering at MIT, and his research team have identified how a missing protein causes tissue to become precancerous. It has been known that most breast and prostate tumors lack the protein 14-3-3 sigma, but its role in tumor growth has remained unclear until now. When the protein is inhibited, dividing cells cannot complete cytokinesis; in other words, they do not fully separate into two cells. The resulting duo-nuclei cells become precancerous. Tissues that contain these cells are termed "dysplatic tissue" and serve as fertile environments for tumor development.

14-3-3 sigma is most active during mitosis, when it helps control protein production for division. It interacts with a translation factor called eIF4B. Translation factors are proteins that help determine what proteins a cell produces. Prior to translation, mRNA carries information encoded by DNA to the ribosomes, which translate the mRNA sequences into proteins. eIF4B forms part of an enzyme that allows mRNA to unwind and bind the ribosome. Without 14-3-3 sigma, eIF4B is not produced, and the mRNA for protein p58 cannot be translated. p58 is crucial in cytokinesis because its absence prevents cells from fully dividing.

Because 14-3-3 sigma is missing in normal tissue surrounding developing tumors, its function is lost early in tumor development. Loss of 14-3-3 sigma in dysplastic tissue could help doctors to better predict whether tumors will develop and to begin early treatments to prevent tumor growth.

—H. Zhou

Source: "MIT IDs Role of Key Protein in Tumor Growth"
http://web.mit.edu/newsoffice/2007/tumorigenesis.html

Modeling the Cellular Response to Drugs

MIT researchers have developed a model that may predict how cells will respond to drugs. The model is based on similarities in the signaling pathways cells use to process information. The researchers, led by Professor Douglas Lauffenburger, head of the MIT Department of Biological Engineering, first attempted to understand the way cells interpret signals and imitate the proper response. These pathways work via a series of signals in which proteins activate other cell machinery to achieve a specific result. The researchers exposed colon epithelial cells to a variety of environmental stimuli and measured activity levels in five major signaling pathways. Cell behavior outcomes, such as cell death and inflammation, were also measured.

Douglas Lauffengurer, Director of the Biological Engineering Department at MIT, pictured with a schematic of the intracellular "information-processing" circuit.
Credit: Donna Coveney.

The data was used to construct a model correlating outcomes with the combined levels of activity in the pathways. The model correctly predicted cellular outcomes when two other types of epithelial cells were exposed to the same stimuli. When tested on a red blood cell, the model did not accurately predict behavioral outcomes; however, this result was not surprising since the model was developed from colon epithelial cells and various cell types process information differently.

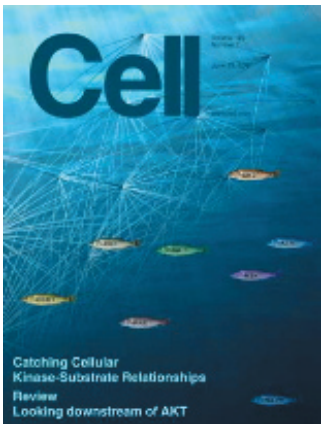
Models based on this approach can help test the effectiveness of drugs on a wide range of diseases. It is important to understand not only a drug's effect on a specific molecule, but also how it works in the context of cell pathways and functions. These models may also help drug developers identify ideal compounds for future research, as well as aid doctors in choosing treatments for patients, who often respond differently to the same drugs.

—H. Zhou

Source: "MIT Model Could Predict Cells' Response to Drugs"
http://web.mit.edu/newsoffice/2007/drugs-0726.html

Computational Model to Understand Protein Networks

An international team, including several researchers at MIT, has designed a new computational model to understand protein networks. The model helps explain the interaction between proteins and enzymes, thereby revealing important details about complex protein networks that influence human diseases such as cancer.



June 29 issue of Cell.

The model, known as NetworKIN, focuses on kinases; enzymes involved in cell signaling pathways, repair of damaged DNA that leads to cancer, and cell division. Kinases primarily work by phosphorylating proteins, effectively telling cells what to do. At a certain time, 30-50% of proteins in a cell are phosphorylated. Mass spectrometry can pinpoint the site of phosphorylation, but the key is finding the location of a specific kinase.

The researchers, in a study published in the June 29 issue of Cell, used a two-step approach. They first identified the family of kinases most likely to act on a given site by using previously developed software that analyzes the amino acid sequence at phosphorylation sites. In order to pinpoint the exact kinase, they designed a computational model that analyzes data on cell signaling pathways and protein interactions in academic literature and databases.

This combined approach of sequencing and contextual information helps create detailed protein networks. This model was developed by researchers from MIT, the Samuel Lunenfeld Research Institute of Mount Sinai Hospital in Canada, and the European Molecular Biology Laboratory in Germany. Michael Yaffe, MIT Associate Professor of Biology and Biological Engineering and member of the MIT Center for Cancer Research, and Rune Linding, a visiting scientist at the MIT Center for Cancer Research and postdoctoral fellow at the Samuel Lunenfeld Research Institute, were the lead authors. Other MIT authors included Gerald Ostheimer, a postdoctoral fellow in biological engineering, Marcel van Vugt, a postdoctoral fellow at the MIT Center for Cancer Research, and Leona Samson, Director of the Center for Environmental Health Sciences and Professor of Biology and Biological Engineering.

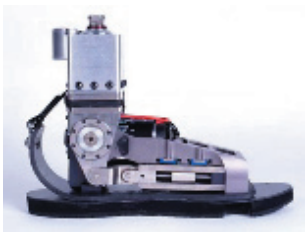
The European Commission FP6 Programme, the Danish Research Council for the Natural Sciences, the Lundbeck Foundation, Genome Canada, and the National Institutes of Health Integrative Cancer Biology Program funded the study.

—B. Sagar

Source: http://web.mit.edu/newsoffice/2007/cancer-0625.html

One-of-a-kind Robotic Ankle

MIT Media Lab Professor Hugh Herr and his team, in collaboration with the Center for Restorative and Regenerative Medicine, have developed the world's first robotic ankle, an important advancement for lower limb amputees. This prototype is novel in that it reduces fatigue, improves balance, and provides amputees with a more fluid gait. Unlike conventional prostheses in which a passive spring response during movement causes an unnatural gait and additional energy expenditure, the new ankle is light and flexible. It contains multiple tendon-like springs that release energy generated by an electric motor to help push the foot off the ground during movement. Herr, a double amputee who demonstrated the invention, said, "This design releases three times the power of a conventional prosthesis to propel you forward and, for the first time, provides amputees with a truly human-like gait." This ankle may become commercially available as early as summer 2008.


The MIT Media Lab's powered robotic ankle-foot prosthesis in action.
Credit: Webb Chappell

—H. Zhou

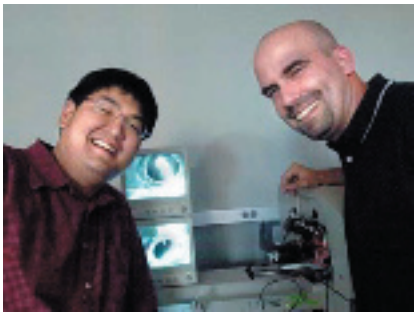
Source: http://web.mit.edu/newsoffice/2007/robot-ankle-0723.html


The robotic ankle-foot prosthesis developed at the MIT Media Lab by Professor Hugh Herr and researchers in the lab's biomechatronics research group.
Credit: Webb Chappell.

Fighting Deadly Tumors

MIT researchers have discovered a link between two proteins that could eventually help treat a form of brain cancer, which kills 99% of patients. Glioblastoma multiform (GBM) is an aggressive brain tumor in adults, which affects 15,000 people in the U.S. annually. It is currently being treated with a combination of surgery, radiation, and chemotherapy; however, these therapies have proven to be ineffective and most patients die within a year.

Associate Professor of Biological Engineering, Forest White, has discovered a connection between two proteins found in GBM tumor cells. Simultaneously attacking both of these proteins kills tumor cells more effectively than targeting either protein individually. White and his team have studied a protein called EGFRvIII, a mutated form of the cell receptor for epidermal growth factor (EGF). This protein is found in a quarter of GBM tumors and persistently stimulates cells to continue growing and dividing. The continuous stimulation of the receptor cannot be curtailed by EGFRvIII-inhibiting drugs. White and his team believe that the action of EGFRvIII and the proteins it stimulates lead to tumor growth.


Paul Huang, graduate student in Biological Engineering, and Professor Forest White of Biological Engineering with a mass spectrometer and liquid chromatography column.
Credit: Donna Coveney.

The group used mass spectrometry to analyze the proteins activated by EGFRvIII. They found that when EGFRvIII is activated, another receptor known as c-Met is also activated. C-Met is normally active in human development but is turned off in most adult cells. It remains unknown how EGFRvIII activates c-Met and whether other proteins are involved. However, this knowledge gives researchers a potential tool for attacking GBM tumors. The group treated tumor cells with drugs that inhibit EGFRvIII and c-Met, and found that this method required a lower number of doses to kill tumor cells than when either drug was used alone.

Furthermore, this work will enable drug developers to produce treatments for GBM, which has been shown to be resistant to all potential drugs. Drug companies have several EGFRvIII inhibitors in clinical trials and are working on c-Met inhibitors as well. Some of these drugs may become available within a decade.

—H. Zhou

Source: http://web.mit.edu/newsoffice/2007/brain-cancer-0718.html

MicroRNA could aid cancer studies

MicroRNAs are small RNAs consisting of approximately 22 nucleotides, and they function by inhibiting gene expression through binding to associated messenger RNA. Although deregulation of microRNAs have been

linked to cancers, few targets of microRNAs have been identified. MIT Institute Professor Phillip Sharp, MIT biology graduate student Margaret Ebert, and colleagues have developed a new technique that could help with studies that aim to identify microRNA targets, which is described in the August 12 online issue of Nature Methods.

The researchers have found a way to inhibit microRNA activity by inserting a gene into cells that causes them to produce a "sponge" that soaks up the microRNA, thereby inhibiting its ability to function. Traditional methods to inhibit microRNA function often used complementary RNA strands called oligos. Inhibiting microRNAs would help determine what their targets may be. There are many advantages to this new sponge method: each sponge can hold up to six microRNAs and can be modified to hold more; the sponge gene includes a gene that fluoresces to signal uptake of the gene in the cell and is never disintegrated. Most importantly, scientists could create transgenic organisms using this technique and control the expression of the sponge to certain tissues and at certain developmental stages. This technique could be used to probe microRNA function in cancers and tumor development and could prove helpful in understanding microRNA activities.

—L. Ong

Source: http://web.mit.edu/newsoffice/2007/cancer-0812.html

Noisy Brain and Redundant Networks

The brain is very active even when it is not learning anything new. This background "noise" helps the brain explore new possibilities. Findings that illustrate this idea were published in the May 24 issue of Neuron, by an MIT research team headed by Sebastian Seung, Professor of Physics and Computational Neuroscience, and Emilio Bizzi, Institute Professor and member of the McGovern Institute for Brain Research. Dr. Uri Rokni, a member of Seung's lab, was the lead author of the study.

An earlier study by Bizzi and his team investigated the motor cortex of monkeys found significant changes in neural activity even when monkeys were doing what they were trained to do, to move cursors to targets on a screen using a handle. Rokni analyzed the data to distinguish learning activity from background changes and generated a theory combining the concepts of redundant neural networks and a "noisy" brain. The theory considers the brain to be similar to a redundant circuitry doing the same thing with different wiring configurations.

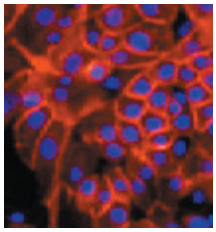
Rokni constructed a mathematical model of redundant cortical networks to simulate the experiment with monkeys. In the model, learning about the connections between neurons was assumed to be a considerably noisy process. The simulation matched the performance levels of the actual experiment, but there were continuous changes in neural representation. This confirmed the "noisy" brain theory. These concepts of redundant networks and "noisy" brains may have great implications for neurobiology, especially for future devices that are operated by brain signals.

—B. Sagar

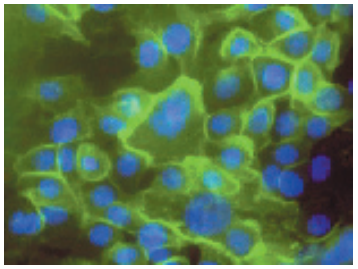
Source: http://web.mit.edu/newsoffice/2007/noisy-brain-0604.html

Creating Cancer Stem Cells

MIT researchers have discovered a method to create cancer stem cells in vitro. In their findings, published in the August 13 issue of Cancer Cell, they also contradict the current assumptions regarding the growth of cancer cells. According to present models, any normal cell can become malignant through a series of alterations and/or mutations leading to uncontrolled growth and subsequent attacks on other tissues. However, the study found that some cells are ten thousands times more susceptible to tumor-initiation.



These normal human breast cells, stained red, form sheets.
Credit: Tan Ince.



Tan Ince transforms normal cells into cancerous cells, stained green. Like tumor cells found in patients, these transformed cells retain their sheet-forming capabilities and possess an enormous potential to create and spread tumors.
Credit: Tan Ince.

The research suggests that tumors resemble beehives; each cell has a specific role to play. A fraction of these tumor cells that remain unspecialized, "queens", seed new tumors and at times, divide to produce worker cells that form the bulk of the tumor. These "queen" cells are the cancer stem cells. Professor Robert Weinberg and his colleagues created these cells by isolating and transforming a particular population of cells from human breast tissue. Mice injected with only one hundred of these cells developed tumors that metastasized. In contrast, infection of one-million cancer cells is normally required for metastasis, which is important considering metastasis is the main cause of cancer mortality.

Weinberg's colleagues for this study were Tan Ince, an independent investigator at Brigham and Women's Hospital and an instructor at Harvard Medical School, Andrea Richardson, George Bell, Maki Saitoh, Samuel Godar, and James Iglehart. This research is funded by the Breast Cancer Research Foundation and the National Institutes of Health.

—B. Sagar

Source: http://web.mit.edu/newsoffice/2007/breast-cancer-0813.html

Protein suppresses prostate cancer

Richard O. Hynes, Daniel K. Ludwig Professor of Cancer Research at MIT and a Howard Hughes Medical Institute Investigator, and colleagues have recently found a protein whose loss of function has been implicated in a variety of cancers, including brain, lung, breast and prostate cancer. This protein, Protein 4.1B, is a member of a family of proteins that link cell membrane proteins to the cell's internal cytoskeleton. They have determined that under normal circumstances, Protein 4.1B suppresses the spread of prostate cancer. As a result, it is possible that testing for the loss of Protein 4.1B can prove a valuable tool for predicting which cancers are likely to spread.

To test the effects of Protein 4.1 loss in living animals, the researchers implanted clumps of human prostate cancer tumors into mice prostates, and then isolated prostate cancer cell variants that showed different metastatic potential. These cells were shown to have lost Protein 4.1B gene activity. The researchers eliminated the Protein 4.1B gene in mice which had been genetically engineered to develop prostate cancer and found that these mice developed more invasive cancers than usual. However, the metastatic cancer cells lacking Protein 4.1B did not divide more rapidly than cells which contained Protein 4.1B. It is thought that Protein 4.1B helps trigger death in metastatic cells, a process that helps prevent the spread of cancer.

Hynes has suggested that the "findings in mice are likely relevant to human prostate cancers" since other researchers have also found reduced levels of Protein 4.1B in metastatic prostate cancers (in comparison to normal prostate tissue). Therefore, a clinical test for Protein 4.1B activity could be used as part of a panel of genetic tests to assess the metastatic potential of prostate cancers.

—A. Chuong

Source: "Protein Suppresses Spread of Prostrate Cancer"
http://web.mit.edu/newsoffice/2007/prostate-0720.html

MicroRNA found to regulate genes in cancer

Researchers at the Whitehead Institute and MIT have found a microRNA that targets a gene linked to many human cancers. MicroRNAs are short RNA strands that help regulate gene expression by targeting the non-protein-coding regions of messenger RNA and inhibiting its activity. They were discovered fourteen years ago in worms. Since then, hundreds of microRNAs have been identified and in many different organisms, including plants, animals, and humans. Although studies have found that over- or under-expression of microRNAs have been linked to cancers, they have yet to find the particular targets of these microRNAs.

Postdoctoral researcher Christine Mayr of the Bartel lab focused on finding a microRNA that targeted the Hmga2 gene, which is often truncated and substituted with DNA from another chromosome in tumors. Interestingly, this replacement of DNA removes the seven let-7 microRNA complementary sites in the deleted non-protein-coding region of the Hmga2 gene. Because these target sites are deleted, let-7 would be unable to bind to and regulate Hmga2 expression. It could then cause an over-expression of Hmga2 and, thus, increase the possibility of tumor formation. In order to test this, Mayr used multiple versions of the Hmga2 gene with different numbers of let-7 binding sites. She found that the fewer let-7 binding sites there were, the more Hmga2 was expressed. To probe let-7's role in tumor formation, she used in vitro testing in mouse cells and found that the cells with damaged let-7 sites grew far more significantly than those with normal or shortened Hmga2. Collaborating with MIT Assistant Professor of Biology Michael Hemann, they found that mice injected with damaged let-7 binding sites developed tumors.

—L. Ong

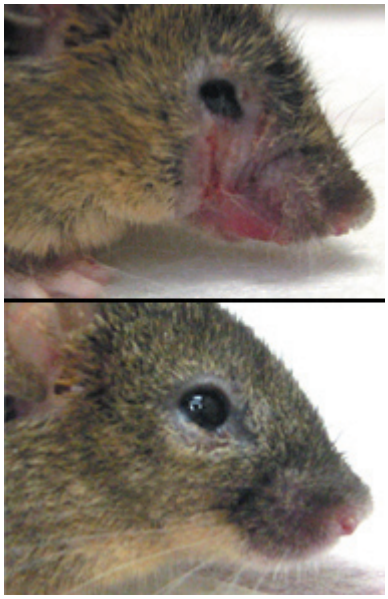
Source: http://web.mit.edu/newsoffice/2007/microrna.html

World Science News In Review

[Biomedical Research]

Compulsive Gene?

A team of neuroscientists at Duke University in Durham, North Carolina recently discovered a link between a gene in mice and human obsessive-compulsive disorder (OCD), a disease that currently afflicts more than two million Americans. The scientists' finding offers valuable insight into the genetic cause of OCD and may help researchers to develop a more effective treatment for the disorder. The Duke team intended to study the link between knocked out genes and abnormal behavior in mice. The researchers soon discovered that mice lacking the synaptic protein SAPAP3 exhibited symptoms of OCD, including excessive facial grooming and heightened anxiety levels. Furthermore, all OCD symptoms were alleviated by Prozac, a drug often used to treat the disorder in humans. Studies of brain slices from the mutant mice revealed a defect in the striatum, part of a circuit involved in controlling actions. The synapses in this circuit appeared unable to properly respond to the neurotransmitter glutamate.



Mice missing the gene for SAPAP3 shown above.
Credit: Jing Lu, Jeff Welch, and Guoping Feng.

The Duke team's finding offers valuable insight into the role of the striatum and glutamate synapses in OCD. The discovery provides hope that scientists may develop more effective OCD treatments which address the underlying cause of the disorder.

—G. Denman

Source: "Missing Gene Makes Mice Compulsive"
http://sciencenow.sciencemag.org/cgi/content/full/2007/822/5l

Combating High Blood Pressure

Despite the existence of powerful drugs that combat high blood pressure, few adults with high blood pressure have been able to achieve target levels. Scientists from King's College in London have discovered a new way to regulate blood pressure, providing hope for the development of new drugs to combat strokes and heart attacks.

The new study involves oxidation, a process that has generally been thought to cause harm rather than good; examples of oxidants include radicals and hydrogen peroxide. However, oxidation plays many important roles in normal cell function. For instance, protein kinase G (PKG) is an important protein that requires nitric oxide produced in blood vessels to regulate blood pressure. The King's College team, led by Dr. Philip Eaton, has found a way in which PKG can be regulated independently of nitric oxide. Oxidants such as hydrogen peroxide create a bond between two amino acids of PKG, which activates the protein and subsequently lowers blood pressure. This finding could lead to the development of drugs that activate this new pathway, as well as research that uses this pathway to investigate the events leading to a heart attack.

—H. Zhou

A doctor measures a patient's blood pressure using a stethoscope.

Source: "New Blood Pressure Control Found"
http://news.bbc.co.uk/2/hi/health/6961911.stm

New Itch Sensation Gene Found

Chronic itching is an extremely disruptive problem—it can be caused by eczema, a skin disorder, or by more serious problems such as kidney failure, cancer therapy side-effects, and liver disease. People suffering from chronic itching are often disrupted during sleep and scratch themselves until they scar. Itch research has been somewhat ignored due to many scientists' belief that itching is a less intense version of pain, resulting in limited effective treatment options for patients.

Researchers at Washington University School of Medicine in St. Louis have identified the first gene for itch sensation in the nervous system. Dr. Zhou-Feng Chen and colleagues found that mice without the gene GRPR (gastrin-releasing peptide receptor) scratched much less than normal mice when subjected to itchy stimuli. GRPR codes for a receptor found in a small number of spinal cord nerve cells, where itch signals are transmitted from the skin to the brain.

Chen's team discovered the GRPR's role in itchiness when they were studying genes in the pain pathway. GRPR did not seem to directly affect pain sensation, as mice lacking GRPR reacted to painful stimuli in the same way normal mice did. However, when post-doctoral fellow Yan-Gang Sun, Ph.D., injected normal mice with a substance that stimulates GRPR, the mice scratched themselves as if they were suffering from a bad itch. Chen's team then studied scratching behavior in normal mice and in GRPR-lacking mice. The mice that lacked GRPR scratched much less; while still responding to painful stimuli as normal mice would, indicating that pain and itch perception are regulated by separate sets of genes in the spinal cord. Moreover, potential therapies should be able to provide itch relief without affecting pain sensation.

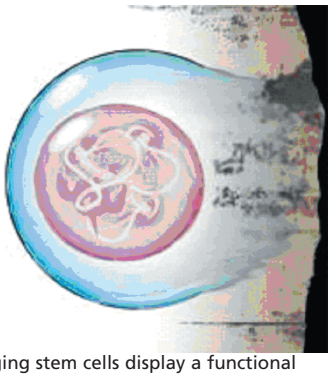
GRPR had been studied for years without the knowledge of the link to itching. Past research has been targeted tumor growth and scientists have synthesized substances to block GRPR activity. Studying the effects of these substances might lead to treatments providing effective relief for severe or chronic itching soon.

—H. Zhou

Source: "Scratch No More: Gene for Itch Sensation Discovered"
http://www.eurekalert.org/pub_releases/2007-07/wuso-snm072407.php

The effects of aging in stem cells

Stem cells fight against aging by replacing old and damaged cells with new ones. However, new evidence suggests that this regenerative capability declines with age.



Aging stem cells display a functional decline, a heightened stress and inflammatory response, and signs of epigenetic erosion.
Credit: S. M. Chambers.

Stuart Chambers, Margaret Goodell, and their colleagues analyzed gene expressions in hematopoietic stem cells (HSCs), which are the precursors of blood cells. They found out that genes involved in the inflammatory and stress response become more active with age, while genes for regulating gene expression and genomic integrity become less active. This suggests that stem cells also deteriorate over time.

To further test the regenerative capacity, Chambers et al. isolated HSCs from young and old mice, which were two and twenty-one months old respectively, and transplanted them separately into two groups of mice that had bone marrow cells previously destroyed by radiation. In both groups, new marrow cells regrew in approximately four weeks. However, the HSC contribution of old mice dropped significantly, proving the decline in thei regrowth capacity. The increase in HSCs caused the blood production to be stable throughout the experiment.

The increase in the inflammatory response correlates to aging in the brain, kidney and arteries. It also suggests why HSCs lose function. While HSCs depend on cell adhesion to colonize the bone marrow, the adhesion function is controlled by the P-selectin gene. The inappropriate up-regulation of genes encoding P-selectin impedes HSC function. Also, a decline in the expression of genes involved in chromatin remodeling, an "epigenetic" regulator of gene expression, leads to erroneous transcription. This view helped to explain the changes at the molecular, cellular and organismal levels. The chromatin dysregulation also resulted in the loss of growth regulation. All these changes were correlated with the increasing risk of cancer and increasing age. The epigenetic regulation, inflammation and the stress response can be combined to further understand the mechanisms underlying aging and the increasing risk of cancer.

—E. Hacisuleyman

Source: "Effects of Aging in Stem Cells"
http://www.eurekalert.org/pub_releases/2007-07/plos-eoa071807.php

A Virus for Obesity

Magdalena Pasarica of Louisiana State University in Baton Rouge and colleagues have discovered a virus that transforms adult stem cells into fat-storing cells, supporting the idea that some cases of obesity may be infectious. This phenomenon was previously known to occur in some animals but had not been observed in people until now. Adenoviruses generally cause colds, but adenovirus-36 has been observed to have this additional effect.

In 2005, researchers reported that 30% of obese people studied showed signs of previous adnovirus-36 infection, compared to only 11% of normal-weight people. To solve this mystery, Pasarica and her team examined adult stem cells that were collected from fat during liposuction. These stem cells can transform into adipocytes (fat-storing cells), bone, and cartilage. The scientists cultured the stem cells and infected half of them with adenovirus-36. Most of the infected cells transformed into adipocytes, while most of the uninfected cells did not. The adipocytes then accumulated fats when grown on standard culture media.

Pasarica also infected stem cells with adenovirus-36 and exposed them to substances that usually transform stem cells into bone. The cells became fat instead, implicating the virus as the cause for adipocyte transformation. During the virus's infectious phase, which lasts for only a few weeks, long-term changes are probably induced in stem cell development, eventually causing fat accumulation. Pasarica and her colleagues are currently attempting to determine the pathway by which the virus transforms the stem cells in hope of discovering pre-obesity treatments.

—H. Zhou

Source: "Infectious Obesity: Adenovirus Fattens Stem Cells"
http://www.sciencenews.org/articles/20070825/fob2.asp

The Bane of Oral Contraceptives

A study of female military cadets at the United States Military Academy has shown that the use of oral contraceptives, along with the loss of normal menstrual function, may have a negative impact on bone health, while greater amounts of exercise and an increase in dairy product consumption are vital. Dr. Jen Nieves of Columbia University and the Helen Hayes Hospital examined correlations between lifestyle, diet, exercise, and bone health in 107 female cadets and found that more fit cadets and those who had a higher milk intake possessed greater bone density and strength, an indication of healthier bones.

According to Nieves, the study found that "a loss of normal menstrual function was found to have a detrimental impact on the skeleton and, in these young adults, oral contraceptives also had a negative impact on the skeleton. Peak bone mass, the maximum bone density a person will ever have, is attained by age 25. It is important to maximize peak bone mass to prevent stress fractures in young adults and osteoporosis in later life."

—A. Chuong

Source: "Oral Contraceptives"
http://www.reuters.com/article/healthNews/idUSCOL46695420070824

A Mouse with Two Mommies

A research team at the Tokyo University of Agriculture in Japan, under the leadership of Tomohiro Kono, has successfully generated normal mice by combining the genomes of two mouse eggs.

Although unfertilized mouse eggs can be prompted to divide in the laboratory, the resulting embryos are unable to make a placenta and therefore die at an early stage of development. It is believed that this early death is due to parental gene imprinting. Under normal circumstances, the genes on a chromosome may be turned on or off depending on which parent the chromosome was inherited from. Expression of both maternal- and paternal-imprinted genes is necessary for mammalian fetal development.

A brood of bimateral mice.
Credit: Kawahara et al., Nature Biotechnology, Advance Online Publication (8/19/07).

The Kono team created a mutant strain of mice with paternally-imprinted genes by deleting the imprinting-control genetic regions and harvesting immature eggs from the resultant mutants. Nuclei from these eggs were then inserted into normal mouse eggs. Once the "fertilized"

eggs had begun to grow and divide, the eggs were inserted into female mice and the pregnancy was allowed to continue to full term. The resulting adult mice were healthy and fertile. Although it is unlikely that this technique will enable two women to produce a biological child, this technology should help scientists gain a better understanding of parental imprinting and the specific ways sperm contribute to development.

—A. Chuong

Source: "Mickey Has Two Mommies"
http://sciencenow.sciencemag.org/cgi/content/full/2007/820/2

Breast Cancer and Estrogen

The cancer biology team at the University of Queenland's Diamantina Institute for Cancer, Immunology, and Metabolic Medicine has found that the female sex hormone estrogen activates a gene linked to breast cancer. This finding may help explain the link between breast cancer and high estrogen levels. The team, led by Professor Tom Gonda, studied a gene known as MYB, a tumor-promoting oncogene found in 70% of all breast cancers. The team's next step is to test the effect of estrogen, utilizing a mouse model of breast cancer. Professor Gonda and his colleagues hope to show that MYB directly induces cancerous changes in normal breast cells. A potential therapy may involve blocking the action of MYB.

—H. Zhou

Source: "Estrogen Linked to Breast Cancer"
http://www.sciencedaily.com/releases/2007/08/070824095749.htm

Death Without Daylight

Scientists recently discovered that a dangerous bacterium depends on blue wavelengths of light for reproduction. The researchers finding, published in the August 24 issue of Science, may enable physicians to eliminate the dangerous and often fatal disease caused by the bacterium.

Brucella is responsible for epidemics of the disease brucellosis throughout the world. It is transmitted to humans primarily through contact with infected animals or consumption of infected dairy products. The research team discovered that the bacterium requires blue-light to activate a component of photoreceptor proteins called LOV, which signals the bacterium to reproduce. When researchers shielded the bacterium from the wavelength, which is present in the sun's rays, the organism's reproductive rate dropped more than 90%.

Researchers' new understanding of the blue-light activation of LOVs may enable them to eradicate brucellosis epidemics by depriving the bacterium of its reproductive ability.

—G. Denman

Source: "Deadly in Daylight"
http://sciencenow.sciencemag.org/cgi/content/full/2007/823/2

Social habits of cells may hold key to fighting diseases

Researchers at the University of Michigan Medical School have found that p53, a gene often called "the guardian of the genome" for the role it plays in preventing cancerous cell development, is involved in the regulation of three members of a gene family known as miRNA34. Micro RNA, or miRNA, is believed to help regulate the levels of mRNA and protein expression, and the U-M research team determined that the miRNA genes not only work in concert with p53 but also have a demonstrated effect on other genes involved in cell cycle timing. Moreover, expression of two of the miRNA34 genes was found to be defective in almost two-thirds of lung adenocarcinomas, the most frequently diagnosed form of lung cancer.

According to Eric Fearon, M.D., Ph.D., senior author of the study and deputy director of the U-M Comprehensive Cancer Center, "The findings in the study offer new insights into specific mechanisms by which the expression of hundreds to thousands of genes and proteins is altered in the roughly 50 percent of cancers that carry mutations in the p53 tumor suppressor gene."

—A. Chuong

Source: "Bits of 'Junk' RNA Aid Master Tumor-Suppressor Gene"
http://www.eurekalert.org/pub_releases/2007-08/uomh-bo082307.php

Immune System's Link to Anthrax

Professor James Whisstock and Dr. Michelle Dunstone from Monash University's School of Biomedical Sciences in Australia have discovered a link between human immune proteins and bacterial toxins that cause anthrax infection and gangrene. The human immune proteins are critical in fighting viruses, bacterial infections, and cancer. According to Professor Whisstock, these proteins, called perforins, most likely developed or evolved in response to bacterial attack. Perforins belong to an ancient toxin family that was previously found only in bacteria. Perforins create tiny holes in cells, causing cell lysis in cancerous cells, bacteria, and virally-infected cells. To determine the structure of one of the perforins called Plu-MACPF, the team used X-ray crystallography. Analysis shows that all perforins in the family work in a similar manner. This knowledge and further research can be utilized to develop new advances in fighting disease.

Artist's rendition of the perforin-like protein, Plu-MACPF. Australian scientists have discovered that this protein belongs to a family of ancient and lethal toxins previously found in bacteria.
Credit: Carla Chinni.

—H. Zhou

Source: "Toxic Shock: Immune System's Anthrax Link"
http://www.eurekalert.org/pub_releases/2007-08/mu-tsi082107.php

Winning by Folding: Protein Structure Formation Demystified

How does the sequence of its component amino acids guide a protein into assuming a precise three-dimensional shape? For half a century, the folding problem has been one of the central unsolved puzzles of biochemistry, but decades of computational, conceptual, and experimental advances have put many of the pieces together.

Adrian Slusarczyk

From protein misfolding disorders such as Alzheimer's and Creutzfeldt-Jakob disease to protein engineering to functional genomics: the list of hot areas in science and technology for which a thorough understanding of protein folding promises major breakthroughs is impressive. And finally, such insight seems within grasp [1].

The Problem

Proteins are synthesized as linear polymers of amino acids by the cell. But their function, for example as enzymatic catalysts or signaling molecules, depends critically on the correct spatial orientation of the chemical groups in the side chains of their amino acids. As Anfinsen showed fifty years ago, a protein achieves its unique and stable three-dimensional shape by spontaneously folding up on itself [2]. The whole process typically takes only milli- or even microseconds to finish and does not require a template. However, an average protein has a near endless number of possible configurations [3]. Obviously, something must be guiding the process to sample only a fraction of conformational space - and that something must be hidden within the protein's amino acid sequence.

Protein structure is organized hierarchically, from the amino acid sequence, or primary structure, upward. The polypeptide backbone forms regular repetitive elements, such as alpha-helices and beta-sheets, by maximizing hydrogen bonding of its C=O and N-H groups in the secondary structure. Tertiary structure consists of the intramolecular interactions of distant amino acid side chains that orient and fix the secondary structural elements in space, and quaternary structure refers to the association of multiple polypeptide chains to form functional units. The protein folding problem can thus be rephrased as two separate questions: How can the secondary and tertiary structure of a protein's active, or native, form be predicted from its amino acid sequence? And by what thermodynamic pathway do hydrogen bonding, the hydrophobic effect, and other interactions fold the linear polypeptide into its secondary and tertiary configuration?

The Thermodynamics of Folding

Just how does the amino acid sequence constrain configuration space enough for a protein to fold rapidly into a unique structure? Early on, it was observed that small peptides spontaneously form secondary structures such as alpha-helices. However, these are neither stable nor unique, and peptides alternate between different members of the available ensemble of structures and a disordered random coil.

The framework model, proposed in the 1980s, suggested that secondary structural elements do form and disappear at random, but can be locked into place by docking onto each other, thus giving rise to tertiary structure [4]. The alternative hydrophobic

collapse model postulated instead that the hydrophobic core of a protein forms first, followed by internal rearrangements that give rise to secondary structure [5].

Better temporal and spatial resolution of the main experimental methods used to investigate folding intermediates, namely, nuclear magnetic resonance (NMR), fast kinetics, and molecular dynamics (MD) simulations, allowed to test both hypotheses. The truth appears to lie in the middle: while some exclusively alpha-helical proteins such as the engrailed homeodomain seem to follow the framework mechanism of "secondary structure first", and pure hydrophobic collapse has not been observed, most proteins including the extensively studied chymotrypsin inhibitor CI2 fold by a "general collapse" in which secondary and tertiary structure form in parallel [6]. Because the stabilization of transient local secondary structure in this model depends on the interactions of side chains, it can account for the specific preference of one fold over any other despite the very similar propensities of different stretches of the chain to form isolated helical, sheet, or coil structures. This mechanism has been named nucleation-condensation by Alan Fersht [7], and the notion that local (secondary) and distant (tertiary) interactions arise in parallel such that the latter stabilize the former at any stage now provides a coherent handle on protein folding [6, 8].

This insight has been accompanied by the growing realization that there is not one thermodynamic pathway, similar to that of a chemical reaction, from the denatured to the native state. Instead, the unfolded chain can assume a vast number of different configurations. Once secondary and tertiary structure have begun to form, the number of possible configurations is reduced, but remains substantial. This is captured by the notion of a folding funnel [9] in the potential energy landscape instead of a one-dimensional reaction coordinate. The polypeptide can take any route down the slope of the funnel, and local minima on the way represent detectable folding intermediates. For practical purposes, constructing the energy function that defines the shape of the funnel remains daunting; at the same time, we now have a very good conceptual understanding of the process by which a unique secondary and tertiary structure arise from the denatured polypeptide chain.

In the near future, the ongoing refinement of experimental techniques including, for example, fluorescence resonance energy transfer (FRET) and real-time NMR promises an unprecedented resolution in time and space for observations of the folding process. Moreover, increased computing power allows longer time-scales and larger systems to be modeled by MD, as well as a more accurate representation of the aqueous solvent. For example, using worldwide distributed computing, the Folding@Home project has succeeded in folding an entire small protein from scratch with an accuracy of the final structure equivalent to what can be obtained by X-ray crystallography [10]. Together, these advances place the complete microscopic description - and prediction - of folding of at least some proteins within reach [1, 11, 12].

Structure Prediction

While such complete simulations of folding are now becoming possible, they are computationally expensive and of limited practical value in obtaining structures for the vast number of protein sequences now deposited on public servers.

Fortunately, computationally faster approaches have turned out very effective at predicting protein structure. It was once assumed that a full understanding of the process of folding would be necessary to make structural predictions, but this has since been thoroughly disproven [8]. In particular, nature's tendency to stick with, and re-use, protein scaffolds has been very helpful: not only do evolutionarily related proteins share similar structures even where their amino acid sequences differ, but also the number of unique overall folds observed across the 46,000 structures currently deposited in the Protein Databank (PDB) is limited to about 1,000.

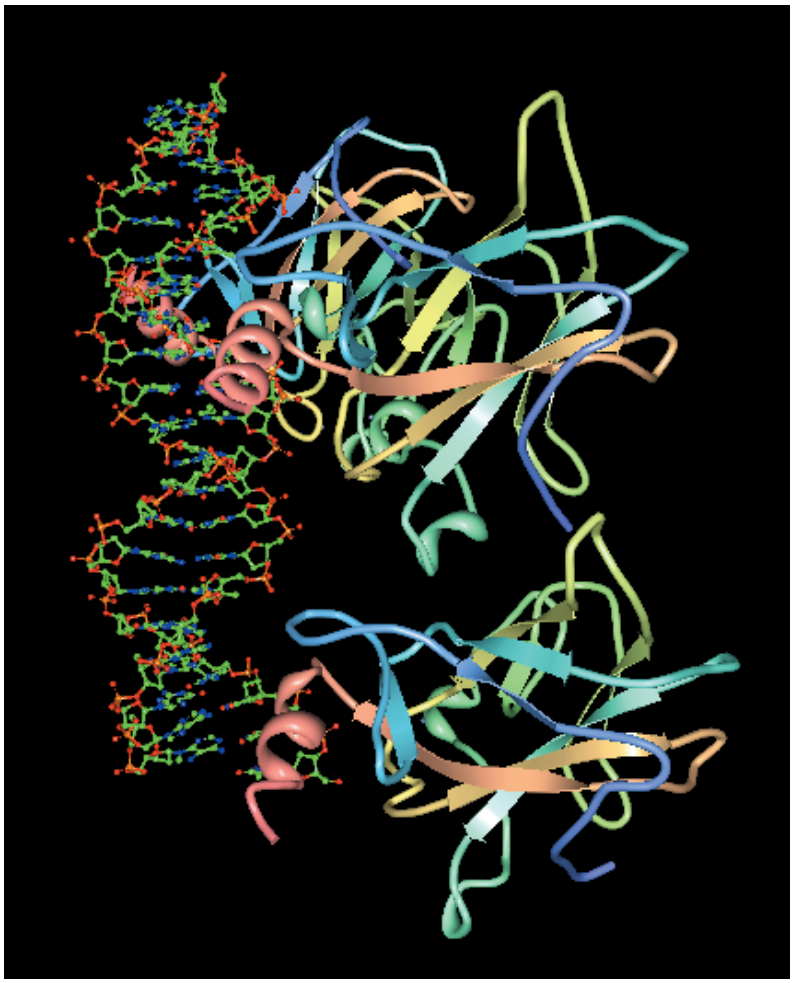


Figure 1. The tumor suppressor p53 (PDB ID 1TUP) bound to a stretch of DNA. For this molecular recognition process to work, the functional groups on the protein have to be precisely oriented in its intricately folded structure.

Homology-based, comparative modeling by programs such as Modeller [13] predicts the structure of a protein by finding a protein of known structure with similar sequence, using its structure as a template and modeling non-homologous regions with refinement methods similar to the template-free algorithms described below.

A more sophisticated approach is used, for example, by the 3D-PSSM software [14] to predict structures for sequences with only distant homologues in public databases. The protein sequence in question is forced into assuming each of a number of protein structures, a process known as "threading," and its compatibility with each structure is scored. This makes it possible to find structural homologues and use them as templates even where the sequences are too divergent to reveal any relationship to the protein of interest.

One of the most successful programs for structure prediction, Rosetta [15], does not rely on finding homologous proteins. Instead, it compares portions of the target sequence to members of a fragment library. These fragments are derived from the Protein Databank (PDB) of known structures, but they are only short stretches of amino acids rather than whole native structures. The underlying assumption is that in a sample as large as the PDB, all conformations that a given short fragment can adopt will be represented, and this allows to narrow down the configuration space for what, in essence, is still a de novo method. The Rosetta@Home project uses the Rosetta algorithm and distributed computing on the home computers of volunteers to predict large numbers of structures for proteins implicated in disease.

Both comparative and de novo algorithms compete and are evaluated in the biannual Critical Assessment of Techniques for Protein Structure Prediction (CASP). Over 250 research groups from around the world participated in the seventh round of this contest in 2006. They were given sequences of protein targets of varying difficulty for the structures had been experimentally solved, but not yet published. The results have been evaluated and will be published in the journal Proteins in late 2007. Since the project was initiated in 1994, impressive progress has been observed [16] due to both better algorithms and computing power. For small proteins under about 90 amino acids with homologous structures available, the predictions rival the accuracy of crude X-ray structures [17] and great improvements have been seen in recent years for sequences of intermediate difficulty. The organizers are confident that another decade of such progress will result in excellent models [16]. However, predicting the structure of large multidomain proteins and of membrane-spanning proteins remains a formidable challenge. Much work now also focuses on predicting how two proteins will interact, for example to relay a signal, and on predicting function as well as structure.

Conclusion

As recently as in the 1980s, it seemed quite possible that the protein folding problem would never be solved. The challenges seemed daunting: instruments were not sensitive enough to follow folding in real time, computer algorithms were capable at best of predicting short stretches of secondary structure, and hypotheses concerning the process as well as the outcomes of folding were hard to prove either false or correct. Fast-forward to 2007, and the picture is a refreshingly different one: a conceptual understanding of the folding of at least globular proteins seems to have been achieved, and improvements in algorithms and the steady gains in computing power enable folding models and structure predictions that were inconceivable a few years ago. Make no mistake: much more work is required across the board to refine the models and extend them to include difficult and unusual proteins. But the science of protein folding has reached a point where structure predictions are being successfully used to design proteins from scratch [18], and thermodynamic folding simulations unveil in detail how certain mutations contribute to cancer, and how these effects can be counteracted [19]. And there are many more pots to scoop as these methods are increasingly applied to real problems.

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Emerging Technologies for Influenza Vaccination

Aniket Schneider

Abstract

Mounting concerns about the pandemic potential of H5N1 avian influenza make the development of more effective vaccines an urgent need. Because of the high pathogenicity of the H5N1 viruses, conventional vaccines cannot be produced against these strains. New vaccines based on reverse genetics will soon become licensed, but suffer from limitations in production capacity. New, more robust techniques, however, are still in the early stages of development. This review discusses the current status of influenza vaccination technology and the drawbacks and benefits of various emerging techniques.

Introduction

The influenza virus spreads across the world in seasonal epidemics, causing approximately 250,000 to 500,000 deaths per year. While most people recover within one to two weeks without medical attention, the very young, the elderly, and people suffering from various co-morbidities experience more serious complications[1]. On rare occasions, influenza strains arise to which humans have no innate immunity. These strains have the potential to create pandemics such as the Spanish Flu pandemic in 1918 which killed over 40 million people[2].

The classification of a strain of influenza virus depends on the various antigenic proteins expressed by the virus. Differences between the nucleoprotein and matrix protein of the virus are used to classify virus strains into three broad categories, types A, B and C, with type A being the most virulent in humans. Type A strains are further subdivided by differences in their surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). These two proteins, particularly hemagglutinin, provide the major targets for the host immune response[3]. Currently, the H1N1 (hemagglutinin 1, neuraminidase 1) and H3N2 strains of influenza A circulate most widely in humans[1].

Recently, however, a new strain of influenza, with many of the characteristics of a pandemic virus, has been discovered in birds. This virus, classified as an H5N1 strain of influenza A, has a very high pathogenicity, and of the 291 confirmed cases of infection to date, 172 (59%) have resulted in the death of the patient[4]. Luckily, the virus cannot yet be transmitted from human to human, but health care officials are concerned that it may reassort with another influenza strain and become a major pandemic threat[5].

Apart from its high pathogenicity and mortality rates, H5N1 avian flu has several features that make it a particularly potent pandemic threat. Because of the strain's novel H5 surface antigen, humans have no preexisting immunity to the virus. Additionally, there are no known H5N1 strains with low pathogenicity from which to produce a vaccine. Finally, due to extensive use of antiviral drugs to control the spread of illness in poultry during a 1997 outbreak of H5N1 in Hong Kong, much of the H5N1 virus in circulation is already resistant to one of the major influenza antiviral drug types, adamantane6. Because of these factors, development of a vaccine against H5N1 avian flu has become a high priority.

Conventional Vaccines

The standard method for creation of influenza vaccines involves growing a virus in embryonated chicken eggs. Allantoic fluids of the eggs are harvested and the virus is purified out, after which it can be injected directly, and function as a live virus vaccine, or it can be chemically inactivated, and function as an inactivated whole virus or inactivated subvirion vaccine[3]. However, the high pathogenicity of H5N1 strains presents a challenge to this traditional approach of vaccine production in an egg: not only does the virus present an unusually great danger to the people working on the vaccine, requiring a higher biosafety level than is present in most production plants, but it also infects the eggs so effectively that it kills the eggs before the virus reaches a sufficiently high concentration for harvesting[5].

Substances called adjuvants can greatly increase the potency of vaccines administered at low doses, a technique that is especially useful when very little vaccine is available[7]. Many types of compounds can be used as adjuvants, but the ones most commonly effective with influenza vaccines are an aluminum salt called alum, and a proprietary emulsion of squalene called MF59[7, 8]. The use of both types of adjuvants in humans is currently being tested in large-scale clinical trials in the US, and MF59 is already in use in European vaccines[7]. Despite their promise, though, adjuvants have not solved the problem of creating a pandemic flu vaccine, and United States regulatory agencies have been slow to approve their use[7].

Attempts to use related low pathogenic avian influenza (LPAI) strains to create vaccines against H5N1 have met with very limited success[9, 10]. These vaccines achieved acceptable immunogenicity when augmented by the use of an adjuvant, demonstrating that adjuvants increase the cross-reactivity of the antibodies induced by a vaccine. Despite their low pathogenicity, however, these strains grew poorly in chicken eggs[10]. This type of vaccine avoids most licensing barriers by making use of previously licensed technology, but production limitations make these vaccines suitable only for high-risk patients.

Reverse Genetics

The most promising body of research into vaccine production involves a technique called reverse genetics, which allows manipulation of the virus genome in cell culture[11-15]. A set of plasmids encoding the entire virus genome is transfected into a eukaryotic cell line, allowing the cells to produce entire virus particles without having been infected[15]. Because of the ease with which plasmid DNA can be modified, this general approach allows controlled manipulation of the features of an influenza strain, and can be used to produce desired characteristics like low pathogenicity and specific surface antigens.

Research has focused on two classes of vaccines produced with reverse genetics, whole virion and subvirion vaccines. In whole virion vaccines the inactivation process preserves the overall structure of the virus particle. Subvirion vaccines, on the other hand, include only specific purified protein subunits. Immunogenicity of whole virion vaccines generally exceeds that of subvirion vaccines[16], making them good candidates for pandemic flu vaccines.

Generally, both types of vaccines are derived from two influenza strains, a pathogenic strain and an attenuated strain. The pathogenic strain supplies genes for the surface glycoproteins, allowing the vaccine to generate a specific immune response against the original strain. Genetic material from the attenuated strain reduces the pathogenicity of the recombinant virus, allowing it to grow efficiently in eggs. Additionally, in the case of H5N1, the H5 antigen actually causes some of the high pathogenicity of the strain, so the sequence of the H5 HA gene must be modified to reduce this effect[3].

Treanor and colleagues developed an inactivated subvirion vaccine as a preliminary step toward a whole virion vaccine[13]. Using reverse genetics, they replaced the surface antigens of a common vaccine platform virus with H5N1 surface antigens, and modified the H5 HA gene to reduce pathogenicity, allowing efficient growth of the seed virus in eggs[13]. The resulting virus was purified out of the eggs and disrupted into its component proteins using a detergent, after which the H5 HA and N1 NA proteins were purified out and administered to patients without adjuvant[13]. While this vaccine was only marginally effective at high doses (two doses of 90 ug induced protective antibody levels in only 58% of patients[13]), use of an adjuvant could potentially increase its effectiveness.

Meanwhile, results of early clinical trials of an inactivated whole virion vaccine have shown promise. Lin and colleagues have created and tested a vaccine derived from a highly pathogenic H5N1 strain and an attenuated H1N1 strain, again with modifications to the H5 HA protein to reduce its pathogenicity[12]. This vaccine was prepared in the conventional way and inactivated using formalin[12], a chemical which disables the virus but preserves its structure. The vaccine, administered with an aluminum-based adjuvant, induced protective levels of antibody in around 80% of patients with a dose of only 10 ug[12]. The trials were conducted in China, however, and approval of this vaccine by foreign regulatory agencies will have to wait for larger scale trials under their jurisdiction.

Novel Alternative Approaches

Reverse genetics-based vaccines rely on well-established technology and tested techniques, and require no changes in the existing vaccine production infrastructure. There are disadvantages, however, to the conventional method. Drug companies base their production capacity on yearly sales, so there is no incentive for them to build the capacity for a pandemic response that may or may not come. The existing limited production capacity cannot be used to stockpile vaccine, because an emerging pandemic strain is likely to have mutated from the original vaccine targets. Finally, practical matters like egg supply and biosafety level requirements place additional limitations on vaccine production.

A number of new ideas are being explored in order to circumvent the weaknesses in conventional and reverse genetics-based vaccines. Increasing production capacity is one important priority, but others include creating broader-spectrum vaccines, increasing the effectiveness of small vaccine doses, and decreasing the lag time between identification of a strain and production of the vaccine. Most of these techniques are still under development, and have not yet undergone clinical trials in humans. Additionally, because they involve developing completely new technology, the licensure process for these vaccines will be longer. Nonetheless, they offer great potential for improving the effectiveness of future vaccines.

One idea is to simplify the manufacturing process by using recombinantly expressed HA protein alone as a vaccine. The gene for H5 HA is transfected into and expressed in cultured

cells, which can then be induced to overexpress the H5 antigen[17]. The resulting vaccine is similar to an inactivated subvirion vaccine, but does not require the cumbersome egg-based system of production. In humans, this vaccine works only at high (90 ug) doses and only in roughly 50% of patients[17], but perhaps with the addition of an adjuvant to increase immunogenicity it could become a viable option.

Other researchers, attempting to broaden their range of protection, have created a vaccine which induces antibodies against the virus matrix protein, M2, which is conserved in all influenza A strains but is normally not immunogenic. The technique involves attaching M2e, the external portion of the M2 protein, to the hepatitis B virus core, which greatly increases its immunogenicity[18]. With the use of various adjuvants and with several copies of the M2e domain per particle, this vaccine successfully conferred protective immunity to an influenza A strain in mice, though the mechanism by which antibody production was induced is not well characterized[18]. This vaccine is still in the early testing stages, but it shows great promise since it would allow stockpiling of vaccine in advance of a pandemic outbreak, as well as immunization against a wide range of influenza strains with a single vaccine.

A very new type of vaccine using an adenovirus as a vector also circumvents many of the problems with conventional influenza vaccines, this time by allowing the host organism's body to do much of the manufacturing work. In this type of vaccine, a replication-defective adenovirus strain is modified to carry an antigen, in this case H5 HA. When the virus infects the host organism, the infected cells start producing large quantities of the antigen instead of producing new virus particles. Additionally, certain strains of adenovirus preferentially infect human antigen-presenting cells, which would amplify the immune response and allows use of a smaller vaccine dose[19].

Adenovirus vector-based vaccines have been tested in mouse and chicken model systems, and they confer protective immunity even against antigenically distinct flu strains, provided that they carry the H5 antigen[19, 20]. Adenovirus vector-based methods have been used in over 100 clinical trials for other purposes, so the technology is well understood[20]. Additionally, Gao and colleagues managed to move from virus sequence to vaccine production in just 36 days, which would facilitate fast response in case of a pandemic[20]. Finally, the vaccine can be produced efficiently in an egg-free system[19, 20]. Unfortunately, this type of vaccine is quite far from being licensed for use, at least in the United States. For now, having been proven in chickens, adenovirus vector-based vaccines may at least be useful for controlling the rampant spread of avian flu in poultry[20].

Conclusions

The FDA recently awarded the first US license for a vaccine against avian flu in humans to Sanofi-Pasteur for an inactivated subvirion vaccine[13, 21]. While this step will greatly enhance the country's ability to respond effectively to an H5N1 flu outbreak, it does not solve many of the systemic issues in conventional vaccine technology. Conventional vaccines such as this one, even those modified with reverse genetics, still suffer from production bottlenecks and shortages, as well as having relatively slow response times when facing a potential pandemic. Thanks to intensive research in recent years, a great many options for new types of vaccines now exist, but the most robust, cheap, and effective vaccines are still under development.

At the moment most governments, lacking a suitable vaccine to invest in, are stockpiling influenza antiviral drugs as their backup plan. H5N1 flu, however, has already developed considerable resistance to the adamantane class of drugs[6, 19], and recently various influenza strains are exhibiting resistance to oseltamivir[22, 23], one of the other two major classes of flu antiviral. This development only serves to underscore the critical importance of continuing vaccine research.

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The Slope of Biology: An Interview with Eric Lander

Melis Anahtar and Paul Baranay

Dr. Eric Lander, a principal leader of the Human Genome Project who is widely regarded as one of the world's leaders in genomics, recently took some time out of his busy schedule to sit down with two MURJ staff writers for a brief interview. What followed was a valuable, thought-provoking discussion about some of the most important topics in biological research today.

Melis: First, we wanted to hear your thoughts on how the biology department has changed since you first arrived at MIT.

Dr. Eric Lander: My goodness, wow. Well, my perspective is that the biology department hasn't changed. The central feature of the MIT biology department is that it has always had very creative young faculty exploring the edge of biology, and it's always been a lively department ever since the days of Salvador Luria [Director of the Center for Cancer Research at MIT and 1969 Nobel Laureate]. The biology department has always been one of the best in the world. It has stayed that way by having a steady stream of young scientists joining the faculty and having older faculty keep reinventing themselves, working on new things. One of the wonderful things about the department is that it's so lively. Nobody gets stuck in their ways, everybody is challenging paradigms, inventing new approaches. I think that aspect of the culture is a constant.

The other constant of the culture is teaching. Undergraduate and graduate teaching have been signatures of the biology department since I've been here and long before, certainly going back to the 60's. MIT has always felt that great researchers should be in classes, even teaching introductory courses. The teaching enterprise and the research enterprise are inextricably linked at MIT, as compared to some other universities where the research faculty don't teach the introductory classes. MIT has always had that as part of its culture. Same for the graduate program. MIT is famous for inventing a graduate class called "Methods and Logic," a paper-reading course, for graduate students to read biology papers and discuss and debate them. This course have been copied at many other universities.

So in that sense I would say that MIT hasn't really changed much because of its two central pillars: that it's all about change and reinvention, staying on the cutting edge, never being satisfied, always being curious, bringing in new people; and that it's all about teaching. Those pillars have been there all along.

Paul: Previously, you were one of the leading collaborators on the Human Genome Project. We were just wondering if you could tell us, on a personal level, what that experience was like.

EL: It varied depending on what day you ask! It was exhilarating, just exhilarating. It is one the most remarkable scientific projects of our generation, perhaps the signature project of our generation. It was an effort larger than any of us, so we all felt that we were contributing to something that we would be very proud to tell our grandchildren we had done. We felt that it would change the face of biology in medicine, and I think it has. On any given day it could be frustrating because machines would blow up on us—metaphorically, they wouldn't be working—or strategies wouldn't work. You'd have reporters calling, you'd have never-ending wars with the private company that was competing to sequence the human genome, or whatever. But I had the pleasure of being part from the very beginning, all the way to its successful completion The overarching feeling is a sense of purpose and satisfaction. We all felt we knew why we were doing this.

There were challenges at every step of the way—nothing was easy. When the project was laid out, it involved trying to do something 10,000 times larger than had been done before. It wasn't just scale-up, it was solving a zillion problems, but it was exhilarating to solve those problems every day and to work with a community of people. As for MIT, we had an extraordinary community of hundreds of people involved in multiple disciplines—from biology, from engineering, from computer science, from chemistry—all working together for a common goal. And we were part of an international network. There were groups in twenty different centers in six different countries. The largest group in the world was here at MIT, but it was just wonderful to feel like we had a community that stretched to my friend Yang Huangming in Beijing, Henry as he's called, who runs the genome center in Beijing; or our colleagues in the Sanger Institute in England, or in St. Louis or in Japan. And so we felt that we were all part of the same goal. There was great pride that the data was released at the end of every day freely on the web. There were,I still remember,

meetings every Friday at 11 A.M., international conference calls for six years. I'll probably never get over that.

It was a great experience, a tremendous challenge. It was very different than my own research lab. I did still have a regular research lab, I still had graduate students and postdocs, though it was a small lab, but on the side, I had about 500 people working together. So it was an interesting balance and it was a tremendous privilege, I have to say.

Paul: Now that the human genome has been sequenced, what keeps you busy now?

EL: So what's the to-do list? [laughs] Here's the to-do list. We sequenced the human genome, but we need to know everything that's encoded in it. 1) We need to know all the functional elements encoded in the genome. All the genes, regulatory elements and structural elements 2) We need know all the genomic variants and how they correlates with the risk of disease. 3) We need to know all the mutations that can occur that can give rise to cancer. 4) We need to be able to recognize all the cellular circuits and how they're read out. So when a cell is doing something, we need to recognize that a certain pathway is turned on. And 5) we need to be able to manipulate or modulate genes in situ. That's pretty much the to-do list. And it's not something for the 21st century, but just for the next ten years, because there's enormous progress being made towards it already.

How do we identify functional elements? We can use evolution—compare the human genome to other species and see what bits evolve slowly, which elements are conserved, in other words. Well, the human genome was finished in 2003, the draft in 2001, and as of now, we have 24 more mammalian genomes sequenced—dogs, mice, rats, elephants, armadillos, etc. We have projects here at MIT to line up all those sequences and figure out what's conserved. We have projects to do large-scale chromatin analysis. Chromatin are proteins that wrap up DNA and are modified in different ways to control genes. In the last 6 months, it's become possible to do large-scale analysis of chromatin structure, build whole maps of the chromatin regulatory states of cells. With regard to human genetic variation, in 1998, there were only a couple well-documented human genetic variants, single nucleotide changes, in the human population. We started a project here to generate some, about 4,000, as a pilot project for a larger project that in two years grew into about a million, which grew into 10 million. And that's actually the majority of human genetic variation. So, most human genetic variation has already been identified, and it's pretty cool.

Correlation with disease: we can look at the genetic variants and see which ones are enriched in people with diabetes or early heart disease or asthma. That only just became possible last summer, with tools that we had been working on. And over this past twelve months, there has just been a huge explosion. For the first time we have genes being discovered all over the world, every week, as risk factors for common disease -- for diabetes, for inflammatory bowel disease, for early myocardial infarction. It's just fantastic. It's something we've been aiming at for ten years and it's finally come to pass.

With regard to cellular circuits and signatures, it's possible to study all the RNAs in a cell and build inventories, catalogues of expression of genes. There are projects here to try to do that. Also there are projects to use RNAi, inhibitory RNAs, to modulate genes. It is sort of like Human Genome Project 2. Human Genome 1 was getting the sequences, Human Genome 2 was breathing meaning into that sequence.

Paul: Apart from what you just mentioned, what do you feel are some of the unsolved problems or "hot topics" within biology?

EL: [sighs] So those weren't impressive enough? Just teasing!

Oh, there are huge, huge problems. They range from the very fundamental problems, like that we still don't really understand development—how it is that a single cell develops into an organism and cells adopt fates in persistent ways. They make career decisions, but we don't really know what that means. What does it mean at the molecular level that a precursor cell is committed to becoming a liver cell? You can buy whole books on development, and read them, and there isn't a meaningful answer to that question at the molecular level. That's a major problem that still is being worked out.

That's a very fundamental question, and at the extreme opposite of the spectrum is an applied question. We still don't really know how to make vaccines. HIV is our most embarrassing case, we can't make a vaccine against HIV. We don't have the deep science of how to make vaccines.

So, the unsolved problems are anywhere from what does it mean for a cell to make a commitment, to how do you actually design and make a vaccine, and many questions in between. But we have tools, and biology is at an amazing time where we can actually begin to take on these huge questions.

Melis: Since this is an undergraduate research journal, what role do you think undergraduates should play in tackling these huge biological problems?

EL: Get involved. The most important thing for an undergraduate to know is that science is changing rapidly. As an undergraduate, you're learning biology for the first time. Maybe you're scrambling just to learn the facts. What you learn in class is: Here are the facts of biology, here are the point estimates of where biology is now. That's useful, I suppose, but it's far more interesting to look at not the point estimate but the derivative. What is the slope of biology? Undergraduates sometimes feel like they should learn just what's in the textbook. But really, what they should do is read the textbook from ten years ago, then read the textbook from today, see how rapidly things are changing and then project forward to what the textbook is going to be twenty years from now when you'll be in the middle of your research career. The best thing to do is work in a laboratory, and see how fast this stuff is changing.

What's the reason to go to a university like MIT? Why not go to a place that is only about teaching? A school that is primarily devoted to teaching can teach you the current state of biology. They can have fabulous teachers. But what they

can't do as well is to teach you how rapidly the field is changing, because only by actually doing the research can you really feel how it's changing. I don't really care if students learn the current state of biology, I care that they're going to develop the next stage of biology. You have to learn the current stage to reinvent it, but I don't want you to take the stuff as received wisdom, I want you to look at it and say, "Well, that's nice, but it's pretty imperfect. There are all sorts of questions can't be answered." Or, there are all sorts questions developed in the last ten years. Think about what can be developed in the next ten years. Edginess, impatience, possibility.

Most undergraduates aren't going to make huge discoveries as an undergraduate: you have a too much classwork to have the time necessary to do it. But what you can do is get the feeling deep in your bones of the exhilaration of being part of such a vast intellectual enterprise. It is addictive, it is an extraordinarily addictive experience to know things that nobody else in the world knows. To know, "Wow, we are the only people who know this so far. The rest of the world will know in a month but right now we're the only ones who know this." Once you do that, then you want to do it again, and again.

Melis: Since you've had many UROP students, you know that we have time constraints, but what are the main qualities you look for in a UROP?

EL: Self-reliance. Often a good UROP is just dropped into the middle of an environment and everyone is busy working, they're busy with their classes, and you know, you learn by encounters with other people. You've got to be flexible, you've got to talk to people. So when I look for a UROP I look for somebody who's curious, gregarious, who will seek others out and ask questions. Flexible—and, in some sense driven—driven to do something, driven to know something. But, of course, most MIT students are like that. And of course, you can only take a handful of UROPs, so it's always tough.

The most legendary UROP that I ever took is Mark Daly. I took him when he was a freshman in 1986. He stayed and continued his UROP all four years, and then came and took a job here, and basically stayed forever. He became known as the most extraordinary genetic analyst in the world. People began writing to him to collaborate with him on different projects. He took up a job as staff scientist at the genome center, and people just began assuming he was a professor. Hhe began writing papers on all sorts of different subjects, and became the world expert and made some very important discoveries, just by basically continuing his UROP for 15 years. And, at some point, it became apparent that he had made such extraordinary contributions to science, people were asking, "Do you want to go to graduate school?" and he would say, "I don't have time to go to graduate school, I'm doing too much science." He was just too productive to want to go to graduate school. So what was arranged was this: the University of Leiden in the Netherlands has an old tradition, going back to the Middle Ages, of being able to award Ph.D.'s to people for work that they have already completed, if they write it up as a thesis. So it was arranged that Mark would write up all his work as a thesis to Leiden and defend it there. He was awarded his Ph.D. from Leiden for the work that he completed, and now has had a faculty position as a professor at the Massachusetts General Hospital for the past couple of years.

But, it was just amazing, it was just a UROP project! And you never know what UROP project will grow into.

Melis: Could you describe some of the projects that UROPs in your lab have undertaken?

EL: Well, Mark was one, whose job was to make a genetic linkage map using DNA polymorphisms and do the analysis of that. Other UROPs have worked on cloning genes in mice, or writing computer programs to analyze gene expression in diabetes, or all sorts of projects.

Paul: Did your UROPs come into your lab already knowing how to do those things?

EL: No, they learned on the job. You come in with some things, you learn some things.

Melis: Graduate schools seem to place a great deal of emphasis on publications. Do you have any publication tips for undergraduates?

EL: Write clearly. I'm serious. The ability to write clearly helps your papers get published far more than you might realize. There are tens of thousands of people doing biology. The ability to get people to stop and pay attention to your work depends on clear communication. You have to know how to give a clear talk, write a clear paper, otherwise people don't have the time to find the gem.

Find papers you love and use them as a model. Emulate writers just as a novelist would emulate some novels as a model. Good scientists should know of some papers that they say, "Wow, that's a model on how to write a paper. It's so crisp, so clear. The sentences flow, the ideas flow. There's a voice."

I hate the traditional way of writing a paper. The usual way they teach in school is tremendously repetitive: give an introduction, give the materials and methods, then give us the results, and only then give a discussion. That's boring, that's incredibly boring. First of all, I don't want to see your materials and methods before I've read your results to see if I'm interested, so put materials and methods at the end. Cell and Nature and a few other journals do that. I don't want to hear the same thing three times, just get to the point. I want to know what the point is. The idea of separating your results from your interpretation is nuts, life doesn't go that way, you don't get a whole bunch of results and then do your interpretations. You say, we got this result, we think it means this, we therefore did something else, we think it means that, we therefore did something else, and so on.

It's a story—writing a paper is telling a story. It's an intellectual story, it's a scientific story, it's a rigorous story, but humans are set up to hear stories. A great scientific paper is a story. Look at "The General Nature of the Genetic Code" by Crick, Brenner, Barnett, and Watts-Tobin, a paper on the fact that the genetic code is in triplet letters. It's a beautiful

paper, it's written in the first person and it's gorgeous, it's really nice.

The passive voice should be killed, there's no excuse for writing in the passive voice. Please write "we"! Science was done by a "we" or an "I," there needs to be a subject to sentences in most cases. Because we thought that it would be a good idea to do this, or we reasoned that. I want to hear that personal voice in there, because I can believe it or disbelieve it, but I want an actor in that story. I don't want some passive hands-off description of things. I want flesh and blood in the paper. Anyway, I have a lot of feelings on how to write a great paper.

Paul: You've done some of your greatest work in the field of genomics. How do you feel your accomplishments are going to affect the lives of everyday people in the near future?

EL: Well, my work has been devoted to making the tools to find the basis of disease. In the 20th century we didn't know the molecular basis of most diseases—diabetes, most kinds of cancer. Pretty much everything I've done, from the beginning of building genetic linkage maps, and physical maps, and sequencing the genome, and determining genetic variation, has been building the tools for finding the cause of disease. How will that affect people? Well, while occasionally it's possible to create therapies for a disease without knowing the cause, it's a pretty shaky business. The most effective approach we have for finding therapies is to start with the cause. So I hope that at some point, in my lifetime or not, there will be hundreds of diseases for which we have more effective diagnostics and therapeutics because the human genome got laid out. That's why we focused on the tools. We apply the tools ourselves to particular problems—I've worked on cancer and other diseases, but I've always had my mind that the real goal is to create the tools that will enable 10,000 people to work on these things, because the multiplier is so much greater than what we can do in our own lab. We work on some such problems ourselves, because that's how we know we're building the right tools, and it's good feedback, but we can't possibility do all that has to be done. But, we can enable the world. That's why feel so strongly about getting the tools out, the information out, freely.

Paul: Finally, what are your thoughts on the growing field of bioengineering?

EL: I think the more we understand cellular circuitry, the more we can try to design cellular circuits. Systems biology is kind of the analysis of circuits, but there's a design component just like there is in electrical engineering. Could we design a new circuit that would make a cell do something for us. It's a challenge, we're nowhere near the point where we have all the parts on the shelf and we just can pull them down say, "Now let's put together a circuit that will do something." I think bioengineering is pretty exciting as a long-term discipline.

Dr. Eric Lander is the Founding Director of the Broad Institute of MIT and Harvard. A mathematics genius turned geneticist, Dr. Lander applied his mathematical prowess to crack the world's most complicated code—the sequence of DNA carried by all human beings. In addition to his extraordinary contributions to the Human Genome Project, Dr. Lander has won the Woodrow Wilson Prize from Princeton University, the City of Medicine Prize, and the Gairdner International Prize. A former MacArthur fellow, Dr. Lander was elected as a member of the U.S. National Academy of Sciences and the U.S. Institute of Medicine. He has served as chair of the Joint Steering Committee for Public Policy. In addition to his work at the Broad Institute, Dr. Lander is a member of the Whitehead Institute and a professor of biology at MIT, where he has won the prestigious Baker Memorial Award for Undergraduate Teaching. He has taught MIT's Introductory Biology course (7.012) for sixteen years.

Prion Diseases: A Review of a Potential Major Risk to Public Health

Mahesh Vidula

A fifty-two year old man stepped into his shower, just as he would on any normal day. But today, something was wrong. He could not recognize anything at all. Even his soap was a stranger. One year later, after losing his ability to speak and see, he died. Shortly after his death, scientists proclaimed that he had died of variant Creutzfeldt-Jakob disease [1].

The prion diseases have recently received universal attention because of their major risk to public health. These diseases, grouped under the category of transmissible spongiform encephalopathies (TSEs), are the result of the misfolding of the normal host-encoded cellular prion protein (PrP^{C}), to its pathogenic isoform, the scrapie protein (PrP^{Sc}) in a post-translational conversion [2]. The PRNP gene, located on the short arm of human chromosome 20, is responsible for the creation of the normal prion protein [3]. Several polymorphisms occur in the PRNP gene, although the most important one is at codon 129, since it appears to affect the susceptibility to prion diseases [4].

Prion diseases have shown to afflict animals as well as humans [5]. Prion diseases include Kuru, Creutzfeldt-Jakob disease (CJD), Fatal Insomnia (FI), and Gerstmann-Sträussler-Scheinker Syndrome (GSS) in humans; Chronic Wasting Disease (CWD) in deer and elk, scrapie in sheep, and Bovine Spongiform Encephalopathy (BSE, commonly known as "mad cow disease") in cows [5]. The most common type of prion disease in humans is the Creutzfeldt-Jakob disease [6].

The most notable feature of prion diseases is their growing importance in today's world. The BSE outbreak captured the attention of many people when almost 200,000 cattle died [7]. New research suggests that BSE can spread to humans, who eat infected meat, after the emergence of variant Creutzfeldt-Jakob disease (vCJD) in humans, since both diseases have been shown to share the same prion strain [8]. Furthermore, out of the 177 cases of vCJD in the world, 156 are from the United Kingdom, where approximately 184,000 cattle have been recorded with BSE [9]. BSE has spread across the world to other countries, such as Japan [10].

PrP^{C} has also enthralled scientists because its function in the body is ambiguous [6]. The normal prion protein aggregates in the central nervous system, specifically in the cerebellar Purkinje cells, spinal motor neurons, and neocortical and hippocampal neurons [11]. PrP^{C} is also found in the glia and neurons of the brain, as well as in the spinal cord [12]. Since the normal prion protein is attached to the cell surface, with a glycophosphatidylinositol (GPI) anchor, it is possible that it has a role in ligand uptake, transmembrane signaling, or cell adhesion and recognition [12]. Furthermore, copper binds at the peptide repeat region of the purified form ofPrP^{C}, which has been shown to improve the endocytosis of PrP^{C} from the plasma membrane, and this introduces the hypothesis that PrP^{C} is involved in the metabolism of copper [12]. Current research has discovered PrP^{C} on the surface of bone marrow stem cells, and has shown that it is important in the self-renewal of hematopoietic stem cells [13]. Moreover, PrP^{C} appears to be important in preventing Alzheimer's, since it inhibits beta-secretase from creating the beta-amyloid plaques that cause the disease [14].

The two isoforms, PrP^{C} and PrP^{Sc}, only differ in conformation and have the same primary amino acid sequence [15]. This, along with the absence of nucleic acid in the prion protein, suggests that the conversion occurs post-translationally [15]. While PrP^{C} is composed of 42% α-helix and only 3% β-sheet, PrP^{Sc} is composed of 30% α-helix and 43% β-sheet [15]. Various models have suggested thatPrP^{C} converts to its pathogenic isoform when the region corresponding to the residues 108-144 fold into β-sheets [15]. Because of these structural differences, the two isoforms have different biochemical characteristics. $PrP^{C}$is very soluble in detergents and easily digested by proteases while the PrP^{Sc} is insoluble in detergents and resistant to protease digestion [15]. Current research hypothesizes that PrP^{Sc} inhibits the 26S proteasome, thus resisting degradation [16]. Therefore, a method used by researchers to differentiate between$PrP^{C}$and PrP^{Sc} is to treat the proteins with a proteinase-K digest.

Prion diseases exist in infectious, sporadic, and genetic forms. The infectious prion diseases are speculated to result from a spontaneous conversion of endogenous $PrP^{C}$to PrP^{Sc} or following the introduction of PrP^{Sc} in the body [17]. The scrapie

prion protein replicates by complexing with and templating its conformation on to the PrP^C [6]. This unique aspect of prions has been demonstrated *in vitro* [18]. Scrapie prion protein can be introduced into the body through consumption of infected cow meat as in the case of Bovine Spongiform Encephalopathy or through the reuse of surgical instruments that are contaminated with infectious prions (infection through this manner is known as iatrogenic CJD) [18, 19]. The etiology of sporadic prion diseases is unknown although it is thought to be the result of randomly misfolding of PrP^C to PrP^{Sc}, which allows for further conversion of normal prion protein to PrP^{Sc} [20]. There are no apparent genetic causes for sporadic PrD since no mutations in the PRNP gene have been detected in sporadic PrD cases [20]. However, there is a clear genetic susceptibility for sporadic CJD in that most cases occur in patients with a homozygosity at codon 129 for either methionine or valine while heterozygous subjects seem to be protected against sporadic CJD [20]. Prion diseases can also be inherited. Studies have shown that prion diseases can also be inherited since 5-10% of CJD cases, and all cases of FI and GSS are linked to germline point mutations in the PRNP gene [21]. These mutations weaken the normal cellular prion protein, which then gives the PrP^C a predisposition to converting into PrP^{Sc} [22]. Prion diseases induce death when the PrP^{Sc} accumulates in the brain, resulting in neuronal death and the creation of microscopic holes, known as vacuoles, in the brain [6].

A common polymorphism occurs at codon 129 of the PRNP gene, where either methionine or valine can be encoded [6]. This codon is thought to have a role in determining the susceptibility of a person to prion diseases [6]. Studies have shown that sCJD is most predominant in subjects who are homozygous for either methionine or valine, while heterozygosity appears to significantly protect against the disease [20]. Furthermore, all patients with variant CJD that results from ingestion of beef tainted with BSE, have been found to have a methionine homozygosity at codon 129 [23].

Table 1: PRNP Codon 129 Genotype in Prion Diseases in Caucasians [23]

Codon 129 genotype %	MM	MV	VV
Normal Population	39	50	11
Sporadic CJD	61	21	18
Variant CJD	100	--	--

Therefore, it has been hypothesized that while homozygosities for methionine or valine may increase the susceptibility to prion diseases, a heterozygote may be protected from them. This is significant since the hypothesis suggests that while 50% of the Caucasian population might be at risk, 50% are not [23]. This implies that the interaction between two homozygous prion proteins is stronger than the interaction between two heterozygous prion proteins.

Furthermore, the homozygotes and heterozygotes at codon 129 experience different disease phenotypes. Those with 129MM normally display a shorter, more rapid course of disease with cognitive distortion, while 129MV and 129VV cases present a longer course of disease, along with ataxia [24]. The polymorphism at codon 129 has been linked to other serious neurological disorders. For example, research has found that Down's syndrome patients who have a valine at codon 129 seem to have a more rapid decline in cognitive performance than compared to methionine homozygotes [25]. Moreover, a methionine homozygote has been reported to show an increased risk to late-onset Alzheimer's disease [26]. These findings suggest that the prion protein may play a role in these disorders, and that its function may soon be elucidated.

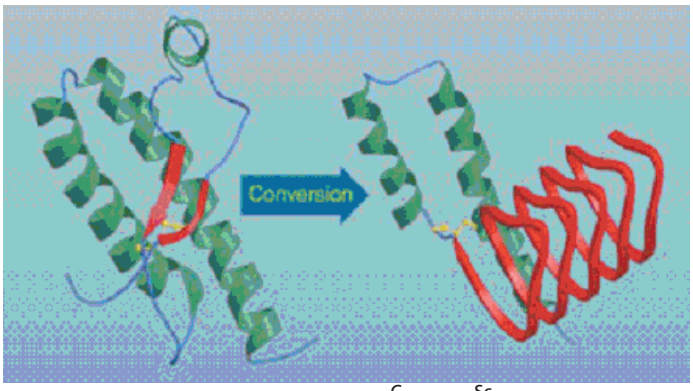

Figure 1: Structural Differences Between PrP^C and PrP^{Sc} [I].
Left: PrP^C with only 3% β-sheet (red) but 42% α-helix. Right: PrPSc with 43% β-sheet and 30% α-helix.

In order to control the spread of prion diseases, effective treatments are crucial. Current research has shown that nonpsychoactive cannabidiol (CBD) inhibits the accumulation of prion proteins, which gives rise to the possibility of an effective treatment [27]. Hopefully, a better understanding of the function of CBD in hindering the aggregation of infectious prions will allow for the development of more treatments that prevent prion diseases. Overall, despite the advances made in prion research, there are still many questions left unanswered.

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UROP Summaries

The effect of p53 on the spontaneous and exogenously induced frequency of homologous recombination in mice

Fall 2006- Present
Engelward Laboratory
Principal Investigator: Bevin P. Engelward
Supervisor: Dominika M. Wiktor-Brown

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Major: Biological Engineering

p53 is one of the most commonly mutated genes in human cancers.[1] It is a tumor suppressor gene that is critical for signaling cell cycle arrest or apoptosis, depending on the extent of cellular damage. If DNA damage is relatively minor, p53 signals for cell cycle arrest and DNA repair. If the damage is extensive, p53 signals for apoptosis to prevent mutations and potential tumorigenesis.[1] We set out to determine whether p53 is involved in regulating a cell's utilization of a specific form of DNA damage repair: homologous recombination (HR). HR is a critical pathway for the repair of DNA double-strand breaks (DSBs), a particularly harmful form of damage. HR repairs DSBs by using homologous sequences, such as sister chromatids, as templates.[2] To minimize deleterious sequence rearrangements between misaligned sequences, HR is upregulated during S phase, when the perfect template, a sister chromatid, is present.[3] In addition to regular DSBs, HR also repairs one-ended DSBs that can result from replication fork breakdown, e.g., when a replication fork encounters a single-strand break or blocking DNA lesion.[2] Given its important role in repairing DNA damage, it is not surprising that HR is critical for maintaining genomic integrity. Misregulation of HR can lead to tumor-promoting genomic rearrangements such as loss of heterozygosity, as well as chromosomal abnormalities such as deletions or insertions. Thus, determining whether p53 status affects utilization of HR is critical for understanding one potential mechanism by which p53 prevents tumorigenesis.

In our study, we used Fluorescent Yellow Direct Repeat (FYDR) mice, which enable the detection of HR events both in vivo and in vitro. FYDR mice carry two truncated copies of the coding sequence for Enhanced Yellow Fluorescent Protein (EYFP) arranged in tandem. An HR event can restore full-length EYFP, resulting in a fluorescent cell that can easily be detected by flow cytometry and in situ imaging.[4,5] To specifically study the effect of p53 on HR, the FYDR mice were crossed with mice that carry a deletion in p53.[6]

We were particularly interested in studying DNA repair within the pancreas, a subject that has not been widely investigated despite the high mortality rate of pancreatic cancer.[7] To determine the effect of p53 on the spontaneous frequency of HR in vivo, pancreata from FYDR; p53 mice were analyzed by both in situ imaging and flow cytometry. In situ imaging enables the quantification of independent HR events, while flow cytometry allows for the determination of recombinant cell frequency. Our results showed no statistically significant difference between the number of HR events and the frequency of recombinant cells in p53 wild-type (WT) versus null cells, suggesting that p53 does not affect the spontaneous frequency of HR in pancreatic cells in vivo.

To further explore the effect of p53 on HR, we analyzed primary ear fibroblasts from FYDR;p53 WT and null mice in vitro. We quantified the frequency of recombinant cells per cell division, which is also known as the rate of recombination. No statistically significant difference was observed in the rate of recombination in p53 WT versus null cells, which was consistent with the in vivo results. These data suggest that p53 may not have a large effect on the spontaneous rate of HR in vitro.

Since it is known that p53 plays an important role in response to DNA damage,[1] we treated cells with a DNA damaging agent (Mitomycin C) to determine the effect of p53 on the damage-induced frequency of recombination. Preliminary results from this study show that both p53 WT and null treated cells have higher frequencies of recombinant cells than control cells, indicating that Mitomycin C induces recombination events in both p53 null and WT cells. In addition, our results show that p53 WT treated cells have a statistically significantly higher frequency of recombinant cells than p53 null treated cells, suggesting that functional p53 may stimulate the repair of exogenously induced DNA damage by HR in vitro.

In summary, p53 does not appear to have an effect on the spontaneous frequency of recombinant cells in vitro or in vivo. However, p53 may affect the frequency of recombinant cells that result from the repair of exogenously induced DNA damage by HR. Thus, the effect of p53 on HR may appear only in the face of extensive DNA damage that does not exist spontaneously.

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Protease Activated Electrostatic Ligand Coating for Targeted Gene Delivery

June 2007- Present
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Major: Chemical Biological Engineering

Gene delivery is the focus of much research as a therapeutic tool in the treatment of diseases, including autoimmune diseases and cancer. It is an ideal therapy with which to treat patients because of the specificity of nucleic acids such as DNA to replace or restore function to diseased tissue without the systemic side effects commonly caused by modern pharmaceuticals. Non-viral, cationic, self-assembling polymer vectors have been devel-

oped to deliver nucleic acid payloads, showing great efficiency in inducing cell uptake.[1] However, these vectors are hampered by the lack of a means to effectively target and systemically deliver genes in an effective manner. The overall effectiveness of current vectors is low, as they are hindered by effects including destabilization due to hydrolysis in blood and nonspecific uptake by cells.

Researchers have added targeting ligands in an effort to address the issues of nonspecific uptake. These ligands usually consist of small peptide sequences that bind to cell surface receptors to promote specific uptake. In addition, stabilization in blood has been shown to be increased by the addition of polyethylene glycol (PEG) to provide steric hindrance, which helps the nanoparticle polyplex evade uptake by the mononuclear phagocyte system and improve accumulation in the tumor. Investigators at the Laboratory for Multiscale Regenerative Technologies (LMRT) previously developed a novel approach for the bioconjugation of a protease cleavable ligand with PEG that is activated in the presence of MMP-2, an enzyme that is upregulated in tumor angiogenesis. This bioconjugated polymer considerably increases circulation time and may be proteolytically cleaved to expose cloaked functions, such as a targeting ligand for specific cell uptake.[2]

Our current study adapts and synthesizes a modified protease cleavable peptide-PEG polymer that works in conjunction with a biodegradable, cationic nucleic acid condensing vector, conferring this cationic polymer with increased circulation times and passive tumor targeting ability.[3] Our goals include the optimization of the nanoparticle formulation in vitro and the analysis of its circulation and localization properties in vivo with GFP as the transfection payload. Preliminary results are promising as we continue to refine our modular nanoparticle design. While currently optimized for DNA delivery, our system is general enough for adaptation to siRNA delivery, and will thereby provide new avenues for the treatment of disease through gene therapy.

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Structure and Function of MEG3

June 2007- Present
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Human pituitary adenomas comprise roughly 10% of all diagnosed brain tumors and affect the levels of hormones produced by the pituitary gland. These tumors can arise from any of the six types of cells found in the pituitary gland: corticotrophs, somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and null cells. Pituitary tumors affect hormone production by either decreasing or increasing the level of hormones. These hormones include prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and growth hormone (GH). Some adenomas are nonfunctional and do not produce any hormones; hence, they are often referred to as null-cell adenomas. Approximately 25-30% of pituitary adenomas are nonfunctional, while the rest are functional and can cause clinical disorders. For example, the overproduction of growth hormone or somatotropins can result in gigantism in children and acromegaly in adults. Similarly, high levels of ACTH in the pituitary gland can cause Cushing's disease. However, Cushing's disease is sometimes independent of ACTH levels and can is affectedbe affected solely by the level of cortisol in the adrenal glands. Since pituitary adenomas can have significant effects on patients and there is currently no effective medical therapy for these tumors, it is critical for scientists to examine their genesis and development so as to find an effective way to suppress the growth of these tumors.

In previous studies conducted at Massachusetts General Hospital's (MGH) Neuroendocrine Unit, a maternally-expressed imprinted gene called MEG3, located on chromosome 14q.32.2, was found to be a potential growth suppressor in tumor cells. A study conducted at MGH by Dr. Xun Zhang et al. showed through cDNA-representational difference analysis that MEG3 was expressed in normal human gonadotrophs, but not in non-functional pituitary tumors. Real-time PCR (RT-PCR) confirmed that a MEG3 isoform, MEG3a, was not expressed in growth-hormone-secreting and nonfunctional pituitary tumors. These results suggested that MEG3 might be involved in controlling rates of cell proliferation.

To test this hypothesis, the cDNA for MEG3a was cloned into the mammalian vector pCI-neo and transfected into several human cancer cell lines that previously did not show MEG3 expression. The transfection reduced the cell clone numbers in these cancer cell lines by approximately 70%, thus confirming the hypothesis that MEG3 inhibits cell proliferation. In another study conducted at MGH by Dr. Jing Zhao et al., MEG3 loss in human tumors was found to be associated with hypermethylation of the promoter region. DNA from normal and tumor pituitary samples was treated with sodium

bisulfite and then analyzed for methylation patterns in its promoter region. The majority of tumor DNA showed high levels of methylation in its promoter region, while normal pituitary DNA was unmethylated. Furthermore, a methylation inhibitor was used to demethylate genomic DNA from the human cancer cell line, MCF7, and this treatment restored MEG3 expression. These results further confirmed that MEG3 plays a key role in the pathogenesis of human tumors.

In the present study, I am working with another researcher at MGH, Dr. Dalia Batista, on several different kinds of pituitary tumors in order to derive their relationship to MEG3 expression. We are investigating corticotropin-secreting adenomas, somatotropin-secreting adenomas, prolactinomas, and non-functioning tumors. Our goals are to discover the structural and functional aspects of MEG3 in these pituitary tumors, to understand the mechanism for loss of MEG3 expression, and to study the effects of MEG3 on growth and hormone production in these tumors. In situ hybridization and immunohistochemical staining will be employed to determine which kinds of pituitary cells express MEG3. Real-time (RT) PCR will then be performed to examine the expression of MEG3 in pituitary tumors, and to determine if a loss of heterozygosity due to a missing allele in the MEG3 gene inhibits MEG3 manifestation in pituitary tumors. Furthermore, bisulfite treatment of DNA, followed by PCR amplification of the promoter region and DNA sequencing, will be used to analyze the methylation patterns of DNA from pituitary tumors. Thus, we will determine whether hypermethylation is responsible for the loss of MEG3 expression in pituitary tumor cells. Later in the experiment, we will transfect pituitary tumor cells with MEG3 in order to study its effect on their growth.

Since there are no effective therapies available, most tumor patients must resort to surgery or radiation therapy. The results of this project will hopefully shed light on the mechanisms underlying pituitary tumorigenesis and provide insight on targeted-therapeutic approaches for the treatment of human pituitary tumors.

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Detection of Nanoparticle Assembly Using Suspended Microchannel Resonators

January 2007 – Present
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The Nanoscale Sensing Group in the MIT Media Lab recently developed fluid-filled suspended microchannel resonators (SMRs) to measure the masses of biomolecules, single cells, and single nanoparticles in fluid.[1] These devices use the basic principle of cantilever detection: changes in the cantilever's resonant frequency are related to the mass of nano- or micro-scale compounds passing within or binding to the channel, enabling continuous, frequency-based observation of mass changes (Δm) in the cantilever. The frequency dependence on the cantilever mass is described by the equation below.

$$f = \left(\frac{1}{2\pi}\right)\sqrt{\frac{k}{m^* + \alpha\Delta m}}$$

Equation 1: "f" is the frequency in Hz, "Δm" is the additional mass added to the cantilever, "k" is the spring constant, "m*" is the effective mass, and "α" is a constant dependent on the geometry of the added mass.

We hypothesized that SMRs could be used to sense the assembly of nanoparticles, mediated by virus-sized polystyrene beads. Spherical gold nanoparticles (NPs) were used in the assembly, as well as polystyrene beads with streptavidin proteins on their surfaces for binding to biotin.[3] A polyethylene glycol polymer (biotin-PEG-thiol) expressing a biotin on one end and a thiol on the other was synthesized for creating a stable, complementary coating on the gold NPs. These polymer coatings were optimized for stability and ligand-mediated biotin-streptavidin assembly was monitored using dynamic light scattering and spectrophotometry. Finally, the assembly of gold NPs and beads was measured with SMRs. Mass histograms demonstrated that over 70% of the original gold NP monomers were able to coalesce through biotin-streptavidin binding into mass aggregates detected by the SMRs. These initial results provide evidence that mass-mediated detection of NP assembly could be used in the future to sense assembly around a variety of pathogenic targets (i.e., viruses, bacteria, etc.) in specimens not amenable to optical analysis.

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Cellular responses to UVA as a source of oxidative stress

Kathy Xu

Background

Oxidation and reduction reactions are important in driving many processes in living organisms. They are chemical reactions that involve the changes in oxidation number between the reactants to products. Pro-oxidants and antioxidants are reagents that drive these reactions, and a balance of them is essential in living organisms. However, when Reactive Oxygen Species, a type of pro-oxidant exceeds the antioxidant capacity, oxidative stress is generated. Reactive Oxygen Species (ROS) in excess can generate oxidative stress that can lead to some major diseases such as cancer, cardiovascular disease, and neurodegenerative diseases.

There are many ways to generate ROS or oxidative stress such as environmental pollution and various forms of radiation. UV light reaches earth in two main forms: UVA (90%) and UVB (10%). Much more is known about UVB and its deleterious effects, such as skin cancer, photodamage, and DNA lesions. Because DNA absorbs at the wavelength of UVB light, when the cells or tissues are irradiated, DNA and other endogeneous photosensitizers in the cells can absorb and mutate. Hence, most of the UV protections used today, such as sunscreens are really focused on blocking out UVB (280-320nm). However, skin cancer and various problems are still prevalent even with such protection. This shows that there are other factors that can contribute to these problems and diseases, such as UVA radiation since about 90% of the UV light that reaches earth's surface is in this form after all. UVA has a slightly higher wavelength (330-395nm) and is known to induce cellular oxidative stress, which can eventually lead to cancer.

It is previously thought that in order to generate oxidative stress, cells must be directly irradiated. However, previous studies in the Wellman Lab at Massachusetts General Hospital have shown that with ionizing radiation and photosensitization, the irradiation not only affects the target cells, but the neighboring cells as well. This effect on the naive neighboring cells is called a bystander effect, in which when cells communicate with one another, the cells and tissues not directly irradiated have also increased in oxidative stress, mutagenesis, and decreased in cell viability and clonogenic survival (the ability of cells to form colonies), the ability of cells to form colonies3. This project investigates whether UVA also generate this bystander effect in cells as well as a look at the mechanisms compared to other means of oxidative insult.

Methods

The preliminary endpoints measured were the cell viability and ROS or oxidative stress levels in cells for both target and bystander cells. The experiment was divided into two main parts: individual or subpopulations and population studies.

Time-Lapse Microscopy

For individual cell studies, EMT-6 breast cancer cells were used. These cells attach to the bottom of the wells, which allows us to monitor and track specific cells. These studies were done with the time-lapse fluorescence microscope. With this microscope, a small region of only a few cells is selected to focus on at a time. Once the target region is selected, the computer can save it such that we can come back to this exact same region at any time. The region is approximately 0.7mm in diameter, and all these cells within the region will be covered by the UVA light. About 5mm away from each region, another region is selected for bystander studies, with no irradiation. Either a 12-well or a 6-well plate was used, and each plate included both a bystander and target region of cells. The cells were approximately 70% confluent in the wells and instead of in regular media, they were incubated in HBSS, which is media that contains mainly salt solution to avoid other variables during irradiation.

Nd:YAG Laser Irradiation

For the population studies, WTK1 human lymphoblastid cells were used. They are suspension cells, and hence do not attach to the bottom of the wells. For bystander experiments, a trans-well system must be used in order to separate the target and bystander cells. This is done by putting bystander cells in a micro-porous insert that sits on top of the target cells in the wells. The pores in the insert allow exchange of cell signals and media. Cells were again incubated in HBSS at about 3 millions cells per milliliter, placed in a 1mm clear square cuvette and irradiated with the Nd:YAG laser.

Cell Viability/Survival

Cell viability in the microscope experiment is measured with the use of propidium iodide (PI), after incubating with 1.7uM of PI. PI is a fluorescent dye that is prohibited by cell membranes, and hence will only go into dead cells. Once it goes into these membranes and into the nucleus, it will show red fluorescence, which can be seen by the microscope. Later, the ratio of red cells to normal, live cells were counted to determine the percent of cells alive. For the WTK1 cells, because there is no microscope used to detect fluorescence, the MTT assay is used to measure cell viability. MTT is a toxic chemical which will go into live cells after 1 hour of incubation, and bind to the mitochondria to form a mitochondria complex. The more mitochondria complexes there are in a sample, the higher the cell viability. In order to detect the amount of mitochondria complexes, DMSO solution is added to dissolve them. Lastly, Spectrmax is used to measure the absorbance of the dissolved solution.

ROS levels/Oxidative Stress

For oxidative stress or ROS levels, the fluorescent dye DCF (2, 7-dichlorodihydrofluorescein diacetate) is used. The green fluorescence of this dye is only turned on when it enters live cells and bind to an active enzyme called oxidase. Oxidase will oxidize the dye and make it fluorescent. The DCF also binds to ROS, and the fluorescent intensity increases with greater levels of ROS.

WTK1 cell viability was measured with different irradiation energies, 0J, 10J, and 20J. Figure 1 shows that the cell viability decreased as the irradiation energies increased. This was true for both direct and bystander cells. The rate seen here for bystander cells is almost the same as for direct cells.

In Figure 2, ROS levels were measured for in both target and bystander cells in the laser experiment. 0J, 10J, and 20J were used and the fluorescence intensity of DCF was taken to measure the oxidative stress.

Figure 3 shows a similar ROS experiment for the time-lapse microcopy. Fluorescence and bright field was taken once every 30 minutes and this experiment ran over night for about 16 hours.

For the ROS experiments, Figures 2 and 3 show that there is an increase in oxidative stress for both direct and bystander cells with increase irradiation energies. In Figure 2a, the ROS levels did not increase after initial DCF incubation for the direct cells, while for the bystander cells, it increased dramatically.

Discussion

From Figure 1, we can see that the bystander cells, which had never been exposed to UVA radiation, showed a decrease in cell viability as well. Hence, we can conclude that UVA does generate a bystander response in terms of cell survival. Previous studies using photosensitization also showed similar results, that the bystander cells near the target cells with higher irradiation energies had much lower (about 15%-30%) cell survival rates1.

From Figures 2 and 3, we can see that oxidative stress in the bystander cells under higher irradiation energies was also increased. This means that there is also a bystander effect in terms of ROS levels. The results also show that for the direct cells, there isn't much change after initial DCF incubation. Studies in photosensitizers showed different results, since for photosensitizers, the DCF intensity level increased after initial incubation, maximize at about 6 hours before it dropped again2. However, for the bystander cells, we see an increase as we increase the incubation time. This is consistent with other oxidative stress experiments because it takes time to generate a bystander effect since cells have to send signals to one another1.

It is also important to note that even though the control cells went up in Figure 2, it is most likely because the cells have all been incubated in HBSS. By placing the cells in HBSS instead of serum media that contains all the essential nutrients, the cells are already under some stress.

In this study, we were able to show that UVA radiation does generate a bystander effect not only in cell viability but also in cellular oxidative stress. High levels of oxidative stress can eventually lead to the development of cancer, not only in the cells that are directly irradiated, but will also spread into neighboring cells. In the future, we would like to investigate the effects of UVA radiation on mutagenesis and clonogenic survival, as well as detailed mechanisms that led to these results compared to other ROS methods.

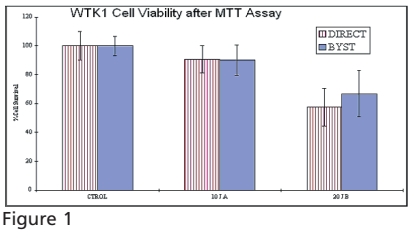

Figure 1

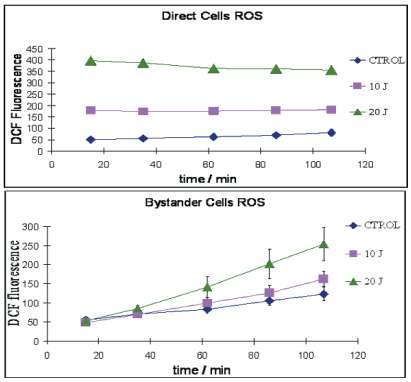

Figure 2

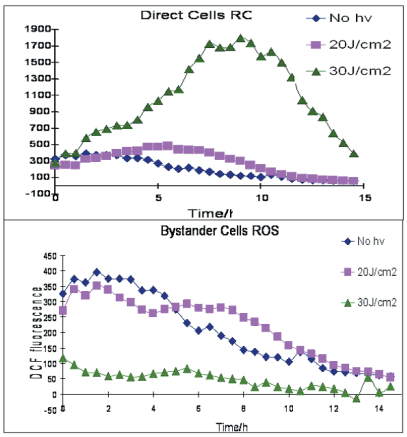

Figure 3

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Differences in Neuronal Responses due to Aging

Wendy Chen

Introduction

Human aging is associated with the decline of many human abilities such as cognitive abilities. It is still unknown how much of cognitive decay is due to pathological reasons and how much is a direct consequence of normal aging. There are some studies indicating that aging causes changes in the brain and in one's neuronal responses. However, other experiments show that there is no relationship between age and neural changes.

One way of looking at neural activity is to use functional magnetic resonance imaging (fMRI) to examine the blood-oxygen level-dependent (BOLD) signal. Since fMRI imaging is safe, noninvasive, and has better resolution and spatial localization than other imaging techniques such as EEG (electroencephalogram) and PET (positron emission tomography), it is the preferred method in mapping neuronal responses. The BOLD mechanism tracks changes in cerebral blood volume, cerebral blood flow, and oxygen consumption to detect neural activation. In response to activation, more oxygenated blood flows to the area of activation, decreasing the ratio of deoxygenated to oxygenated hemoglobin in that area and increasing its BOLD signal (Logothetis & Wandell, 2004).

A problem with the BOLD response is that both age and disease can affect the integrity of the hemodynamic (amount of blood flow) response (Nielson et al., 2004). Aging causes a decreased signal-to-noise ratio in the BOLD signal and a reduction in both the signal amplitude and the number of activated voxels (the 3D equivalent of a pixel) within the sensorimotor cortex. Cerebrovascular diseases, such as strokes, cause a decrease in the rate of rise and maximal BOLD hemodynamic response function (HRF) (D'Esposito, Deouell & Gazzaley, 2003). Since there are additional factors that affect the BOLD signal besides age, fMRI is not a flawless method of tracking age-dependent changes in the brain.

Many studies have been conducted to identify the role that age plays in neural activity. Some scientists believe that neural responses do in fact correspond with age. Scott Huettel and researchers at Duke University Medical School used checkerboard stimuli to influence the hemodynamic response. They found that young people had twice as many activated voxels and reached peak activation later than the older participants, but the hemodynamic responses had a similar onset time, rate of rise, and peak amplitude in both groups (Huettel et al., 2001). Other studies also show that prefrontal activation is similar for both groups, but older people tend to have greater deep gray matter activation. Older adults place greater emphasis on the attentional control of response regulation since their task performance is more influenced by deep gray matter structures (Madden et al., 2004). These studies show a clear correlation between aging and brain response.

Other studies suggest that aging only affects certain parts of the brain, primarily the prefrontal cortex. Researchers at the National Institute of Aging reported that older participants performed more slowly in their tasks and also had greater activation in the prefrontal cortex during location matching, suggesting that aging affects spatial vision (Grady et al., 1994). Calautti and his colleagues also found that older people had more activation in the superior frontal cortex, hinting that older people treat stimuli as more complex. Furthermore, younger people had higher perfusion in anterior brain regions, allowing them to have less activation in those areas. Aging causes a decline in the resting-state brain perfusion and glucose consumption that leads to the need for more control during tasks, which in turn increases prefrontal cortex activation (Calautti et al., 2001).

In addition, there are studies showing that neural changes have very little correlation with aging. Dr. McConnell recorded that aging causes no significant change in motor threshold, percent signal change, and volume of activation produced by transcranial magnetic stimulation (TMS), a noninvasive method of using changing magnetic fields to excite neurons in the brain. His study suggested that age-related increases in the BOLD signal during tasks might actually be secondary to changes in the peripheral systems. In addition, he argued that cortical physiology does not actually decline with normal aging (McConnell, 2003). Dr. Nielson also found that old and young adults had similar hemodynamic responses, thus suggesting that differences in activation during cognitive inhibition are not due to vascular coupling. Instead, some researchers blamed the differences in BOLD responses on less-significant or negative voxels. The elderly have a greater percentage of negative voxels in the visual region, suggesting that they may have more unconstrained visual processing (Aizenstein et al., 2004).

Different studies have divergent findings on neuronal differences between older and younger adults. Since this issue is important in learning more about the effects of aging on the human brain and its mental capabilities, the present study was conducted similarly to previous studies in order to compare the results. In this experiment, different stimuli were simultaneously presented to college students and older adults. Using BOLD fMRI imaging, the participants' brain activation levels were recorded via their hemodynamic responses. Scans were performed in two different sessions to ensure reliability. By comparing data such as the time to reach activation peaks and the amount and length of activation, the extent to which aging affects the human brain would be better understood.

Methods

Participants:

Stanford University's Internal Review Board (IRB) approved this experiment before it was conducted. Twelve young individuals (8 males, 4 females, 18-28 years old) and twelve elderly individuals (7 males, 5 females, 65-75 years old) recruited from Stanford University and the surrounding communities voluntarily participated in this study. All gave informed consent and reported no neurological or psychiatric disorders, drug abuse, or other abnormalities that might distort the results. Functional MRI conducted on a 3.0-Tesla GE scanner (General Electric Medical Systems Signa, Waukesha, Wisconsin) paired with a whole-head coil was used to detect neural activation in the participants' brains when presented simultaneously with visual, motor, and auditory stimuli. Flashing checkerboard patterns and a sequence of ascending and descending tones were presented concurrently to them while they tapped their fingers. Head movement was minimized with a bite-bar. They were each scanned twice to make sure the results were consistent and therefore reliable. The scanning took approximately an hour and the participants had to return after two weeks for the second scan. Functional data consisting of 220 volumes used an interleaved T2*-weighted spiral in-out acquisition sequence to measure the BOLD response (TR = 1500ms, TE = 30ms, flip angle=70°, FOV=24, 64 x 64 matrix). Twenty-two axial-oblique slices of 5mm thickness were acquired parallel to the AC-PC line and covering the whole brain. Co-planar and high-resolution T1-weighted volumes were collected for each subject.

Preprocessing:

The first step of the analysis involved preprocessing the raw functional images using Matlab. The first 4 images were dropped to allow for equilibrium to be reached in the magnetic field. The orientation and appearance of both the anatomical and functional images were checked for any irregularities. Next, a time course for global signal and its relation to movement was plotted to make sure that no signal changes were more than 1.5% or above 4 standard deviations (SDs) and that the range of mean signal was less than 50. This ensured that the signals were relatively constant and did not correspond with movement.

After making sure that there were no clear artifacts, the data was slice timed to correct for different slices of the brain being collected at slightly different times. Then the images were coregistered to align the first functional images of the two scan sessions. The first anatomical image was then coregistered with the first functional image of the first scan to make sure that the anatomical images were aligned with the functional ones. Next, the images were transformed again through normalization to match the first anatomical and first functional images with that of the Montreal Neurological Institute's (MNI) brain template to ensure that everything was well aligned. The functionals from the two scans were also normalized to align with the first anatomical. The last step of the preprocessing involved 6 mm smoothing of the functional images in both scan sessions to blur the data and increase the signals.

HRF Estimation:

After the preprocessing, the images for both scan sessions were reviewed to make sure that there was activation in regions of the brain corresponding to the stimuli presented to the participants. Since motor, auditory, and visual stimuli were presented to the two groups, activation in the left and right motor, auditory (temporal), and visual (occipital) regions of the brain was expected and confirmed in all the participants. Then, the timecourses of each region of interest (ROI) for every individual were extracted.

Regions of Activation

Cluster Size	Region	MNI Coordinates (x, y, z)
226	Left Motor	(-51, -27, 51)
210	Right Motor	(45, -18, 57)
149	Left Auditory	(-54, -18, 0)
133	Right Auditory	(69, -21, 0)
555	Left Visual	(-12, -90, -9)
	Right Visual	(6, -87, -6)

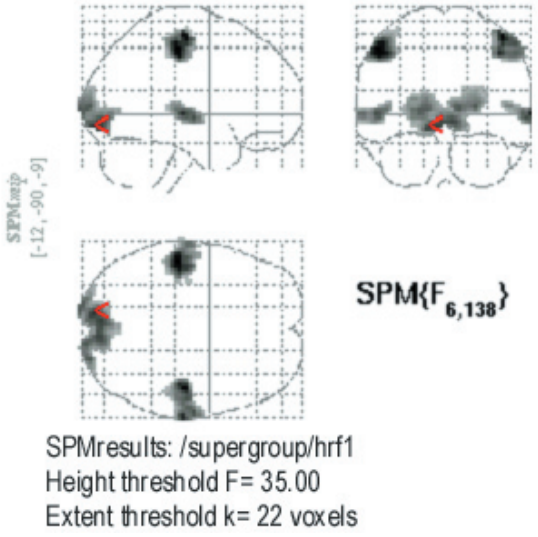


Figure 1: Glassbrain image showing the 6 major regions of activation. Red arrow points to the peak activation in the left visual cortex, which occurs at coordinates (-12, -90, -9).

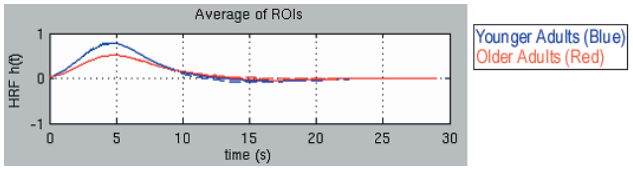
After the timecourses were extracted, the individual HRFs for each ROI were estimated using first- and second-order functions with a program that performed Fourier analysis. The functions were checked for relatively high fit correlations with the raw timecourse data (0.7 or above) for both first- and second-order fits. After saving the data, a Matlab-based custom program was used to combine the timecourses for the young and old participants so that the results could be compared and analyzed to discover the differences in the HRFs due to age.

Next, instead of analyzing the expected regions of interest, the timecourses for only the peak voxels from each participant's six ROIs were extracted. These six voxels were located in the left and right motor, auditory, and visual regions of the brain. Due to individual differences, some adults might not show as much activation in expected regions of interests but still have the same amount of peak activation, thus the peak voxels were examined in addition to the ROIs. The HRFs for each voxel of interest (VOI) were estimated and the graphs were checked for high correlation (>0.7) with the raw data for both first- and second-order fits. Then, the graphs for each VOI of the young and old adults were combined to compare the results of the two groups.

Statistical comparisons were conducted at an alpha level of .05.

Results and Discussion

The data collected from this study showed very reliable results. The estimation of the HRFs for the ROIs using a second order function had an acceptable correlation with the raw data. The average fit correlations of the HRFs with the raw timecourse data for the two scan sessions were high for both groups (0.90 for the younger adults and 0.82 for the older adults). However, the older adults had a lower correlation, indicating that their raw timecourse data was more irregular than that of the younger participants. The variability in this group could be due to the fact that aging might have different



effects on different adults, with some older adults having less cognitive decay than others. Some older participants might also have health problems affecting their hemodynamic responses. Since younger participants were experiencing fewer effects due to aging or pathological reasons, their hemodynamic responses were more consistent. Nonetheless,

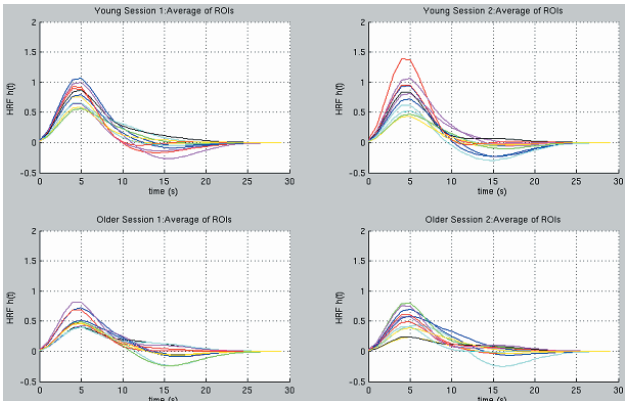


Figure 3: Individual differences in HRFs within each group for scan 1 and 2

the fitted HRFs were generally accurate in predicting the neuronal responses for the two groups.

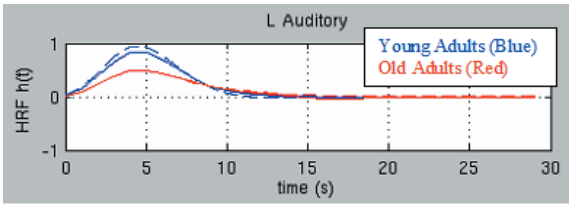
For the HRFs estimated from group-specified ROIs, the mean individual test-retest correlation between the two scan sessions was 0.98 for the young participants and 0.96 for the old. For the raw data, the mean individual test-retest correlation was 0.81 for the young and 0.73 for the old. The raw data showed some discrepancy across the two scan sessions, but nonetheless still displayed very high correlations, indicating that the data collected from this study was consistent for the two scan sessions and thus reliable. In comparison, the HRFs improved upon the correlations from the raw data and appeared to provide a more reliable measure of the hemodynamic responses of the two groups.

The correlations between the HRFs for the regions of activation and the canonical were also very good, averaging 0.94 for the young adults and 0.91 for the older adults. Since

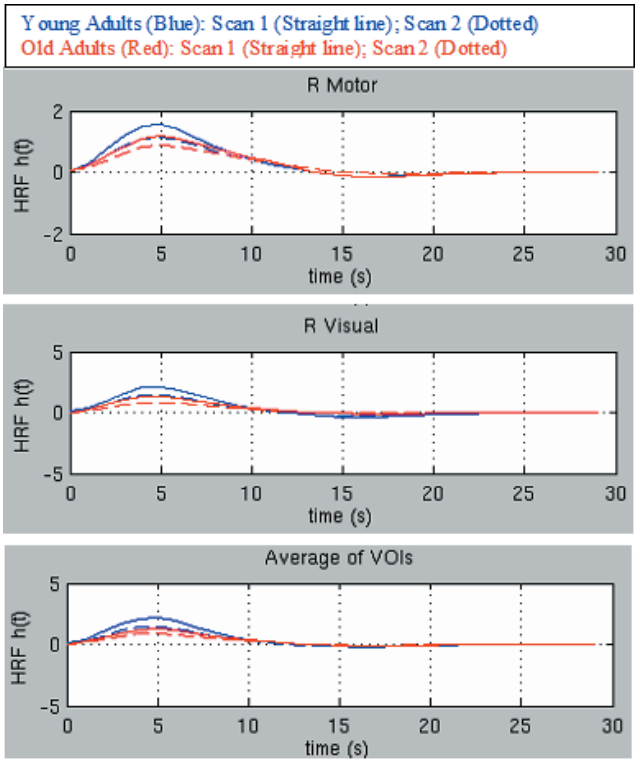
they were similar and high for the two participating groups, the canonical HRF is a good predictor of the HRFs in the different regions, thus implying that that the canonical is useful in research such as adult developmental studies. In addition, the correlations between the HRFs for the different regions of activation were also good, averaging about 0.97 for the young participants and 0.93 for the old. This showed that the neuronal response is similar in different parts of the brain.

All the HRFs exhibited a similar bell-shaped curve that first rises to the peak activation and then slowly dips down and levels off to equilibrium. This was consistent between the old and young adults and also across different brain regions. The similarity in the HRF shape showed that the human hemodynamic response follows a similar trend.

There were individual differences across both groups in peak activation and length of activation. The range in peak



amplitude for the first scan was about 0.5 for the young adults and 0.6 for the old adults. The range for the second scan was around 1.0 for the young and 0.6 for the old. In addition, some participants in both groups reached equilibrium sooner than others. Some also had a small dip in activation after approaching their equilibrium state.



ROI- Peak Amplitudes

Young Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	0.616	0.614	0.874	0.743	0.826	1.03	0.793
Scan 2	0.629	0.622	0.958	0.779	0.799	0.971	0.793
Old Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	0.437	0.430	0.497	0.492	0.577	0.655	0.521
Scan 2	0.452	0.422	0.497	0.482	0.574	0.[illegible]1	0.520

ROI- Time to Peak (seconds)

Young Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	5.50	5.83	5.58	5.50	5.83	5.83	5.75
Scan 2	5.58	5.67	5.50	5.25	5.75	5.83	5.83
Old Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	6.00	6.33	6.00	6.67	5.75	5.92	6.00
Scan 2	6.25	5.92	5.83	6.50	5.83	5.83	5.92

VOI- Peak Amplitudes

Young Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	1.53	1.56	2.89	2.73	2.68	2.08	2.18
Scan 2	1.20	1.22	1.66	1.84	1.73	1.50	1.48
Old Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	1.13	1.17	1.23	1.28	1.46	1.34	1.24
Scan 2	0.954	0.918	0.914	0.879	0.941	0.840	0.886

VOI- Time to Peak (seconds)

Young Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	6.25	6.00	5.75	5.58	5.92	5.92	5.92
Scan 2	6.17	6.17	5.58	5.50	5.83	6.00	5.83
Old Adults							
	Left Motor	Right Motor	Left	Right	Left Visual	Right	
Scan 1	6.33	6.33	5.75	5.83	5.92	5.92	5.92
Scan 2	6.33	6.50	5.83	7.83	5.92	6.17	5.92

Despite the similarity in shape and the individual differences in HRFs within each group, the old and young adults differed in the time to reach peak activation and in the amount and length of activation. In the estimation of the individual HRFs for the ROIs, the older participants took a longer time to reach peak activation, averaging about 6.00 seconds for scan one and 5.92 seconds for scan two, while the young adults took 5.75 seconds for the first scan and 5.83 seconds for the second, although these differences were not statistically significant. The older participants also had a lower peak of activation, indicating that their response to the stimuli was not as high. The average peak amplitude for both scans was 0.52 for the old and 0.78 for the young, which was a significant difference, $t(22) = 3.44$, $p = .002$. In the left auditory cortex, the younger adults had about two times more peak activation than the older adults. The younger adults also reached equilibrium sooner, indicating that their length of activation was shorter. The length of activation was around 11 seconds for the younger adults and 15 seconds for the older adults. This was consistent for both scan sessions.

However, the data collected from the peak voxels within each ROI implied that aging has less influence on hemodynamic responses. The time to reach peak activation was similar and the peak amplitude was also very close for the two groups. For example, in the right motor and visual VOIs for the two groups, the older adult HRFs were very similar to those of the younger participants. The peak activation for older adults in the first scan session (1.24) was close to the peak activation for younger adults in the second scan session (1.48). This could be due to sensory adaptation since the younger participants might have adapted to the stimuli and responded less to them during the second scan. However, the older participants also showed this adaptation since their second scan peak activation was lower than their first scan. Therefore, one can still conclude that aging lowers the amount of activation and increases the length of activation.

Conclusion

This study confirmed the findings of several other similar studies by showing that aging affects indirect measurements of neuronal response. Younger adults in this study had a higher peak of activation and reached peak activation and equilibrium sooner when presented with visual, auditory, and motor stimuli. Older adults had a lower and longer response, suggesting that their mental responsiveness was reduced relative to those of their younger counterparts. However, as discussed in the introduction of this paper, pathological influences related to aging can also affect one's neuronal response. Although the participants in this study were all healthy adults, the older adults might have experienced more small "strokes" or other health issues throughout their lives that reduced the rate of rise and peak hemodynamic responses.

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Computational Modeling and Analysis of the Effects of Retinal Injury on the Formation of Response Feature Maps in the Primary Visual Cortex

Robert Chen

Abstract

Retinal diseases impair vision, making the visual field appear blurry. As a result, properties of visual stimuli are manipulated. The representation of response features of neurons in the primary visual cortex (V1) changes as well. In this investigation, the Kohonen self-organizing map algorithm was used to model the formation of response feature maps under normal conditions as well as under diseased conditions. The following response features were included in the computational models: retinotopy, orientation preference, orientation selectivity, spatial frequency, and ocular dominance. In injured maps formed with disease of radius 2.5 retinotopic units (2.5 pixels, same length as the retinotopy tuning width), gradient magnitude comparison showed no significant difference in the feature maps of normal and injured conditions for maps of orientation selectivity, orientation preference, and ocular dominance. Furthermore, continuity was retained in all maps formed under disease of radii 5 units and 2.5 units. These observations suggest that retinal diseases with radii close in length to the retinotopy tuning width may not create a significant impact on response feature map formation. Also, it was demonstrated that in cases of retinal injury, the visual cortex is able to reorganize itself in areas of the visual cortex analogous to injured areas of the retina. In addition, response feature map formation was modeled for a condition in which stimuli values for orientation selectivity and spatial frequency were chosen from a weighted probability distribution using a Monte Carlo sampling method.

1 Introduction

Computational models can be used to simulate the interactions between neurons in specific parts f the brain; these computational models help us gain insight on the ways in which the brain operates. Many computational models analyze the neocortex, the area of the brain involved in high-level functions such as sensory perception, spatial reasoning, and conscious thought [8]. The primary visual cortex, the area of the neocortex which receives visual input from the retina, is of particular interest.

The primary visual cortex (V1) is one of five main areas of the visual cortex. After V1 receives visual input from the retina, it transmits the information primarily to two pathways: the ventral stream, which is associated with form recognition and object representation (i.e. form vision) and the dorsal stream, which is associated with motion and representation of object locations (i.e. spatial vision) [13]. The neurons in the V1 region act as receptive fields for visual stimuli which are transduced by the retina and relayed to the primary visual cortex. In the brain, the V1 region exists as an intricately folded sheet of interconnected neurons. However, for the purposes of computational modeling, the region is represented by an "unfolded" two-dimensional sheet of neurons.

Neurons in V1 fire after receiving visual input from the retina; each neuron fires at a different intensity in response to a specific stimulus. Cortical maps of neuron firing intensities can be formed. A dimension-reduction model helps explain the organization of neurons according to their response properties. Dimension-reduction models seek to map multi-dimensional cortical response features (e.g. response features to visual stimuli) to a simpler, two-dimensional area. One significant goal of many dimension-reduction models is to bring closer the neighboring neurons which share similar properties [3, 6]. This is achieved by reducing the connection length between neurons. Cortical feature maps are formed based on the intensity of responses to visual input from the retina. Response features which are coded in V1 include: azimuth retinotopic position, elevation retinotopic position, orientation selectivity, orientation preference, ocular dominance, and spatial frequency preference[1].

The mapping of response features on the neocortex is simulated by a self-organizing map.

This is a neural network which provides a visual representation in low dimensions (usually 2 or 3 dimensions) for topological properties which may involve several dimensions [4]. Self-organizing maps are commonly used for modeling of the visual cortex because they selectively update the neurons in the neighborhood region of a given neuron, thus working under the dimension-reduction model. A self-organizing map of V1 gives a representation of the visual field as seen through the eyes.

The stimuli and response features represented in the self-organizing map work to model data from real-life electrophysiological experiments, primarily involving ferret and cat V1 regions. Response feature maps are created via optical imaging of intrinsic signals [5].

Although computational models have been developed for a variety of situations involving cat and ferret V1 regions [3, 6, 7, 10–12], they do not take into account possible situations involving retinal pathology. Retinal diseases involve blurring of vision in certain areas of the visual field. Retinal diseases directly impact the spatial frequency and orientation selectivity response features, as these features are involved in changing the clarity of vision perceived through the retina. Examples of retinal diseases include glaucoma and macular degeneration. Glaucoma involves blurriness in peripheral vision while macular degeneration involves blurriness in certain spots across the visual field.

In this study, computational models for retinal injury were developed and implemented with the computational model of the cortical response feature maps. Several cases involving the retinal injury model were implemented with the self-organizing map algorithm, and it was found that cortical maps can "repair"[2] themselves only if small areas of the retina were affected by the disease (mapping is changed for areas of the visual cortex analogous to the injured region of the retina). In these disease cases, the cortex does not receive visual input from certain areas of the visual field.

Furthermore, it was hypothesized that representation of orientation selectivity and spatial frequency would be different between normal and injured maps (due to blurriness of vision in retinal disease, which implies a reduced ability to discern objects of high orientation selectivity and spatial frequency). This condition was modeled with a Monte Carlo (MC) distribution of stimulus values for the features of orientation selectivity and spatial frequency. In the MC disease case, the cortex receives visual input from the entire visual field; however, there was not a uniform distribution of stimulus values for the aforementioned features (relatively low stimulus values for orientation selectivity and spatial frequency are presented more than are higher values).

2 Methods

2.1 Computational Model - Formation of Cortical Maps

For this investigation, a modified version of the Kohonen self-organizing map algorithm was used [3]. Each stimulus and each receptive field are represented as multicomponent vectors. It is assumed that the stimuli and receptive fields exist in a multi-dimensional feature space.

Let x represent the azimuth retinotopic position; y, elevation retinotopic position; q, orientation selectivity; ϕ, orientation preference; z, ocular dominance; and f, spatial frequency preference.

Each stimulus is represented by the vector

$$V_S = (x_S, y_S, q_S \cos(2\Theta_S), q_S \sin(2\phi_S), z_S, f_S).$$

The stimuli are mapped onto a cortical surface, where each neuron and its receptive field is represented by the vector

$$V_r = (x_r, y_r, q_r \cos(2\phi_r), q_r \sin(2\phi_r), z_r, f_r).$$

In these vectors, the feature x ranges from (0,X), y from (0,Y), q from (0,Q), ϕ from (0,π), q from (0,Z), and f from (0,F). The stimuli are mapped onto a cortical surface with dimensions N x N. Each cortical point was identified as r = (i, j). At the beginning of the simulation, the following parameters were set: $x_r = i$, $y_r = j$, $q_r = Q/2$, $\phi_r = \pi/2$, $z_r = Z/2$, and $f_r = F/2$.

To form the maps, iterations of three steps each are performed. (1) A stimulus is chosen at random from the feature space. (2) The cortical point whose features are most similar to those of the stimulus is identified as the "winner neuron". The winner neuron is determined by finding the neuron which is closest to the stimulus in terms of Euclidean distance. Euclidean distance was determined by the expression $|V_S - W_r|^2$. (3) The winner neuron and the neurons around it are updated by the equation

$$\Delta W_r = \alpha h(r)(V_S - W_r),$$

where α is the learning rate, r is the cortical distance between the winner neuron and any given cortical point (i,j), and h(r) is the neighborhood function. The neighborhood function modifies all neurons on the cortical map. The neurons closest (in terms of cortical distance) to the winner neuron are

updated the most, while neurons farther away from the winner are updated less. The neighborhood function is

$$h(r) = e^{-r^2/\sigma^2}$$

which restricts modifications in receptive fields to cortical points nearby the winner. Usually, the neighborhood function modifies only the area within a radius of σ (in terms of cortical distance) from the winner neuron. σ is referred to as the retinotopy tuning width.

The following parameters were used for the simulation: N=65, $\sigma = 2.5$, $\alpha = 0.02$, X = N, Y = N, Q = 10, Z = 15, F = 15. 100,000 iterations of the algorithm were completed. Periodic boundary conditions were implemented for all cortical maps. Each pixel was analogous to one cortical neuron and each pixel represented one retinotopic unit. The resulting map represented a 65 x 65 sheet of neurons.

2.2 Computational Model - Retinal Injury

Two different disease cases were implemented in this study. Both disease cases model the reorganization of the cortical maps after injury in areas of the retina. For all disease simulations, the same parameters as those used in creating the normal maps were used. All disease simulations were run for 100,000 iterations. In generating the response feature maps, results were averaged over 10 simulations, using a different random number seed for each simulation. For disease cases 1 and 2, a uniform probability distribution of stimulus values for all response features is assumed.

2.2.1 Disease Case 1: Center

Case 1 simulates damage to the center of the retina. A circular area in the center of the visual field does not receive input (the circular area about the cortical point r(33,33). Case 1 uses the same method of map formation as that of the formation of the original cortical maps (see section 2.1). Case 1 was run using the following possible radii: 2.5, 5, 10, 15, 20 units.

2.2.2 Disease Case 2: Scattered

Case 2 simulates damage to several areas of the retina. Circular areas on the retina do not receive visual input. Areas affected are the circular areas within a given radius from cortical points r(16, 16), r(16, 49), r(49, 16), r(49, 49), and r(33, 33). Otherwise, the Case 2 simulation was run under the same conditions as was the normal simulation. Case 2 was run using the following possible radii: 2.5, 5, 15, and 20 units.

2.2.3 Disease with Monte Carlo (MC) Stimulus Sampling

It was predicted that during advanced forms of retinal disease, the eye is unable to discern stimuli with high orientation selectivity and spatial frequency (properties which contribute to the clarity of visual stimuli). The MC disease case models a highly advanced form of retinal disease (as in macular degeneration), in which all associated V1 neurons are affected. To account for the inability of the visual cortex to register stimuli with high values for these features, a Gaussian normal distribution was used to select stimulus values. The stimulus probability distribution was given by

$$p(v) = \left| \frac{e^{-x^2/2\sigma^2}}{\sigma\sqrt{2\pi}} \right|$$

where p(v) represents the probability of choosing a stimulus parameter of v. For orientation selectivity, $\sigma = 3$; for spatial frequency, $\sigma = 5$. Uniform sampling of stimuli values was used for the following properties: retinotopy, orientation preference, and ocular dominance.

2.3 Analysis of Cortical Response Feature Maps

Gradient analysis was performed on the cortical maps. Gradient maps were computed for the response features of orientation preference, orientation selectivity, ocular dominance, and spatial frequency. The gradient vector magnitude for each cortical point was calculated with the expression

$$\sqrt{dx^2 + dy^2}$$

If A(x,y) represents the gradient value at the pixel (x,y), $\delta x = ((A(x+1), y) - A(x, y))$ while $\delta y = (A(x, y + 1) - A(x, y))$. To quantitatively assess the affect of the retinal disease cases on the formation of response feature maps, affected areas of the injured maps were compared against the corresponding areas of normal maps. T-tests were conducted to assess the differences between normal and injured maps for both disease cases and all disease radii. For the t-tests[3], the difference in average gradient magnitude was analyzed for the affected areas of the injured map and the corresponding areas of the normal map. For example, for disease case 1, with a disease of radius of 15, the gradient magnitudes in the circular area lying in the center of the map with radius 15 pixels would be compared with the gradient magnitudes in the analogous area of the normal map.

Gradient relationships between the organizations of normal and injured maps were plotted. A significant difference between normal and injured maps is represented by a strong negative correlation between the gradient percentile of the normal map and the number of pixels of the injured map per each percentile bin. A strong negative correlation signifies that the maps of the two features being analyzed intersect at near-perpendicular angles (hence the inverse relationship). Gradient relationships between maps of different features were analyzed as well. For gradient relationships analyzed, power regression was performed to compare the strength of correlations between normal and affected maps (disease case 1 and 2) and between different feature representations during disease modeled by Monte Carlo disease stimuli sampling.

Coverage uniformity (c') was calculated for all maps. c' was defined as follows. Let $n_{i,j} \in \{-1, 1\}$ be the ocular dominance value of cortical point (i, j); $m_{i,j} \in \{-1, 1\}$ be the

3 See Appendix B

spatial frequency value; $\theta_{i,j}$ the orientation preference in degrees and $x_{i,j}$, $y_{i,j}$ the receptive field position in degrees of visual angle. Let the vector $v = (n_S, m_S, \theta_S, x_S, y_S)$ represent a particular stimulus, and let the vector $w_{i,j} = (n_{i,j}, m_{i,j}, \theta_{i,j}, x_{i,j}, y_{i,j})$ represent the center receptive field values of cortical point (i, j). The total amount of activity, A, evoked in the cortex by a stimulus v, can be written as

$$A(v) = \sum_{i,j \in C} f(v - w_{i,j})$$

where f defines the receptive field of point (i,j) (i.e. the amount of activity evoked as a function of the difference between the stimulus value and the center receptive field values. Next, let $n = ns - ni,j$,$m = ms - mi,j$, $\theta = \theta_S - \theta_{i,j}$, $x = x_S - x_{i,j}$, and $y = y_S - y_{i,j}$. The receptive field was defined as

$$f(n, m, \varphi, x, y) = g(n)g(m)e^{-\varphi^2/2\sigma_\varphi^2} e^{-(x^2+y^2)/2\sigma_r^2}$$

where $g(n) = 1$ if $n = 0$ and $g(n) = 0$ if $n \neq 0$. $\sigma\theta$ and σ_r define the orientation tuning width and the retinotopy tuning width, respectively. A(v) was calculated for a representative set of stimulus values and then coverage uniformity was calculated as c' = standard deviation(A) / mean(A).

Values of A were calculated for all combinations of $n_s \varepsilon \{-1, 1\}$, $m_S \varepsilon \{-1, 1\}$, $\theta_S \varepsilon \{0, 30, 60, 90, 120, 150\}$, $x_S \varepsilon \{1, 5, 9... i_{max}\}$, ys $\varepsilon \{1, 5, 9... j_{max}\}$, where i_{max} and j_{max} are the size in pixels of the map (65 pixels).

Good coverage uniformity is shown by a small value for c'. A small c' value shows that the total amount of activity evoked by the visual cortex is relatively independent of the particular combination of values (i.e. all response features are mapped evenly across the cortex) [14].

3 Results

Quantitative analysis involving the comparison of gradient magnitudes was conducted for all response features in all disease settings. However, in the results section, only the maps for orientation selectivity are presented for all disease situations (Figure 2). It should be noted that the maps of orientation preference, spatial frequency, and ocular dominance all exhibited similar graphical differences in response to retinal injury as did the map of orientation selectivity.

3.1 Normal Conditions

Under normal conditions, the cortical maps exhibit excellent continuity (Figure 1).

3.2 Disease Case 1: Center

The formation of feature maps was simulated in the presence of retinal injuries with radii of 2.5, 5, 10, and 20 units. Cortical maps that were simulated with retinal injuries of radius 10, 15, and 20 units were significantly different from cortical maps simulated in the presence retinal injuries of radius 2.5 units and injuries of radius 5 units. Orientation selectivity maps that were simulated with retinal injuries of radius 2.5 and radius 5 show properties that are similar to the maps formed under normal conditions, suggesting the ability of the visual cortex to reorganize to normal conditions. Cortical maps that were simulated with larger areas of retinal injury did not reform to their normal states. For example, (Figure 2, C) shows that a major portion of the orientation selectivity map analogous to the diseased area of the retina remained the same color as during initial conditions.

Tables 1-4[4] show the differences between gradient magnitudes of injured and normal maps for each disease size. Results of unpaired t-tests show that for disease radii 5 and 15, all features exhibited significantly different gradient magnitudes between the normal and injured feature maps. For disease radius 2.5, only the spatial frequency map exhibited significantly different gradient magnitudes from the normal map.

When gradient relationships between the affected areas of injured and normal maps (see Methods section), a clearly negative correlation was observed in all response features for disease radius 15. For disease radius 5 and 2.5, plots for

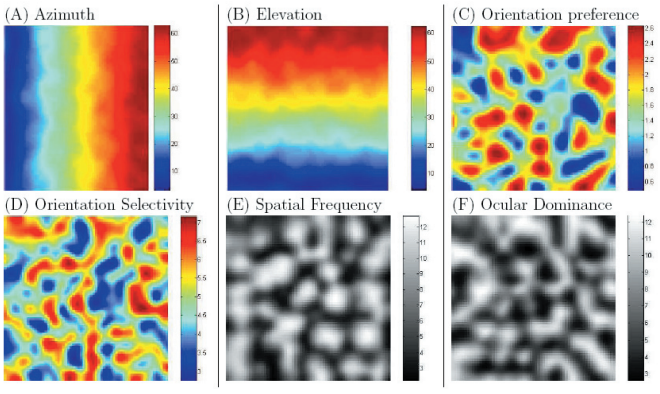


Figure 1: Response feature maps under normal conditions. (A) Azimuth position preference map. (B) Elevation position preference map. (C) Orientation preference map, which ranges from 0 radians to π radians. (F) Low values for ocular dominance indicate preference for contralateral eye, while high values indicate preference for ipsilateral eye.

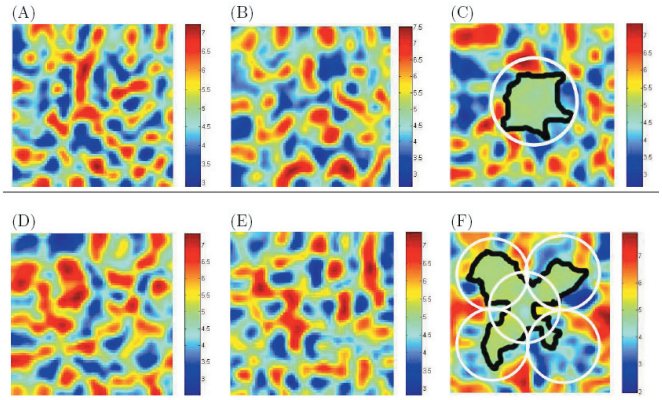


Figure 2: Maps of orientation selectivity formed in disease case 1 (A-C) and disease case 2 (D-F). Maps were formed with disease areas of radius 2.5 (A, D), 5 (B,E), 10 (C,F). Areas of the cortical maps unable to reform after retinal disease are outlined in (C, F). The areas of the maps analogous to injured areas of the retina are outlined in white (C, F).

4 See Appendix B

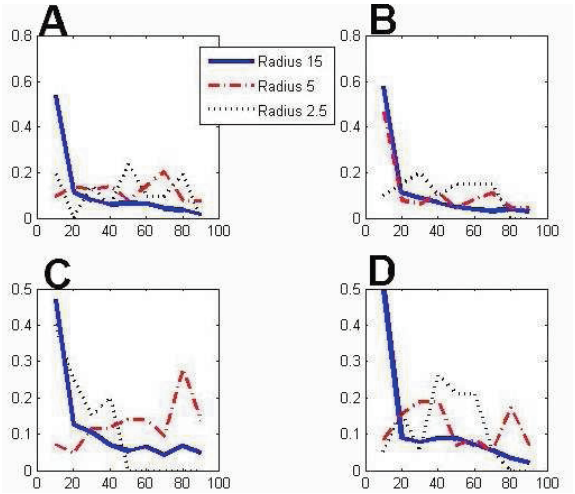


Figure 3: Disease Case 1: Pixels of normal map are grouped into ten bins according to their gradient percentile (x-axis), and the percentage of pixels of the injured map for each percentile group is indicated (y-axis, shown as a fractional value out of 1). A: Orientation selectivity; B: Orientation preference; C: Spatial frequency; D: Ocular dominance.

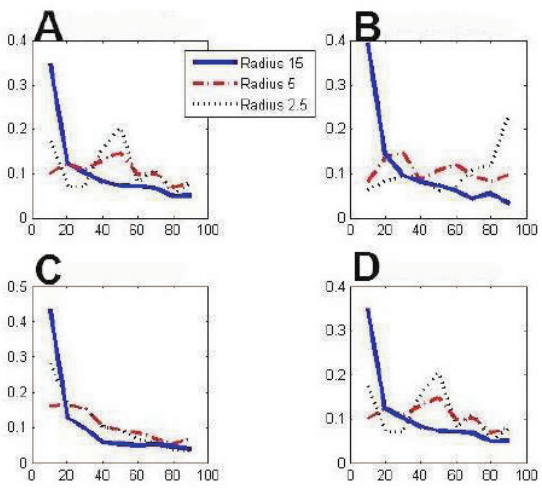


Figure 4: Disease Case 2: Pixels of normal map are grouped into ten bins according to their gradient percentile (x-axis), and the percentage of pixels of injured map for each percentile group is indicated (y-axis, shown as a fractional value out of 1). A: Orientation selectivity; B: Orientation preference; C: Spatial frequency; D: Ocular dominance.

orientation selectivity, orientation preference, and ocular dominance did not show a clear relationship. However, a weak correlation was observed in the spatial frequency plots for disease radius 5 and 2.5, suggesting that the spatial frequency feature is strongly affected by retinal disease, even in cases of small disease size.

3.3 Disease Case 2: Scattered

The formation of cortical response feature maps was simulated with retinal injuries with radii of 2.5, 5, and 20 units. Cortical maps that were simulated with retinal injuries of radius 15 units were significantly different from cortical maps simulated with retinal injuries of radius 2.5 units and radius 5 units (Figure 2 D-F).

The map of orientation selectivity with retinal injury of radius 15 was able to form with a smaller affected area than the initial affected area. However, the injured area did not reform completely, as parts of the affected areas retained the same color as during initial conditions. Also, the affected area of the area of the cortical map was not uniform (Figure 2, F). The injured area around the point r(49, 49) reorganized to a greater extent than the injured areas around the other injury center points.

Tables 5-8[5] show the differences between gradient magnitudes of injured and normal maps for each disease case. As with disease case 1, spatial frequency did not show a significant difference between gradient magnitudes of normal and injured maps; all other features exhibited a significant difference between gradient magnitudes normal and injured maps. This observation is paralleled by the plotting of gradient relationship between normal and injured maps for spatial frequency, which shows that the gradient relationship is similar for all disease sizes. Plots for all other features show that normal and injured maps are significantly different for disease radius 15; for disease radius 2.5 and 5, no clear correlation suggested a significant difference between normal and injured maps (figure 4). For spatial frequency plots, maps from all disease sizes exhibited strong negative correlations between gradient percentile of normal maps and affected percent gradient values per normal percentile bin (power regression; $r^2 > 0.900$ for all disease sizes). For the features of orientation selectivity, orientation preference, and ocular dominance, the plots implied strong differences between gradient percentile plots of normal and affected maps for disease radius 15 only ($r^2 = 0.8038$, 0.8770, and 0.9552, respectively).

3.4 Gradient relationships between response features: case 2

Plotting gradient relationships between different response features, it was shown that spatial frequency and ocular dominance maps do not coincide with each other in a clear relationship. In the normal map, it is suggested that the maps of orientation selectivity and spatial frequency tend to intersect at near-perpendicular angles. The same observation is supported for the following combinations of features: orientation preference vs. orientation selectivity, orientation selectivity vs. ocular dominance (Figure 5).

The plot of orientation preference vs. orientation selectivity is similar in all situations, which suggests that the relationship between orientation preference and orientation selectivity remain unchanged after retinal injury. On the other hand, the plot of orientation selectivity vs. spatial frequency for radius 15 is significantly different from the plots for radius 2.5, radius 5, and normal map. A similar observation was found in the plot of orientation selectivity vs. ocular dominance. Therefore, it is suggested that the relationship between orientation selectivity and spatial frequency as well as the relationship between orientation selectivity and ocular dominance are changed during advanced levels of retinal injury (Figure 5).

5 See Appendix B
6 See Appendix C for maps of other response features

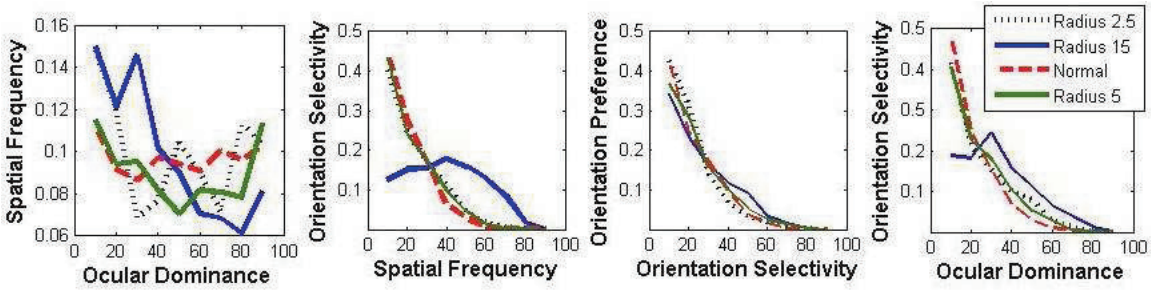


Figure 5: Disease Case 2: Pixels of one specific feature map are grouped into ten bins according to their gradient percentile (x-axis), and the percentage of pixels (of a different feature map) for each percentile group is indicated (y-axis, shown as a fractional value out of 1).

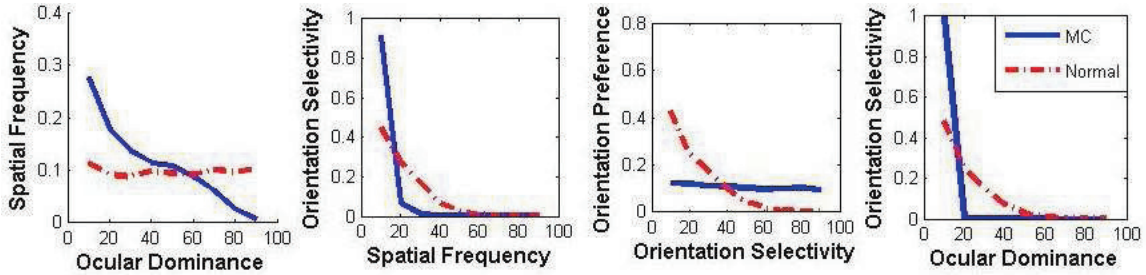


Figure 8: Pixels of one specific feature map are grouped into ten bins according to their gradient percentile (x-axis), and the percentage of pixels (of a different feature map) for each percentile group is indicated (y-axis, shown as a fractional value out of 1).

3.5 General Observations: Disease Case 1 and 2

The injured maps of ocular dominance exhibited similar patterns in organization as did those of orientation selectivity and spatial frequency. However, the maps of orientation preference exhibited more uniform areas affected by retinal injury (Figure 6). For disease case 1 and 2, azimuth and elevation retinotopic position maps did not exhibit any significant differences as compared to the retinotopic position maps formed under normal conditions[6].

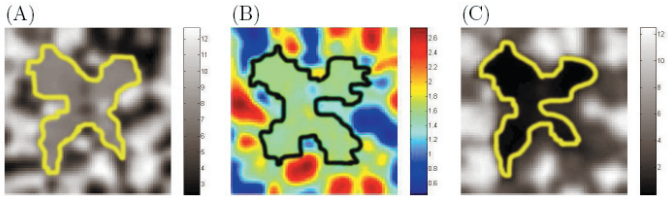
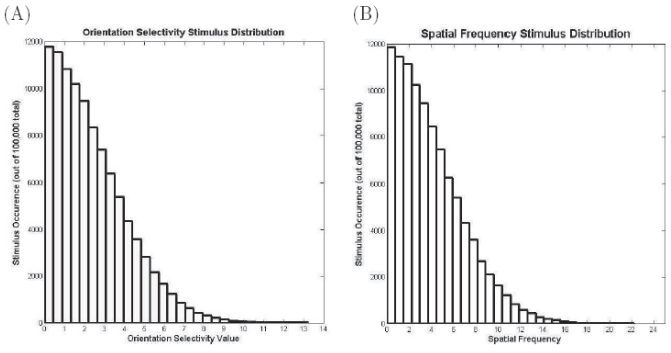


Figure 6: Cortical maps of (A) ocular dominance, (B) orientation preference, and (C) spatial frequency. Diseased areas in (A,B) are outlined. The diseased ae in (C) is shaded. All images are from Disease Case 2, scattered, with injury radius of 15.



(Figure 7: MC disease case: Stimulus value distributions for (A) orientation selectivity and (B) spatial frequency. This distribution shows as the number of occurrences per interval out of 100,000 total stimuli values.

3.6 Disease Model with Monte Carlo (MC) Stimulus Sampling

The response features of orientation selectivity and spatial frequency were sampled according to a Gaussian normal probability distribution. This was done to model the prediction that during cases of retinal injury, relatively high values for orientation selectivity and spatial frequency would be represented less than lower values during feature map formation. The stimulus distribution values for orientation selectivity and spatial frequency are shown in figure 7.

3.7 Gradient relationships between response features: MC

Compared with gradient relationships for normal maps, in MC maps the same gradient relationships between certain response features are significantly different (Figure 8). This suggests that during cases of retinal injury in which spatial frequency and orientation selectivity map formation are affected for the entire visual field (and thereby affecting representation of these features for all associated neurons), certain gradient relationships between response features are affected as well. All of the gradient relationships analyzed are significantly changed in the MC model as compared with the normal model. The data strongly suggests that in cases where orientation selectivity and spatial frequency representation are affected, normal relationships among response features are affected significantly.

7 See Appendix C for maps of other response features

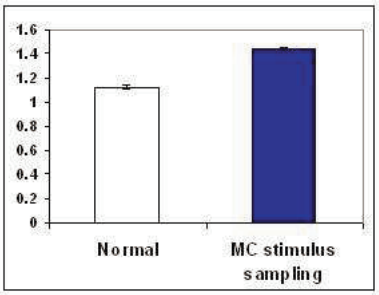


Figure 9: MC disease case: coverage uniformity comparison between normal and MC maps.

3.8 Coverage Uniformity Comparisons

Response feature maps formed under the MC disease case exhibited significantly higher coverage uniformity (c') values than did those formed under normal conditions ($P < 0.0001$, n=10, c'=1.44008 for MC map, c'=1.12508 for normal map) (Figure 9). The data suggests that the MC disease map is not optimized for uniform coverage. Coverage uniformity analysis was performed for disease cases 1 and 2, but the results did not exhibit significant differences between maps of different disease sizes[7].

4 Discussion

In this investigation, it was shown that normal cortical maps can form even in the absence of visual input from small areas of the retina. It was found that retinal injuries with a radius less than 5 units did not impact the continuity in the formation of the cortical map. Cortical maps that were formed under both disease cases with radii of 2.5 units and 5 units retained similar structure to the map formed under normal conditions. Although there was a difference in the arrangement of intensity "blotches" (enclosed areas of the cortical map which represent neurons firing at similar intensities (e.g. blotches of red which represent neurons firing at high intensity) within the cortical maps of orientation selectivity for normal and diseased cases, the overall structure of diseased maps (disease radius 2.5 and 5) retained continuity.

Through analysis of differences between gradient magnitudes of normal and injured maps, it can be confirmed that the features of orientation selectivity, orientation preference, and ocular dominance are not affected dramatically with a disease of radius 2.5. However, with a disease of radius 2.5, spatial frequency maps are still affected significantly as compared with the normal feature map. This observation suggests that the spatial frequency feature map is affected even in situations in which the retinal disease size is small. Furthermore, it is evident that the spatial frequency feature is affected significantly following retinal injury. The results of gradient magnitude comparisons show significant differences in all features between the maps of disease radius 5 versus those of the normal map (which suggests that injury of radius 5 significantly affects response feature map formation. However, gradient plots do not suggest a clear distinction between the normal and affected maps of disease radius 5. Further analysis is necessary to determine the effects of retinal disease of radius 5.

The concept of continuity is pivotal in interpreting the results of this investigation. In this investigation, continuity refers to "the desire to represent all input features smoothly" [2]. In (Figure 2A) the center area is continuous because the neurons in the center primarily fire at intensities on the higher end or the lower end; this pattern is represented throughout the entire cortical map. However, in (Figure 2C) the center area is not continuous because the neurons primarily fire at the same intensity as during initial conditions. These neurons primarily fire in the middle of the range of possible intensities; this pattern is different from those which are exhibited in other areas of the map.

Coverage uniformity is also important in interpreting the effects of retinal disease [14]. Since it has been shown that cortical maps optimize (i.e. minimize) coverage uniformity, we predicted that diseased maps would show a higher, less optimal coverage uniformity. Our hypothesis was substantiated in the MC disease case but not in disease cases 1 and 2, for which only certain areas of the visual cortex were affected.

Previous studies have shown that certain response features bear strong relationships with each other [1, 5, 7, 11, 12]. It has been shown that there is a strong negative correlation between the map formations of orientation preference and spatial frequency. Furthermore, it has been found that the representation of orientation preference map formation influenced the representation of ocular dominance map formation [3]. Based on these data, Yu et al. proposed that the following combinations of response features are represented such that one feature influences the other: orientation preference and orientation selectivity, orientation selectivity and spatial frequency, orientation selectivity and ocular dominance. It can be hypothesized that since certain features influence the formation of response feature maps of other features, the elimination of a feature from the study could impact the maps of other response features. It can also be proposed that the feature maps which formed as a result of elimination of other features will form differently in the presence of a retinal injury. Future studies should investigate these concepts.

Future models of retinal disease should manipulate the features of orientation selectivity and spatial frequency. Although it was hypothesized that orientation selectivity representation would be strongly affected by retinal disease, results of analysis for disease cases 1 and 2 do not provide conclusive evidence that orientation selectivity is substantially affected by retinal disease. The MC disease case models the prediction that representation of orientation selectivity and spatial frequency would be strongly affected by retinal disease. Since results of disease cases 1 and 2 suggest that orientation selectivity representation is not strongly affected by retinal disease, experimental studies should be conducted which confirm this observation.

7 For disease cases 1 and 2, since only the affected areas were analyzed for coverage uniformity, we propose that the lack of a general trend in coverage uniformity differences was due in part to the lack of variability of represented stimuli values in the injured areas.

5 Conclusion

This study suggests that small retinal injuries may not necessarily impact the function of the primary visual cortex in adapting to visual input. This study also suggests that certain response features are represented differently in terms of neuronal activity in the primary visual cortex. Lastly, this study shows that certain relationships between response features are preserved after retinal injury. The results of this study can be confirmed by studies analyzing the electrophysiological responses in areas of the visual cortex analogous to the affected visual field. This investigation serves as a predicative model for future experiments regarding response feature map formation in response to stimuli distributions as well as stimuli locations as exhibited in retinal disease. Furthermore, the results of this study can be applied to the development of possible treatments for disease in the primary visual cortex as well as in the broader neocortex.

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Appendices

A Definitions

The response features of the visual cortex include: orientation preference, orientation selectivity, spatial frequency, and ocular dominance.

Orientation selectivity is a feature which involves the detection of local bars and edges in the processed visual images, as well as their orientations [10].

Orientation preference refers to the angle of the presented stimulus at which the neurons respond most.

Spatial frequency refers to how much a periodic structure repeats per unit of distance in the visual field. For example, the sine-wave grating is used as a means for assessing spatial frequency. Sine-wave gratings which appear blurry have low spatial frequency; distinct, clear sine-wave gratings have high spatial frequency [13].

Ocular dominance refers to the preference to receive visual input from a particular eye. Low values for ocular dominance indicate the preference to receive input from the contralateral eye, while high values indicate preference to receive input from the ipsilateral eye. [13].

B Gradient Magnitude Comparison Results

Tables 1-8 show the gradient magnitude comparisons, which were performed with unpaired t-tests. A * denotes a significant difference between normal and injured maps. Tables 1-4 display results from disease case 1, while tables 5-8 display results from disease case 2.

Table 1: Disease Case 1: Orientation Selectivity

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	0.6983	0.2498	0.0755
5	0.5175	0.4115	0.0425*
15	0.4370	0.2032	$P < 0.0001*$

Table 2: Disease Case 1: Orientation Preference

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	0.2270	0.1857	0.1170
5	0.2167	0.1471	$P < 0.0001*$
15	0.1672	0.0762	$P < 0.0001*$

Table 3: Disease Case 1: Spatial Frequency

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	1.5463	0.1471	$P < 0.0001*$
5	1.1631	1.5569	0.0004*
15	1.2174	0.7707	$P < 0.0001*$

Table 4: Disease Case 1: Ocular Dominance

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	0.7884	0.6838	0.2067
5	0.7977	1.5569	$P < 0.0001*$
15	1.0450	0.5457	$P < 0.0001*$

Table 5: Disease Case 2: Orientation Selectivitv

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	0.3724	0.3960	0.4422
5	0.4021	0.3506	0.0010*
15	0.4191	0.3104	$P < 0.0001*$

Table 6: Disease Case 2: Orientation Preference

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	0.1418	0.1765	0.0623
5	0.1678	0.1402	0.0071*
15	0.1748	0.1014	$P < 0.0001*$

Table 7: Disease Case 2: Spatial Frequency

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	1.3190	0.9359	$P < 0.0001*$
5	1.1443	0.9413	$P < 0.0001*$
15	1.0827	0.6916	$P < 0.0001*$

Table 8: Disease Case 2: Ocular Dominance

Disease Radius	Average Gradient Magnitude - Normal	Average Gradient Magnitude - Injured	P-value
2.5	0.9562	0.6770	0.0590
5	0.8595	0.6702	0.0235*
15	1.0309	0.6971	$P < 0.0001*$

C Response Feature Maps After Injury

The following response feature maps were obtained for disease cases 1 and 2. The response feature maps for all features exhibited similar patterns. All injuries of radius 2.5 and 5 did not significantly impact continuity in the formation of cortical response feature maps.

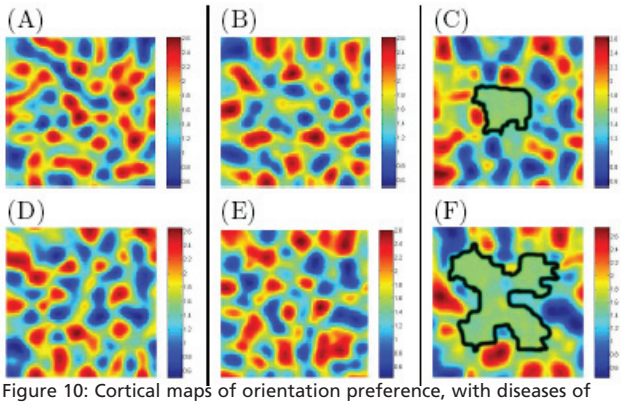

Figure 10: Cortical maps of orientation preference, with diseases of radius 2.5 (A,D), 5 (B,E), and 15 units(C,F).

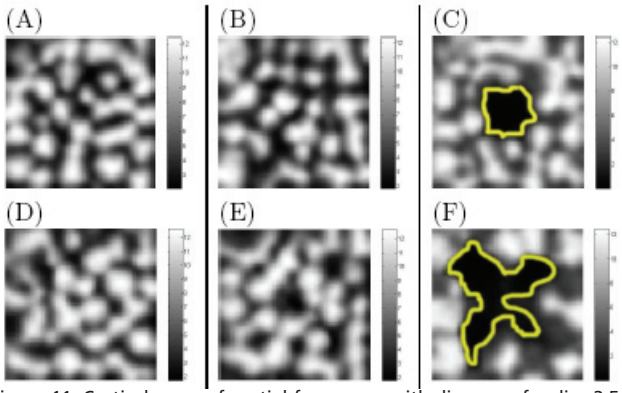

Figure 11: Cortical maps of spatial frequency, with diseases of radius 2.5 (A,D), 5 (B,E), and 15 units(C,F).

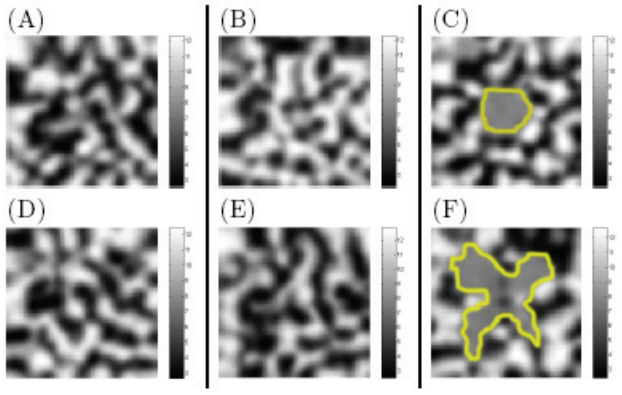

Figure 12: Cortical maps of ocular dominance, with diseases of radius 2.5 (A, D), 5 (B, E), and 15 units (C, F).